EFFECTS OF UV RADIATION ON ANTARCTIC BENTHIC ALGAE – WITH EMPHASIS ON EARLY SUCCESSIONAL STAGES AND COMMUNITIES

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EFFECTS OF UV RADIATION ON ANTARCTIC BENTHIC ALGAE – WITH EMPHASIS ON EARLY SUCCESSIONAL STAGES AND COMMUNITIES

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A journey of a thousand miles begins with a single step – Lao Tsu

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
C	closed cage
CPD(s)	cyclobutane pyrimidine dimer(s)
CPD Mb^{-1}	amount of cyclobutane pyrimidine dimers per million bases
DNA	deoxyribonucleic acid
ETR_{max}	maximal relative electron transport rate
FS	full sunlight control treatment
F_v/F_m	optimal quantum yield of PSII in dark-acclimated state
$\Delta F/F_m'$	effective quantum yield of PSII in light-acclimated state
HC	half cage control treatment
HPLC	high performance liquid chromatography
J	Joule
λ_{max}	absorption maximum
MAA	mycosporine-like amino acid
nm	nanometer
O	open cage
PAR	photosynthetically active radiation (400 - 700 nm)
P	PAR
PA	PAR + UV-A
PAB	PAR + UV-A + UV-B
PAM	pulse-amplitude modulated fluorometer
PFD	photon flux density
P-I curve	photosynthesis-irradiance curve
PS I	photosystem I
PSII	photosystem II
PSCs	polar stratospheric clouds
SD	standard deviation
SE	standard error
UVR	ultraviolet radiation
UV-A	ultraviolet-A (315 nm - 400 nm)
UV-B	ultraviolet-B (280 nm - 315 nm)
UV-C	ultraviolet-C (100 nm - 280 nm)
UV_{ery}	erythema weighted UV dose
µm	micrometer
µmol	micromoles
Water-PAM	Water pulse-amplitude modulated fluorometer
W	Watt
λ	wavelength

SUMMARY

Benthic marine macro- and microalgae are very important primary producers in coastal ecosystems, serving as habitat for many organisms and as food for different types of animals. Especially intertidal communities are exposed to a variety of stressful conditions during the change of the tide levels (desiccation, changing light climate and temperature etc.). Particularly Antarctic organisms suffer from strong seasonal changes in the radiation climate. After a long period of darkness during the austral winter, they are suddenly exposed to high solar radiation after sea ice break-up in spring. The water transparency is highest at this time of the year and solar radiation can penetrate quite deep into the water column, thereby affecting also subtidal organisms. This is further intensified by the fact that the stratospheric ozone depletion, increasing the UV-B on the Earth's surface coincides with spring conditions, additionally stressing the organisms. For example, in spring in the study area photosynthetically active radiation (PAR = 400 – 700 nm), ultraviolet- A (UV-A = 315 – 400 nm) and UV-B radiation (280 – 315 nm) penetrated down to > 20, 19 and 16 m (1% of surface values), respectively, into the water body.

UV radiation (UVR = 280 – 400 nm) impairs a variety of biological processes in algae, causing e.g. DNA and protein damage and a reduction in photosynthetic efficiency which can result in reduced growth and reproduction. It was therefore hypothesized that UVR also affects ecosystem structure. However, UVR research on marine algae has hitherto focussed mainly on physiological effects at the organism level (macroalgae) or on soft-bottom communities and phytoplankton (microalgae). Field-experiments on benthic hard bottom communities are scarce, particularly in the Antarctic region, where ozone depletion and consequently the increase in UV-B radiation are highest. The present thesis aims to detect UVR effects on benthic algal communities at King George Island, Antarctica, by combining laboratory approaches and field-experiments. The study focused on the UV susceptibility of the early successional stages of macro- and microalgae.

In field experiments the interactive effects of UVR and grazing on early life stages of a hard bottom algal community were studied. In a two-factorial design, experimental units (1. ambient radiation, >280 nm; 2. ambient minus UV-B, >320 nm; 3. ambient minus UVR, >400 nm vs. grazer – no grazer) were installed for 2.5 and 3.5 months in the field, respectively (n = 4 plus controls). The results showed a reduction in both, macro- and microalgal biomass due to grazing. The most important grazer was the limpet *Nacella concinna* which on the other hand increased macroalgal richness and diversity due to an enlarged spatial heterogeneity of the system.

While microalgal biomass and species composition were unaffected by UVR, both UV-A and UV-B radiation negatively affected macroalgal succession. UVR effects were species-specific and changed over time. UVR decreased the density of green algal recruits in the first 10 weeks of the experiment, whereas the density of red algal recruits was significantly depressed by UVR at the end of the study. Strongest effects were found at the end of the experimental period when macroalgal diversity and species richness were significantly higher in UV depleted than in UV exposed assemblages. Furthermore, species composition differed significantly between the UV depleted and the UV exposed treatment. Different species in the assemblage possess different adaptation mechanisms, leading to species-specific responses to UVR.

Laboratory experiments with subtidal microalgal soft-bottom communities showed transient negative effects on photosynthetic efficiency and cell number, which, however, disappeared after 2 weeks of repeated exposure or after 4 h under darkness. A decrease in the photosynthetic efficiency seems to be the most frequently observed short-term effect for benthic microalgae, also in the presented studies. But microalgae apparently developed acclimation and recovery strategies to cope with high irradiance and especially high UVR as no permanent negative effects could be observed. Parameters like growth and biomass were generally unaffected by UVR. The ability to acclimate to UVR induced stress seems to be crucial for organisms inhabiting a wide range of water depths.

The negative effects of UVR and possible recovery mechanisms have previously been investigated on adult macroalgae or propagules from Arctic or cold-temperate species but not on Antarctic reproductive cells. The unicellular propagules were shown to be the most UV sensitive stage in the life history of seaweeds. In the present study, effects of UVR on the DNA and photosynthesis of intertidal and subtidal macroalgal propagules were tested in the laboratory during exposure to different light treatments (PAR, PAR + UV-A and PAR + UV-A + UV-B). Furthermore, their ability to recover after UV induced stress was investigated.
All Antarctic macroalgal propagules were low light adapted, a feature generally observed in reproductive cells from other geographical regions. Propagules of the intertidal species *Adenocystis utricularis* (brown alga), *Monostroma hariotii* (green alga), *Porphyra endiviifolium* (red algae) and *Iridaea cordata* (red algae), as well as of the subtidal species *Ascoseira mirabilis* (brown alga) and *I. cordata* (tetraspores) were investigated. In all but one species (*A. utricularis*) an exposure to PAR alone already reduced photosynthetic efficiency. UVR further decreased optimum quantum yield in all species with the species from the upper intertidal (*P. endiviifolium*) being least affected by UVR. Intertidal species generally showed higher recovery rates than subtidal ones especially after pre-exposure to UV-B radiation. Photosynthetic efficiency of *A. mirabilis* and *I. cordata* propagules did not fully recover after 48 h under dim

white light after exposure to 4 and 8 h of UV-B while spores and gametes from the intertidal species did not show any significant differences between the treatments after recovery.
DNA damage was minimal in intertidal species and highest in zoospores of *A. mirabilis* from the subtidal. However, also this species had a high ability to repair DNA damage.

Generally, UVR effects on macroalgal propagules were species-specific and followed the zonation patterns of the respective macrothallus at the coastline. Variations in responses to UV exposure within one species was also shown for *I. cordata* tetraspores. Tetraspores from the intertidal had a better photoprotection compared to its subtidal counterpart and showed a better recovery after UV-B exposure. Adaptation to the extreme environmental condition in the intertidal might be responsible for the broader vertical distribution pattern (from the eulittoral to sublittoral zone) of this species. Propagules of the intertidal species obviously possess good repair and protective mechanisms. Adult brown and red macroalgae can produce UV absorbing phlorotannins or mycosporine-like amino acids (MAAs) providing partial protection against harmful UVR. In *I. cordata* tetraspores two kinds of MAAs were found (shinorine and palythine). The MAA concentrations were higher in tetraspores treated with UVR than in spores exposed to PAR only, indicating a possible protective role of these substances already in early life-stages. DNA damage can be repaired e.g. by photolyase and the ability of the propagules to cope with UV-B induced DNA damage seems to be crucial for the vertical zonation of the macrothalli at the coastline. If not repaired, DNA lesions can disrupt metabolism, cell division and impair growth and germination. Reproductive cells from intertidal species were well adapted to the UV doses applied in the laboratory. Adaptation and acclimation to the extreme environmental conditions are a precondition for the ecological success of macroalgal species in the intertidal, while spores from the subtidal are more sensitive to UVR but are also more protected by the absorption of the water body.

In conclusion, the results showed that the ambient UVR do not seem to be a threat to benthic marine Antarctic diatoms while grazers acted as important drivers on the complete intertidal algal community structure. In contrast, UV-B radiation significantly shaped macroalgal diversity and species composition. Limpets could mediate negative effects of ambient UVR on species richness and diversity to a certain level. On the basis of these results we hypothesise that ambient UV-B radiation, and a potential further increase of these wavelengths has the ability to affect the zonation, composition and diversity of Antarctic intertidal macroalgae altering trophic interactions in this system. Whether these findings persist during later succession requires further studies.

ZUSAMMENFASSUNG

Benthische, marine Makro- und Mikroalgen sind wichtige Primärproduzenten in Küstenökosystemen. Sie stellen sowohl Habitate, als auch Nahrungsquelle für verschiedene Tierarten dar. For allem eulitorale Gemeinschaften sind durch den Gezeitenhub einer Vielzahl von Stressfaktoren ausgeliefert (Austrocknung, schwankende Lichtbedingungen, Temperaturen etc.). Antarktische Organismen sind besonders starken saisonalen Schwankungen des Lichtklimas ausgesetzt. Nach einer langen Dunkelphase im Südwinter werden sie plötzlich einer hohen Strahlung nach Aufbrechen des See-Eises im Frühling ausgesetzt. Die Wassertransparenz ist zu dieser Jahreszeit am größten, dadurch kann die solare Strahlung tief in die Wassersäule eindringen und somit auch sublitorale Organismen beeinflussen. Diese Tatsache wird durch den stratosphärischen Ozonabbau und die damit verbundene Zunahme an UV-B Strahlung auf der Erdoberfläche zusätzlich verstärkt. Im Untersuchungsgebiet konnte z.B. die photosynthetisch aktive Strahlung (PAR = 400 – 700 nm), die Ultraviolet-A (UV-A = 315 – 400 nm) und die UV-B Strahlung (280 – 315 nm) bis zu > 20, 19 bzw. 16 m (1% der Oberflächenwerte), in den Wasserkörper eindringen.

UV-Strahlung (UVR = 280-400 nm) beeinträchtigt eine Vielzahl von biologischen Prozessen in Algen, so werden z.B. die DNA und Proteine geschädigt und die Photosynthese-Effizienz nimmt ab, was wiederum in einer reduzierten Wachstums- und Fortpflanzungsrate resultieren kann. Es ist daher anzunehmen, dass UV-Strahlung auch die Struktur von Ökosystemen verändert. In der Vergangenheit hat sich die Erforschung der Einflüsse von UV-Strahlung auf marine Algen hautsächlich mit physiologischen Effekten auf Organismus-Level (Makroalgen) oder mit Weichboden-Gemeinschaften und Phytoplankton (Mikroalgen) befasst. Feld-Experimente mit benthischen Hartboden-Gemeinschaften sind selten, vor allem in der Antarktis, wo der Ozonabbau und die Erhöhung der UV-B Strahlung am größten sind. Die vorliegende Arbeit hat sich zum Ziel gesetzt UV-Effekte auf benthische Algen-Gemeinschaften auf der King George Insel (Antarktis) zu erforschen. Dabei wurden Labor- mit Feldexperimenten kombiniert. Der Schwerpunkt der Arbeit lag auf der UV-Empfindlichkeit junger Makro- und Mikroalgen-Gemeinschaften und früher Entwicklungsstadien.

In Feldexperimenten wurden interaktive Effekte von UV-Strahlung und Fraßdruck (grazing) auf junge Stadien einer Hartboden-Gemeinschaft untersucht. In einem zweifaktoriellen Ansatz wurden 32 Versuchseinheiten (n = 4 plus Kontrollen) für 2,5 und 3,5 Monate im Feld ausgebracht (1. natürliche Strahlung, >280 nm; 2. natürliche minus UV-B Strahlung, >320 nm; 3. natürliche minus UV-Strahlung, >400 nm; *versus* Grazer – keine Grazer). Die Ergebnisse zeigten eine Verringerung der Gesamtalgen-Biomasse

durch grazing. Der wichtigste Grazer war die Napfschnecke *Nacella concinna*, die wiederum die Diversität und den Artenreichtum der Makroalgen vergrößerte, indem sie die Heterogenität des Systems erhöhte.

Während die Mikroalgen-Biomasse und deren Artzusammensetzung nicht von der UV-Strahlung beeinflusst waren, führten UV-A und UV-B Strahlung zu einer negativen Beeinflussung der Sukzession der Makroalgen. Die UV-Effekte waren artabhängig und veränderten sich über die Zeit. Die Dichte der Grünalgenkeimlinge wurde innerhalb der ersten 10 Wochen des Experiments durch UV-Strahlung verringert, während die Dichte der Rotalgenkeimlinge am Ende des Experiments reduziert wurde. Die stärksten UV-Effekte wurden am Ende der Studie gefunden. Die Diversität und der Artenreichtum der Makroalgen waren in der Behandlung ohne UV-Strahlung im Vergleich mit den Gemeinschaften die UV-Strahlung ausgesetzt waren signifikant höher. Weiterhin war die Artzusammensetzung zwischen den UV und nicht-UV Behandlungen signifikant verschieden. Einzelne Arten in der Gemeinschaft besitzen unterschiedliche Adaptionsmechanismen, welche zu den artspezifischen Unterschieden nach UV-Behandlung führten.

Die Laborexperimente mit sublitoralen Mikroalgen-Weichboden-Gemeinschaften zeigten vorübergehende negative Effekte auf die Photosynthese-Effizienz und die Zellzahl. Diese verschwanden allerdings nach 2 Wochen wiederholter UV-Bestrahlung, bzw. nach 4 h Erholung in Dunkelheit. Abnahmen in der Photosynthese-Effizienz scheinen die am meisten beobachteten Kurzzeit-Effekte durch UV-Strahlung zu sein. Dies wurde auch durch die vorliegende Arbeit bestätigt. Mikroalgen besitzen offensichtlich gute Akklimatisations- und Erholungsmechanismen gegen hohe Strahlungsintensitäten (im speziellen hohe UV-Strahlung), da keine dauerhaften negativen Effekte auf die Mikroalgen-Gemeinschaften festgestellt werden konnten. Parameter wie Wachstum und Biomasse wurden prinzipiell nicht von UV-Strahlung beeinflusst. Diese Fähigkeit der Akklimatisation an durch UV-Strahlung ausgelösten Stress, scheint für Organismen, die einen großen Tiefenbereich besiedeln, entscheidend zu sein.

Die negativen UV-Effekte und mögliche Erholungsmechanismen wurden bisher hauptsächlich an adulten Makroalgaen oder Keimlingen aus der Arktis und kalt-gemäßigten Zonen durchgeführt, aber nicht an Fortpflanzungszellen antarktischer Algen. Die einzelligen Fortpflanzungszellen sind die am UV-sensitivsten Stadien im Lebenszyklus von Makroalgen. Daher wurden in der vorliegenden Arbeit die Effekte von UV-Strahlung auf die DNA und die Photosynthese von eu- und sublitoralen Makroalgen-Keimlingen in Laborversuchen getestet. Dafür wurden diese verschiedenen Lichtbedingungen ausgesetzt (PAR, PAR + UV-A und PAR + UV-A + UV-B). Weiterhin wurde ihre Fähigkeit, sich von UV-induziertem Stress zu erholen, untersucht.

Prinzipiell zeigte sich eine Schwachlicht-Adaptation für alle untersuchten Arten. Dies wurde grundsätzlich auch für Fortpflanzungszellen anderer geographischer Regionen festgestellt. Keimlinge der eulitoralen Arten *Adenocystis utricularis* (Braunalge), *Monostroma hariotii* (Grünalge), *Porphyra endiviifolium* (Rotalge) und *Iridaea cordata* (Rotalge) sowie der sublitoralen Arten *Ascoseira mirabilis* (Braunalge) und *I. cordata* (Rotalge) wurden untersucht. In allen Arten außer *A. utricularis* hatte bereits eine Exposition im PAR-Licht eine Reduktion der Photosynthese-Effizienz zur Folge. In allen Arten wurde die optimale Quantenausbeute zusätzlich durch UV-Strahlung inhibiert, dabei waren Monosporen von *P. endiviifolium* aus dem oberen Eulitoral am wenigsten beeinträchtigt. Generell zeigten die eulitoralen Arten eine höhere Erholungsrate als sublitorale Arten, vor allem nach vorangehender Bestrahlung mit UV-B Licht. Nach 48 h unter Schwachlicht erholte sich die Photosynthese-Effizienz von *A. mirabilis* und *I. cordata* Fortpflanzungszellen aus dem Sublitoral nach vorausgegangener UV-B Bestrahlung für 4 und 8 h nicht vollständig. Sporen und Gameten von eulitoralen Algen hingegen zeigten keinen signifikanten Unterschied zwischen den einzelnen Behandlungen nach der Erholungsphase. DNA-Schäden in eulitoralen Algenarten waren gering und am höchsten in Zoosporen der sublitoralen Braunalge *A. mirabilis*. Allerdings zeigt diese Art eine hohe DNA-Reparaturrate.

Grundsätzlich waren UV-Effekte auf Makroalgenkeimlinge artspezifisch und folgten der Zonierung der adulten Thalli an der Küstenlinie. Tetrasporen von *I. cordata* zeigten unterschiedliche Antworten auf UV-Bestrahlung innerhalb einer Art. Tetrasporen aus dem Eulitoral hatten einen besseren Schutz und zeigten eine bessere Erholung im Vergleich zu den Individuen aus dem Sublitoral nach UV-B Bestrahlung. Die Adaptation an die extremen Bedingungen im Eulitoral könnten für die größere vertikale Verbreitung dieser Art verantwortlich sein (vom Eulitoral bis ins Sublitoral). Keimlinge aus dem Eulitoral besitzen offensichtlich gute Reparatur- und Schutzmechanismen. Adulte Braun- oder Rotalgen können UV-absorbierende Substanzen wie Phlorotanninen oder Mykosporin-ähnliche Amisnosäuren (MAAs) bilden, die sie teilweise gegen schädliche UV-Strahlung schützen können. In Tetrasporen von *I. cordata* wurden zwei Klassen MAAs gefunden (Shinorine und Palythine). Die Konzentration der MAAs war in mit UV-bestrahlten Tetrasporen höher als in denen, die nur PAR ausgesetzt waren. Dies spricht für eine mögliche Schutzfunktion der MAAs schon während der frühen Lebensphase der Algensporen. DNA-Schäden können z.B. durch Photolyase repariert werden und die Fähigkeit der Keimlinge auf durch UV-B Strahlung induzierte DNA-Schäden zu reagieren scheint entscheidend für die vertikale Zonierung der Makrothalli an der Küstenlinie zu sein. Wenn DNA-Schäden nicht repariert werden, kommt es zu einem gestörten Metabolismus und Zellteilung, dadurch können Wachstum und Keimung beeinträchtigt werden. Fortpflanzungszellen von eulitoralen Arten waren prinzipiell gut and die im Versuch angewandten UV-Dosen angepasst. Adaptation und Akklimatisation an extreme Umweltbedingungen sind eine entscheidende

Voraussetzung für den ökologischen Erfolg der verschiedenen eulitoralen Makroalgenarten. Keimlinge aus dem Sublitoral sind UV-sensitiver, da sie durch die Absorption des Wasserköpers geschützt sind. Eine Zunahme der UV-Strahlung und ein tieferes Eindringen in den Wasserkörper könnte aber Auswirkungen auf das Überleben der einzelnen Arten haben.

Prinzipiell scheint die natürliche UV-Strahlung keine Gefahr für die benthischen Mikroalgengemeinschaften darzustellen. Grazer hingegen beeinflussten die gesamte eulitorale Gemeinschaftsstruktur. Im Gegensatz zu den Mikroalgen wurden die Diversität und die Artzusammensetzung der Makroalgen signifikant von UV-Strahlung reduziert. Napfschnecken konnten diesem negativen UV-Effekt bis zu einem gewissen Grad entgegenwirken. Aufgrund dieser Ergebnisse nehmen wir an, dass die aktuelle UV-B Strahlung sowie eine potentielle weitere Erhöhung dieser Wellenlängen die Fähigkeit besitzt die Zonierung, Zusammensetzung und Diversität antarktischer Makroalgen zu beeinflussen. Dies führt zu einer Veränderung der trophischen Interaktionen in diesem System. Ob diese Ergebnisse auch für ältere antarktische Gemeinschaften Bestand haben bedarf weiterer Forschung.

1 INTRODUCTION

1.1 MARINE ALGAL COMMUNITIES

Benthic primary producers (e.g. macrophytes and benthic microalgae) are of great importance for the stability of coastal ecosystems and are responsible for ca. 10% of the global total carbon production (Mann & Chapman 1975). Habitats of macroalgae (multicellular Rhodophyta, Chlorophyta and Heterokontophyta) are rocky intertidal and subtidal shores all over the world where they can form dense underwater forests. The largest macroalgae can reach a length of around 60 m. On the other hand, the autotrophic component of the microbenthic community, the microphytobenthos, consists of unicellular algae living on or in the sediment. This communities are usually dominated by diatoms and cyanobacteria. In shallow water areas (estuaries) benthic microalgal communities can account for a substantial part (ca. 50%) of the total primary productivity (Underwood & Kronkamp 1999). Marine benthic algae (both macro- and microalgae) play further an essential role for many marine animals, providing food and shelter (Hay & Fencial 1992; Mallin et al. 1992). They are directly consumed by grazers, which can thus alter the structure and species composition of algal communities (Duffy & Hay 2000). Algal exudates can be used by bacteria and decomposing algae can e.g. effect survival of animals indirectly by producing anoxic conditions (Bischof et al. 2006).

Especially intertidal algae can be exposed regularly to extreme changes in abiotic parameters due to e.g. tidal influences (reviewed in Davison & Pearson 1996). During low tide organisms are exposed to high solar irradiance, atmospheric temperatures, low salinities and desiccation. Furthermore, mechanical stress due to tide currents can be very high. In the subtidal zone benthic algae encounter a more stable habitat, as the water column above works as a buffer against strong changes in abiotic conditions (Lüning 1985; Bischof et al. 2006).

In Antarctic habitats tidal algal assemblages suffer additionally from ice-disturbance causing shading and ice-scour thereby affecting the upper distribution of susceptible species (Klöser et al. 1996). Species richness of Antarctic macroalgae is low in comparison with temperate or tropical regions with a high level of endemism (33 %) especially in the taxa of Rhodophyta and Heterokontophyta (Wiencke & Clayton 2002). Benthic algae play an important role in the benthic food web and the diet of herbivores (Iken 1996).

The marine microphytobenthos forms an essential food source for both benthic and pelagic heterotrophs. Particularly in Antarctic ecosystems, a poor development of

pelagic microalgae (Hapter et al. 1983; Schloss et al. 1998) but an important contribution of resuspended benthic diatoms to the phytoplankton has been suggested and/or observed (e.g. Ahn et al. 1994; Gilbert 1991a,b).

Due to the extreme importance of the benthic primary producers within coastal ecosystems any change or decrease in their abundance related to environmental changes (e.g. under increased UV-B irradiance) can have dramatic consequences for the whole community and especially to the coastal ecosystem.

1.2 STRATOSPHERIC OZONE, PHOTOSYNTHETICALLY ACTIVE RADIATION AND UV RADIATION

Solar radiation is the most important prerequisite for life on Earth. The sun radiates energy over a broad spectrum of wavelengths. The "visible light" corresponding to photosynthetically active radiation (PAR, 400-700 nm) is used for photosynthesis where light is converted into chemically bound energy, the basis for biomass production. Thereby molecular oxygen is generated which is needed by heterotrophic organisms. Furthermore, the more energetic ultraviolet radiation (UVR, 280-400 nm) reaches the Earth's surface with the potential of affecting organisms negatively.

The solar radiation measured at the Earth's surface is subject to atmospheric absorption and scattering by gas molecules, aerosols and clouds. The UV radiation is divided into UV-C (190-280 nm), UV-B and UV-A. The Commission Internationale De l'Éclairage (CIE) has defined UV-B (ultraviolet-B radiation) as wavelength of 280-315 nm and UV-A as 315-400 nm. Many aquatic scientists, however, accept 320 nm as the border between UV-A and UV-B (Franklin & Forster 2003).

While UV-C radiation is absorbed completely in the atmosphere UV-B is only partly absorbed by the stratospheric ozone layer (ca. 20-40 km above the Earth's surface) and UV-A is almost unaffected by ozone. With the thinning of the stratospheric ozone layer (see e.g. Nardi et al. 1999), more of the highly energetic and biologically effective UV-B radiation reaches the surface threatening life on Earth (Environmental Effects Assessment Panel 2006). For example, a 10% decline in stratospheric ozone results in a 5% increase of surface irradiance at 320 nm while the same decline would result in a 100% increase at 300 nm (Frederick et al. 1989).

The ozone layer also plays a key role in controlling the temperature of Earth's atmosphere by absorbing UV-B radiation which is a source of heat production in the stratosphere (WMO 2003).

Ozone (O_3) is a gas naturally present in the stratosphere that rapidly reacts with many chemical compounds. Total ozone is not evenly distributed over the globe and generally highest at middle and high latitudes and lowest over the equator (WMO 2003). The differences are caused by stratospheric winds and the chemical production and destruction of ozone. In the last decades an anthropogenically caused reduction of stratospheric ozone was observed due to the emission of ozone-depleting gases. Especially manufactured gases containing chlorine and bromine released into the atmosphere by human activities are converted by UVR in the stratosphere thereby forming reactive halogen gases. These reactive gases (e.g. ClO, BrO, Br, Cl) chemically destroy ozone in the stratosphere.

Stratospheric ozone depletion over the Antarctic continent was first observed in the early 1980s (Farman et al. 1985) with a significant decline until the mid-1990s (Weatherhead & Andersen 2006). Late winter/early spring stratospheric ozone losses of up to 60 % - the "ozone hole" - were observed over this continent each year, leading to enhanced UV-B radiation at the Earth's surface. This extreme situation is caused by the low stratospheric temperatures (< 78 °C) over the Antarctic continent which favour the formation of polar stratospheric clouds (PSCs). PSCs form when nitric acid and sulfur-containing gases condense with water vapour and form solid and liquid PSC particles. Reactions on the surface of the cloud particles conveys the formation of reactive halogen gases. Chlorine gases are converted to the most reactive form, ClO. With elevated ClO concentrations additional catalytic cycles accelerate in the chemical destruction of ozone when exposed to solar radiation in Antarctic spring. During winter the air in the stratospheric regions above the Antarctic continent is relatively isolated from other regions due to strong circular winds so that an accumulation of ClO can take place. With the warming of the temperatures in spring, PSCs decline and the production of ClO ends. As a result, the intense period of ozone depletion finishes and ozone values return to the normal values (WMO 2003).

The overall question is, when do spring ozone levels recover and if so do they ever reach pre-1980 values? Numerous computer-models tried to estimate the time of recovery, now that most of the ozone-depleting substances are banned due to the Montreal protocol from 1987. It is, however, a difficult task, because of high natural variability in ozone levels, e.g. due to the solar cycle, total column ozone fluctuates over timescales of a few years. These fluctuations can obscure long-term changes and offer false indications of recovery (Weatherhead & Andersen 2006). For example only half of the models compared in this study predict that column ozone will rise above 1980 levels when the abundance of ozone-depleting substances returns to 1980 concentrations. Furthermore, total column ozone, carbon dioxide emissions, stratospheric temperatures and circulation patterns are closely linked, and changes in one of these variables can affect the others (Weatherhead & Andersen 2006 and references therein).

Generally, little improvement is expected for total column ozone in the Antarctic for the next several decades with the significance that terrestrial and aquatic organisms go on suffering from an increased UV-B radiation in the future.

1.3 UV RADIATION IN THE AQUATIC ENVIRONMENT

UVR can penetrate into the water body to considerable depth thereby also affecting marine organisms. It is therefore very important to measure the UVR entering the water column while performing any kind of field experiment regarding UVR effects. The UV penetration into the water not only depends on the atmospheric conditions given (e.g latitude and altitude, sun elevation due to season and time of the day, cloud cover, ozone concentration) but also strongly on the optical properties of the water body (Kirk 1994; Hanelt et al. 2001). In Antarctic oceanic waters UV-B was recorded to a depth of 60-70 m (Smith et al. 1992). In coastal zones, however, UVR enters much less due to a higher amount of e.g. dissolved organic matter (DOM; Kirk 1994). Its impact depends largely on the input from the terrestrial ecosystem and is especially high in polar regions when meltwater form the glaciers enters in high quantities into the coastal waters (Hanelt et al. 2001). In Antarctica UVR can penetrate quite deep into the water column. UV transparency of the water body at the experimental site of this study was e.g. highest in spring with a maximal measured 1 % depth at 16 m for UV-B radiation, 19 m for UV-A radiation, and >20 m for PAR (Paper I & II) in summer decreasing strongly (Paper X). UVR was further shown to penetrate ca. 1 mm into the sediment (Wulff et al. 1999). Thus, UVR should be considered a very important environmental factor in the tidal fringe that can affect different metabolic and physiological processes in organisms living in the water column and in the benthos.

1.4 IMPACT OF UV RADIATION ON ALGAE

UVR negatively influences not only algae but a wide range of biological systems, from humans over terrestrial plants to fishes, phytoplankton and bacteria. UVR impacts on organisms are manifold and reach from the molecular to the organism level. UV-B is considered the most dangerous part of the UV range reaching the Earth's surface due to its short wavelength with high energy content as it is absorbed directly by biomolecules (Vass 1997). UV-A has been shown to have less pronounced effects, although there is some debate about this.

UV-B effects on macroalgae are thoroughly described in the review by Bischof et al. (2006; Paper IX). Benthic microalgae have not been as extensively studied as e.g.

phytoplankton but the work of Wulff (1999) provides a good overview about UVR effects on microphytobenthic communities.

The DNA is one of the most UV-B sensitive molecules and damage is shown directly by e.g. the formation of cyclobutane dimers (Lois & Buchanan 1994) or indirectly due to free oxygen radicals produced by UVR (Mitchel & Karentz 1993). DNA damage inhibits replication and gene expression severely. Furthermore, aromatic residues absorb UV-B, thus inducing damage of proteins. Lipids might be destroyed by UV-B in the presence of oxygen. In higher plants and algae the pigments of the photosynthetic apparatus are another target of UV-B radiation (Strid et al. 1990). As a consequence of these effects a number of physiological processes are affected, e.g. photosynthesis (Strid et al. 1990) and nutrient uptake (Goméz et al. 1998). On the organism level, the molecular effects can result in reduced growth and reproduction (Wulff 1999; Wiencke et al. 2000; Roleda 2006a).

Consequently, UVR might also affect ecosystem structures. Little information on how UVR influences communities is available to date. The few studies existing show that the early successional stages (within the first weeks of development) are the most susceptible to UV radiation and effects were mostly transient disappearing at later stages of succession (Wulff 1999; Lotze et al. 2002; Wahl et al. 2004). However, these results were not consistent and in different habitats and climatic regions different results were obtained indicating a lack of latitudinal patterns for UVR effects on community level (Dobretsov et al. 2005; Wahl et al. 2004).

Moreover, species do not exist in isolation in their habitat and interactions occur on an intra- and interspecific level. UVR might affect some species more than others, or effects are indirect, e.g. affecting herbivores more than the algae thereby increasing the biomass of the primary producers (e.g. Bothwell et al. 1994). Final answers on how UVR influences the life of organisms on earth can therefore not be made on single trophic assessments including only one species, but rather on community level testing for interactions with other important biotic and abiotic parameters.

It is proposed that an increased UV-B radiation might lead to a decrease in primary productivity and a downward shift of the upper depth-distribution of the macroalgae at least for single species and therefore to a shift in species composition of the ecosystem (Bischof et al. 2006 and references therein). However, effects on community level are still mostly hypothetical and various acclimation processes can counteract radiation stress.

1.5 PROTECTIVE AND REPAIR MECHANISMS AGAINST UV RADIATION

Micro- and macroalgae have different mechanisms of avoidance, genetically fixed adaptation, and physiological acclimation to protect themselves against harmful UVR. However, protection against UVR requires in most cases additional energy, which may result in reduced growth and primary productivity (Roleda et al. 2006a). Generally, UVR effects and the ability to recover form UV induced stress were shown to be species-specific (reviewed in Bischof et al. 2006).

Some benthic microalgae possess the ability of downward migration into the substrata to avoid excessive light. Most of them have receptors for PAR and an UV-B mediated downward migration has been proposed for the benthic diatom *Gyrosigma balticum* (Underwood et al. 1999). Macroalgae can avoid UVR by growing in deeper waters or under canopy algae. However, this is not an active process like in some microalgal species.

Morphological features seem to play an important role in UV protection as bigger cells and species with thicker thalli generally seem to be better shielded against harmful radiation. This might be due to an increasing pathway for UV penetration through the cytoplasm (filtering, absorption, and scattering of UVR; Garcia-Pichel 1994; Franklin & Forster 1997; Swanson & Druehl 2000). Furthermore, some micro- and macroalgal species are able to produce UV absorbing compounds such as mycosporine-like amino acids (MAAs) or phenolic compounds (e.g. phlorotannins). Certain carotenoids and flavonoids may also have a photoprotective role.

MAAs are water-soluble compounds with an absorption maxima between 310 and 360 nm and were mainly observed in Rhodophyta and several groups of microalgae (Hoyer et al. 2001; Karentz et al. 1991). Their concentration was shown to decrease with increasing water depth with high contents in supra- and eulittoral species and low or no MAAs in subtidal species (Hoyer et al. 2001). The induction, synthesis and accumulation of the MAAs is a highly flexible and species-specific process.

Many brown algae on the other hand, are able to produce photoprotective phlorotannins under UVR exposure (Pavia et al. 1997). A variety of functions have been reported for phlorotannins in brown algae, such as herbivore deterrents, a role in adhesion, antibacterial agents, strengthening role in cell walls and UV screens (Schoenwaelder 2002). Phlorotannins are secondary metabolites and can occur in high concentrations in the algae and have their absorption maxima at 195 and 265 nm (Pavia et al. 1997). They possess a high antioxidant activity and are therefore important for scavenging toxic reactive oxygen species (ROS) produced by UV-B radiation.

DNA damage can be repaired by different mechanisms (i) under photo-reactivating light, i.e. an enzyme system that reverse the UV-B induced damage in the presence of UV-A and/or blue-light (van de Poll et al. 2002), (ii) nucleotide and base excision repair (dark repair), an enzymatic process involving removal and resynthesis of damaged DNA, and (iii) postreplication repair that corrects the DNA defect on the basis of information contained in the undamaged strand (Roy 2000).

For algae inhabiting shallow water zones an adjustment of the photosynthetic performance to variations in light intensity and spectral quality is important (Bischof et al. 2006). Photosynthesis is a dynamic process and short time light fluctuations cause fast and reversible reactions, such as fluorescence or heat dissipation via the xanthophyll cycle which is regarded as the major photoprotective process, or energy redistribution between the two photosystems (Hall & Rao 1994). Acclimation to repeated UV exposure in some brown algal species showed two different responses of photosynthetic activity. First, the rate of recovery from UV induced photoinhibition increases and second, the degree of inhibition becomes smaller (Bischof et al. 1998; 1999).

1.6 AIMS OF THE THESIS

There is a big counterbalance in UV research between field and laboratory experiments. Statements made on the impact of UVR on algae are mostly based on laboratory studies with single species. Especially for macroalgae surprisingly few experiments on community level exist (but see e.g. Lotze et al. 2002; Wahl et al. 2004; Dobretsov et al. 2005). Furthermore, stratospheric ozone depletion is highest over Antarctica (WMO 2003) but only few UV studies on Antarctic species exist. Macroalgal propagules and new developing algal communities are proposed as the most susceptible to UVR (Wulff 1999; Coelho et al. 2000; Roleda et al. 2004; 2005; 2006b; Roleda 2006; Bischof et al. 2006; Wiencke et al. 2006). This study aims to detect consequences of UVR on early successional stages of Antarctic benthic primary producers by combining field and laboratory approaches.

In my thesis I tried to answer the following questions:

1. How do UVR and grazing affect the succession of an intertidal macroalgal assemblage in Antarctica during a long-term field study (Paper I)?

2. How do UVR and grazing affect the succession of an intertidal microalgal assemblage in Antarctica during a long-term field study (Paper II)?

3. Do Antarctic subtidal and intertidal algal communities react differently to UVR and grazing pressure (Paper III)?

4. What is the response of Antarctic microphytobenthos to UVR under short- and mid-term exposure in the laboratory (Paper IV & V)?

5. Do propagules of different macroalgal species from Antarctica differ in there response to UVR applied in the laboratory and how well will they recover from UV induced stress (Paper VI, VII & VIII)?

The main focus of this thesis was the field-study including two long-term experiments on community level (Paper I, II & III). The significance of interactions between climatic (e.g. temperature, UVR) and ecological factors (e.g. grazing) as important drivers on algal recruitment were demonstrated earlier (Lotze & Worm 2002). Consequently, questions concerning community level are difficult to answer as a single-trophic assessment.

Therefore, we combined the effects of UVR and grazers on the developing communities. To elucidate the major mechanisms behind results found in the field, laboratory studies were performed to complement the field studies, giving a more mechanistic approach (Paper IV to VIII). Paper IX is a state of the art of how UVR influences macroalgae and Paper X deals with the light climate at the study area at the surface and in the water column.

2 METHODOLOGICAL CONSIDERATIONS

The experiments and field studies in this thesis were performed between October 2003 and March 2005. In the following an overview of the experiments in general, some measuring principles and problems encountered are given. Detailed descriptions are made in the material and method section of each paper.

2.1 STUDY AREA

All studies were performed close to the Dallmann Laboratory, King George Island, South Shetland Islands, Antarctica (62°14´S, 58°40´W, Fig. 1a-c). The Dallmann Laboratory is a summer base maintained by Argentina, Germany and The Netherlands and is an annex to the Argentine permanent station Teniente Jubany.

The maximal tidal range in this region is about 2 m and the sea surface temperature varies between -1.8°C (spring) and 2°C (summer). Water transparency is strongly variable, depending on glacial freshwater input and wind direction (Paper X). Minimum concentrations of nitrate, phosphate, and silicate were recorded in February at non-limiting algal growth levels of 15, 2, and 47 µmol/l, respectively (Schloss et al. 2002).

The field experiments were conducted at a rocky intertidal platform (Peñón Uno, 62°14´S, 58°41´W). The substratum consists of andensitic bedrock (Kleinschmidt, personal communication) and boulder fields. Intertidal Antarctic seaweed communities are dominated by annual or pseudoperennial species of Rhodophyta (e.g. *Iridaea cordata* Turner (Bory), Heterokontophyta (e.g. *Adenocystis utricularis* (Bory) Skottsberg) and Chlorophyta (e.g. *Monostroma hariotii* Gain, Iken 1996; Kim 2001) as well as mobile consumers, mostly gastropods and amphipods (Ferraz Nonato et al. 2000).

Fertile algal material for the macroalgal spore experiments in the laboratory was collected around Peñón Uno (eulittoral to upper sublittoral; Paper VI, VII & VIII) and at Peñón de Pesca (sublittoral, only *Iridaea cordata*; Paper VIII).
Benthic microalgae for the laboratory experiments were collected in the subtidal from soft-bottom substrata in the Potter Cove around 5 to 7 m depth (62°15´S, 58°41´W; Paper IV and V).

All samplings during high tide or in the subtidal were performed using SCUBA diving.
Sampling areas and experimental sites are shown in Fig. 1c.

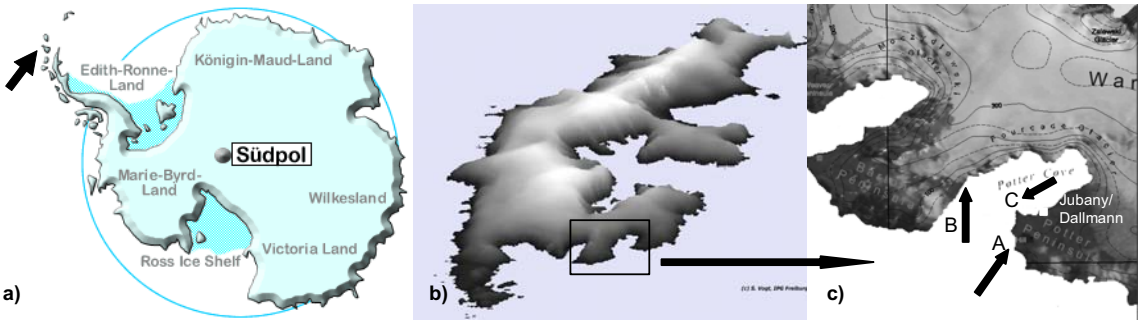

Fig 1 a-c. (a) Antarctica, **(b)** King George Island, **(c)** Sampling areas close to the Dallmann Laboratory: A. Peñón Uno (field-experiment and fertile macroalgae), B. Peñón de Pesca (fertile macroalgae), C. Potter Cove (microlagae). Maps b) and c) partly S. Vogt, IPG Freiburg.

2.2 THE FIELD EXPERIMENTS – AN OVERVIEW

In two sets of field experiments performed in 2003/2004 and 2004/2005 (four sampling occasions, n = 4) the response of early successional stages of macroalgal and microalgal assemblages to the combined effects of UVR and grazing were studied (Paper I, II & III, Table 1). 32 experimental units (cages) were fixed in the intertidal (Fig. 2). The different treatments applied with the help of different cut-off filters were: (i) ambient radiation (PAR + UV-A + UV-B = PAB), (ii) ambient radiation minus UV-B (PAR + UV-A = PA) and (iii) ambient radiation minus UV-A and UV-B (PAR only = P) *vs.* grazer and no-grazer treatments. Methods are described in detail in Paper I and II. Variables investigated are shown in Table 1.

Table 1. Summary of variables measured in the field-experiments

	Paper I	***Paper II***	***Paper III***
Duration of experiment	2.5 & 3.5 months	2.5 & 3.5 months	2.5 months
Assemblage studied	macroalgae	microalgae	both
Depth zonation	eulittoral	eulittoral	eu-and sublittoral
Algal biomass	•	•	•
Algal abundance	•	•	•
Algal composition	•	•	•
Algal diversity + richness	•		
Macrofaunal composition	•	•	
Photosynthetic efficiency			•

In field experiments it is very important to use "controls" to check for possible artefacts introduced by the experimental set-up. However, it is hardly possible to perfectly mimic natural conditions in a field study and some compromises have to be made between the most natural and the set-up best to handle. In the present studies, controls for filter (full sunlight treatment) and for cage artefacts (half cage) were used.

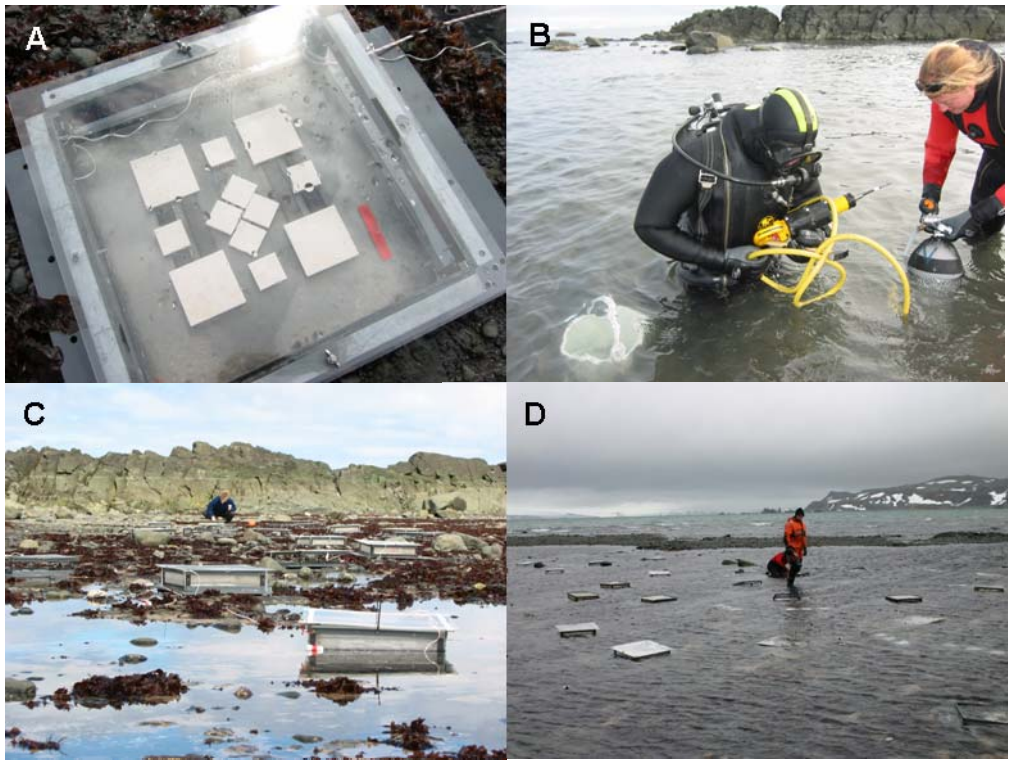

Fig 2 A-D. (A) Experimental unit (cage) with ceramic tiles and filter. **(B)** Fixation of the cages with an underwater drilling machine. **(C)** Cages at Peñón Uno during extreme low tide. **(D)** Cages at Peñón Uno during low tide.

2.2.1 Measuring diversity and species richness

There is a variability of diversity indices described in the literature. Their main aim is to reduce the multivariate (multispecies) complexity of assemblage data into a single index in order to compare it by univariate analyses. Diversity is the number of different species in a particular area (i.e. species richness) weighted by some measure of abundance such as number of individuals or biomass. In the present study, the widely used index, the Shannon diversity index (H') was applied for macroalgal diversity (counts of macroalgal species) on the experimental tiles:

$$H' = -\sum_i p_i \log(p_i)$$

where p_i is the proportion of the total counts arising from the ith species. The higher the calculated value the higher the diversity of the system. Care should be taken, however, when comparing diversity indices from different studies, because it is sensible to the degree of sampling effort. Hence H' should only be compared across equivalent sampling designs (Clarke & Warwick 1994).

Species richness can either be simply the total number of species present in the study, what is obviously very dependent on sampling size or an index which incorporates the total number of individuals (*N*). The Margalef's index (*d*):

$$d = (S-1) / \log N$$

a measure of the total number of species (S) present for a given number of individuals as used in the present study (Clarke & Warwick 1994).

2.3 THE LABORATORY EXPERIMENTS – AN OVERVIEW

The impact of UVR on the physiology of Antarctic benthic primary producers was tested in laboratory experiments. In Paper IV and V benthic microalgal communities from the subtidal were exposed to different radiation regimes and in Paper VI to VIII the UV susceptibility of reproductive cells of macroalgae was tested. The different treatments applied were (i) PAR + UV-A + UV-B (PAB), (ii) PAR + UV-A (PA) and PAR only (P), generated by the use of various cut-off filter foils. Methods are described in detail in the respective papers as well as irradiances applied. Variables measured are shown in Table 2.

Table 2. Summary of variables measured for the different laboratory experiments

	Paper IV	***Paper V***	***Paper VI***	***Paper VII***	***Paper VIII***
Duration of experiment	2 x 3 wks	4 x 2 days	3 x 4 d & 5 d	4 days	2 x 4 d
Species studied	microalgae	microalgae	*A. utricularis* *M. hariotii* *P. endiviifolium*	*A. mirabilis*	*I. cordata*
Depth zonation	sublittoral	sublittoral	eulittoral	upper sublittoral	both
Algal biomass	•	•			
Algal composition	•	•			
MAAs					•
DNA damage & repair			•	•	•
Photosynthetic efficiency	•	•	•	•	•

2.3.1 MEASURING PHOTOSYNTHESIS

Photosynthetic activity was recorded by measuring the emission of variable chlorophyll fluorescence of PS II with a pulse-amplitude modulated fluorometer (PAM 2000/PAM 2100 and Water PAM, Walz, Germany). Measuring with the PAM is a non-invasive method; measurements can therefore take place without damaging the algae. In the Water-PAM a cuvette with the algal solution is used, it is therefore possible to measure photosynthetic efficiencies of small cells (e.g. microalgae) and reproductive cells of macroalgae. The PAM 2000/PAM 2100 possess a fiberoptic which is directly held above the sample.

The general principle of this measurement is described by Schreiber et al. (1994). The optimum quantum yield is expressed as the ratio of variable to maximal chlorophyll fluorescence (F_v/F_m). It indicates the efficiency of energy transfer from the antennae systems to the reaction centers of PS II. Chlorophyll fluorescence is complementary to the alternative pathways of de-excitation (photochemistry and heat dissipation), so the fluorescence yield is highest when the yields of photochemistry and heat dissipation are lowest and changes in fluorescence reflect changes in the other two pathways. In a dark adapted sample fluorescence is measured before (initial fluorescence = F_0; all reaction centers of PSII are fully oxidised) and during a saturation pulse of white light (maximal fluorescence = F_m; all reaction centers are closed and photochemical quenching is zero). Consequently, with these two measurements the optimum quantum yield of PSII can be calculated (F_0 to F_m = F_v = F_v/F_m). The optimum quantum yield corresponds to the photosynthetic efficiency of the sample because in darkness absorbed energy can be only dissipated by heat or fluorescence. In this case the fluorescence yield is related to the rate of photochemistry which would occur under actinic light irradiation. The maximal values for optimum quantum yield vary in the different algal groups due to their different composition of the photosynthetic apparatus. In the Chlorophyta values up to 0.83 can be measured, for Phaeophyta values from 0.7 to 0.8 and for Rhodophyta from 0.6 to 0.7 can be recorded in unstressed adult algae (Büchel & Wilhelm 1993). For benthic diatoms values up to 0.8 were measured (Wulff et al. unpublished).

In contrast to the optimum quantum yield, the effective quantum yield ($\Delta F/F_m$') reflects the actual light utilisation during illumination of samples. In order to obtain reliable data with this technique it has to be considered that changes in ambient radiation result in changes in $\Delta F/F_m$' (ration decrease with increasing irradiation) and ambient radiation has to be carefully recorded.

To estimate overall photosynthetic capacity, maximal relative electron transport rates (ETR_{max}) were measured with photosynthesis vs. irradiance curves (P-I curves) also described in Schreiber et al. (1994). They were recorded while measuring effective

quantum yield of PSII during a stepwise increase of actinic light (see Bischof et al. 1999). Different parameters can be measured with the P-I curve: The saturating irradiance (I_k) demonstrates the point when photosynthetic performance starts to become saturated without light limitation. Higher values are characteristic for intertidal and lower for subtidal species. Alpha (α) is the initial slope in the light limited part of the P-I curve (as a measure for the electron transport efficiency, i.e. the quality and quantity how light energy is absorbed, conducted and converted into charge separation). A steep slope (high α) corresponds to a more shade adapted algae and a low α to a more light adapted algae or plant.

2.3.2 MEASURING DNA DAMAGE

UV-B can cause DNA lesion involving dimerization of pyrimidine bases, resulting in cyclobutane-pyrimidine dimers (CPDs). CPDs can be detected in a two-step antibody assay modified after Vink et al. (1994) and van de Poll et al. (2001). Extracted DNA is treated with a specific antibody binding at the CPDs. The detection of the primary antibody is followed by an incubation with horseradish peroxidase-conjugated secondary antibody (rabbit anti-mouse serum). After adding a detection reagent a luminescence reaction starts and samples are exposed to photosensitive films. The intensities of the grey scale values of the respective samples were compared with calibrated DNA with a known amount of CPDs to quantify the amount of DNA damage. Details are described in the respective papers and in Roleda et al. (2004).

2.3.3 MACROALGAL PROPAGULES

Propagules (spores and gametes) of five macroalgal species from the intertidal and the upper subtidal were tested regarding their UV susceptibility (Paper VI, VII & VIII). Table 3 gives an overview of the species tested and some general information. Wiencke & Clayton (1997; 2002) and Wiencke (1990a; b) describe in detail the life cycle of the respective algae.

Macroalgal life-histories are very variable: Sometimes the only existing free-living thallus is diploid and the zygote develops directly into a new individual (e.g. *Ascoseira mirabilis*). Other species have only a haploid generation with the only diploid stage being the zygote (e.g. *Ulothrix sp*.) or an alternation between haploid and diploid stage exists. Between these forms many variations exist and in many algae the complete life-history it is not yet known.

In the presented experiments, spores of two different red algal species were tested. Monospores from *Porphyra endiviifolium* were released from the asexual thallus. They are yellowish-green, ca. 15-20 µm in diameter and can develop directly into another leafy asexual thallus or a leafy gametophyte (Wiencke & Clayton 1997). Tetraspores (haploid meiospores, released from the diploid tetrasporophyte) from *Iridaea cordata* have a size of ca. 20 µm in diameter and develop into the gametophyte. Furthermore, gametes of the green algae *Monostroma hariotii* were exposed to UVR. Gametes are released from the haploid gametophyte and possess, in contrast to the red algal spores, flagella. Flagellated propagules of the brown algal species *Ascoseira mirabilis* and *Adenocystis utricularis* were isolated (Table 3). *A. mirabilis* possesses only one diploid generation, while *A. utricularis* has a heteromorphic life-history with a microscopic filamentous gametophyte and a macroscopical sporophyte generation (Wiencke & Clayton 2002).

Table 3. Summary of macroalgal species and their respective propagules tested in the different laboratory experiments

Species	Phylum	Reproductive cells	Depth (m)	Life-history	Distribution	Life-form
Porphyra endiviifolium (A. Gepp & E.S. Gepp) Y.M. Chamberlain	Rhodophyta	monospores	upper eulittoral	hetero-morphic	endemic to Antarctica	annual
Iridaea cordata (Turner) Bory de Saint-Vincent	Rhodophyta	tetraspores	0-30	triphasic, isomorph	West and East Antarctica, sub-Antarctic regions	pseudo-perennial
Monostroma hariotii Gain	Chlorophyta	gametes	0-20	hetero-morphic	Antarctica and sub-Antarctic regions	annual
Adenocystis utricularis (Bory de Saint-Vincent) Skottsberg	Phaeophyta	zoospores	0-20	hetero-morphic	Antarctic peninsula, sub-Antarctic islands, cold temperate regions	annual
Ascoseira mirabilis Skottsberg	Phaeophyta	gametes	1-12	one diploid generation only	endemic to Antarctica	perennial

2.3.4 MICROALGAL COMMUNITIES

In the microalgal experiments the most frequently observed genera or species in the sampled and cultured sediment were the benthic pennate diatoms *Navicula cancellata*, *Navicula spp.*, *Cylindrotheca closterium*, *Nitzschia spp.*, *Petroneis plagiostoma*, and *Gyrosigma fasciola* (Paper IV & V).

2.4 About the challenges of performing field-experiments in Antarctica

The Antarctic continent can be cold and unfriendly, especially for biologists working in the field. Winds over 20 knots and groups of sea leopards can impede diving, cold fingers detain you from picking up more samples, not to talk about the logistical challenge getting all the equipment in one piece and in time to the experimental site. Despite these difficulties it is worth all the work and the cold when experiments finally work and do not get destroyed by floating ice or by the 4 tons heavy sea-elephants.

Fig 3 A-D. (A) Peñón Uno full of floating ice with the cages underneath. **(B)** A couple of days later collection of the destroyed cages. **(C)** At the Dallmann repairing the destroyed cages. Cages were fixed again end of November 2004 at Peñón Uno.

2.5 Set-up, design and time scale

The sampling of the material for the laboratory experiments was only a matter of weather, time, sea leopards around, finding fertile algal material and coldness and generally did not cause much trouble (Paper IV, V, VI, VII & VIII).

The more difficult task was the field-experiment at Peñón Uno (Paper I, II & III). One problem we were confronted with was the entrance of smaller amphipods in the planned non-macrograzer treatments. There was nothing that could be done about it in the first year but changes of the cages were made for the second season together with the help from the work-shop in Bremerhaven and the crew in Antarctica.

Unfortunately, it still didn't work and amphipods entered. The problem was caused by the design of the cages in general and is matter of discussion in Paper I & II.

Other problems were caused by the extreme climatic and geographically isolated situation. Floating ice completely destroyed the set-up in the second year (November 2004, Fig. 3). Everything had to be rebuilt and reinstalled, thus reducing the exposure time of the field-experiment. Nevertheless, the aim to increase the exposure time compared with the first year (2.5 months) has been reached in the second year (3.5 months). The longer exposure time was necessary due to the slow settlement and growth rates of the macroalgal recruits. A further limitation was the number of replicates (n = 4). Sampling of the in total 32 experimental units (cages) took around 2 hours in ~0 °C water. Working and diving for longer periods at this temperature was hardly possible.

2.6 LIGHT MEASUREMENTS

In field-experiments investigating UVR effects on benthic marine organisms it is absolutely necessary to measure radiation regimes in the air and underwater. Also in laboratory experiments the radiation needs to be controlled to be able to quantify the actual irradiance reaching the experimental organisms.
For this purpose various types of instruments were used, including broad-band sensors measuring UVR and PAR (air and underwater), but also spectroradiometers giving detailed information about the incoming radiation at each wavelength. The advantage of spectrally resolved measurements of UV irradiance is that any type of action spectrum can be applied to the data. An action spectrum describes the measure of a biological effect as a function of wavelength (e.g. DNA and erythemal weighted action spectrum). Different weighing functions have their effects at different wavelengths, mostly in the UV-B range of the spectrum. A small resolution of the spectroradiometer of about 1 nm with high sensitivity and wavelength accuracy is desirable, especially in the UV-B range. Due to the strong ozone absorption of UV-B irradiance in the atmosphere, and consequently, a strong decrease of UV irradiance towards shorter wavelength a high magnitude/dynamic of UV sensitivity of the measuring instrument is necessary which covers 6 orders of magnitude. Thus, spectroradiometers have to be capable of detecting high intensities without saturating the detector and of suppressing noise and stray light well enough to provide reliable data for the shorter wavelengths down to the detection limit. However, these instruments need careful calibration in the laboratory and in the field. Paper X gives an overview about the challenges which have to be tackled measuring spectral UV irradiance under water and the problems encountered. International organisations such as WMO (World Meterological Organisation) or the

NDACC (Network for the Detection of Atmospheric Composition Change) have established instrumental requirements to precisely detect spectral UV irradiance and provide guidelines for instrumental operation in the field (McKenzie et al. 1997; Seckmeyer et al. 2001). In the experiments a land based UV spectroradiometer was equipped with a single monochromator to measure UV-A and a double monochromator with a 32 channel photomultiplier plate which is necessary to decrease the quantity of stray light (ISITEC GmbH, Bremerhaven, Germany; Hanken & Tüg 2002). The instrument was installed on the roof of the Dallmann Laboratory and logged the incoming mean irradiance in 5 min intervals. The UV-A sensor of the underwater instrument covered also the PAR region of the spectrum (Kruse, Germany). The main problems measuring UVR under water using the spectroradiometer were (i) the size and weight of the instrument which had to be operated from a small inflatable boat (the big monochromator for detection of UV-B radiation and the additional instrument for UV-A and PAR plus energy supply with batteries had to be transported), (ii) technical problems of the instrument, and (iii) the weather conditions (boats could only be operated at a wind speed of less than 20 knots). Therefore, in the second year additional underwater broad-band sensors were used which were easier to handle and to read out the data, however, supplying less detailed information.

Furthermore, for ecologically relevant laboratory studies on UV effects, it is of importance that the ratios between PAR, UV-A and UV-B are realistic. However, due to a low emission by the lamps (especially PAR) under low temperatures, these ratios were violated in the laboratory experiments. The UV doses applied was kept as natural as possible (compared with measurement made in the field) with a relatively lower PAR, excluding UV or UV-B with filter foils to see the differences between the wavelengths ranges. Thus, the studies were not designed to perfectly mimic natural conditions and should be considered more mechanistic and complementing the results of the field-studies.

2.7 STATISTICS

Statistics in ecological studies are always a difficult and sometimes contradictory task due to their complex design and high number of statistical tests applied. Because all of our experiments were designed to test for effects between treatments, we decided to use the widely used and accepted analysis of variance (ANOVA, one-way, two-way and repeated measures). The ANOVA was designed to solve the problem of trying to compare more than two populations and are used for tests of means of several populations (Underwood 1997). They are very suitable for planned, manipulative experiments and the underlying hypothesis of all ANOVA is that some difference is

predicted to occur among the means of a set of populations (Underwood 1997). Due to the four sampling occasions in the field-experiments some procedures to correct the p-value were performed. However, tests like Mauchley's test of sphericity or Bonferroni correcting (both applied in Paper I & II) are highly conservative. For example, the Bonferroni correction should lower the probability of making a type I error by dividing the α value (usually 0.05) by the number of samplings (in our studies 4 samplings so $p < 0.0125$, Quinn & Keough 2004). At this point the work of Moran (2003) might help ecologists not to get overrun by statistical procedures. In his opinion researchers should use the accepted $p < 0.05$ cut-off and make reasonable interpretations based on design, power analysis, differences between control and treatment groups, and most important basic logic. Detailed and complex investigations should be encouraged and not be inhibited by unreasonably low p-values making it very unlikely to find statistical significances and often impedes publishing of data.

In ecological studies it is further equally important and interesting to find and publish "not significant" results and not only the significant ones (e.g. Paper II).

Additionally, PRIMER statistic (PRIMER ™ 5 software package, Plymouth Marine Laboratory) was used to analyse data on community level, taking each species and its importance regarding the different variables (UV and consumers) into account (Paper I & II). Changes or shifts in species composition due to different treatments can be detected.

3 SUMMARY OF RESULTS

3.1 UV RADIATION AT KING GEORGE ISLAND, ANTARCTICA

The results of the two years UVR measurements in the field (air and under water) are summarized in Paper I, II & X (Richter et al. in press). Generally, surface UVR was highest in December and lowest from June to August when the sun rises over the horizon only for a few hours at King George Island (Fig. 4). Maximal irradiance measured mid- December 2004 was 2130 µmol m^{-2} s^{-1}, 44 W m^{-2}, 2.3 W m^{-2} for PAR, UV-A and UV-B, respectively. Day to day and within one day variation was high due to changes in cloud cover.

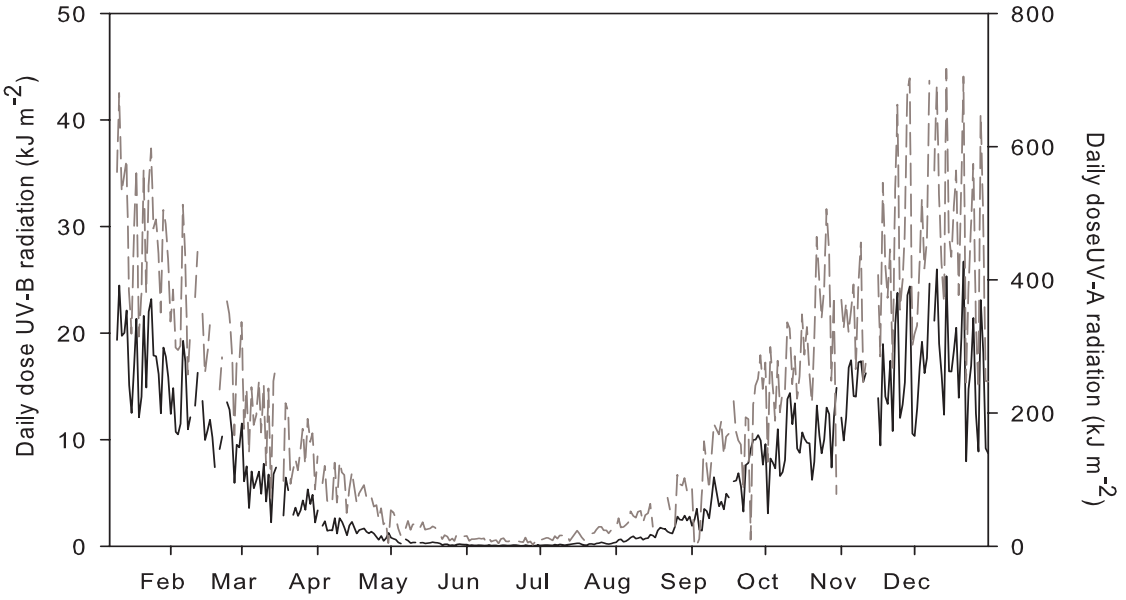

Fig 4. One year of surface UVR measurements at the Dallmann Laboratory: Daily UV-A and UV-B doses from January 2004 to December 2004 (UV-A dashed grey line, UV-B black solid line). Note secondary axis for UV-A doses.

The penetration into the water column depended strongly on the input of turbid melt water, the wind direction and the general circulation pattern in the bay. When temperatures rise above zero the melting process starts and sediment rich fresh water enters the Potter Cove. From end of December the water transparency decreased strongly in the Potter Cove and at Peñón Uno whereas the site Peñón de Pesca remained mostly unaffected because clear oceanic water enters the bay from this direction (see Klöser et al. 1994; Roese & Drabble 1998). In spring and early summer (October to end of December), when ozone depletion and water transparency are highest UVR can penetrate relatively deep into the water column (1% of surface PAR > 20 m, UV-A 19 m and UV-B 16 m). Lowest underwater UVR values were measured in February and March (see Paper I & X).

3.2 UV RADIATION EFFECTS ON INTERTIDAL ALGAL ASSEMBLAGES

3.2.1 FIELD-EXPERIMENT MACROALGAE

Generally the experiments of both seasons in 2003/2004 and 2004/2005 showed a very similar outcome although the second experiment was one month longer (Table 4). The sampling occasions (1 to 4) from the two years are not directly comparable because sampling periods were different. In the first year (2003/2004) sampling took place 26, 40, 58 and 74 days after starting the experiment (20.12.2003) and in the second year (2004/2005) 43, 71, 88 and 106 days after starting the experiment (28.11.2004).

Biomass was affected by consumers in both years, as well as density of green algae (Table 4, Fig. 5 and 6A). UVR effects on green algae were found only during the early successional stage (58 to 71 days old assemblage, Table 4). Red algal recruits and diversity were significantly affected by UVR at the end of the experiments (74 to 106 days old assemblages) and species richness was significantly different due to UVR and grazing at later succession only in the second, longer experiment (106 days, Table 4).

Table 4. Two-factorial ANOVA or non-parametric Kruskal-Wallis test on UVR and consumer (C) effects on biomass, density of Chlorophyta and Rhodophyta, species richness d and diversity H' for the four sampling dates (denoted by numbers one to four in the first column of the table) of both experiments in 2003/2004 (samplings took place in 2004) and 2004/2005 (samplings took place in 2005; - not significant, **+** significant), p-values Bonferroni corrected (significance level $p < 0.0125$). Note that samplings one to four did not take place in the same time interval in 2004 and 2005 (see Paper I)

		biomass		***density Chlorophyta***		***density Rhodophyta***		***species richness***		***diversity***	
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
1	UV	-	-	-	**+**	-	-	-	-	-	-
	C	**+**	**+**	-	-	-	-	-	-	-	-
	UV:C	-	-	-	-	-	-	-	-	-	-
2	UV	-	-	-	**+**	-	-	-	-	-	-
	C	**+**	**+**	**+**	**+**	-	-	-	-	-	**+**
	UV:C	-	-	-	-	-	-	-	-	-	-
3	UV	-	-	**+**	-	-	-	-	-	-	-
	C	**+**	**+**	**+**	**+**	-	-	-	-	-	-
	UV:C	-	-	-	-	-	-	-	-	-	-
4	UV	-	-	-	-	**+**	**+**	-	**+**	**+**	**+**
	C	**+**	**+**	**+**	**+**	**+**	-	-	**+**	-	-
	UV:C	-	-	-	-	-	-	-	-	-	-

In the following sections some graphs from the 2003/2004 experiment are presented to illustrate the above mentioned results. These results are not published in Paper I which mostly refers to the second experiment in 2004/2005.
The controls for full sunlight and half cages (FS and HC, respectively) were not significantly different compared to the open cage with the ambient spectrum (OPAB) during the experiments, indicating that no cage artefacts were present.
As mentioned above biomass was significantly reduced by grazing due to limpets but did not change much over time in the respective treatment (Fig. 5).

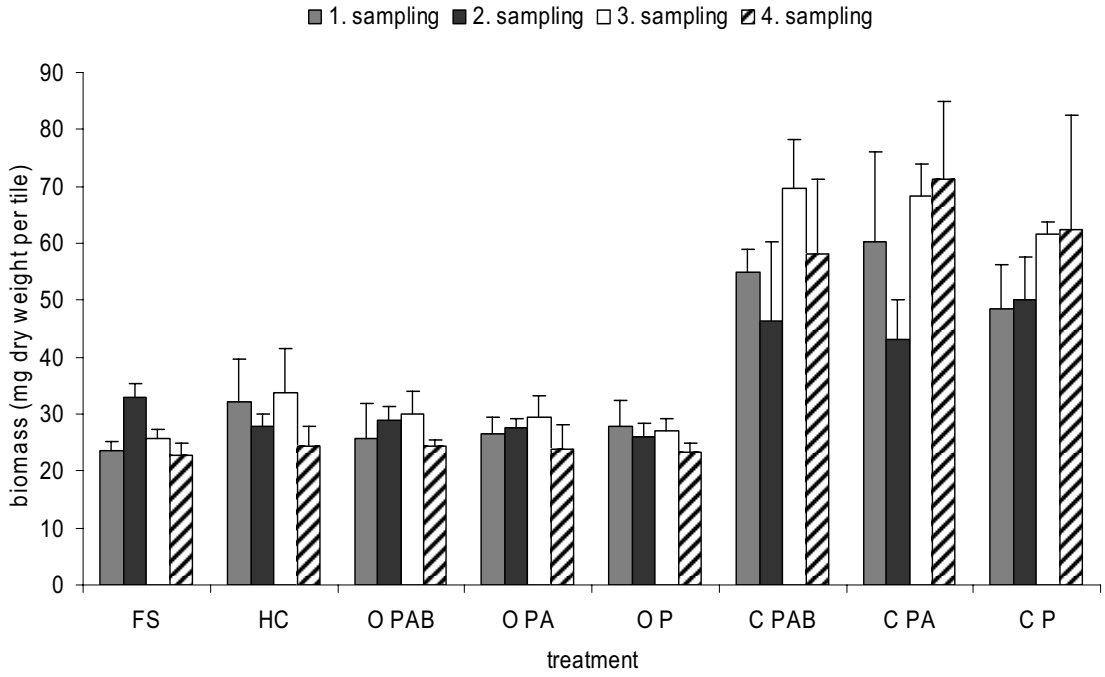

Fig 5. Mean biomass (as dry weight per tile) ± SD under the different treatments during the four sampling occasions in the first experiment 2003/2004 (FS = full sunlight control, HC = half cage control, O = open cage, C = closed cage; PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR).

The density of green and red algal recruits on the tiles increased from sampling 1 to 4 (Fig. 6A, B). The number of green algae recruits was significantly reduced by grazing at the last three sampling dates. An increase under the treatment without UV-B in relation to the one with the ambient spectrum and the one with UV-A was observed at the first two sampling occasions (see Table 4, Fig. 6A). Additional UV-A (PA treatment) negatively affected the recruitment of red algae at the last sampling in comparison with the one without UVR (P treatment) while the additional UV-B did not exhibit a further significant negative effect (Fig. 6B).

Macroalgal diversity was higher in treatments shielded from UVR at the last sampling occasion (see Table 4).

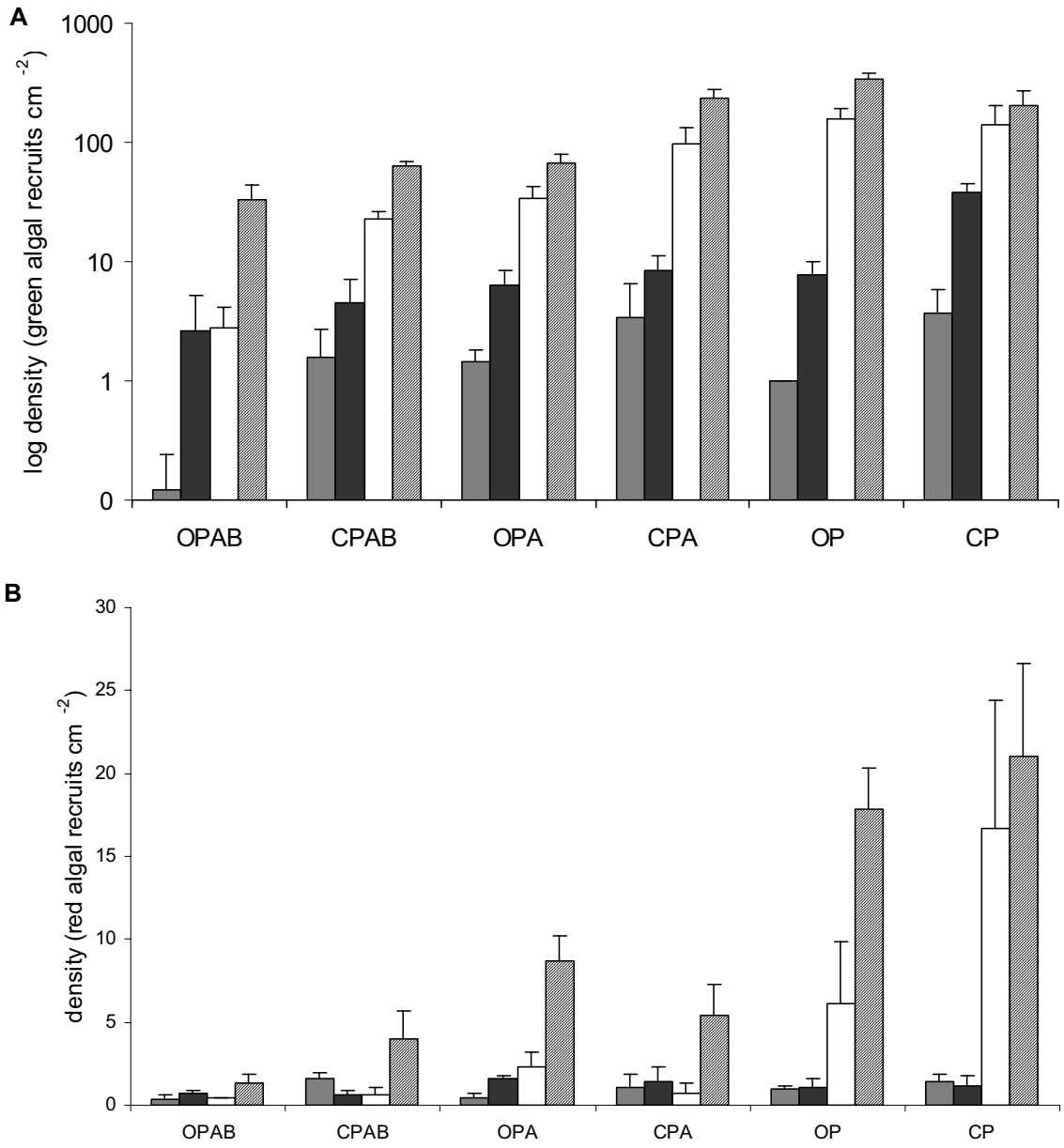

Fig 6A, B. Mean density of green (**A**) and red algal (**B**) recruits (cm^{-2}) ± SE under the different treatments during the four sampling occasions in the first experiment 2003/2004 (O = open cage, C = closed cage; PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR). Legend as in Fig. 5.

3.2.2 FIELD-EXPERIMENT MICROALGAE

Generally microalgal biomass was low on the tiles and few species settled during the experiment (Paper I), an observation generally made in the intertidal of King George Island. During various sampling occasions at intertidal sites in the area (soft-bottom and hard-bottom) hardly any microalgae were found while in the subtidal a high density of microphytobenthos was observed.

The assemblage on our tiles was dominated by the benthic diatoms *Navicula* cf *perminuta* and *Cocconeis costata* and *C. pinnata*. Less abundant were the genera *Licmophora* and *Fragilariopsis*. Biomass was significantly reduced by grazing limpets (mostly *Nacella concinna*) throughout the experiment. Absence of this grazer particularly favoured diatoms of the genus *Cocconeis* thereby affecting species composition. No negative UVR effects were found on cell number, biomass and species composition. The number of small *Navicula* increased under the ambient spectrum (PAB). No interactive effects between grazing and UVR could be observed.

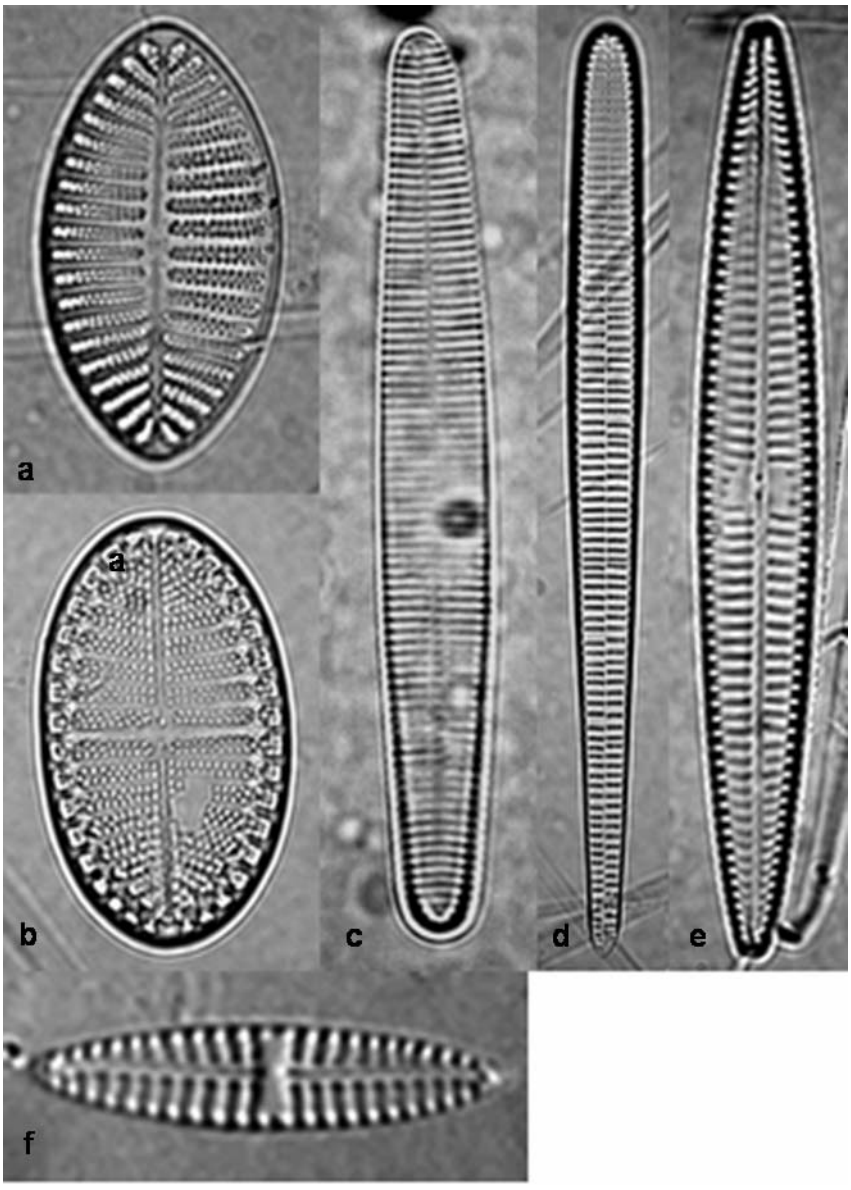

Fig 7a-f. Light microscopically pictures of the most dominant species found on the tiles. **a)** *Cocconeis pinnata* (27 x 16 µm), **b)** *Cocconeis costata* (27 x 16 µm), **c)** *Fragilariopsis* sp. (60 x 10 µm), **d)** *Licmophora antarctica* (28 x 10 µm), **e)** *Navicula directa* (60 to 100 µm length), **f)** *Navicula* cf *perminuta* (13 x 3.5 µm). Pictures were kindly provided by Dr. A. Al-Handal.

3.2.3 COMPARISON BETWEEN INTERTIDAL AND SUBTIDAL ALGAL ASSEMBLAGES

First results of a comparison between the effects of UV radiation and grazing on the succession of benthic algae are presented in Paper III. Because results were shown to be species-specific and species composition differed between the intertidal and the subtidal site, a variation among some results could be found. Analysis revealed a significant reduction of biomass due to grazing and a negative effect of UVR on *Palmaria decipiens* (Rhodophyta) recruits at both sites. A general difference was the higher biomass in the subtidal (due to colonize-forming diatoms) and a different composition in red algal recruits.

3.3 UV RADIATION EFFECTS ON MACRO- AND MICROALGAE IN LABORATORY EXPERIMENTS

3.3.1 MACROALGAE

3.3.1.1 PHOTOSYNTHETIC PERFORMANCE

The I_k values of propagules investigated varied between species, reproductive cell type and habitat. Saturating irradiance (I_k) was highest in gametes of the eulittoral green macroalga *Monostroma hariotii* and lowest in monospores of supralittoral red macroalga *Porphyra endiviifolium* (Table 5). Comparison between different groups of algae showed a generally higher I_k in green followed by brown and lowest in red macroalgae.

The slope alpha (α), a parameter for the performance of both light-harvesting and photosynthetic conversion efficiency, varies between $\alpha = 0.06$ in gametes of *M. hariotii* and $\alpha = 0.14$ in zoospores of *A. utricularis*. The other species lie in between these values (Table 5). Photosynthetic capacity, expressed as $rETR_{max}$, was highest in the brown eulittoral species *A. utricularis* (9.04) and lowest in the red subtidal species *I. cordata* (2.1; Table 5).

Optimum quantum yield of the PSII (F_v/F_m) of freshly released reproductive cells was highest in *P. endiviifolium* (0.488 ± 0.04) and lowest in *M. hariottii* (0.288 ± 0.04). In brown and red macroalgae, higher F_v/F_m was observed in supra- and eulittoral (*A. utricularis*, *P. endiviifolium* and *I. cordata*) compared to sublittoral (*A. mirabilis, I. cordata*) species (Table 5). Post cultivation under dim white light (4 µmol photons m^{-2} s^{-1}) generally showed an increase in the photosynthetic efficiencies of germinating cells except for *P. endiviifolium* (decrease) and *I. cordata* from the eulittoral (equal, Table 5).

Exposure to different light treatments consisting of PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) showed species-specific responses in F_v/F_m, expressed as percent of control (Fig. 8a-c). All species except *A. utricularis* were already inhibited by exposure to PAR only (20 to 90% under 22 µmol photons m^{-2} s^{-1}). The species most strongly inhibited in their photosynthetic efficiency were the sublittoral *I. cordata* and *A. mirabilis* (70 and 90% inhibition after 8 h exposure, respectively; Fig. 8a).

Table 5. Photosynthesis-irradiance curve parameters estimated using the hyperbolic tangent equation of Jassby and Platt 1976, and mean optimum quantum yield (F_v/F_m) of propagules immediately after release and after post cultivation under dim white light (4 ± 1 µmol photons m^{-2} s^{-1}) for 48 h

Class/Species	*Cell size (µm)*	*P-I curve parameters*			*F_v/F_m*	
		I_k	Alpha	$rETR_{max}$	After release	After 48h postculture
ULVOPHYCEAE						
M. hariotii	7‡	83	0.06	5.41	0.288±0.04	0.397±0.15
PHAEOPHYCEAE						
A. utricularis	4‡	64	0.14	9.04	0.462±0.11	0.601±0.04
A. mirabilis	3	52	0.10	4.99	0.400±0.06	0.446±0.05
BANGIOPHYCEAE						
P. endiviifolium	15	33	0.12	4.07	0.488±0.04	0.249±0.02
FLORIDEOPHYCEAE						
***I. cordata* (eu.)**	22	57	0.12	6.9	0.476±0.04	0.448±0.07
***I. cordata* (sub.)**	20	31	0.07	2.1	0.445±0.04	0.523±0.02

I_k (µmol photon m^{-2} s^{-1}) is the light intensity at which the initial slope of the curve intercepts the horizontal asymptote of the maximum relative electron transport rate ($rETR_{max}$). Cell sizes are in diameter, ‡ cell length

Additional UV-A further decreased F_v/F_m of propagules by 5 to 60% (Fig. 8b). The highest photoinhibition due to UV-A relatively to the P treatments occurred in *A. utricularis* (30 to 60%) and was lowest in *P. endiviifolium* (5 to 18%). A further, but small decline of F_v/F_m in relation to the PA treatment could be seen in all species and most exposure times if UV-B was added to the spectrum (1 to 20%; Fig. 8c).

Post cultivation for 2 d under dim white light (4 µmol photon m^{-2} s^{-1}) allowed reproductive cells of all macroalgal species to at least some extent recover their photosynthetic efficiencies. Complete recovery after all treatments and exposure times was only observed in *P. endiviifolium* monospores and *A. utricularis* zoospores (Fig. 8d-f). Recovery from PAB exposure was relatively more efficient in supra- and eulittoral species (*P. endiviifolium*, *M. hariotii*, *A. utricularis* and *I. cordata*) compared to sublittoral (*A. mirabilis* and *I. cordata*) species where an especially reduced recovery after 8 h exposure to PAB was observed (Fig. 8f).

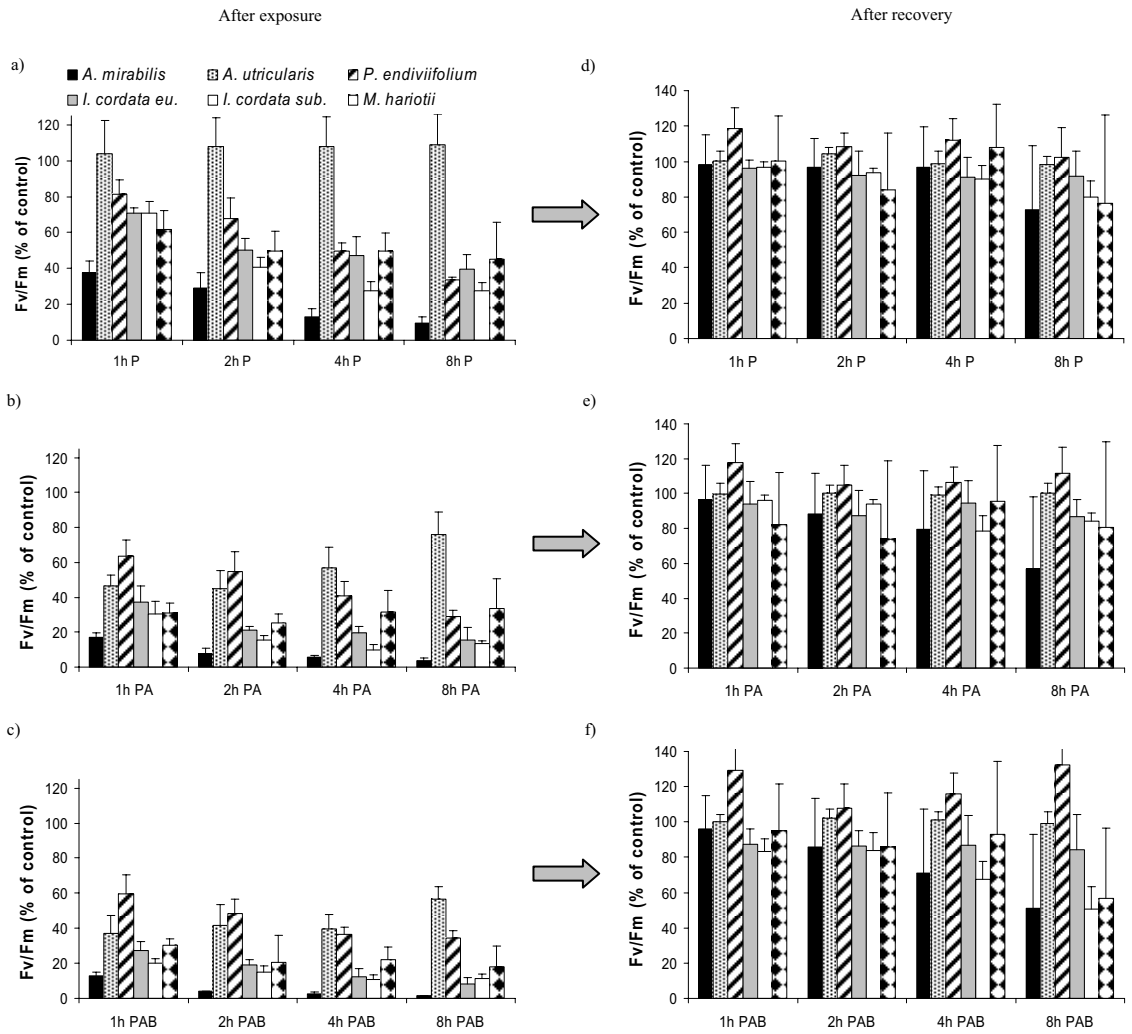

Fig 8a-f. Mean optimum quantum yield ± SD (F_v/F_m) of propagules during exposure (1 to 8 h, left column) to (i) PAR (P), (ii) PAR + UV-A (PA) and (iii) PAR + UV-A + UV-B (PAB) and after 48 h of recovery under dim white light (right column) expressed as percentage of the respective control measurements. Applied doses (in kJ m^{-2}) were: PAR 17, 34, 68, 136; UV-A 16, 31, 63, 125 and UV-B 1.3, 2.5, 5.0, 10.1 for 1, 2, 4 and 8 h of exposure, respectively.

3.3.1.2 DNA DAMAGE AND REPAIR

UVR induced DNA damage was lowest in species from the supra- and eulittoral and highest in sublittoral species. The supralittoral *P. endiviifolium* did not show any DNA damage at all, *M. hariotii*, *I. cordata* and *A. utricularis* from the upper to mid-intertidal showed little DNA damage, whereas CPD formation in *A. mirabilis* from the upper sublittoral was significantly higher (Fig. 9). DNA damage was also shown to be dose dependent with highest CPD formation under longer exposure times. DNA damage of *M. hariotii* and *A. utricularis* propagules was completely repaired after 48 h exposure to dim white light (Paper VI). In *I. cordata* and *A. mirabilis* DNA repair was still incomplete after 48 h of recovery (Paper VII & VIII).

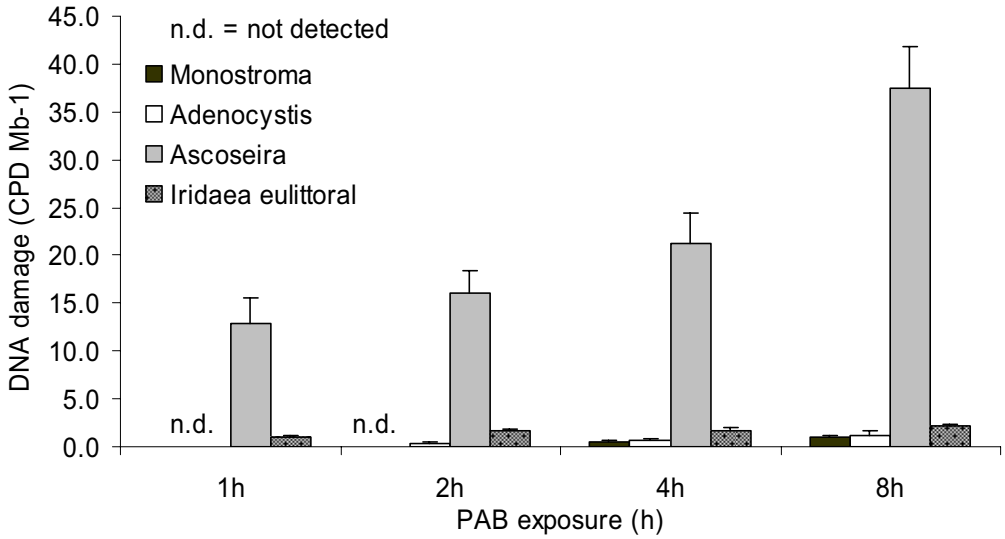

Fig 9. UV-B induced DNA damage (mean ± SD, n = 3, CPD concentration per million nucleotides) in *M. hariotii*, *I.* cordata, *A. utricularis* and *A. mirabilis* after exposure to 1, 2, 4 and 8 h to PAR + UV-A + UV-B (PAB). CPDs were not detected in *P. endiviifolium* after exposure.

3.3.1.3 Mycosporine-like amino acids

Mycosporine-like amino acids (MAAs) were determined in *Iridaea cordata* tetraspores (Paper VIII). Tetraspores contained two different MAAs, shinorine (λ_{max} = 334 nm) and palythine (λ_{max} = 320 nm). The most abundant was palythine. The concentrations of both MAAs, shinorine and palythine increased from the P to the PA and to the PAB treatment during 8 h exposure (Fig. 10).

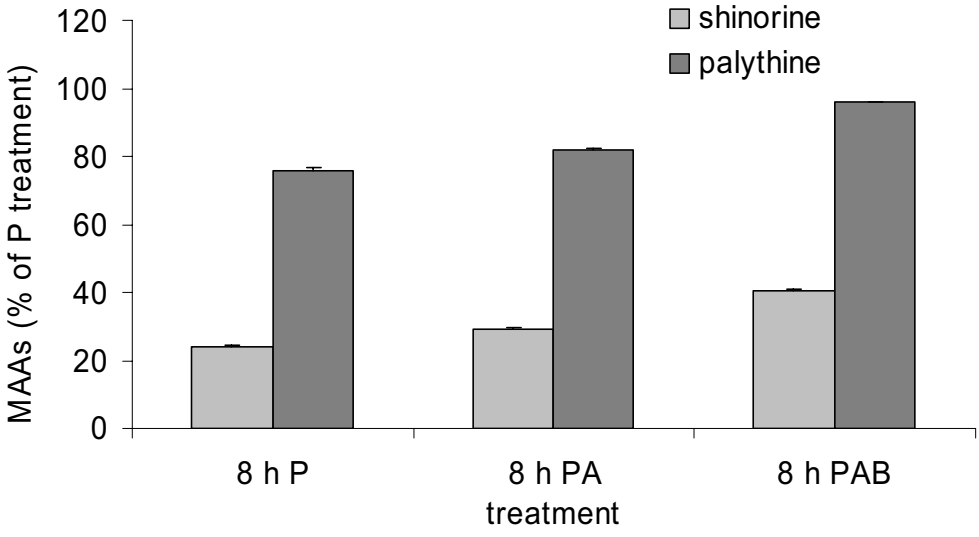

Fig 10. MAAs relative between treatments (all MAAs in the P treatment = 100 %) after 8 h of exposure to P, PA and PAB. Analysis performed with HPLC.

3.3.2 MICROALGAE

3.3.2.1 MID-TERM UV RADIATION EFFECTS ON BENTHIC DIATOMS

The studied benthic Antarctic diatoms were low light adapted as shown by the respective P-I curves (Paper IV). The maximum I_k value measured was 184 µmol photons m^{-2} s^{-1}. Optimum quantum yields (F_v/F_m) were initially > 0.6 after that a decrease in F_v/F_m over time at non-saturating irradiances occurred probably due to nutrient limitation. Transient significant negative UV-B effects were found on optimum quantum yield and total cell number, disappearing after 13 or 16 days, respectively. No effects on species composition or specific growth rate were found.

A general succession irrespective of treatment occurred in these microalgal experiments. Some species grew better under culturing condition than others leading to a shift in species composition over time.

3.3.2.2 SHORT-TERM UV RADIATION EFFECTS ON BENTHIC DIATOMS

Two types of set-up were used to test for UVR effects on photosynthetic efficiency of microalgae in the short-term experiments (Paper V): intact diatom mats (measured with PAM 2000) and diatom suspensions (measured with Water PAM). The mats were not disturbed during the measurements, the fiberoptic was placed directly above the sediment (diatoms could escape UVR by downward migration into the sediment) whereas the diatom suspension was shaken and filled into a quartz cuvette for measurements (diatoms could not hide from UVR).

Maximal initial values of effective quantum yield (no dark adaptation) was 0.601 (mat experiments) and maximal initial optimum quantum yield (with dark adaptation) was 0.573 (suspension). Significant negative UV effects on F_v/F_m under UV exposure and recovery under light were observed in the mats but not in the suspensions. In darkness treatment, the effects disappeared and diatom photosynthesis recovered.

4 SUMMARY OF THE DISCUSSION

4.1 EXPERIMENTAL RADIATION CONDITIONS

The presented field-experiments were conducted under the ambient radiation regime excluding the UV wavelength with cut-off filters under the natural ratios of PAR, UV-A and UV-B radiation. A comparison is hence possible between the different treatments. It is consequently the ecologically most relevant design in order to see a wavelength dependent effect on growth, survival, biomass etc. (Franklin & Forster 1997). However, field-experiments on the impact of UVR on organisms are still scarce because they are logistically difficult to perform and control compared to laboratory experiments.

In contrast to field experiments, laboratory experiments ensure a controlled set-up. A critical point always coming up in UVR studies performed in the laboratory is the artificial light spectrum and the ratios between PAR, UV-A and UV-B radiation. The spectra emitted by the lamps (in our experiments: Osram, L65 Watt/25S and Q-Panel UV-A) do not match the spectrum emitted by solar radiation and the ratios of PAR, UV-A and UV-B radiation are mostly strongly violated. For example the Q-panel emits a ratio of UV-A to UV-B around 10 to 1 while in the field the proportion is around 20 to 1. Even stronger is the violation taking the PAR part of the spectrum into account. In the study area a mean ratio in the field (n = 112) between PAR:UV-A:UV-B was 790:19:1 but only 13.5:12.4:1 in the laboratory light set-up used for the spore experiments. Results obtained in laboratory experiments may therefore overestimate the effectiveness of UVR induced damage (see e.g. Fiscus & Booker 1995; Molina & Montecino 1996, Franklin & Forster 1997). In the presented laboratory studies a variety of different light set-ups was therefore used. In the experiments with macroalgal propagules a lower UV dose than occurring in the field was chosen in order to avoid an overestimation of effects (see Paper VI). The microalgal studies were performed with natural to higher UV doses compared to values measured in the field (Paper IV) and using a different type of lamp (400 W Metallogen lamp, Philips) emitting higher PAR resulting in an ecologically more relevant ratio between PAR, UV-A and UV-B of around 353:11.5:1, respectively (Paper V).

Another important point is how algal material was grown prior to the experiments. It has been shown that the PAR levels during cultivation are important for the sensitivity of the samples during UVR exposure (Teramura 1986; Franklin & Forster 1997; Swanson & Druehl 2000). Samples previously cultured under PAR only were more stressed by UVR. In the experiments presented here, however, algal material was directly collected from the field and the time period before spore release was short (< 2 d), while the microalgae were cultured for about two to three weeks under low light

conditions which could have led to an acclimation to low light and a higher sensitivity towards UVR.
Taking into account these restrictions, laboratory studies (giving a more mechanistic approach) *together* with field-experiments can provide valuable insights in specific mechanisms involved in UVR induced stress of single species or communities.

4.2 GRAZING EFFECTS ON BENTHIC INTERTIDAL ALGAL COMMUNITIES

Grazing significantly reduced algal biomass and altered the species composition in the field-experiments. In the presented studies the absence of grazers generally favoured the abundance of the diatom genus *Cocconeis* and grazers preferred green algae over red algal recruits.

Meiofauna can have a grazing impact on benthic microalgae but they were not included in our experimental design, however, only very few e.g. copepods or polychaets were observed. Therefore, it is likely that their impact in relation to the grazing impact by macrograzers was negligible.

The analysis showed that the reduced algal biomass was not caused by amphipods (not excluded from the closed cages). Many Antarctic amphipods e.g. *Gondogneia antarctica* are omnivorus and feed on micro- and macroalgae (Jazdzewski et al. 2001; Huang et al. 2006) but they might have difficulties to graze on diatoms and early successional stages of macroalgae firmly attached to the substrata. In support of our results, Sommer (2000 and references therein) showed that a tight attachment to the substratum offered protection against grazing by the isopod *Idothea chelipes*. The firm attachment of the diatoms and propagules was further emphasised by the difficulties to detach the cells from the tiles during sampling. Moreover, Hillebrand et al. (2000 and references therein) found an herbivore preference of erect, chain-building microalgal species over strongly attached species such as *Cocconeis* sp. In this context, the impact of amphipods on the early successional algal stages growing in our experiment seems to be negligible.
Consequently, biomass effects were mainly caused by larger gastropods, e.g. *Nacella concinna*, successfully excluded by cages. Similar results were found in laboratory experiments with the green alga *Enteromorpha intestinalis* where snails had strong negative effects on macroalgal recruitment, whereas amphipods did not feed on *Enteromorpha* recruits but instead consumed adult *Enteromorpha* pieces (Lotze & Worm 2002). The limpet *N. concinna* is clearly the largest (mean length 20 - 30 mm) and most important grazer at our study site and can reach densities from 28 to 131 ind.

m^{-2} in the Antarctic intertidal (Brêthes et al. 1994). *Nacella concinna* feeds on macroalgal propagules and benthic microalgae (Iken 1996; Kim 2001; Peck and Veal 2001), whereas the smaller snail *Laevilacunaria antarctica* was shown to feed on *Monostroma hariotii*, the most dominant green alga on our tiles (Iken 1999). At the experimental site (Peñon Uno), a negative correlation between the density of *N. concinna* and macroalgae was also detected by Kim (2001), indicating effective grazing of this species. For example, *N. concinna* and *L. antarctica* contributed up to 47% of the biomass of macroalgae-associated herbivores at the study site (Iken 1996). Due to the availability of important energy resources in form of benthic micro- and macroalgae, *Nacella* migrates to the intertidal in spring (Brêthes et al. 1994). In winter it migrates back into the subtidal zone.

In our study, e.g. small diatoms belonging to the genera *Navicula* and *Cocconeis* were more heavily consumed by gastropods than other species. This happens most likely by passively ingesting more of the species which are structurally easier available (passive preference; Hillebrand et al. 2000) thereby shaping the species composition of the community. Macroalgal diversity was higher in plots grazed by snails than in ungrazed ones due to an increase in the spatial heterogeneity of the system (Sommer 2000).

All these findings demonstrate the important trophic link between microphytobenthos, macroalgae and grazers and the importance of snails, especially *N. concinna*, as driver on community structure in the Antarctic intertidal during early succession of the benthic algal community.

4.3 UV RADIATION EFFECTS ON BENTHIC ALGAL ASSEMBLAGES

Intertidal benthic algal assemblages at King George Island react differently to UVR. While the microalgal assemblage was unaffected by ambient and even enhanced UV-A and UV-B levels for the tested parameters (Paper II, IV & V), macroalgal recruits showed a species-specific and age specific inhibition due to UVR (Paper I). A comparison of intertidal and subtidal assemblages demonstrated that the UVR reaching the subtidal macroalgae assemblage still had negative impacts on some species while subtidal microalgae were unaffected by UVR as shown in the intertidal experiments (Paper III).

4.3.1 CAN UV RADIATION SHAPE INTERTIDAL MACROALGAL ASSEMBLAGES?

The filed-experiment in the first year (over 2.5 months) showed the importance of choosing an adequate experimental period due to the slow growth of the recruits. Therefore, in the second year a longer exposure time (3.5 months) was chosen. However, the general outcome of the two experiments was very similar.

UV effects changed over time showing species-specific differences. Strongest impacts on the community structure were observed at the end of the experiments in contrast to other studies (Santas et al. 1998; Lotze et al. 2002; Molis & Wahl 2004; Wahl et al. 2004 but see also Dobretsov et al. 2005). UV-A radiation was mainly responsible for a decrease in recruit density and species richness whereas additional UV-B had a significant negative influence on species composition and diversity. The different effects of UV-B and UV-A (with UV-A exceeding UV-B by a factor around 20 on a daily dose) demonstrated that UV-B radiation was more damaging per unit irradiance, but that UV-A was damaging due to the higher daily doses received (see also Cullen & Neale 1994; Wahl et al. 2004; Wiencke et al. 2006b).

The green algal recruit density was decreased by UVR at the start of the experiment whereas the red algal recruit density was most affected at the end with impacts on diversity, species richness and species composition. Several explanations for the changing nature of UV effects on the assemblage level are conceivable: (i) UV effects may match with changing radiation fluxes during the experiments, (ii) shading effects, where less UV-sensitive canopy species allow colonization of more UV-sensitive species as understorey algae, (iii) different adaptation strategies (e.g. morphology, protective substances such as MAAs or phlorotannins, DNA repair mechanisms) leading to a species-specific response to UVR (Lotze et al. 2002; Molis & Wahl 2004).
In our study, a correlation between diminishing UV effects and a decrease in UV doses over time (model i) was shown for the density of green algae recruits i.e. the most dominant representative *Monostroma hariotii*. An adaptation to UVR over time together with decreasing UV doses is a possible explanation for diminishing UV effects on this species. The macrothallus of *M. hariotii* occurs in high abundance in the Antarctic intertidal. Early life stages, however, are shown to be more sensitive to UV stress compared with adults of the same species (reviewed by Coelho et al. 2000), but have the capacity to acclimate as they mature (Lotze et al. 2002).
In contrast to the green algal recruits, red algal recruits were more sensitive to UVR during later stages of succession but early negative UV effects on red algal germlings might have been masked by low densities at the beginning of the experiment (few individuals and species settled in the first weeks and the variance between replicates was high; see also Dobretsov et al. 2005). Most red algae are fertile in late summer

whereas green algae like *M. hariotii* release spores earlier in the season (Wiencke & Clayton 2002). Especially one unidentified Gigartinales recruit, occurring only at the end of the experiment was highly UV susceptible and mainly responsible for the strong UV effects on red algal recruits. Macrothalli of some Antarctic red algal species (e.g. *Palmaria decipiens* and *I. cordata*) produce MAAs which enable them to grow in the intertidal (Hoyer et al. 2001). However, little is known about MAAs production in spores and germlings, but just recently MAAs were found in tetraspores of a red alga (Paper VIII) indicating a protective role already during early succession.
In temperate and tropical regions, some UV-tolerant species provide protective shading and allow colonization of more UV-sensitive species (model ii, Wahl et al. 2004; Lotze et al. 2002; Molis & Wahl 2004). In our experiment, however, these shading effects were lacking because propagules were still very small at the end of the experimental period. The macrothalli of many species develop in the winter period or in early spring of the following season. UVR could therefore directly inhibit growth and influence negatively species richness and diversity. Species occurrence on the tiles was therefore most likely a function of species-specific UV susceptibility and the ability to recover from UV induced stress (model iii, species-specific responses).

Species composition was significantly affected by both UVR and grazers due to different species and group-specific responses to radiation and grazing treatments, especially at the last sampling dates. Whereas UVR suppressed recruit density after 106 days of colonization, grazers favoured the density of some leathery red algal recruits (*Palmaria decipiens* and *I. cordata*). Therefore, at least in some cases consumers had the potential to counteract negative UV effects. On the other hand, UV and grazing effects on *M. hariotii* and one unidentified red alga worked in the same direction further decreasing their density.

The results show that Antarctic macroalgal recruits are particularly sensitive to UVR and grazing pressure and changes in these variables might cause seasonal and/or spatial shifts in species composition and community structure (see also Lotze et al. 2002; Dobretsov et al. 2005). While UV-B radiation had a significant negative influence on macroalgal composition and diversity a further increase, due to stratospheric ozone depletion, would influence these variables most, while species richness and biomass would be less affected. On the basis of these results we hypothesise that ambient actual UV-B radiation, and a potential further increase of these wavelengths has the ability to affect the zonation, composition and diversity of Antarctic intertidal seaweeds altering trophic interactions in this system. Whether the significant negative impact of ambient UVR at the end of the experiments is persistent when recruits develop into macrothalli in the next spring requires further studies.

4.3.2 NO PERMANENT UV RADIATION EFFECTS ON MIRCOPHYTOBENTHIC COMMUNITES?

In various mid- and short-term laboratory (Paper IV & V) and long-term field experiments (Paper II) the impact of UVR on the photosynthesis, cell number, biomass and species composition of subtidal and intertidal benthic diatoms was investigated. Generally, the results from the different experiments (laboratory and field) and communities (hard and soft bottom) did not show pronounced negative effects of UVR in the long term. Transient UVR effects were only observed in functional variables such as photosynthetic efficiency and cell number but not on variables like biomass or species composition. In all experiments diatoms recovered effectively from UVR induced stress, indicating a high capability to cope with even enhanced UV-B radiation.

The P-I curves measured in the laboratory on subtidal microphytobenthos showed, that the Antarctic microalgal community can be considered as low light adapted. The maximum I_k value (ca 184 µmol photons m^{-2} s^{-1}) was within values observed in other studies on benthic diatoms (e.g. Blanchard & Cariou-Le Gall 1994)

The exposure of benthic Antarctic diatoms to UVR resulted in a decreased optimum quantum yield and cell density mainly due to the UV-B part of the spectrum. Decreased photosynthetic efficiency appears to be the most frequently observed short-term effect for benthic microalgae (Villafañe et al. 2003). However, these treatment effects diminished after 13 and 16 days, respectively, or after an exposure to darkness for > 4 h. The microalgae seemed to have a capability to acclimate to the repeated UV exposure with time (see also Waring 2006); similar effects have also been reported for macroalgae (Bischof et al. 1999).

Vertical migration has been suggested to be a key mechanism for epipelic benthic diatoms to avoid UV-B radiation (Underwood et al. 1999). In the study by Underwood et al. (1999), the epipelic diatom *Gyrosigma balticum* responded to UV-B by vertical migration, but a significant damage to PSII was still apparent after 5 days of repeated UV-B exposure. UV-B radiation has been shown to penetrate down to 0.6 mm sediment depth (Wulff et al. 1999), and the diatoms might have escaped the UV-B through downward migration. In the short-term experiments (6 h UV exposure), an indication of UV-induced vertical migration was found (Paper V). However, photosynthetic efficiency of the algae in the field-experiment did not show any decrease in optimum and effective quantum yield due to UVR although vertical migration was not possible in this hard-bottom diatom community.

Other possible explanations are an effective shielding of the cell due to UV protective mechanisms, such as the production of photoprotective carotenoids, e.g. beta-carotene.

In planktonic Antarctic diatoms, UV absorbing compounds such as mycosporine-like amino acids (MAAs) could be a protective mechanism against harmful UV-B radiation (Hernando et al. 2002). But the benthic diatoms in the present study were analyzed for the presence of MAAs and no MAAs were found (Campana et al. unpublished). We conclude that this mechanism is not the cause of the UV resistance in our diatom assemblage. Furthermore, de-epoxidation of diadinoxanthin to diatoxanthin is known to occur in excessive light (PAR) as a protection against photooxidation (Arsalane et al. 1994), but the effect of UV-B on the de-epoxidation process is not yet clear. DNA repair is an important process minimizing UV stress (Buma et al. 2001), but no DNA damage could be found in benthic Antarctic diatoms from the same study area (Wulff et al. unpublished).

UVR effects on microalgae are often species-specific (e.g. Karentz et al. 1991). For example, UVR can affect the photosynthetic efficiency of some species more negatively than others (Waring et al. 2006). Over a longer experimental time this could lead to a shift in the microalgal composition towards more UV resistant species (Worrest et al. 1981, Wängberg & Selmer 1997, Vinebrooke & Leavitt 1999). However, in the present studies no shift in species composition due to UVR could be observed, indicating a good adaptation of all species of the microalgae communities used in the experiments. One of the dominating species in the experiments was the diatom *Cylindrotheca closterium* which was found to be unaffected by UV-B in a 7 day microcosm study (Wängberg et al. 1999). Many of the species in our mixed diatom community presumably have a good ability to recover from UV-B induced stress, later confirmed by further experiments performed in the study area (Wulff et al. unpublished). It should be pointed out, however, in a few studies transient UV effects on e.g. biomass and species composition have been found in an early stage of microalgal succession (Wulff et al. 2000). The authors related these effects to a species-specific response to UV-B radiation. Particularly *Navicula perminuta*, the most common species in our study, was shown to adapt and recover rapidly from UV-B induced stress (Warning et al. 2006).

However, the lack of UV effects found on biomass and abundance of benthic Antarctic diatoms generally confirmed former studies at different geographical sites (e.g. Hill et al. 1997). Earlier field-studies on UV effects on benthic microalgae have mainly focussed on soft bottom communities where benthic diatoms seem to be very tolerant to UV-B radiation (Peletier 1996; Wulff 1999 and references therein). The main variable to some extent affected in these communities was primary productivity while structural variables such as the overall biomass and microalgal pigments appeared unaffected (Odmark et al. 1998; Sundbäck et al. 1997; Wulff et al. 1999; Wulff et al. 2000). Wulff (1999) hypothesized that the lack of UV effects on structural variables in earlier field-experiments was due to the study of already established diatom communities and inadequate (too short) experimental periods. Microbenthic assemblages during early

succession, as in our study, should therefore be more UV susceptible (Wulff et al. 2000). Nevertheless, no UV effects were found on any of the tested structural variables during the 3.5 month sampling period.

4.4 SHORT-TERM RADIATION EFFECTS ON ANTARCTIC MACROALGAL PROPAGULES

In various laboratory studies the impact of UVR on isolated macroalgal propagules (Paper VI to VIII) was studied. Laboratory experiments should, however, not be extrapolated to determine community responses but they still provide valuable information of underlying mechanistic processes.

Generally, the impact of UVR to different macroalgal propagules reflected the zonation patterns of the adult algae on the shore. Reproductive cells of eulittoral algae were less affected and recovered better from UVR induced stress. Propagules of sublittoral algae on the other hand showed a higher degree of photodamage and DNA damage, also seen in slower or less effective recovery and repair mechanisms.

Photosynthesis-irradiance (P-I) curves

The P-I curves measured in the different species showed that the photosynthesis of reproductive cells of Antarctic macroalgae is shade adapted with saturating irradiances varying from 33 to 83 μmol m^{-2} s^{-1}. Low light adaptation of photosynthesis is observed to be the general characteristic feature of reproductive cells of macroalgae (Amsler & Neushul 1991; Roleda et al. 2004; 2005; 2006b). This might be related to the chlorophyll antenna size and number of chloroplasts present in reproductive cells compared to multicellular macroscopic stages. Red algal spores had lower I_k values than brown or green algae gametes or spores. These results are in agreement with measured I_k values for the adult thalli. Weykam et al. (1996) showed that the I_k values of adult Antarctic Rhodophyta are low compared to Chlorophyta or Heterokontophyta.

PAR effects on photosynthesis

Optimum quantum yield (F_v/F_m) of zoospores of *Adenocystis utricularis* was not affected by 8 h exposure to PAR (a dose of 136 kJ m^{-2}). Investigations of spores and gametes of the other studied species revealed a reduction of photosynthetic efficiency with increasing dose. Strongest inhibition was found in subtidal species (*A. mirabilis* and *I. cordata*). Reduction of photosynthetic efficiency while exposed to PAR is a protective mechanism to dissipate energy absorbed by PSII as heat via the xanthophyll cycle to avoid photodamage (dynamic photoinhibition; Osmond 1994). This process occurs mostly in algae from the intertidal or the upper subtidal and enables them to recover rapidly after the offset of the stressful condition (Hanelt et al. 1994). In contrast,

impairment of the D1 protein leading to a decrease in photosynthetic capacity is called chronic photoinhibition. This occurs in shade-adapted macroalgae growing in the lower sublittoral zone when exposed to high irradiances and is only reversible by a replacement of this protein which can take several hours (Matteo et al. 1984). These species have a lower ability to down-regulate photosynthesis through the protective dynamic photoinhibitory process (Hanelt et al. 2003). Reproductive cells of all macroalgae can be exposed to high PAR values during their planktonic phase while they stay in the euphotic zone. The potential for acclimation and recovery of the photosynthetic apparatus to high PAR conditions is therefore an important pre-requisite for the recruitment and ecological success of a shallow water alga (Roleda et al. 2006b).

UVR effects on photosynthesis

UVR exhibited an additional negative effect on the photosynthetic efficiency in all species tested. Monospores of the supralittoral species *P. endiviifolium* were the most tolerant to UVR. Although the measurable effects of both PAR and UVR in the reduction of photosynthetic efficiency are similar, the mechanisms behind are different. UVR damage of the photosynthetic apparatus occurs in a more direct way, due to its absorption by biomolecules (Vass 1997; Franklin et al. 2003). Photosynthetic performance may be additionally depressed in light treatments supplemented with UVR by possible damage to the oxidizing site and reaction center of PS II (Grzymski et al. 2001; Turcsányi & Vass 2002). Especially Antarctic macroalgae can therefore suffer from the increased UV-B radiation during spring (due to stratospheric ozone depletion) exhibiting adverse effects on photosynthesis (Hanelt et al. 2003).

Recovery of photosynthesis

After photoinhibition, recovery of photosynthesis often requires exposure to low white light (Hanelt et al. 1992). Optimum quantum yield of all eulittoral species recovered completely after 48 hours post-cultivation under low white light. An incomplete recovery was observed in sublittoral propagules, especially when pre-exposed to UVR.
Recovery of photosynthetic efficiency of zoospores of different kelp species varied between 8 and 24 hours in upper and lower sublittoral species, respectively (Roleda et al. 2006b). Exposure to UVR was further observed to delay photosynthetic recovery of Arctic kelp zoospores (Roleda et al. 2006b). Comparison between species showed that intertidal *I. cordata* tetraspores had higher recovery rates compared to tetraspores isolated from subtidal algae, especially when pre-exposed to UV-B. Depth related sensitivity of reproductive cells was previously reported in kelp zoospores isolated from sporophytes collected at different depth gradient (Swanson & Druehl 2000).

DNA damage and repair

The absence of DNA damage in *P. endiviifolium* spores and minimal CPD formation in *A. utricularis*, *I. cordata* and *M. hariotii* propagules indicate effective shielding of the DNA and/or fast repair mechanism in the Antarctic intertidal propagules. Damage in subtidal *A. mirabilis* was significantly higher and no complete DNA repair was observed.

The degree of damage due to UVR was observed to be related to the zonation of the adult algae at the coastline. An effective DNA repair mechanism was also observed in spores of Arctic and temperate Laminariales and Gigartinales (Roleda et al. 2004, 2005). DNA damage can be repaired through photolyase enzyme (light-dependent), nucleotide excision and recombination repair (light-independent; van de Poll et al. 2002). The small amount of DNA damage in the tested intertidal Antarctic species might therefore be related to a high photolyase activity. Another possibility is shielding due to UV absorbing compounds. Brown algae are known to be able to produce phlorotannins which absorb in the UV-B range of the spectrum (Pavia et al. 1997). If phlorotannins occur in the exposed brown algal spores remains to be studied. On the other hand adult *I. cordata* was shown to produce two different types of MAAs (shinorine and palythine; Hoyer et al. 2001; Karsten et al. in press), absorbing in the UV-A wavelengths, both of these were also found in tetraspores indicating a protective role of MAAs already in reproductive cells. The ability of the propagules to cope with UV-B induced DNA damage seems to be crucial for the vertical zonation of the macrothalli at the coastline. If not repaired, DNA lesions can disrupt metabolism, cell division and impair growth and germination.

In general, exposure to the UV doses used in our laboratory experiment should not affect the survival and success of the investigated intertidal algae on short term view as all species recovered effectively from UV induced damage. Subtidal species on the other hand, were more affected and might especially suffer from an increasing UV-B penetration into the water body due to stratospheric ozone depletion. In the field, maximal light intensities can be much higher than the ones applied in the laboratory experiments, especially when low tide coincides with times of highest ozone depletion, noon, cloudless weather conditions and a high water transparency. All these factors can occur together in austral spring, when most macroalgae start to grow and reproduce. Moreover, longer exposure times to ambient radiation over more than 8 h are possible in the austral summer when cells are suspended within the euphotic layer of the water column.

4.5 CONCLUSIONS

Grazing significantly shaped the early successional benthic algal community in the Antarctic intertidal. The limpet *Nacella concinna* plays a key role in the system, feeding on both micro- and macroalgae, thereby not only reducing biomass but also shaping species composition and increasing diversity of the algal assemblage. Potential changes in the structure of the algal assemblages will therefore most likely affect intertidal grazers with consequences for higher trophic level organisms as well.

Benthic Antarctic diatoms were found to be mostly unaffected by UVR, both in the field and in the laboratory experiments. Even if the applied doses in the laboratory experiments were much higher than in the field (ca. 5 times) they recovered completely from transient negative effects on photosynthesis and cell number. Other variables such as species composition, biomass and specific-growth rate showed no negative influence of UVR at all. We therefore conclude that natural and even enhanced UVR does not effect the growth of Antarctic benthic diatoms in our study area. From an evolutionary perspective, it is possible that these species are capable to endure UVR. During the course of evolution, they have been exposed to high irradiance levels and UVR exerted a selective pressure. Therefore, it is plausible that the cause of this "endurance" is due to not yet established key mechanisms. In contrast to the macroalgal experiments transient negative effects found in the laboratory were not reflected on community level.

The sensitivity of reproductive cells of Antarctic macroalgae to PAR and UVR was shown to be related to the observed zonation pattern of the adult plants. This response was also reported in the early life stages of macroalgae from the northern Hemisphere (Roleda 2006). Intertidal species showed good recovery and repair mechanisms but an increase in stratospheric ozone depletion and the corresponding increase in irradiance of UV-B on the biosphere might, however, re-shape the structure of algal communities especially if UVR penetrates deeper into the water column as sensitive subtidal species were more affected than intertidal ones (see also Bischof et al. 2006 and references therein). This could lead to a shift in species composition or susceptible species have to escape into deeper waters.

As still only few data are available it is difficult to predict the consequences of enhanced UV-B radiation on community level and ecosystem structure. Our results show that microphytobenthic Antarctic communities are not affected by ambient UVR levels while macroalgal communities were inhibited by UVR already under actual ambient light conditions. The field-experiments on young successional stages revealed species-specific reaction to UVR. Although reproductive cells of species like *M. hariotii* and *I. cordata* showed good recovery mechanism in the laboratory experiments, recruit densities were lower already under the natural UVR regime in the field indicating a non-

optimal condition for the algae at least at some stages of succession. Maximal irradiance intensities in the field could be much higher than the intensities applied in the laboratory approach. Furthermore the ratio between PAR:UV-A:UV-B and intra- and interspecific competition can lead to different results in comparison with laboratory studies. These findings demonstrate again that it is not sufficient to measure only physiological parameters in the laboratory to predict changes on community level. In this context it has to be mentioned that UV-B doses in Antarctica increased already during the past two decades. No long-term studies exist for this area but Karentz (2003) speculated that subtle shifts in community structure to more UV resistant species have already occurred and continue as a result of increased UV exposure. More UV susceptible species might therefore be already excluded from the intertidal habitat, which was supported by our results as at least one species exclusively grew under the no-UV-B treatment while all other intertidal species tested showed good recovery abilities.

4.6 FOR THE FUTURE

In my opinion future research on the UV effects on organisms should focus on two main directions:
Firstly, molecular mechanisms behind the physiological responses are still mostly unknown (see also Bischof et al. 2006). We can only speculate about e.g. the regulation of enzyme activity through changes in gene expression etc. of different species under UV exposure.

Secondly, field-experiments on UVR effects would give valuable information on how organisms react to a natural light regime. Outdoor experiments on e.g. macroalgal propagules are of great importance also taking into account parameters like germination and growth as integrative parameters of all physiological processes. More comparative studies on related species and their reproductive cells from different geographical regions but similar zonation would improve our knowledge about the species-specific reactions and adaptations to (elevated) UVR.

The small amount of field-studies available on macroalgal community level did not show a consistent pattern in their response to UVR (Wahl et al. 2004). More field-studies in this area are necessary to broaden the knowledge on how UVR may alter species composition and the ecosystem structure of benthic algal communities. Furthermore, determinations of UV effects on natural Antarctic microphytobenthos requires more *in situ* measurements of the photosynthetic activity and productivity as already stated by Wulff (1999). Combining ecological (e.g grazing) and abiotic factors (e.g UVR, temperature, nutrients) would moreover increase our understanding of the

integrated response of Antarctic species, communities and ecosystems to their changing environment, especially under the threat of global climate change (Karentz 2003; Bischof et al. 2006).

For future research in the Antarctic region it is necessary to include long-term monitoring studies considering the community development during the Antarctic winter and early spring. Hardly any long-term monitoring program exists to date, especially from the Antarctic region including benthic primary producers. Our conclusions are therefore mainly based on short-term data but to predict changes on community level long-term studies in the time span of years are necessary, including multiple abiotic and biotic variables. However, these types of experiments are, due to the extreme climatic situation in this region, difficult to perform and require logistically difficult maintenance throughout the entire year.

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LIST OF PUBLICATIONS

This thesis is based on the following publications, referred to by their Roman numbers:

I Zacher K, Wulff A, Molis M, Hanelt D, Wiencke C (in press) UV radiation and consumers effects on field-grown intertidal macroalgal assemblages in Antarctica. Global Change Biology

II Zacher K, Hanelt D, Wiencke C, Wulff A (in press) Grazing and UV radiation effects on an Antarctic intertidal microalgal assemblage – a long term field study. Polar Biology

III Zacher K, Campana G (in press) UV radiation and consumer effects on an intertidal and subtidal macroalgal assemblage: a comparative study. Wiencke C, Ferreyra G, Abele D, Marenssi S (eds) In: The Potter Cove coastal ecosystem, Antarctica. Synopsis of research performed 1999-2006 at the Dallmann Laboratory and Jubany Station, King George Island (Isla 25 de Mayo). Berichte zur Polar- und Meeresforschung

IV Wulff A, Zacher K, Hanelt D, Wiencke C (accepted) UV radiation- a threat to Antarctic benthic marine diatoms? Antarctic Science

V Wulff A, Zacher K (in press) Short-term effects of UV radiation on benthic diatoms. Wiencke C, Ferreyra G, Abele D, Marenssi S (eds) In: The Potter Cove coastal ecosystem, Antarctica. Synopsis of research performed 1999-2006 at the Dallmann Laboratory and Jubany Station, King George Island (Isla 25 de Mayo). Berichte zur Polar- und Meeresforschung

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VII Roleda MY, Zacher K, Wulff A, Hanelt D, Wiencke C (accepted) Photosynthetic performance, DNA damage and repair in gametes of the endemic Antarctic brown alga *Ascoseira mirabilis* exposed to ultraviolet radiation. Austral Ecology

VIII Zacher K, Roleda MY, Wulff A, Hanelt D, Wiencke C (manuscript) Physiological and biochemical responses of Antarctic *Iridaea cordata* tetraspores exposed to ultraviolet radiation

IX Bischof K, Gomez I, Molis M, Hanelt D, Karsten U, Lüder U, Roleda MY, Zacher K, Wiencke C (2006) Ultraviolet radiation shapes seaweed communities. Reviews in Environmental Sciences and Bio-Technology, 5: 141-166

X Richter A, Wuttke S, Zacher K (in press) Two years of in situ UV measurements at Dallmann Laboratory/ Jubany Base. Wiencke C, Ferreyra G, Abele D, Marenssi S (eds) In: The Potter Cove coastal ecosystem, Antarctica. Synopsis of research performed 1999-2006 at the Dallmann Laboratory and Jubany Station, King George Island (Isla 25 de Mayo). Berichte zur Polar- und Meeresforschung

UV radiation and consumer effects on a field-grown intertidal macroalgal assemblage in Antarctica

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Running Title: UV and consumer effects on macroalgae succession

Abstract

Ultraviolet radiation (UVR) research on marine macroalgae has hithero focussed on physiological effects at the organism level, while little is known on the impact of UV radiation on macroalgal assemblages and even less on interactive effects with other community drivers, e.g. consumers. Field-experiments on macrobenthos are scarce, particularly in the Antarctic region. Therefore the effects of UVR and consumers (mainly limpets were excluded) on early successional stages of a hard bottom macroalgal community on King George Island, Antarctica, were studied. In a two-factorial design experimental units (1. ambient radiation, 280 to 700nm; 2. ambient minus UV-B, 320 to 700nm and 3. ambient minus UVR, 400 to 700nm vs. consumer – no consumer) were installed between November 2004 and March 2005 (n = 4 plus controls). Dry mass, species richness, diversity and composition of macroalgal assemblages developing on ceramic tiles were followed. Consumers significantly suppressed green algal recruits and total algal biomass but increased macroalgal richness and diversity. Both UV-A and UV-B radiation negatively affected macroalgal succession. UVR decreased the density of *Monostroma hariotii* germlings in the first 10 weeks of the experiment, whereas the density of red algal recruits was significantly depressed by UVR at the end of the study. After 106 days macroalgal diversity was significantly higher in UV depleted than in UV exposed assemblages. Furthermore, species richness was significantly lower in the UV treatments and species composition differed significantly between the UV depleted and the UV exposed treatment. Marine macroalgae are very important primary producers in coastal ecosystems, serving as food for herbivores and as habitat for many organisms. Both, UVR and consumers significantly shape macroalgal succession in the Antarctic intertidal. Consumers, particularly limpets can mediate negative effects of ambient UVR

on richness and diversity till a certain level. UV-B radiation in general and an increase of this short wavelength due to stratospheric ozone depletion in particular may have the potential to affect the zonation, composition and diversity of Antarctic intertidal seaweeds altering trophic interactions in this system.

Introduction

The ozone layer protects all living organisms from excessive ultraviolet-B radiation (UV-B, 280-320 nm). Due to anthropogenic emission of ozone-depleting substances a decline in stratospheric ozone concentrations was detected in the early1980s (Farman *et al*., 1985). During Antarctic spring, the ozone concentration can decrease by >50%, consequently increasing the UV-B radiation reaching the Earth's surface (WMO, 2003). Little improvement is expected for total column ozone in that region for the next several decades (Weatherhead & Andersen, 2006). Although the release of ozone-depleting substances is declining, whether or not ozone levels will ever recover to pre-1980s values is unknown (Weatherhead & Andersen, 2006).

The timing of the ozone depletion over Antarctica is crucial for aquatic organisms, as it coincides with the break up of sea ice, i.e. the phase of highest water transparency (Karentz, 2003), and the season with strongest growth and reproduction for most macroalgal species (Wiencke *et al*., 2006a). Macroalgae are the major primary producers on intertidal rocky shores, providing food and shelter for a variety of associated species (Iken, 1996). Changes in macroalgal productivity or diversity are known to severely affect the structure of coastal marine food webs (Santas *et al.*, 1998). Compared to algae from subtidal habitats, specimens from the intertidal are exposed to higher UV-B regimes. Consequently, changes in species composition and species interactions due to UVR should firstly be recognized within eulittoral communities (Wahl *et al*., 2004).

Most UVR studies on marine macroalgae have been conducted in the laboratory, using artificial irradiance and focusing on physiological effects at the organism level. These studies indicate adverse UV-B effects on macroalgal DNA (van de Poll *et al.*, 2001; Roleda *et al.*, 2004, 2005), growth (reviewed in Franklin & Forster, 1997), photosynthe-

sis (Dring *et al.*, 1996; Hanelt *et al.*, 1997), and an influence on the vertical zonation of macroalgae (e.g. Wiencke *et al.*, 2004; Bischof *et al.*, 2006 for a review). Early developmental stages of macroalgae are regarded as most susceptible to UV stress (reviewed in Coelho *et al*., 2000) and therefore, harmful UV effects should be most severe during early succession.

However, in laboratory studies with single species it is not possible to detect synergistic or indirect UV effects on community level. Furthermore, in laboratory studies unnatural ratios of UV-B, UV-A and photosynthetically active radiation (PAR, 400-700 nm) have been applied with a possible overestimation of UV-B effects. Predictions of ecosystem response to UVR cannot be made by single trophic-level assessments. Different UV sensitivities of e.g. algae and consumers may lead to strong interactive effects as shown by Bothwell *et al*. (1994). In the marine environment, only few studies on interactive effects exist, demonstrating the significance of climatic (e.g. temperature, UVR) and ecological factors (e.g. grazing) as important drivers on macroalgal recruitment (Lotze & Worm, 2002; Lotze *et al.*, 1999). Recently, the effects of UVR on the succession of field grown marine macrobenthic communities were investigated in temperate and tropical regions. In these experiments, UVR was identified as a significant, but non-persistent driver of community structure during early successional stages in macrobenthic assemblages (Lotze *et al.*, 2002; Molis & Wahl, 2004 but see Dobretsov *et al.*, 2005).

Studying UV effects on Antarctic macroalgal assemblages is particularly important due to the severe ozone depletion over this region (WMO, 2003). However, to our knowledge only few field-studies investigated effects of UVR on Antarctic microalgal assemblages (Wahl *et al.*, 2004, Fairhead *et al.* 2006). To date, studies testing for interactions between UV effects and other ecologically important factors are missing.

In the light of this we designed a two-factorial field-experiment to test the separate and combined effects of UVR and consumers on the early succession of an Antarctic intertidal macroalgal assemblage. The main questions were (1) whether UVR and consumer treatments influence biomass, the structure, and diversity of the macroalgal assemblage, (2) whether there is a difference between UV-A and UV-B radiation effects, and (3) whether interactive effects of UV radiation and consumers affected macroalgal community structure.

Materials and Methods

Study site

The field experiment was conducted at a rocky intertidal platform at Peñon Uno, Maxwell Bay, King George Island, Antarctica (62°14 S, 58°41 W). The substratum consists of andensitic bedrock (Kleinschmidt, personal communication) and boulder fields. Intertidal Antarctic seaweed communities consist mainly of annual or pseudoperennial species and richness is low in comparison to temperate or tropical ecosystems (Wiencke & Clayton, 2002). Epibenthic communities are characterized by Rhodophyta (e.g. *Iridaea cordata* Turner (Bory), Heterokontophyta (e.g. *Adenocystis utricularis* (Bory) Skottsberg) and Chlorophyta (e.g. *Monostroma hariotii* Gain, Iken, 1996) as well as mobile consumers, mostly gastropods and amphipods (Ferraz Nonato *et al.*, 2000). In the present study, the gastropod *Nacella concinna* Strebel among other, smaller gastropods like *Laevilacunaria antarctica* Martens and *Laevilitorina umbilicata* Pfeffer was found very frequently and was according to its biomass the most important grazer on macrolagae in the intertidal. Dominant amphipod species in the area are *Gondogeneia*

antarctica Chevreux and *Djerboa furcipes* Chevreux (Jazdzewski *et al.*, 2001; Obermüller personal communication). During the sampling period, the maximal tidal range was about 2 m at a sea surface temperature between -1.8°C (spring) and 2°C (summer). Water transparency is strongly variable, depending on glacial freshwater input and wind direction. UV-transparency of the water body was highest in spring (e.g. 28 November 2003) with a maximal 1 % depth at 16 m for UV-B radiation, 19 m for UV-A radiation, and >20 m for PAR (photosynthetically active radiation 400-700 nm). Minimum concentrations of nitrate, phosphate, and silicate were recorded in February at non-limiting algal growth levels of 15, 2, and 47 µmol, respectively (Schloss *et al.*, 2002).

Experimental design and set-up

Using a randomized block design, we tested in a two-factorial experiment the effects of consumers (two levels, fixed) and UV radiation (three levels, fixed) on the succession of a macroalgal assemblage (n = 4).

The experiment was run from 28 November 2004 to 14 March 2005 (106 days). A pilot-study was performed the year before from 20 December 2003 to 9 March 2004 (74 days). Thirty-two PVC cages (50 x 50 x 12 cm, including the control treatments) were fixed to the substratum at Peñon Uno at a minimal distance of 1 m to each other in the lower eulittoral (Fig. 1). Consequently, cages were submerged at a maximum depth of 2 m. Cages were either open to all sides (open cage) or closed with plastic mesh (1 mm mesh size) to exclude macrograzers, mainly limpets (closed cage). To test for cage artefacts, partially open cages (half cages, equipped with PAB filters, n = 4) were deployed by cutting two holes (~ 15 x 5 cm = 25%) into each sidewall. Using cut-off filters as cage tops, ambient UV radiation levels were manipulated (see below for details). Open

cages without filter (= full sunlight, n = 4) were used as procedural controls to test for filter artefacts.

Unglazed ceramic tiles served as settlement substrata and were attached with Velcro to cage bottoms (Fig. 1). Each cage contained four large (10 x 10 cm) and eight small tiles (5 x 5 cm). At each of four sampling events, one large and one small tile were randomly withdrawn from each cage to determine treatment effects on the macroalgal and microalgal community, respectively. The results from the microalgal experiment will be presented elsewhere. At the end of the experiment four small tiles remained and were returned to the laboratory at Bremerhaven, Germany for cultivation (see below).

UV radiation treatments

Cut-off filters manipulated the ambient light regime in three ways. (1) P = PAR (photosynthetically active radiation) treatment (>400 nm): using a 3 mm thick Perspex sheet (GS 231, Röhm, Germany), radiation <400 nm was blocked, while filters were transparent for 91% of PAR. (2) PA = PAR + UV-A treatment (>320 nm): using a 3 mm thick Perspex sheet (GS 2458, Röhm, Germany) and a 0.13 mm transparent polyester film (Folanorm-SF/AS, folex imaging GmbH, Germany), radiation <320 nm was blocked, while 89% of PAR and UV-A passed the filter. (3) PAB = PAR + UV-A + UV-B treatment (>280 nm): using a 3 mm thick Perspex sheet (GS 2458, Röhm, Germany) transmitting 92 % of PAR and UV radiation. Transparency of the GS 231 and GS 2458 Perspex filters decreased on average by 1.11% (SD ± 0.01) and 1.31% (SD ± 0.01) per a month, respectively. Therefore only damaged filters were exchanged. Polyester films were exchanged bi-weekly to minimize aging and fouling effects on transparency. Filters were cleaned once or twice per week.

Radiation measurements

Weekly to bi-weekly, the radiation regime above the water surface, at 10 and 200 cm depth was recorded at a distance ~50 m to the experimental site with a LiCor data logger (LI-1400, Li-Cor, Lincoln, USA) equipped with an underwater PAR sensor (LI-192) and a Solar Light (PMA2100, Solar Light Co. Inc., USA) equipped with a UV-B (PMA2106-UW) and a UV-A radiation (PMA2110-UW) broad-band sensor. Readings were taken ± 1 h of local noon. Ambient UV-A + UV-B radiation was continuously recorded at the nearby (1.5 km) Dallmann Laboratory with a 32-channel single-photon counting spectroradiometer (Isitec, Germany). In addition, the weighted irradiance (minimal erythemal dose, UV_{ery}) was measured continuously next to the cages with two ELUV-14 UV-dosimeters (El Naggar *et al.*, 1995) to follow the underwater UV-regime and its relative changes during the experiment.

Consumer abundance

Macrobenthic consumer density in each cage was estimated in January and March 2005 (by SCUBA diving). In each cage, the individuals of each gastropod species were counted and the density of amphipods estimated in categories of tens. Consumers inside closed cages were also counted and occasionally found gastropods were removed. Amphipods entering or recruiting in the closed cages could not be removed and remained inside.

Sampling of macroalgae

The density (number/cm^2) of each macroalgal species was estimated on 15 and 29 January, 16 February, and 3 March 2004 (i.e. 26, 40, 58, and 74 days after starting the pilot-study) and 10 January, 7 and 24 February, and 14 March 2005 (i.e. 43, 71, 88, and 106

days after starting the experiment). At the final sampling, four small tiles from each cage were transported in seawater filled plastic bags to Bremerhaven, Germany and cultivated under fluctuating Antarctic daylength (10 to 30 µmol m^{-2} s^{-1}) at 0° C in a constant temperature room until most macroalgal germlings could be identified. Species identified after postcultivation served as qualitative data only and not for the statistical tests. All large tiles were sampled immediately after collection from the field at the Dallmann Laboratory. Recruit density of macroalgae was determined by counting individual germlings in five sub-samples per tile (~50 mm^2) using a stereomicroscope (16x magnification), leaving a border of 1 cm unsampled to avoid edge effects. Biomass of the community was measured as dry mass, by removing and drying (48 h at 80°C) all organisms from the tile. We calculated Shannon diversity *H'* and Margalef species richness *d* (PRIMER ™ 5 software package, Plymouth Marine Laboratory).

Data analysis

A t-test was performed to test for differences between two independent groups (e.g. test for cage or filter artefacts). Repeated measures (RM) ANOVA was used to test for the overall effects of consumers and UV radiation over time. Because the assumption of sphericity was not met (Mauchley's test) adjusted univariate *F*-ratios (Greenhouse-Geyser and Huynh-Feldt) were used (Quinn & Keough, 2002). Outcome was the same as in the RM ANOVA therefore we refer in the following to the former test. For separate sampling dates, a two-way ANOVA was performed to test for the effects of consumers and UV radiation on biomass, density of red and green algae recruits, species richness *d* and diversity *H'* at a Bonferroni corrected significance level ($\alpha = 0.0125$) in order to lower the probability of making a type I error (Quinn & Keough, 2002). Prior to analysis, data were tested for homogeneity of variances (Cochran´s test). Heterosce-

dastic data after ln- or square-root transformation were analyzed by the non-parametric Kruskal-Wallis test. Post-hoc comparisons were performed with Newman-Keuls test using Statistica ™ 6.0 software package. Species composition of communities was compared by ANOSIM, and in case of significance, followed by SIMPER to quantify the relative contribution of species to observed dissimilarities among treatments (PRIMER ™ 5 software package, Plymouth Marine Laboratory). ANOSIM used a Bray-Curtis similarity matrix based on fourth root transformed density data. Results from ANOSIM were illustrated with MDS-plots.

Results

Radiation measurements

Figure 2 shows the maximal UV-A and UV-B irradiances measured during April 2004 and April 2005. Peak values of UV-A and UV-B radiation in the air were recorded in December (Fig. 2), coinciding with the highest values of underwater UV-B irradiance determined as UV_{ery} (Fig. 3). Lowest underwater UV-B values during the experiment were measured in February and March 2005 (Fig. 3). Maximum UV-exposure on the tiles was reached during low tide on the 14^{th} of December 2004 (around noon) were the cages were exposed to 44 W m^{-2} UV-A and 2.3 W m^{-2} UV-B, respectively. On average, 7.3 ± 5.7% (mean ± S.D.) of surface UV-B, 13 ± 9.8 % of UV-A and 30 ± 11.4 % of PAR reached 200 cm water depth close to the experimental site around noon (Table 1).

Consumer abundance

The most abundant consumers during the experiment were amphipods (Table 2). Amphipod density in January was higher in half cages (n = 4) than in open cages (t-test =

2.78, p = 0.032), indicating cage artefacts. Furthermore their density was significantly higher (about 100%) in closed cages in relation to open cages (t-test = -3.30, p = 0.003). In January, *Nacella concinna* and other gastropod densities in open and half cages showed no significant differences (t-test = 2.41, p > 0.05), thus no cage artefact was observed. Gastropod densities in closed cages were significantly lower (96%) in comparison to open cages (t-test = 6.20, p < 0.001).

In March, amphipod density was again higher in half cages than in open cages (t-test = 3.66, p = 0.011, Table 2). Their density was significantly higher (about 240%) in closed cages in relation to open cages (t-test = -4.66, p < 0.001). For gastropod densities (open and half cages) no significant differences were found (t-test = 1.62, p > 0.05); densities in closed cages was 40% lower compared with open cages (t-test = 1.79, p > 0.05).

No UV effects on total consumer density were detected (RM ANOVA, radiation effect, $F_{2,18} = 1.69$, p = 0.213).

UVR and consumer effects

In general both experiments (the pilot study in 2004 and the longer experiment in 2005) gave very similar outcomes. Table 3 gives an overview of the significant results of the two seasons. The following sections refer to the second, longer experiment.

In general, neither significant differences between open and half cages, nor between PAB and full sunlight treatments were detected for all tested parameters (t-test, p > 0.05), showing that there were no cage or filter artefacts.

UVR and consumer effects on biomass and abundance

Overall, both consumers and the interaction of UV radiation and consumers had a significant effect on biomass over the whole time span. These effects did not change over

the duration of the experiment, shown by a non-significant time x treatment interaction (RM ANOVA, Table 4). For single sampling dates, no significant treatment effects on biomass were observed for either UV radiation or the interaction of UVR and consumers (Table 3). Consumers significantly reduced biomass on all sampling events (ANOVA or Kruskal-Wallis, January, $F_{1,18} = 70.31$, $p < 0.001$; early February, $H_{1,24} = 16.80$, $p < 0.001$; late February, $F_{1,18} = 298.03$, $p < 0.001$; March, $H_{1,24} = 17.29$, $p < 0.001$, correspondingly, Fig. 4).

The most abundant colonizer throughout the experiment was the green alga *Monostroma hariotii* Gain, reaching a total of 92 to 99 % of all germlings on the tiles. Green algal recruitment was suppressed by UV radiation after 43 (ANOVA, $F_{2,18} = 14.58$, $p < 0.001$) and 71 days (ANOVA, $F_{2,18} = 7.69$, $p = 0.004$, Table 3, Fig. 5), but not at later samplings. During the last three sampling events, the density of green algal recruits was significantly reduced when consumers were present (Day 71: ANOVA, $F_{1,18} = 23.69$, $p = 0.004$, Day 88: $F_{1,18} = 31.51$, $p < 0.001$, Day 106: ANOVA, $F_{1,18} = 41.50$, $p < 0.001$, Table 3, Fig. 5).

At the beginning of the experiment very few red algal recruits settled but the density increased towards the end of the study (Fig. 5). UV radiation significantly reduced the red algal density at the end of the experiment (Kruskal-Wallis, $H_{2,24} = 15.14$, $p = 0.001$, Table 3) mostly due to UV-A rather than UV-B (Newman Keuls, P:PAB and P:PA, $p < 0.05$; PAB:PA, $p > 0.05$). The density of red algal recruits was not affected by consumers.

UVR and consumer effects on species composition and diversity

Eight macroalgal species were found on the experimental tiles throughout the experiment (see Fig. 6). Three belonged to Chlorophyta (*Monostroma hariotii* Gain, *Urospora*

penicilliformis (Roth) Areschoug, and *Ulothrix sp*.) and the remaining five belonged to Rhodophyta (*Iridaea cordata* Turner (Bory), *Palmaria decipiens* (Reinsch) Ricker plus three unidentified Gigartinales). During postcultivation in the laboratory, four Heterokontophyta were encountered (*Petalonia fascia* (Müller) Kuntze, *Adenocystis utricularis* (Bory) Skottsberg, *Geminocarpus geminatus* (Hooker *et* Harvey) Skottsberg, and one unidentified microthallus). Their young germlings were not detectable under the dissection microscope in Antarctica and could only be seen after being held in culture for an additional period of time. In sum, after cultivation 12 different macroalgal species were identified.

Overall, UV x consumer interactions on species richness were dependent on sampling dates (Table 4). Only at the final sampling, species richness was significantly increased by consumers (ANOVA, $F_{1,18} = 11.48$, $p = 0.003$) and decreased by UV (ANOVA, $F_{2,18} = 6.51$, $p = 0.007$; Table 3, Fig. 7). This was an effect of UV-A rather than UV-B (Newman-Keuls, P:PAB and P:PA, $p < 0.05$; PAB:PA, $p > 0.05$, Fig. 7).

UV x consumer interactions and consumer effects on diversity significantly changed over time (Table 4). At day 71, the presence of consumers increased diversity significantly (ANOVA, $F_{1,18} = 11.41$, $p = 0.003$, Table 3, Fig. 7). At day 106, UV radiation suppressed diversity significantly (Kruskal-Wallis, $H_{2,24} = 11.96$, $p = 0.003$, Table 3). Diversity under the PAB-treatment was significantly lower than under P-treatment, with PA-regimes resulting in intermediate levels of diversity (Newman-Keuls, P:PAB, $p < 0.05$; PAB:PA and P:PA, $p > 0.05$, Fig. 7).

UV radiation affected species composition at later stages of succession (Fig. 8 for sampling 4). At day 71, species composition was significantly different between PAB and P treatments. This difference was mainly due to the strong decline in the density of recruits of the green alga *Monostroma hariotii* and one unidentified Gigartinales recruit

(Red 1) under the PAB treatments which explained together 70% of the dissimilarity between the treatments (Table 5). Again, at day 106, species composition was significantly different between PAB and P treatments. This difference was mostly due to the negative UV-impact on the density of one unidentified Gigartinales recruit (Red 2) and *M. hariotii* under the PAB treatment which explained together 60% of the dissimilarity between the treatments (Table 5). The PA treatments took an intermediate position between the P and the PAB treatments (Fig. 8).

Consumer affected species composition significantly during the last three samplings (e.g. Fig. 8 for sampling 4). SIMPER analysis showed that *M. hariotii* and *P. decipiens* recruits together explained 60, 60 and 40 % of the dissimilarities between the open and closed cages at the three samplings, respectively. Thereby consumers decreased *M. hariotii* density, whereas *P. decipiens* density was favoured by consumer presence (or inconsistent at sampling 3, Table 5).

Discussion

Overall, the experiments revealed significant negative effects of ambient levels of UV radiation and consumers on the intertidal Antarctic macroalgal assemblage. The treatment effects were more pronounced at the end of the study. In general, consumer effects (mainly on biomass and recruit density) were more often observed than UV effects (affecting mainly diversity and species composition).

The pilot study showed the importance of choosing an adequate experimental period due to the slow growth of the recruits. Therefore, in the second year a maximal experimental exposure time was chosen (from sea ice break up until the end of summer). However, the general outcome of the two experiments was similar.

Consumer effects

Consumers reduced biomass of macroalgal assemblages throughout the experiment. Herbivores preferred green algae over red seaweeds, decreasing the density of green algal recruits in open and half cages compared to closed cages. This effect on biomass was not caused by the small-sized amphipods, as they were not excluded by cages. Antarctic amphipods e.g. *Gondogneia antarctica* feed on some macroalgae, such as *I. cordata* and *P. decipiens* (Huang *et al.*, 2006), but are apparently not able to graze on macroalgae during early succession were recruits are very small and well attached to the ground. Similar results were found in laboratory experiments with the green alga *Enteromorpha intestinalis* where snails had strong negative effects on macroalgal recruitment, whereas amphipods did not feed on *Enteromorpha* recruits but consumed adult *Enteromorpha* pieces (Lotze & Worm, 2002). The firm attachment of recruits made it difficult to detach them, even with a brush. Thus, the impact of amphipods on early successional stages of the macroalgae species growing on our experimental tiles seems to be negligible. Other species might have been grazed by amphipods from the start and therefore don't grow in the field but later in culture (e.g. *Geminocarpus*). Consequently, biomass effects in our set-up were mainly caused by larger limpets, e.g. *Nacella concinna*, which were successfully excluded by cages. In contrast to amphipods, *N. cocinna* is clearly the largest (length ≤ 46 mm) and most important grazer at our study site and can reach densities from 28 to 131 ind. m^{-2} in the Antarctic intertidal (Brêthes *et al.* 1994). *Nacella concinna* mostly feeds on macroalgal propagules and benthic microalgae (Kim, 2001; Iken, 1996), whereas the smaller snail *Laevilacunaria antarctica* was shown to feed on *Monostroma hariotii*, the most dominant green alga on our tiles (Iken, 1999). At the experimental site (Peñon Uno), a negative correlation between the density of *N. concinna* and macroalgae was also detected by Kim (2001), indicating effective

grazing of this species. This further demonstrates the importance of gastropods, especially *N. concinna* as driver on community structure in the intertidal during early macroalgae succession. For example, *N. concinna* and *L. antarctica* contributed to 47% of the biomass of macroalgae-associated herbivores at the study site (Iken, 1996). Grazers can also influence the diversity by e.g. increasing or decreasing the spatial heterogeneity of the system (Sommer, 2000). Gastropods, like *Littorina littorea* were shown to increase the diversity by creating a diverse mosaic of microhabitats (Sommer, 2000). In our study, feeding tracks alternate with untouched biofilm (due to snail grazing) and species richness and diversity were generally higher in cages where gastropods were present.

UV radiation effects

UV effects changed over time showing species-specific differences. Strongest impacts on the community structure were observed at the end of the experiment (after 3.5 months) in contrast to other studies (Santas *et al.*, 1998; Lotze *et al*., 2002; Molis & Wahl, 2004; Wahl *et al*. 2004 but see also Wulff *et al.*, 1999 and Dobretsov *et al*., 2005). UV-A radiation was mainly responsible for a decrease in recruit density and species richness whereas additional UV-B had a significant negative influence on species composition and diversity. The different effects of UV-B and UV-A (with UV-A exceeding UV-B by a factor around 20 on a daily dose) demonstrated that UV-B radiation was more damaging per unit irradiance, but that UV-A is more damaging at the actual daily doses received (Cullen & Neale, 1994; Wahl *et al.*, 2004; Wiencke *et al*., 2006b). Green algal recruit density was decreased by UV radiation at the start of the experiment whereas red algal recruit density was most affected at the end with impacts on diversity, species richness and species composition. Several explanations for the changing nature

of UV effects on the assemblage level are conceivable: (i) UV effects may match with changing radiation fluxes during the experiments, (ii) shading effects, where less UV-sensitive canopy species allow colonization of more UV-sensitive species as understorey algae, (iii) different adaptation strategies (e.g. morphology, protective substances like MAAs or phlorotannins, DNA repair mechanisms) leading to species-specific response to UV radiation (Lotze *et al.*, 2002; Molis & Wahl, 2004).

In our study, a correlation between diminishing UV effects and a decrease in UV doses over time (model i) was shown for the density of green algae recruits i.e. its most dominant representative *Monostroma hariotii*. An adaptation to UV radiation over time together with decreasing UV doses are possible explanations. The macrothallus of *M. hariotii* occurs in high abundance in the Antarctic intertidal. Early life stages, however, are shown to be more sensitive to UV stress compared with adults of the same species (reviewed by Coelho *et al.*, 2000), but have the capacity to acclimate as they mature (Lotze *et al.*, 2002).

In contrast to the green algal recruits, red algal recruits were more sensible to UV radiation during later stages of succession but early negative UV effects on red algal germlings might have been masked by low densities at the beginning of the experiment (few individuals and species settled in the first weeks and the variance between replicates was high (Dobretsov *et al.*, 2005). Most red algae are fertile in late summer whereas green algae like *M. hariotii* release spores earlier in the season (Wiencke & Clayton, 2002). Especially one unidentified Gigartinales recruit, occurring only at the end of the experiment was highly UV susceptible and mainly responsible for the strong UV effects on red algal recruits. Macrothalli of some Antarctic red algae species (e.g. *P. decipiens* and *I. cordata*) produce MAAs which enables them to grow in the intertidal (Hoyer *et al*., 2001). However, little is known about MAA production in spores and germlings. In

temperate and tropical regions, some UV-tolerant species provide protective shading and allow colonization of more UV-sensitive species (model ii, Wahl *et al.*, 2004; Lotze *et al.*, 2002; Molis & Wahl, 2004). In our experiment, however, these shading effects were lacking because propagules were still very small at the end of the experimental period. The macrothalli of many species develop in the winter period or in early spring of the following season. The UV radiation could therefore directly inhibit growth and influence negatively species richness and diversity.

UV-B doses in Antarctica have increased for more than two decades. No long-term studies exist for this area but Karentz (2003) speculated that subtle shifts in community structure to more UV resistant species have already occurred and is continuing as a result of increased UV exposure. Species encountered in the intertidal nowadays should therefore be well adapted to UV radiation. However, our results show that this is only partly true for macroalgal recruits which are species-specifically inhibited by UV radiation.

Interactive UV and consumers effects

Overall interactive effects of UV x consumer were found on biomass but not for single sampling dates. Interactions between UV radiation and consumers can occur when UV induces changes in the chemical composition of algae thereby altering consumption patterns (Lotze *et al.*, 2002). On the other hand, UV radiation can have a direct negative effect on consumers, resulting in an enhanced algal productivity (Bothwell *et al*., 1994). From the second to the last sampling date, the biomass was lower in the PAB treatment when consumers were absent, but this effect was not significant for the single sampling dates. Since there was no UV effect on biomass and no UV effect on consumers, we assume this to be a spurious effect.

Species composition was significantly affected by both UV and consumers due to different species and group-specific responses to radiation and consumer treatments, especially at the last sampling dates. Whereas UV radiation suppressed recruit density after 106 days, consumers favoured the density of some leathery red algal recruits (*P. decipiens* and *I. cordata*). Therefore, at least in some cases consumers have the potential to counteract negative UV effects. On the other hand, UV and consumer effects on *M. hariotii* and one unidentified red alga worked in the same direction further decreasing their density. In general, changes in UV radiation and consumer pressures might cause seasonal and/or spatial shifts in species composition and community structure (see also Lotze *et al*., 2002; Dobretsov *et al*., 2005).

In conclusion, our results show that Antarctic macroalgal recruits are particularly sensitive to UV radiation and consumer pressure. Consumers, especially snails, can compensate for negative effects of ambient UV on richness and diversity up to a certain level, but never reach the same level as without UV radiation. While UV-B radiation had a significant negative influence on macroalgal composition and diversity a further increase, due to stratospheric ozone depletion, would influence these variables most, whereas species richness and biomass would be less affected. Therefore we hypothesise that UV-B radiation in general, and an increase of these wavelengths in particular has the potential to affect the zonation, composition and diversity of Antarctic intertidal seaweeds altering trophic interactions in this system. Whether the significant negative impact of ambient UV radiation at the end of the experiments is persistent when recruits develop into macrothalli in the next spring requires further studies. Therefore we suggest that future research in Antarctic region should include long-term monitoring studies considering the community development during the Antarctic winter and early spring. Combining ecological and abiotic factors would further increase our understanding of

the integrated response of Antarctic species, communities and ecosystems to their changing environment (Karentz, 2003; Bischof *et al.*, 2006; Molis & Wahl, 2004). However, these types of experiments are, due to the extreme climatic situation in this region, difficult to perform and would require logistically difficult maintenance throughout the entire year.

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Table 1 Mean irradiance (± SD) above the water surface, at 10 and 200 cm water depth and the percentage of the irradiance relative to surface values (100%). All measurements ± 1 hour around local noon for three solar wavebands: (1) PAR (400 to 700 nm, n = 7); (2) UV-A (320 to 400 nm, n = 12); (3) UV-B (280 to 320 nm, n = 12); measured with a broad-band sensor from December 2004 until February 2005.

	PAR (µmol m^{-2} s^{-1})		UV-A (W m^{-2})		UV-B (W m^{-2})	
	mean	S.D.	mean	S.D.	mean	S.D.
above surface	1136	327	24.1	12.6	1.4	0.7
% irradiance in 10 cm	64	14.4	55	15.4	60	7.3
10 cm	734	291	13.7	8.4	0.8	0.4
% irradiance in 200 cm	30	11.4	13	9.8	7	5.7
200 cm	314	150	2.9	2.7	0.1	0.1

Table 2 Consumer density (number of individuals) in cages from different consumer treatments.

	closed cages (n = 12)		open cage (n = 12)		half cage (n = 4)	
	mean	SE	mean	SE	mean	SE
January						
Nacella cocinna	0	0	3.00	0.82	2.25	0.95
other Gastropods	0.58	0.43	13.33	2.24	28.80	8.61
Amphipods	28.75	3.15	14.58	2.92	22.50	4.79
March						
Nacella cocinna	0	0	1.67	0.47	3.25	1.03
other Gastropods	2.58	0.74	2.67	0.58	3.50	1.89
Amphipods	25.42	3.61	7.50	1.31	22.50	4.79

Table 3 Two-factorial ANOVA or non-parametric Kruskal-Wallis test on UV radiation and consumer (C) effects on biomass, density of Chlorophyta and Rhodophyta, species richness *d* and diversity *H'* for the sampling dates (numbers one to four in the table) of both studies 2004 and 2005 (- not significant, + significant), p-values Bonferroni corrected (significance level $p < 0.0125$). Note that samplings one to four did not take place in the same time interval in 2004 and 2005 (see materials and methods).

		biomass		density Chlorophyta		density Rhodophyta		species richness		diversity	
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
1	UV	-	-	-	+	-	-	-	-	-	-
	C	+	+	-	-	-	-	-	-	-	-
	UV:C	-	-	-	-	-	-	-	-	-	-
2	UV	-	-	-	+	-	-	-	-	-	-
	C	+	+	+	+	-	-	-	-	-	+
	UV:C	-	-	-	-	-	-	-	-	-	-
3	UV	-	-	+	-	-	-	-	-	-	-
	C	+	+	+	+	-	-	-	-	-	-
	UV:C	-	-	-	-	-	-	-	-	-	-
4	UV	-	-	-	-	+	+	-	+	+	+
	C	+	+	+	+	+	-	-	+	-	-
	UV:C	-	-	-	-	-	-	-	-	-	-

Table 4 RM ANOVA on UV radiation and consumer (C) effects on biomass, species richness *d* and diversity *H'* (four sampling events between January and March 2005, n = 4).

Source		biomass		species richness		diversity	
	df	*F*	p	*F*	p	*F*	p
UV	2	2.84	0.085	0.77	0.480	1.99	0.165
C	1	33.95	**<0.001**	0.17	0.681	0.56	0.464
UV:C	2	39.96	**<0.001**	1.52	0.246	1.27	0.305
Residuals	18						
Time	3	2.36	0.081	4.35	**0.008**	10.87	**<0.001**
Time:UVR	6	1.50	0.197	0.34	0.915	1.78	0.120
Time:C	3	2.19	0.010	2.19	0.099	3.28	**0.028**
Time:UVR:C	6	1.66	0.148	4.53	**<0.001**	6.94	**<0.001**
Residuals	54						

Table 5 Results of ANOSIM (pairwise test and Global R, p) on species composition for all sampling events, and results of SIMPER for significant results, indicating the contribution of single species to total dissimilarity in species composition due to treatment effects. Data were 4th root transformed, p-values Bonferronie corrected (significance level $p < 0.0125$), PAB = PAR + UV-A + UV-B, P = PAR; nt = not tested. The direction of the effect is given as + positive UV or consumer effect, - negative UV or consumer effect, ± inconsistent.

	UV PAB:P	**Consumer**
After 43 days	R = 0.286, p = 0.018	R = 0.015, p = 0.300
After 71 days	**R = 0.323, p = 0.006**	**R = 0.406, p < 0.001**
M. hariotii	41.5% -	35.0% -
P. decipiens	18.8% -	23.8% +
Red 1	27.1% -	24.1% -
I. cordata	12.6% -	10.9% -
After 88 days	R = 0.073, p = 0.261	**R = 0.291, p = 0.001**
M. hariotii	nt	39.0% -
P. decipiens	nt	19.2% ±
Ulothrix sp.	nt	19.2% +
I. cordata	nt	14.7% -
After 106 days	**R = 0.792, p = 0.001**	**R = 0.331, p = 0.001**
M. hariotii	17.7% -	27.2% -
Red 2	40.3% -	25.6% -
P. decipiens	11.5% -	15.2% +
I. cordata	15.0% -	17.4% +

List of Figures

Fig. 1 Open cage allowing free access for consumers. Spatial arrangement of large and small ceramic tiles for the macro- and microalgal assemblage, respectively. Large tiles were used for macroalgae recruit identification and biomass measurements, half of the remaining small tiles for post-cultivation of macroalgae and the other half for the assessment of the microalgal assemblage (to be published elsewhere).

Fig. 2 Daily maximum UV-A and UV-B irradiance from April 2004 to April 2005 measured at the Dallmann Laboratory (UV-A grey line, UV-B black line).

Fig. 3 Erythema weighted UV-B irradiance (UV_{ery}) during the duration of the experiment at Peñon Uno from December 2004 to March 2005. The sensor was located close to the cages with a maximal water column on top of 200 cm during high tide.

Fig. 4 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and consumers (open and closed cages) on the biomass at the four samplings (mean of total biomass of each tile = 100 cm^2 ± 1 SE, n = 4). Capitals indicate significant differences between consumer treatments, i.e. A is significant different from B (as mean of the UV treatments).

Fig. 5 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and consumers (open and closed cages) on density of red (diagonal hatched) and green algal (grey) recruits at the four samplings (mean ± 1 SE, n = 4). Note logarithmic scale. Lower case letters indicate significant differences between different UV treatments (as

mean of closed and open treatments, respectively) and capitals significant differences between consumer treatments (as mean of the UV treatments, here only for green algal density, different letters demonstrate significant differences). If no letters were used no significant difference was found.

Fig. 6 Macroalgal germlings on postcultivated tiles. (first row: left *Ulothrix sp*., middle *Urospora peniciliiformis*, right *Monostroma hariottii*; second row: left *Geminocarpus geminatus*, middle *Adenocystis utricularis*, right *Petalonia fascia*; third row: left *Iridaea cordata*, middle *Palmaria decipiens*, right postcultured tile).

Fig. 7 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and consumers (open and closed cages) on species richness *d* (black) and diversity *H'* (grey) of red and green algal recruits at the four samplings (mean ± 1 SE, n = 4). Letters indicate significant differences between different UV treatments, a (A) is significant different from b (B), AB is not significantly different from A or B (as mean of closed and open treatments, respectively). Consumer effects on diversity were found on Day 71 and for species richness for Day 106 with the open cages having higher values than the closed ones.

Fig. 8 MDS plot of macroalgal assemblages after 106 days (sampling 4). The species composition is different from P to PAB but also differs between grazed and non-grazed plots. The PA treatment does not show a clear pattern. Key: white circle = open PAB treatments; hatched circle = closed PAB treatments; grey triangle = open PA treatments; black triangle = closed PA treatments; white square = open P treatments; hatched square = closed P treatments; n = 4; Stress = 0.17.

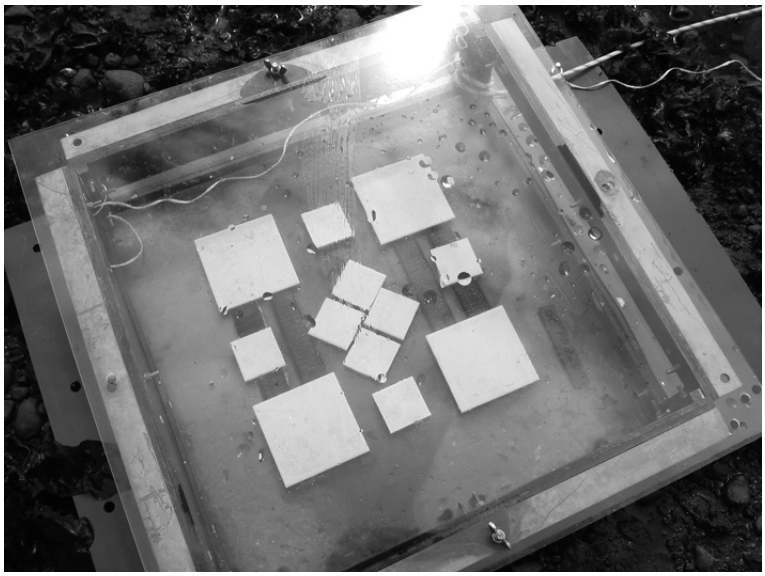

Fig. 1

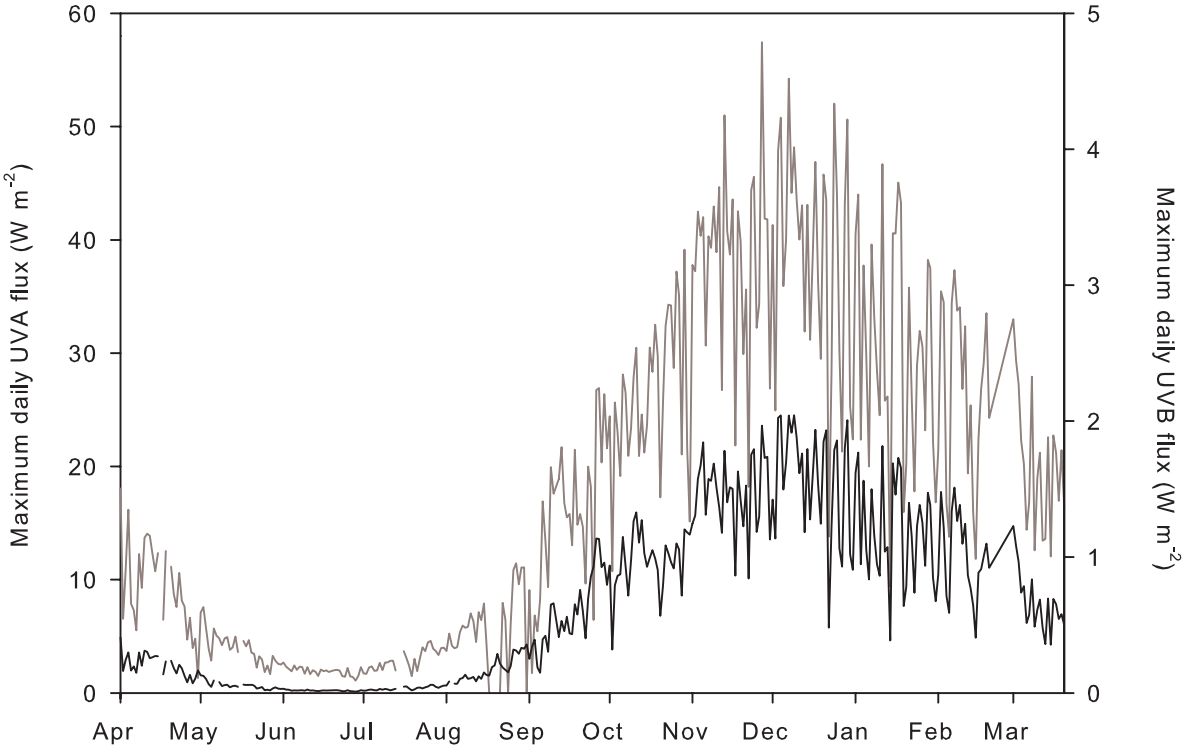
Maximum daily UVA flux (W m-2)
Maximum daily UVB flux (W m-2)
60
50
40
30
20
10
0
5
4
3
2
1
0
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar

Fig. 2

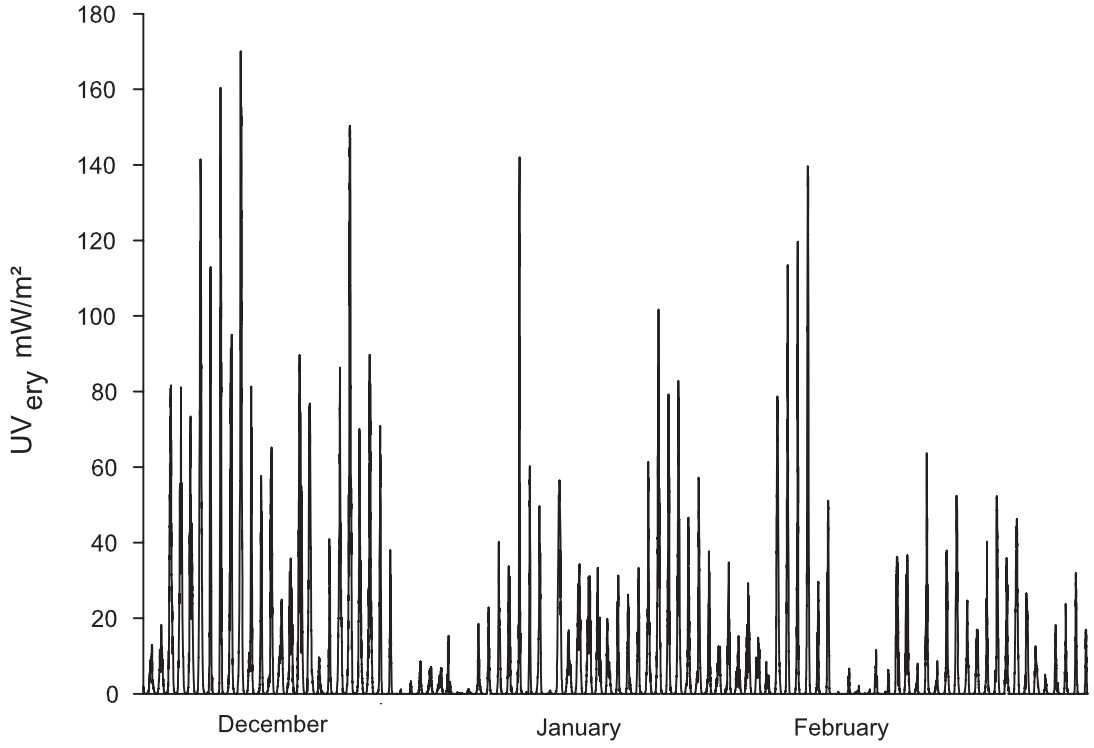
180
160
140
120
100
80
60
40
20
0
UV ery mW/m²
December
January
February

Fig. 3

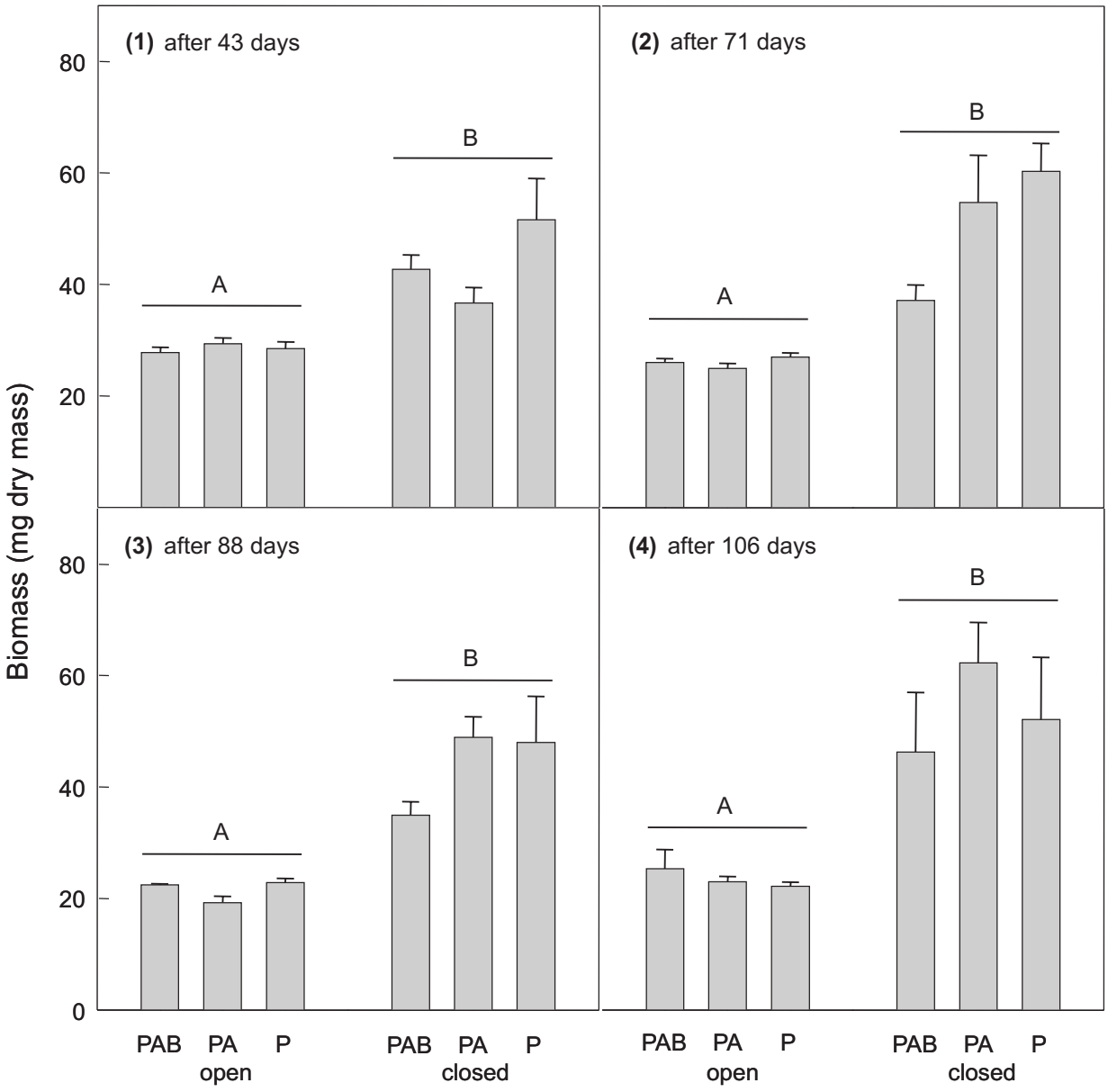
(1) after 43 days
(2) after 71 days
(3) after 88 days
(4) after 106 days
Biomass (mg dry mass)
0
20
40
60
80
A
B
PAB
PA open
P
PA closed

Fig. 4

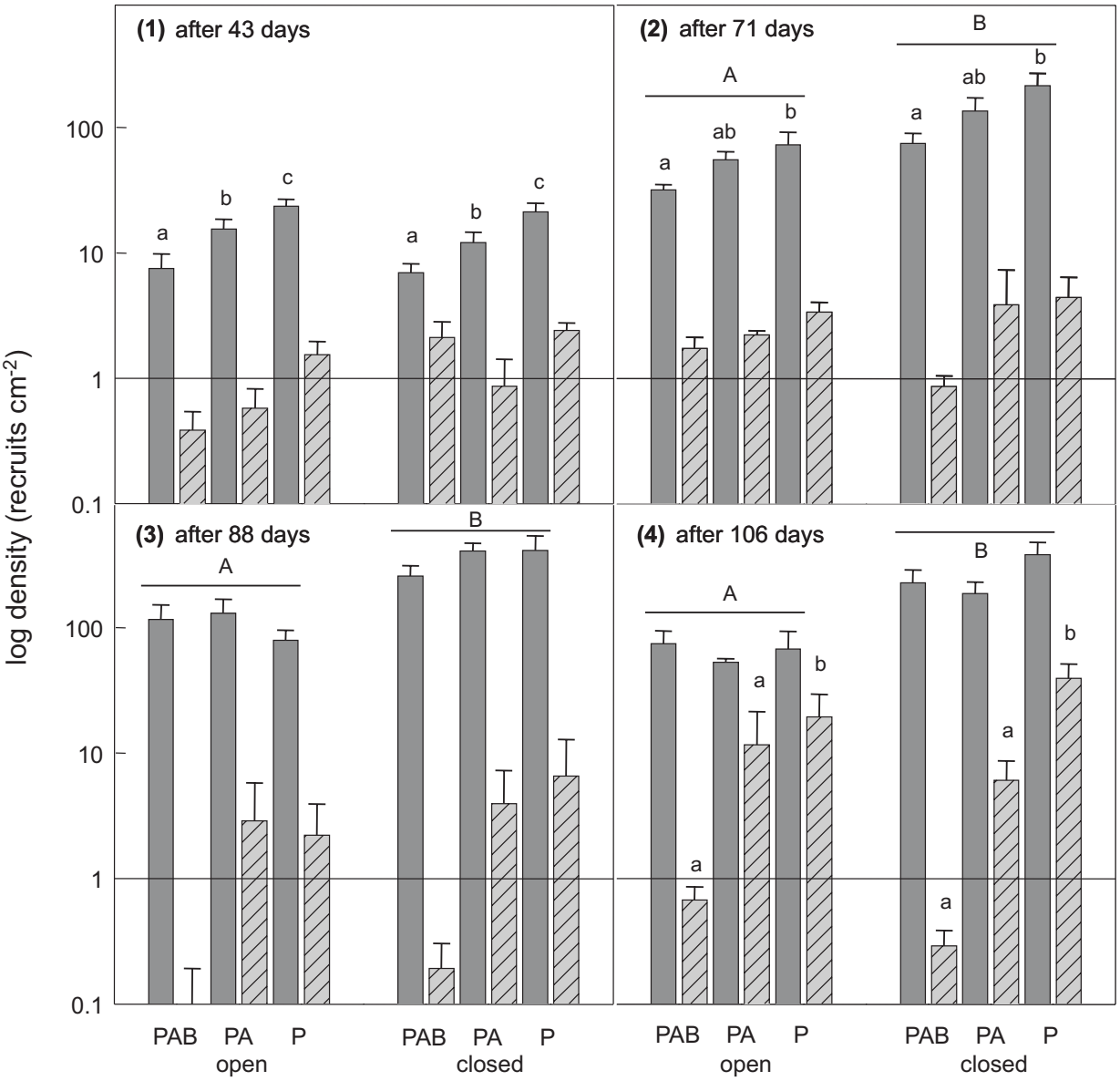
(1) after 43 days
(2) after 71 days
(3) after 88 days
(4) after 106 days
log density (recruits cm-2)
100
10
1
0.1
A
B
a
ab
b
c
PAB
PA
P
open
closed

Fig. 5

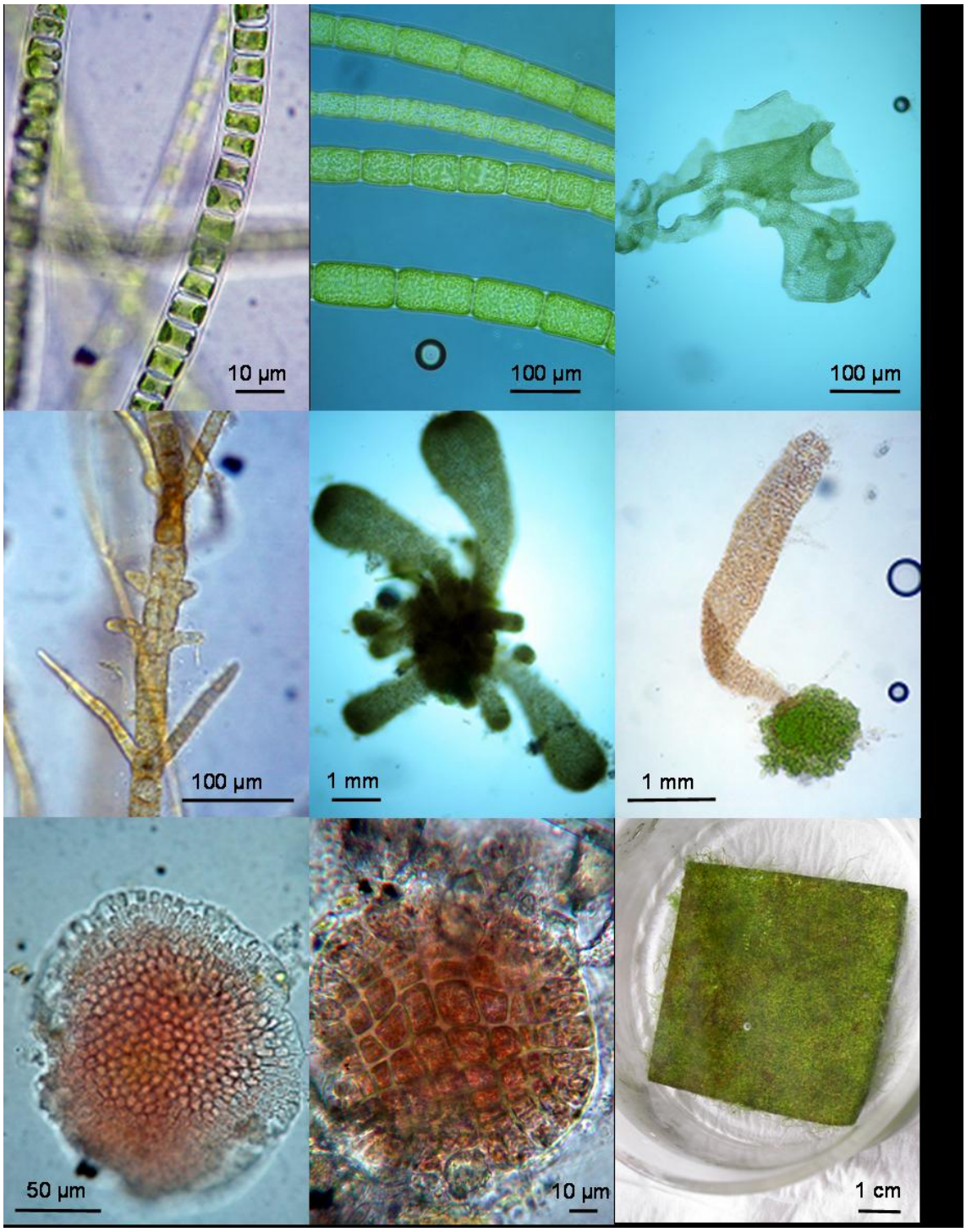

Fig. 6

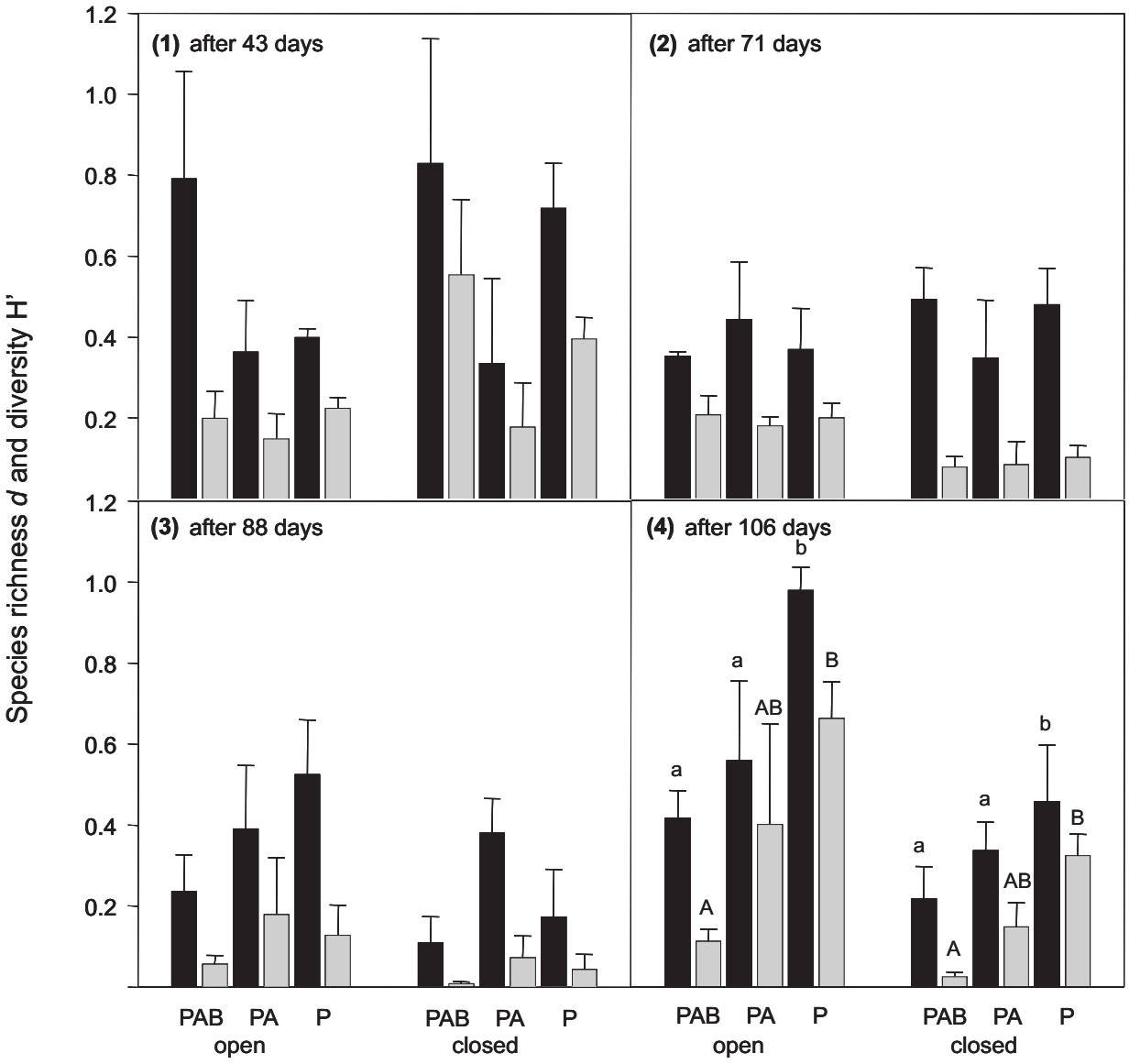
Species richness d and diversity H'
(1) after 43 days
(2) after 71 days
(3) after 88 days
(4) after 106 days
1.2
1.0
0.8
0.6
0.4
0.2
PAB PA P
open
closed
a
A
AB
B
b

Fig. 7

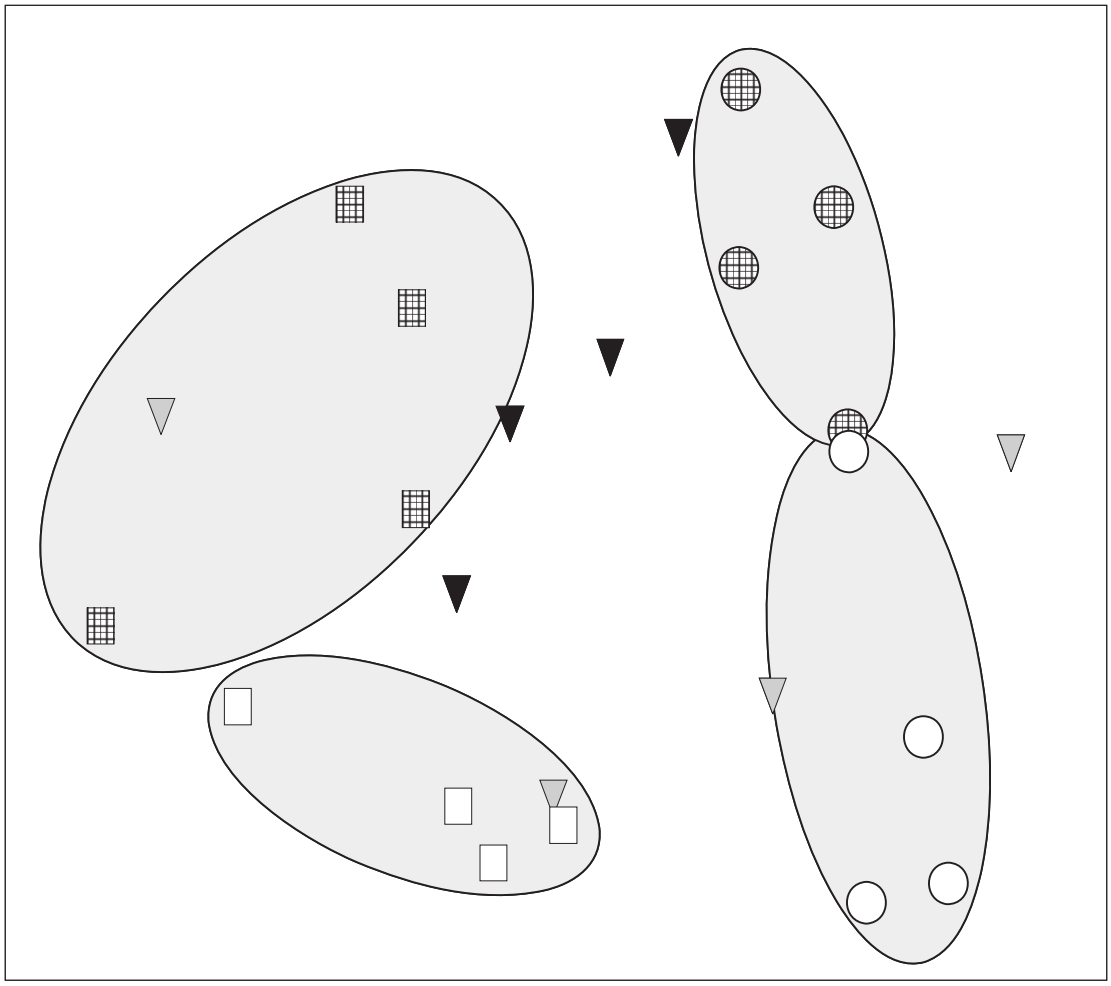

Fig. 8

Grazing and UV radiation effects on an Antarctic intertidal microalgal assemblage – a long-term field study

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Abstract A 15 wks field experiment (austral summer Nov to Mar) was carried out in an intertidal hard bottom platform in Antarctica (King George Island). To test whether grazing and ultraviolet radiation (UVR) influenced the succession of a benthic microalgal assemblage, a two-factorial design was used (1. ambient radiation, >280 nm; 2. ambient minus UV-B, >320 nm; 3. ambient minus UVR, >400 nm vs. grazer – no grazer). On four sampling occasions microalgae were identified, counted and carbon contents were calculated. The assemblage was dominated by the diatom genera *Navicula* and *Cocconeis*. Biomass was generally low in all treatments but was significantly reduced by grazing throughout the experiment. No significant UV effects were found. Grazer absence particularly favoured diatoms of the genus *Cocconeis*. We conclude that the Antarctic microalgal assemblage was unaffected by present day UVR whereas grazers acted as important drivers on the intertidal microalgal community structure.

Keywords: benthic diatoms, diversity, grazing, hard bottom community, microalgae, UV radiation

Introduction

Benthic microalgal communities, on hard and soft bottom substrata, are of vital importance for the ecological function in marine habitats. They constitute the local basis of the food webs in shallow areas, which are recognized to have a high secondary production (e.g. of fish and fish prey; Villafañe et al. 2003). The benthic heterotrophic community is depending on a sufficient primary production. However, in polar areas only a minor fraction of the gross pelagic primary production reaches the sediment, due to efficient carbon and nutrient recycling in the upper water column (e.g. Rysgaard et al. 1999). The importance of benthic microalgae for ecosystem primary production on a global scale based on 85 worldwide studies was reviewed by Cahoon (1999). Of these, only four studies were performed in polar regions. In Antarctic coastal ecosystems, poor development of pelagic microalgae (Hapter et al. 1983; Schloss et al. 1998) but an important contribution of resuspended benthic diatoms to the phytoplankton have been suggested and/or observed (e.g. Ahn et al. 1994; Gilbert 1991a; Gilbert 1991b). In the Arctic, a detailed study of carbon cycling in a fjord system showed that the primary production of phytoplankton and ice algae could not account for the carbon input required by the benthic community (Glud et al. 2000; Rysgaard et al. 2001). Thus, benthic microalgae are likely to provide an important food source for both benthic and pelagic heterotrophs.

The Antarctic “ozone hole” and the consequent increase of UV-B radiation (280-320 nm) is a well known fact and despite efforts to minimize the anthropogenic impact on stratospheric ozone destruction little improvement is expected for total column ozone in the Antarctic for the next several decades (Weatherhead and Andersen 2006). One

reason is that many factors influencing ozone levels (e.g. carbon dioxide emission, stratospheric temperature and circulation patterns) are also changing, and even if the anthropogenic impact in terms of ozone depleting compounds were removed, the ozone levels might never reach pre-1980 values (Weatherhead and Andersen 2006).

In the present study, UV-B radiation penetrated down to 16 m (1% of surface intensity). Thus, the tidal microalgal assemblages were exposed to high irradiances of both photosynthetic active radiation (PAR, 400-700 nm) and UV-B radiation. There are basically two ways for the microorganisms to react to UV-B radiation: adaptation or avoidance. Adaptation processes include protection and repair mechanisms. Avoidance of UV-B includes the ability to move away from the harmful radiation (Underwood et al. 1999) and, on community level, "self-shading" i.e. cells deeper in the assemblages get protection through light absorption by cells at the surface (Blanchard and Gall 1994).

The complexity of links – trophic and non-trophic – inherent in most natural systems (cf. (Polis and Strong 1996) makes it difficult to interpret treatment effects in natural experimental systems. However, the more we study intact communities under different environmental conditions, in addition to mechanistic experiments to identify certain key mechanisms, the closer we get to an understanding of what is happening in complex communities. Thus, short term experiments could give an insight about the worst case scenario for UV radiation effects on e.g. photosynthesis but long-term studies under ambient radiation are more ecologically relevant (Wulff et al. 1999; Zacher et al. unpublished).

Field experiments about the UV radiation impact on microalgae community structure are scant in general, but even more in Antarctica (but see Wahl et al. 2004). Studies on the interactive effects of herbivory and UV radiation considering successional processes of benthic microalgae are to our knowledge completely missing for Antarctic communities.

In the present study, the succession of an intertidal microalgal community in Antarctica was investigated in a field experiment to answer the questions: (1) how UV radiation and consumers influence biomass and abundance of a field-grown microalgal assemblage and (2) whether there are any interactive effects of UV radiation and consumers on the microalgal assemblage structure.

Materials and methods

Study site

The field experiment was conducted at a rocky intertidal platform at Peñón Uno, King George Island, Antarctica (62°14 S, 58°41 W). Common grazers at the study site are the gastropod *Nacella concinna* Strebel and other, smaller gastropods like *Laevilacunaria antarctica* Martens and *Laevilitorina umbilicata* Pfeffer. Dominant amphipod species in the area are *Gondogeneia antarctica* Chevreux and *Djerboa furcipes* Chevreux (Jazdzewski et al. 2001; Obermüller personal communication). During the sampling period, the maximal tidal range was ca. 2 m and the sea surface temperature ranged between -1.8°C (spring) and 2°C (summer). UV-transparency of the water body is highest in spring decreasing with higher temperatures due to the entrance of glacial freshwater and sediment. Minimum concentrations of nitrate, phosphate, and silicate

were previously recorded in February at non-limiting algal growth levels of 15, 2, and 47 μM, respectively (Schloss et al. 2002).

Experimental design and set-up

Using a randomized block design, we tested in a two-factorial experiment the effects of grazers (two levels, fixed) and UV radiation (three levels, fixed) on the succession of a microalgal assemblage (n = 4). The experiment was run from 28 November 2004 to 14 March 2005 (106 days). A pilot-study was performed from 20 December 2003 to 9 March 2004 (74 days). Results from both years were similar therefore, only data from the second longer experiment are shown here. Thirty-two PVC cages (50 x 50 x 12 cm, n = 4, including eight control treatments) were fixed to the substratum in the lower eulittoral at a minimal distance of 1 m to each other. Cages were submerged at a maximum depth of 2 m during highest tide levels or exposed during lowest tide levels. Cages were either open to all sides (open cage) or closed with plastic mesh (1 mm mesh size) to exclude macrograzers (closed cage). To test for cage artefacts, partially open cages (half cages, equipped with PAB filters, n = 4) were deployed by cutting two holes (~ 15 x 5 cm = 25%) into each side wall. Using cut-off filters as cage tops, ambient UV radiation levels were manipulated (see below for details). Open cages without filter (= full sunlight, n = 4) were used as procedural controls to test for filter artefacts. Unglazed ceramic tiles served as settlement substrata and were attached with Velcro to cage bottoms. Each cage contained four large (10 x 10 cm) and eight small tiles (5 x 5 cm). At each of four sampling events, one small tile was randomly withdrawn from each cage to determine treatment effects on the microalgal community. The other tiles were used to detect treatment effects on the macroalgal community (Zacher et al. unpublished).

UV radiation treatments

Cut-off filters manipulated the ambient light regime in three ways. (1) P = PAR (photosynthetically active radiation, >400 nm): using a 3 mm thick Plexiglas sheet (GS 231, Röhm, Germany), radiation <400 nm was blocked, while filters were transparent for 91 % of PAR. (2) PA = PAR + UV-A (>320 nm): using a 3 mm thick Plexiglas sheet (GS 2458, Röhm, Germany) and a 0.13 mm transparent polyester film (Folanorm-SF/AS, folex imaging GmbH, Germany), radiation <320 nm was blocked, while 89 % of PAR and UV-A passed the filter. (3) PAB = PAR + UV-A + UV-B (>280 nm): using a 3 mm thick Plexiglas sheet (GS 2458, Röhm, Germany) transmitting 92 % of PAR and UV radiation. Transparency of the GS 231 and GS 2458 Plexiglas filters decreased on average by 1.11 % (SD ± 0.01) and 1.31 % (SD ± 0.01) per a month, respectively. Therefore only damaged filters were exchanged. Polyester films were exchanged bi-weekly to minimize aging and fouling effects on transparency. Filters were cleaned once or twice per week.

Radiation measurements

Weekly to bi-weekly, the radiation regime above the water surface, at 10 and 200 cm depth was recorded at a distance ~50 m to the experimental site with a LiCor data logger (LI-1400, Li-Cor, Lincoln, USA) equipped with an underwater PAR sensor (LI-192) and a Solar Light (PMA2100, Solar Light Co. Inc., USA) equipped with a UV-B (PMA2106-UW) and a UV-A radiation (PMA2110-UW) broad-band sensor. Readings were taken ± 1 h of local noon. Ambient UV-A + UV-B radiation was continuously recorded at the nearby (1.5 km) Dallmann Laboratory with a 32-channel single-quantum counting spectroradiometer (Isitec, Germany). In addition, the weighted irradiance (minimal erythemal dose, UV_{ery}) was measured continuously next to the cages with two

ELUV-14 UV-dosimeters (El Naggar et al. 1995) to follow the relative changes of the underwater UV-B regime during the experiment.

Grazer abundance

Macrobenthic grazer density in each cage was estimated in January and March 2005 (by SCUBA diving). In each cage, the individuals of each gastropod species were counted and the density of amphipods estimated in categories of tens. Grazers inside closed cages were also counted and occasionally captured gastropods were removed. Amphipods entering or recruiting in the closed cages could not be removed and remained inside.

Sampling of microalgae

Sampling of the microalgal assemblage on the tiles occurred on 10 January, 7 and 24 February, and 14 March 2005 (i.e. 43, 71, 88, and 106 days after starting the experiment). Tiles were brought to the laboratory, immediately frozen (-20 °C) and brought to Göteborg, Sweden for further analysis. Microalgae were sampled and resuspended in 2.5% glutaraldehyde. After shaking each sample for 20 seconds a 20 µl subsample was put on an object slide and cell numbers were counted until a total of 300 cells under a known number of light fields in the microscope (various subsamples were used for each sample). Cells were counted in different size classes. Seven size classes of frequently found species were chosen according to their size and genus: (1) navicoloid cells <12.5 µm, (2) navicoloid cells >12.5 µm, (3) *Cocconeis* < 25µm, (4) *Cocconeis* > 25 µm, (5) *Licmophora* narrow, (6) *Licmophora* broad, (7) *Fragilariopsis*. All of theses groups were found in almost all counted subsamples. The diatom genus *Cocconeis* was

affected by freezing of the tiles (for conservation) sometimes causing cell ruptures. This was taken into consideration while counting.

Microalgal biomass

For conversion to carbon, averaged cell volumes were calculated by measuring biovolumes for each size class (in μm^3) and multiplying it with a factor of 10^{-12} x 0.089 g C $cell^{-1}$ (Edler 1979). For calculating biovolumes, cell dimensions of a minimum of 30 cells per species or size classes and per light treatment were measured and average cell volumes calculated from geometric formula (Edler 1979; Hillebrand et al. 1999).

Species identification

Naphrax mounted slides were prepared for diatom species identification. Samples were washed with distilled water to remove the salts and then boiled with 30% H_2O_2 to remove organic matter. 1-2 drops of 50 % HCl were added to remove carbonates and to eliminate H_2O_2. After washing, diatom suspensions were allowed to settle on a cover slip and left to dry before mounted. For species identification, differential interference contrast and phase contrast microscopy (100 x magnification) were used (Axioplan 2 imaging, Zeiss, Germany). Diatoms were identified following Hustedt (1961-1966), Krammer and Lange-Bertalot (1986, 1988), Hendey (1952; 1964) and Witkowski et al. (2000). The nomenclature was updated with the help of Round et al. (1990).

Data analysis

A t-test was performed to test for differences between two independent groups (e.g. test for cage or filter artefacts). For separate sampling dates, a two-way ANOVA was performed to test for the effects of consumers and UV radiation on density (number m^{-2})

and carbon content at a Bonferroni corrected significance level ($\alpha = 0.0125$) in order to lower the probability of making a type I error (Quinn and Keough 2004). Prior to analysis, data were tested for homogeneity of variances (Cochran´s test). Heteroscedastic data after ln- or square-root transformation were analyzed by the non-parametric Kruskal-Wallis test. Post-hoc comparisons were performed with Newman-Keuls test using Statistica ™ 6.0 software package. Species composition of communities was compared by ANOSIM, and in case of significance, followed by SIMPER to quantify the relative contribution of species to observed dissimilarities among treatments (PRIMER ™ 5 software package, Plymouth Marine Laboratory). ANOSIM used a Bray-Curtis similarity matrix based on root transformed density data.

Results

UV measurements

Figure 1 shows the daily doses of UV-A and UV-B radiation measured during January 2004 and April 2005. Peak values of UV-A and UV-B radiation (air) were recorded in December, coinciding with the highest values of underwater UV-B irradiance determined as UV_{ery} (Fig. 2). Lowest underwater UV-B values during the experiment were measured in February and March 2005 (Fig. 2). Maximum UV exposure on the tiles was reached during low tide on the 14th of December 2004 (around noon) were the cages were fully exposed to 44 W m^{-2} UV-A and 2.3 W m^{-2} UV-B, respectively (irradiance in air). Table 1 shows the minimal and maximal values measured with a broad-band sensor at the experimental site during the experiment. The depth range between 10 and 200 cm water depth covers the range from very high tide to almost low tide. Maximal values were encountered in December/January and minimal in February.

Grazer abundance

In January and March, gastropod densities in open and half cages showed no significant differences (January: t-test = 2.41, $P > 0.05$; March: t-test = 1.62, $P > 0.05$), thus no cage artefact was observed. Gastropod densities in closed cages were significantly lower in comparison to open cages in January (t-test = 6.20, $P < 0.001$) and equal in March (t-test = 1.79, $P > 0.05$). The gastropod *N. concinna* was successfully excluded from the closed cages throughout the experiment.

Amphipod density in January and March was higher in half cages (n = 4) than in open cages (January: t-test = 2.78, $P = 0.032$; March: t-test = 3.66, $P = 0.011$), indicating cage artefacts. Furthermore, their density was significantly higher in closed cages in relation to open cages (January: t-test = -3.30, $P = 0.003$; March: t-test = -4.66, $P < 0.001$).

No UV effects on total consumer density were detected (RM ANOVA, radiation effect, $F_{2,18 = 1.69,}$ $P = 0.213$).

Grazing and UVR effects

Overall, few species settled on the tiles (Table 2). Dominant species were *Navicula perminuta*, *Cocconeis costata* and *C. pennata*.

In general, no significant differences between open and half cages, or between PAB and full sunlight treatments were detected for any of the tested parameters (t-test, $P > 0.05$), showing no cage or filter artefacts. There were two exceptions for the second and the last sampling date were cage artefacts were found (t-test, Carbon content, t = 4.64, $P = 0.003$; abundance, t = -4.62, $P = 0.004$, respectively).

The most dominant diatom group throughout the experiment was the group of small naviculoid cells (mostly *Navicula perminuta*; Fig. 3). In the closed cages generally a

significantly higher diatom abundance was found, from the second sampling date onward (Fig. 3). In particular the density of small naviculoid cells, and cells of the genus *Cocconeis* increased. The genera *Licmophora* sp. and *Fragilariopsis* sp. were observed in almost all samples but were less abundant. No UV effects on the cell numbers were found.

Biomass was lowest at the first sampling date (Day 43), highest at the second (Day 71) and declined again until the last sampling date (Day 106, Fig. 4). In the closed cages, a significantly higher biomass was found from the second to the last sampling date (Fig. 4). Although the genus *Cocconeis* did not dominate in cell numbers this group prevailed in terms of biomass. No UV effects on biomass were observed.

Grazers significantly affected species composition at all sampling occasions with strongest effects found at the last three sampling dates (Table 3). SIMPER analysis showed that the naviculoid cells and *Cocconeis* together explained more than 90 % of the dissimilarities between the open and closed cages. Thus, grazers decreased the diatom density in almost all cases (except from naviculoid cells at the first sampling date).

UVR had a significant effect on species composition at two sampling dates; PAB differed from PA (first sampling date) and PAB differed from P (fourth sampling date). The differences were mainly due to the increase in the numbers of the small naviculoid cells under the PAB treatments, explaining ca. 70 % of the dissimilarity between the treatments (Table 3). However, average dissimilarity between these treatments was relatively low (17.75 and 32.53 % at sampling date1 and 4, respectively).

Discussion

In the experimental area, the biomass of benthic diatoms was lower in the intertidal compared with the subtidal (Campana et al. unpublished), probably due to ice disturbance (e.g. spring) and high grazing pressure (e.g. summer). Overall, the experiments revealed strong significant grazing effects on the intertidal Antarctic microalgal assemblage. Negative UV effects could not be observed, indicating efficient adaptation and/or acclimation processes of the microphytobenthic assemblage. However, some positive UV effects were found on small naviculoid cells.

Methodological considerations

In our experiment, as observed by others (e.g. Hillebrand et al. 2000), some inconsistent cage artefacts were found; a higher biomass but a lower abundance in half cages compared with open ones at the 2^{nd} and 4^{th} sampling, respectively. These findings are most likely a consequence of the high natural variability in these types of field-experiments. Nevertheless, we believe, that field-experiments on community level, like ours, still give valuable insights to complex ecological interactions, impossible to test in the laboratory. Meiofauna can have a grazing impact on benthic microalgae but they were not included in our experimental design, however, only very few e.g. copepods or polychaets were observed. Therefore, it is likely that their impact in relation to the grazing impact by macrograzers was negligible.

Grazing effects

From 71 days onwards, grazing significantly reduced benthic diatom biomass and altered the species composition. These findings are in agreement with previous studies on benthic diatoms from marine (e.g Hillebrand et al. 2000) and freshwater (Steinman

1996; Hill et al. 1997) habitats. In our study the absence of grazers generally favoured the abundance of the diatom genus *Cocconeis*.

The reduced diatom biomass was not caused by amphipods (not excluded from the closed cages). Many Antarctic amphipods e.g. *Gondogneia antarctica* are omnivorus and feed on microalgae (Jazdzewski et al. 2001) but they might have difficulties to graze on firmly attached diatoms. The latter was confirmed by our results where the biomass in closed cages was higher than in open ones although amphipods were present. The firm attachment of the diatoms was further emphasised by our difficulties to detach the cells from the tiles during sampling. In support of our results, Sommer (2000 and references therein) showed that a tight attachment to the substratum offered protection against grazing by the isopod *Idothea chelipes.* Furthermore, Hillebrand et al. (2000 and references therein) found an herbivore preference of erect, chain-building microalgal species over more attached species such as *Cocconeis* sp. and, moreover, diatoms might be more accessible for grazers when growing as epiphytes on macroalgae. In this context, the impact of amphipods on the microalgal assemblage growing in our experiment seems to be negligible. Consequently, biomass effects were mainly caused by larger gastropods, e.g. *Nacella cocinna*, successfully excluded by cages. This limpet is clearly the largest (mean length 20 - 30 mm) and most important grazer at our study site and can reach densities from 28 to 131 ind. m^{-2} in the Antarctic intertidal (Brêthes et al. 1994). *Nacella concinna* feeds on macroalgal propagules and benthic microalgae (Iken 1996; Kim 2001; Peck and Veal 2001). Due to the availability of important energy resources in form of benthic micro- and macroalgae, *Nacella* migrates to the intertidal in spring (Brêthes et al. 1994). In winter it migrates back into the subtidal zone. The abundance of microphytobenthos is correlated with the soma and

gonad mass of *Nacella* (Brêthes et al. 1994) which further demonstrates the important trophic link between microphytobenthos and this limpet.

In the absence of gut analysis and other direct measurements, selectivity of grazers for different algal species or growth types can only be inferred from the species composition of the prey community. Herbivores can alter species composition by actively preferring food items (active choice), by passively ingesting more of the species which are structurally more available (passive preference), or by changing the competitive outcome between prey species by increasing mortality rates or nutrient supply via regeneration (Hillebrand et al. 2000).

In our study, species composition was mainly shaped by the consumption of small diatoms belonging to the genera *Navicula* and *Cocconeis* (open cages). A selective choice of the main grazer *N. concinna* and other, smaller gastropods, is most unlikely because they fed on all algae available (as shown by their feeding track). Usually herbivores are known to prefer loosely attached, upperstory microalgae (Hillebrand et al. 2000). However, such algae did not grow in our experiment and grazers like *N. concinna* were apparently successful in removing firmly attached diatoms. Therefore, we conclude that gastropods can act as important drivers on community structure in the Antarctic intertidal during microalgal succession.

UV effects

The relative increase in UV-B radiation over the last 25 years has been highest in Antarctica compared with other regions of the world. Thus, any UV effects on benthic diatoms should most likely be found here (particularly in the intertidal). However, the lack of UV effects found on biomass and abundance of benthic Antarctic diatoms

confirmed former studies at different geographical sites (e.g. Hill et al. 1997). Earlier field-studies on UV effects on benthic microalgae have mainly focussed on soft bottom communities where benthic diatoms seem to be very tolerant to UV-B radiation (Peletier 1996; Wulff 1999 and references therein). The main variable to some extent affected in these communities was primary productivity while structural variables such as the overall biomass and microalgal pigments appeared unaffected (Odmark et al. 1998; Sundbäck et al. 1997; Wulff et al. 1999; Wulff et al. 2000). Wulff (1999) hypothesized that the lack of UV effects on structural variables in earlier field-experiments was due to the study of already established diatom communities and inadequate (too short) experimental periods. Microbenthic assemblages during early succession, as in our study, should therefore be more UV susceptible (Wulff et al. 2000). Nevertheless, no UV effects were found on any of the tested structural variables during the 3.5 month sampling period. It should be pointed out, however, in a few studies transient UV effects on e.g. biomass and species composition have been found in an early stage of microalgal succession (Santas et al. 1998; Wulff et al. 2000). The authors related these effects to a species-specific response to UV-B radiation. Particularly *Navicula perminuta*, the most common species in our study, was shown to adapt and recover rapidly from UV-B induced stress (Warning et al. 2006). Furthermore to a species-specific effect a habitat-specific effect of UV radiation on microphytobenthic communities was described by Villafañe et al. (2003). However, in this review UV-B was expected to be a more important controlling factor in cold climate habitat in relation to habitats in lower latitudes due to more frequent colonization effects (ice and scouring). Our findings are in contradiction to this study showing that hard bottom intertidal microphytobenthos in Antarctica was unaffected by UV radiation for the tested parameter.

Vertical migration has been suggested to be a key mechanism for epipelic benthic diatoms to avoid UV-B radiation (Underwood et al. 1999). However, in the present study vertical migration was not possible. In planktonic Antarctic diatoms, UV absorbing compounds such as mycosporine-like amino acids (MAAs) could be a protective mechanism against harmful UV-B radiation (Hernando et al. 2002). But the benthic diatoms in the present study were analyzed for the presence of MAAs and no MAAs were found (Campana et al. unpublished). We conclude that this mechanism is not the cause of the UV resistance in our diatom assemblage. Other UV protective mechanisms are the production of photoprotective carotenoids, such as beta-carotene. Furthermore, de-epoxidation of diadinoxanthin to diatoxanthin is known to occur in excessive light (PAR) as a protection against photooxidation (Arsalane et al. 1994), but the effect of UV-B on the de-epoxidation process is not clear. DNA repair has been proposed as an UV protective mechanism (Buma et al. 2001), but no DNA damage could be found in benthic Antarctic diatoms (Wulff et al. unpublished).

Interactive grazing and UV effects

Interactions between UV radiation and consumers can e.g. occur when UV induces changes in the chemical composition of the algae, thereby altering consumption patterns (Lotze et al. 2002). On the other hand, UV radiation can have a direct negative effect on the grazers, resulting in an enhanced algal productivity (Bothwell et al. 1994). However, no grazer – UV interactions were found in our study as also observed by Hill et al. (1997).

In conclusion, the intertidal benthic diatoms were found to be an important food source for the Antarctic gastropod community. With respect to UV radiation the benthic

diatoms were unaffected. However, only few species were found in the intertidal, maybe only the ones best adapted for this extreme environment. From an evolutionary perspective, it might be that these species have a capacity to endure UV-B radiation. During the course of evolution, they have been exposed to high irradiance levels and UV radiation exerted a selective pressure. Therefore, it is plausible that the cause of this "endurance" is due to not yet established key mechanisms.

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Figure legends:

Figure 1. Daily UV-A and UV-B doses from January 2004 to April 2005 measured at the Dallmann Laboratory (UV-A dashed grey line, UV-B black solid line).

Figure 2. Erythema weighted UV-B irradiance (UV_{ery}) during the duration of the experiment at Peñon Uno from December 2004 to March 2005. The sensor was located close to the cages with a maximal water column on top of 200 cm during high tide.

Figure 3a-d. Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and consumers (open and closed cages) on density of the most dominant diatoms groups and genus at the four samplings (a to d). Note that the first sampling has another scaling on the y-axe. Capitals indicate significant differences between consumer treatments. NAV = *Navicula sp*., COC = *Cocconeis sp*., LIC = *Licmophora sp*., FRA = *Fragilariopsis sp*.).

Figure 4a-d. Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and consumers (open and closed cages) on biomass (g C m^{-2}) of the most dominant diatoms groups at the four samplings (a to d). Note that the first sampling has another scaling on the y-axe. Capitals indicate significant differences between consumer treatments. NAV = *Navicula sp*., COC = *Cocconeis sp*., LIC = *Licmophora sp*., FRA = *Fragilariopsis sp*.).

Table 1. Minimal and maximal irradiance values at 10 and 200 cm water depth measured at the experimental site. All measurements ± 1 hour around local noon for three solar wavebands: (1) PAR (400 to 700 nm, n = 7); (2) UV-A (320 to 400 nm, n = 12); (3) UV-B (280 to 320 nm, n = 12); measured with a broad-band sensor from December 2004 until February 2005.

		PAR (µmol m^{-2} s^{-1})	UV-A (W m^{-2})	UV-B (W m^{-2})
Minimal values	10 cm	360	3	0.16
	200 cm	100	0.12	0
Maximal values	10 cm	1178	26	1.5
	200 cm	513	8.6	0.26

Table 2. Species list for identified benthic diatoms on the tiles according to their abundance. Dominant >50 %; frequent = 10 to 50 %; rare = < 5 %.

Dominant	**Frequent**	**Rare**
Navicula perminuta	*Cocconeis costata var costata*	*Odontella sp.*
Cocconeis costata var	*Navicula directa*	*Gyrosigma*
Cocconeis pinnata	*Licmophora gracilis*	*Pleurosigma sp.*
	Licmophora antarctica	*Thalassiosira sp.*
	Licmophora sp.	*Achnanthes sp.*
	Pseudogomphonema	*Nitzschia distans*
	Fragilariopsis sp.	*Nitzschia hybrida*
		Nitzschia sp.
		Amphora sp.
		Cocconeis schütti
		Rhicospheria sp.

Table 3. Results of ANOSIM (pairwise test and Global R, *P*) on species composition for all sampling events, and results of SIMPER for significant results, indicating the contribution of single species to total dissimilarity in species composition due to treatment effects. Data were square root transformed, PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR; nt = not tested. The direction of the effect is given as + positive UV or consumer effect, - negative UV (-B) or grazing effect. Significant results are bold.

	UV PAB:P	**UV PAB:PA**	**Grazing**
After 43 days	R = 0.198, *P* = 0.078	**R = 0.260, p = 0.003**	**R = 0.382, *P* = 0.001**
NAV <12.5 µm	nt	73.14% +	70.18% +
COC <25 µm	nt	<10.0% -	<10.0% -
COC > 25 µm	nt	/	<10.0% -
After 71 days	R = 0.307, *P* = 0.037	R = 0.328, *P* = 0.018	**R = 0.990, *P* = 0.001**
NAV <12.5 µm	nt	nt	28.71% -
COC <25 µm	nt	nt	46.50% -
COC > 25 µm	nt	nt	20.43% -
After 88 days	R = -0.016, *P* = 4.91	R = 0.281, *P* = 0.033	**R = 0.931, *P* < 0.001**
NAV <12.5 µm	nt	nt	46.67% -
COC <25 µm	nt	nt	26.11% -
COC > 25 µm	nt	nt	22.18% -
After 106 days	**R = 0.422, *P* = 0.002**	R = 0.266, *P* = 0.082	**R = 0.792, *P* < 0.001**
NAV <12.5 µm	67.16% +	nt	66.09% -
COC <25 µm	18.14% -	nt	19.10% -
COC > 25 µm	<10.0% -	nt	<10.0% -

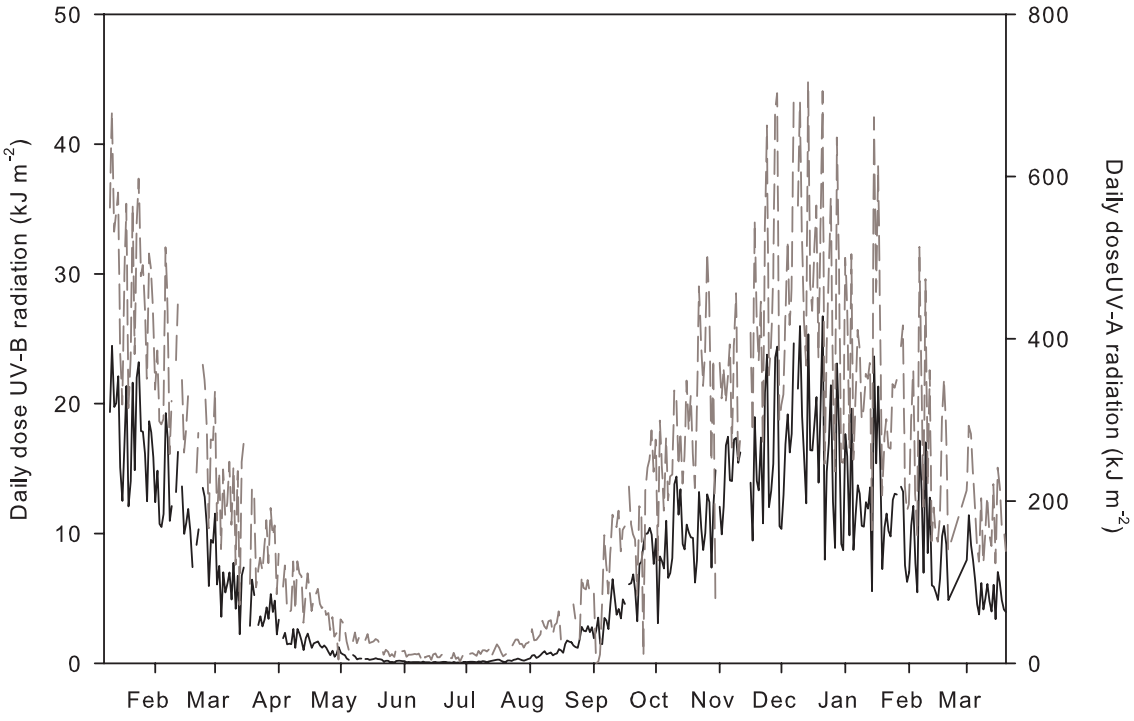
50
40
30
20
10
0
800
600
400
200
0
Daily dose UV-B radiation (kJ m-2)
Daily doseUV-A radiation (kJ m-2)
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar

Figure 1.

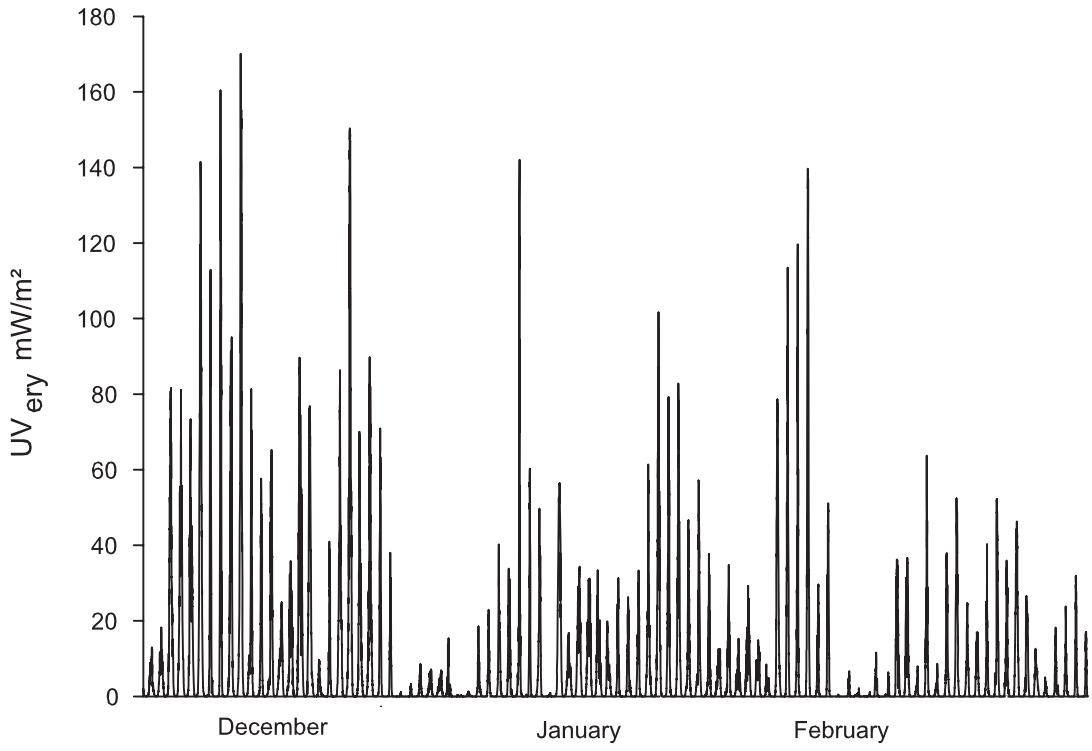
180
160
140
120
100
80
60
40
20
0
UV ery mW/m²
December
January
February

Figure 2.

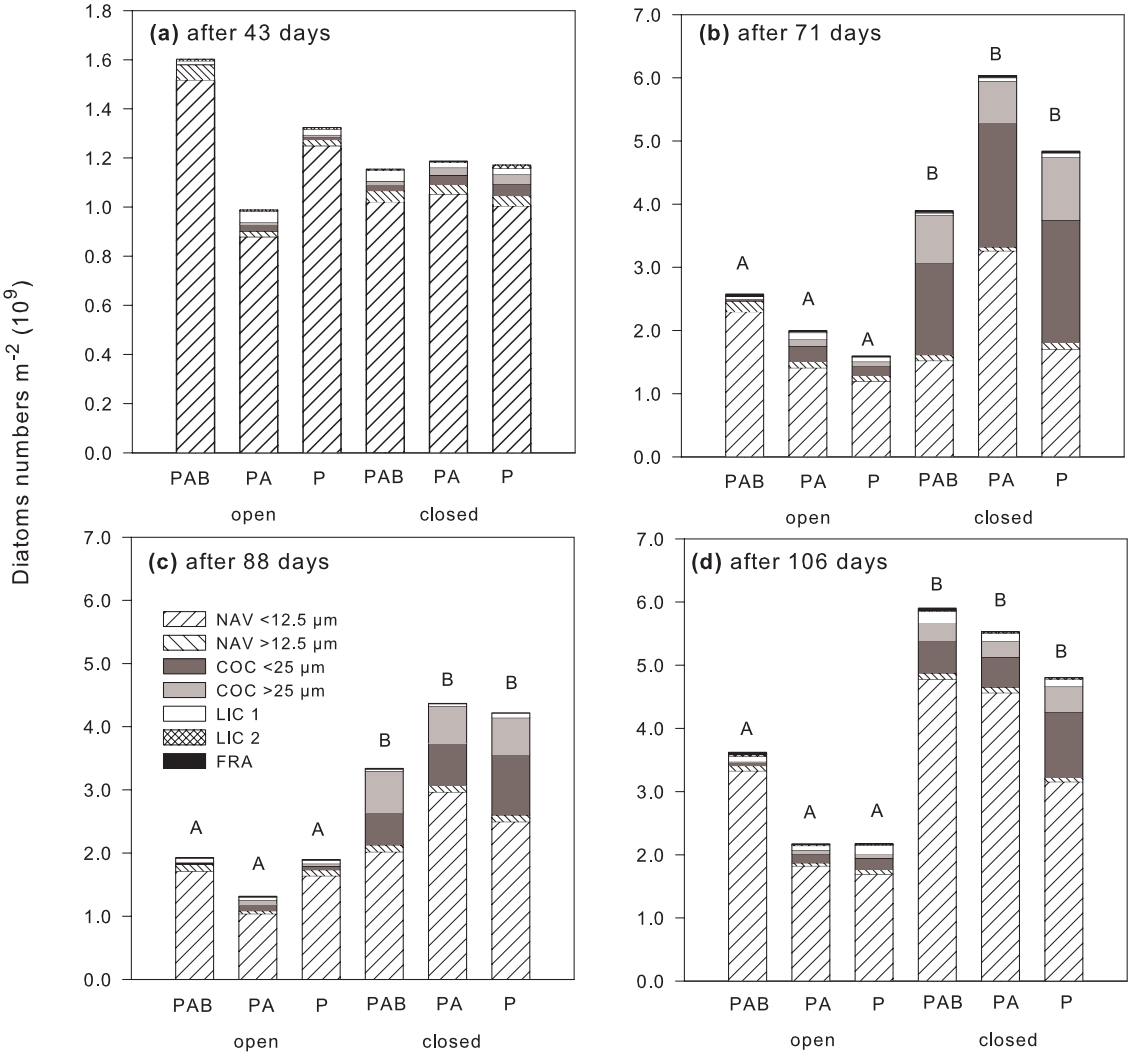
Diatoms numbers m-2 (10^9)
(a) after 43 days
(b) after 71 days
(c) after 88 days
(d) after 106 days
NAV <12.5 µm
NAV >12.5 µm
COC <25 µm
COC >25 µm
LIC 1
LIC 2
FRA
PAB
PA
P
open
closed

Figure 3a-d.

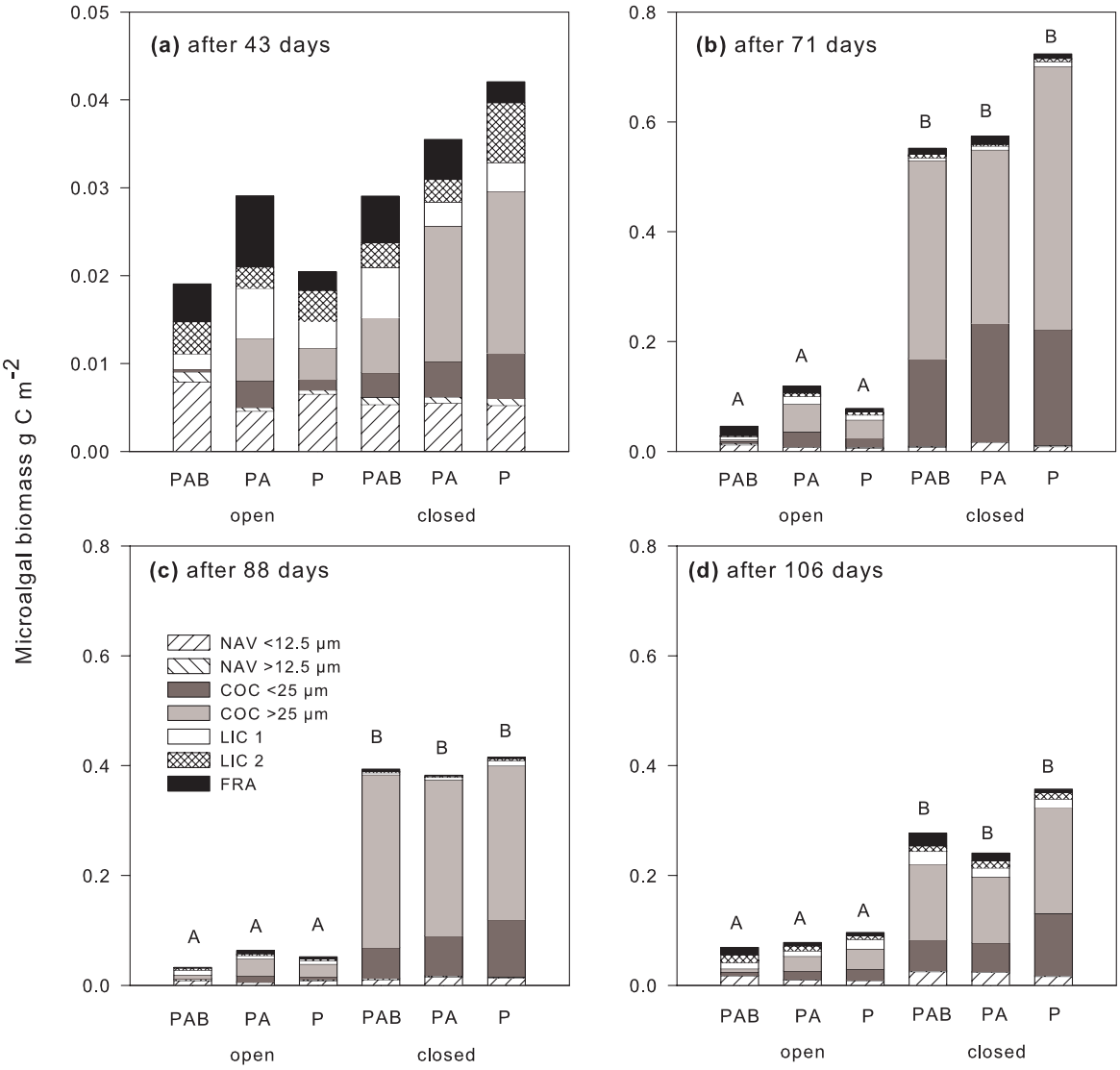
Microalgal biomass g C m-2
(a) after 43 days
(b) after 71 days
(c) after 88 days
(d) after 106 days
NAV <12.5 µm
NAV >12.5 µm
COC <25 µm
COC >25 µm
LIC 1
LIC 2
FRA
PAB
PA
P
open
closed

Figure 4a-d.

UV radiation and grazing effects on an intertidal and subtidal algal assemblage: a comparative study

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UV radiation and grazing effects on an intertidal and subtidal algal assemblage: a comparative study

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Introduction

Benthic marine macro- and microalgae are the major primary producers in coastal ecosystems, providing food and shelter for a variety of associated species (Iken 1996; Pinckney and Zingmark 1993). Therefore, changes in algal productivity or diversity can severely affect the structure of coastal marine food webs (Santas et al. 1998). A well known phenomenon over the Antarctic continent is the strong decline of stratospheric ozone (>50%) in spring (Farman et al. 1985; WMO 2003), thereby increasing the irradiance of ultraviolet-B radiation (UV-B 280-315 nm) on the Earth's surface. The timing of the ozone depletion over Antarctica is crucial as it coincides with the break up of sea ice, i.e. the phase of highest water transparency and highest growth rates of most algal species (Karentz 2003). Thus, tidal algal assemblages can be exposed to high UV-B radiation during the austral spring and summer months.

UV radiation (UVR, 280-400 nm) can negatively effect algae in various ways (reviewed in Franklin and Forster 1997; Wulff 1999; Bischof et al. 2006). Most harmful effects were found in laboratory studies, using artificial irradiance and focusing on physiological effects at the single organism level. However, autecological studies with single species are not able to detect synergistic or indirect UV effects on community level and predictions of ecosystem response to UVR cannot be made on single trophic-level assessments (Bothwell et al. 1994). In the marine environment, only few studies on interactive effects exist, demonstrating the significance of climatic (e.g. temperature, UVR) and ecological factors (e.g. grazing) as important drivers on algal recruitment (Lotze and Worm 2002).

Field-studies regarding UV effects on marine intertidal communities have been carried out mostly in temperate and tropical regions. In these experiments, UVR was identified as a significant driver on community structure during early succession (Lotze et al. 2002; Molis and Wahl 2004; Dobretsov et al. 2005).

Studying UV effects on Antarctic algal assemblages is particularly important due to the severe ozone depletion over this region (WMO, 2003). However, to our knowledge only one field-study on UV effects on an Antarctic microalgal assemblage exists (Wahl et al. 2004). To date, experiments studying interactions between UV effects and other ecologically important factors, e.g. grazing are missing. Furthermore, comparisons of benthic assemblages at

different tidal zones can help us identify a more general impact of UVR and grazing on the colonization process in marine hard bottom benthos in Antarctica.

In the light of this we designed a two-factorial field-experiment to study the effects of UVR and grazing on an intertidal and a subtidal algal assemblage in Antarctica. The main questions were (1) whether UVR and consumer treatments influence dry weight and recruit density at the two sites, (2) whether the developing assemblages at the two sites react differently to UVR and grazing.

Materials and Methods

Study sites. The field experiments were conducted close to the Dallmann Laboratory/Jubany Base, King George Island, Antarctica at two different sites. One on a rocky intertidal platform (lower eulittoral, Peñón Uno, 62°14 S, 58°41 W) and another one in a sheltered, hard bottom subtidal area (Peñón de Pesca, 62° 14´S, 58° 40´W) at 2 m water depth (Fig. 1).
In our study area the intertidal algal communities are characterized by Rhodophyta (e.g. *Iridaea cordata* Turner (Bory), Phaeophyta (e.g. *Adenocystis utricularis* (Bory) Skottsberg) and Chlorophyta (e.g. *Monostroma hariotii* Gain, (Iken 1996) and the subtidal communities by Phaeophyta (*Ascoceira mirabilis*, *Desmarestia sp.*), Rhodophyta (e.g. *Iridaea cordata* Turner (Bory), *Gigartina skottsbergii*) and benthic diatoms (Klöser et al. 1996; 1998).
The gastropod *Nacella concinna* Strebel, among other smaller gastropods like *Laevilacunaria antarctica* Martens and *Laevilitorina umbilicata* Pfeffer, was found very frequently at both sites (Kim 2001; Ferraz Nonato et al. 2000). Dominant amphipod species in the area are *Gondogeneia antarctica* Chevreux and *Djerboa furcipes* Chevreux (Momo et al. 1998; Obermüller et al. 2003). During the sampling period, the maximal tidal range was around 2 m at a sea surface temperature between -1.8°C (spring) and 2°C (summer). Water transparency is strongly variable, depending on glacial freshwater input and wind direction. UV-transparency of the water body was highest in spring (e.g. 28 November 2003) with a maximal 1 % depth at 16 m for UV-B radiation (280-315 nm), 19 m for UV-A radiation (315-400 nm), and >20 m for PAR (400-700 nm). Minimum concentrations of nitrate, phosphate, and silicate were recorded in February at non-limiting algal growth levels of 15, 2, and 47 µM, respectively (Schloss et al. 2002).

Experimental design and set-up. Using a randomized block design, we studied in a two-factorial experiment the effects of grazers (two levels, grazer vs. no grazer) and UVR (three levels, PAR+UV-A+UV-B, PAR+UV-A and PAR only,) on the succession of algal assemblages (n = 4) in the intertidal and the subtidal. For details of the design see Campana et al. (this issue).The experiments were run from 20 December 2003 to 9 March 2004 (74 days) in the intertidal and from 20 December 2003 to 8 March 2004 (73 days) in the subtidal. In this study we only refer to the third sampling date after 8 weeks of exposure.

Radiation measurements. For detailed radiation measurements and the differences of the two sites see Richter et al. (this issue).

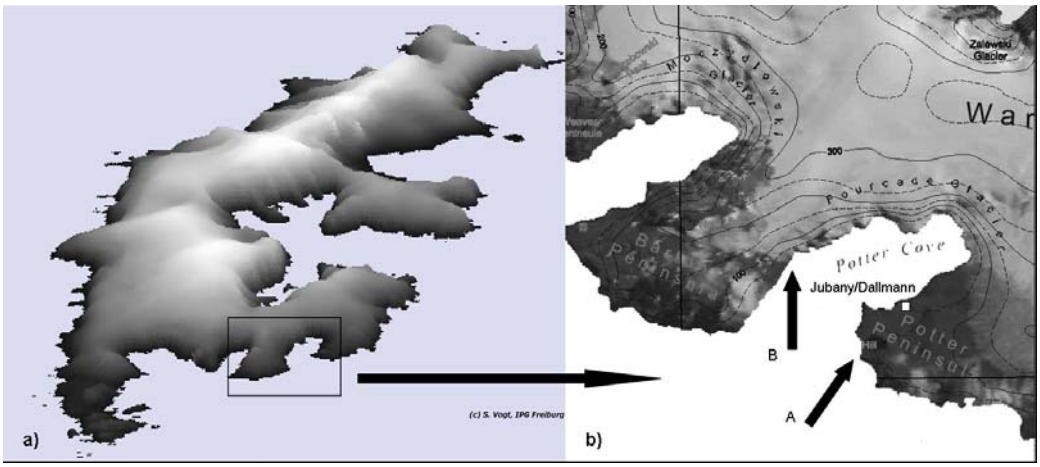

Fig. 1 a) King George Island, Antarctica (S. Vogt, IPG Freiburg). b) Experimental sites close to the Dallmann Laboratory. A: Peñón Uno; B: Peñón de Pesca.

Sampling of macroalgae. The collection of the tiles took place on 16 February (i.e. 58 days after starting the experiment) at Peñón Uno (lower intertidal) and 17 February 2004 (i.e. 59 days after starting the experiment) at Peñón de Pesca (subtidal). All tiles were processed at the Dallmann Laboratory immediately after collection from the field. Photosynthetic efficiency as optimum quantum yield (F_v/F_m) was measured with a PAM 2000 (Walz, Germany) after 5 minutes dark adaption directly on the tiles. Percent cover of all species (>2 mm length) was estimated with a Plexiglas sheet marked with 50 random dots (1 dot = 2 % cover). Recruit density (number cm^{-2}) of macroalgae < 2 mm was determined by counting individual germlings in four sub-samples per tile (~50 mm^2) using a stereomicroscope (16x magnification), leaving a border of 1 cm unsampled to avoid edge effects. Dry weight (as dry mass) of the community was measured by removing and drying (48 h at 80 °C) all organisms from the tile.

Data analysis. A t-test was performed to test for differences between two independent groups (e.g. test for cage or filter artefacts). A two-way ANOVA was performed to test for the effects of consumers and UV radiation on biomass, density of red and green algae recruits and photosynthetic efficiency with a significance level of P <0.05. Prior to analysis, data were tested for homogeneity of variances (Cochran´s test). Heteroscedastic data after ln- or square-root transformation were analyzed by the non-parametric Kruskal-Wallis test. Post-Hoc comparisons were performed with Newman-Keuls test using Statistica ™ 6.0 software package.

Results

Overall, four macroalgal species were found in the intertidal and three in the subtidal after 58 to 59 days of exposure, respectively (Table 1). At Peñón Uno (intertidal) the new developing assemblage was formed by very small stages of the green alga *Monostroma hariotii* Gain (more than 95% of the total macroalgal recruits on the tiles), pennate diatoms and red algal recruits. At Peñón de Pesca (subtidal) the assemblage was dominated by green algal filaments of *Urospora*

penicilliformis (Roth) Areschoug and colony-forming diatoms, accompanied by fewer red algal recruits Table 1).

Table 1. Algae found after 58 and 59 days of exposure in the inter- and subtidal. Dominant species are bold.

	Intertidal	Subtidal
Chlorophyceae	***Monostroma hariotii***	***Urospora penicilliformis***
	Ulothrix sp.	
Rhodophyceae	*Palmaria decipiens*	*Palmaria decipiens*
	Red int	Red sub, Delesseriaceae
Bacillariophyceae	**Diatoms**	**Diatoms (colonies)**

In general, no cage or filter artefacts were detected (t-test P >0.05, between open and half cages and PAB and full sunlight treatment, respectively).
In the closed cages optimum quantum yield (F_v/F_m) was significantly lower at the intertidal site (P = 0.0001, Table 2). Optimum quantum yield was not significantly influenced by UVR neither in the intertidal nor the subtidal site.
The dry weight was generally higher at the subtidal than at the intertidal site. Both sites showed a significant higher dry weight in the closed cages (intertidal: P <0.0001; subtidal: P = 0.012). No significant effects of UVR on dry weight were found in any of both sites (Table 2).

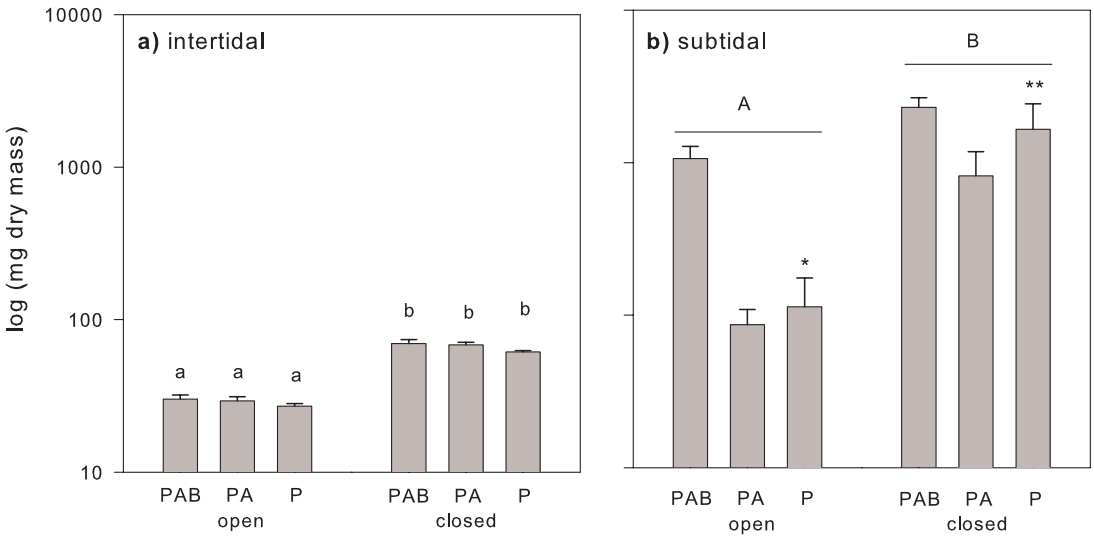

Fig. 2 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and grazers (open and closed cages) on dry weight at the two samplings sites (mean ± 1 SE, n = 4). Note logarhythmic scale. Different letters indicate significant differences between grazer treatments. Lower case letter were used to indicate significant differences in the intertidal and capitals for significant differences in the subtidal (as mean of the grazer treatments). (*,**) indicates the significant difference found between P open and closed treatment (Newman-Keuls-test)

Overall, at the intertidal site only microscopically identifiable green algal recruits (<2 mm) were present (mostly *Monostroma hariotii* and few *Ulothrix sp.*) while at the subtidal site bigger green algal filaments (>2 mm) of *Urospora penicilliformis* were identified.
UVR and grazers significantly reduced green algal recruits in the intertidal (UV: P <0.002; grazer: P <0.0001). Highest effects (minimal green algal recruits)

were found in the treatment with the ambient spectrum and grazers present (PAB open) resulting in an UV*grazer interaction (*P* <0.024; Table 2, Fig. 3).
In the subtidal site no significant UV or grazer effects on the green algal filaments were found after 8 weeks of exposure (Table 2, Fig. 3).

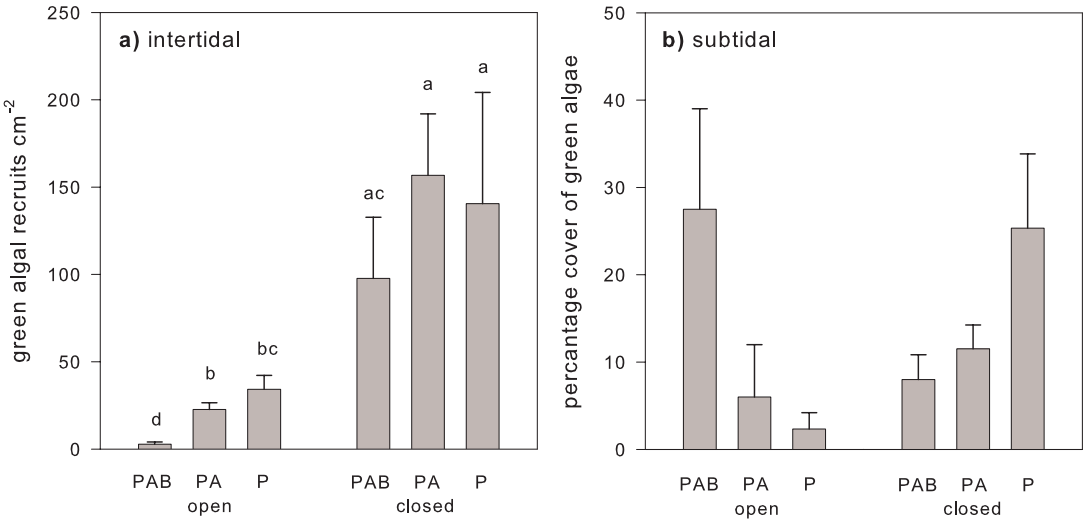

Fig. 3 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and grazers (open and closed cages) on green algal recruits at the two samplings sites (mean ± 1 SE, n = 4). Note that in the intertidal only small recruits were found (<2mm) using individuals cm^{-2} whereas in the subtidal the percent cover of bigger filaments (>2mm) was used for the analysis. Different letters indicate significant differences between treatments.

Table 2. Two-factorial ANOVA or non-parametric Kruskal-Wallis test on UV radiation and grazer (G) effects on photosynthetic efficiency, biomass, density of Chlorophyta and Rhodophyta at both sampling sites.+ significant; - not significant, nf not found.

	Intertidal			Subtidal		
	UV	G	UV*G	UV	G	UV*G
Photosynthetic efficiency	-	+	-	-	-	-
Dry mass	-	+	-	-	+	-
Green algae density	+	+	+	-	-	-
Palmaria decipiens density	+	-	-	+	+	+
Red int density	-	-	-	nf	nf	Nf
Red sub density	nf	nf	nf	-	+	-

In general more red algal recruits grew in the intertidal than in the subtidal (Fig. 4). At both sites two species of red algal recruits settled. Dominant among these was *Palmaria decipiens* (Reinsch) Ricker in the intertidal and *P. decipiens* as well as a member of the family Delesseriaceae in the subtidal. However, *P. decipiens* was only growing in the open cages in the subtidal.
UVR significantly reduced the density of *P. decipiens* in the intertidal and in the subtidal (intertidal: *P* = 0.037; subtidal: *P* = 0.042; Table 2). The density of *P. decipiens* was not affected by grazers in the intertidal but was favoured by herbivores in the subtidal (*P* = 0.024) revealing in a UV*grazer interaction (*P* = 0.042; Table 2). The density of a second red algal recruit (Red int) from the intertidal did not show any treatment effect whereas the species from the Delesseriaceae was depressed due to grazer presence (*P* = 0.044, Table 2, Fig. 4).

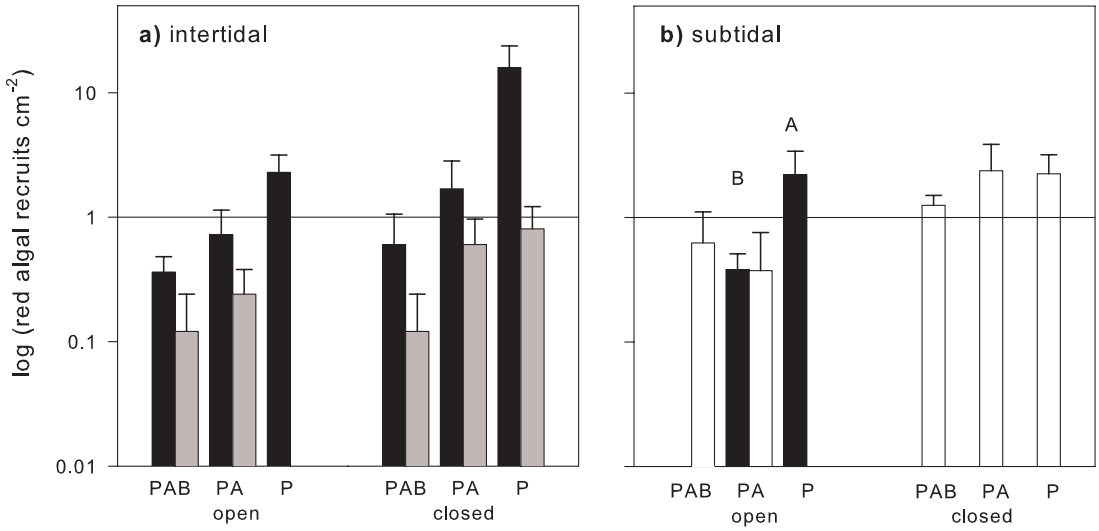

Fig. 4 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and grazers (open and closed cages) on red algal recruits at the two samplings sites (mean ± 1 SE, n = 4). Black bar *Palmaria decipiens*, grey bar unidentified red algae recruit in the intertidal (Red int), white bar unidentified red algae from the family Delesseriaceae in the subtidal (Red sub). Note logarhythmic scale. Different letters indicate significant differences between treatments. Kruskal-Wallis showed a significant UV effect on *P. decipiens* in the intertidal and ANOVA a significant grazer effect on Red sub, however following post-hoc test did not give any significant results.

Discussion

Main differences between the two sites were found in the species composition and in the dry weight due to general differences of the two locations. However, for this particular sampling date and year, effects of UVR and grazing on the respective assemblages followed a similar pattern at both sites. We would like to note that results presented here are only a small part of the complete experiment (one sampling out of four and only one season out of two studied).

The cages at Peñón Uno were installed at a shallower water depth than at Peñón de Pesca, therefore one might expect more pronounced UV effects at the intertidal site. However, the water body at Peñón de Pesca was clearer, due to the circulation patterns in the Potter Cove (see Richter et al. this issue). The water reaching Peñón Uno was richer in sediments from the nearby glacier, whereas Peñón de Pesca was influenced mainly by oceanic water entering from Maxwell Bay (Roese and Drabble 1998). Therefore, the UV doses actually reaching the respective algal assemblages were relatively equal (Richter et al. this issue) and responses to UV did not show pronounced differences at both sites. Nevertheless, intertidal communities are exposed to more stressors than subtidal ones, such as desiccation, temperature changes, wind, ice and higher maximal irradiance if local noon and low tide coincide.

Generally, dry weight was lower in the intertidal than in the subtidal. This was mainly due to a higher biomass of benthic diatoms in the subtidal (see also Campana et al. this issue). A higher exposure to mechanical forces at the intertidal site, (ice abrasion, strong wave action), among other stressors (e.g. desiccation) may have impeded growth of colony-forming diatoms in the

intertidal which were dominant at the more sheltered Peñón de Pesca. We don't ascribe this to UV effects as the diatom assemblages were not affected by UVR (Campana et al this issue). Nevertheless, both assemblages were top-down controlled by grazing. The main effects were caused by gastropods, like *Nacella concinna* and *Laevilacunaria antarctica* which were shown to feed effectively on macroalgae and microphytobenthos (Iken 1996; Kim 2001), as well as amphipods in the subtidal. Grazing effects in the subtidal resulted stronger in UV shielded assemblages (open P treatment). A reduction of the algal palatability of UV exposed assemblages, the algal composition or even a direct UV effect on grazers might explain this trend (Bothwell et al. 1994; see also Campana et al. 2005).

UV and grazing effects on green algal recruits were species-specific as shown by different results for the intertidal (mainly microscopically *Monostroma hariotii*) and the subtidal (mainly *Urospora penicilliformis* filaments) green algae. However, green algal recruits at an earlier successional stage were also shown to be UV sensitive in the subtidal (Campana et al. 2005). Generally early stages of succession were shown to be more vulnerable to UV stress than adults of the same species (Coelho et al. 2000) but have the possibility to adapt as they mature (Lotze et al. 2002).

At both sites the red algal recruit *Palmaria decipiens* was UV sensitive, especially to the UV-B part of the spectrum. A total lack of *P. decipiens* recruits in the closed cages in the subtidal was probably due to the dense settlement of colony-forming diatoms (not being present in the intertidal) which limited growth of *Palmaria* due to interspecific competition (see Huang & Boney 1985). Moreover, the density of *Palmaria* recruits has been shown to be favoured by grazer presence at longer times scales (Campana et al. 2006; Zacher et al. in press).

Overall, UVR and grazers significantly shaped both, the intertidal and the subtidal site in Antarctica. Especially red and green algal recruits showed species-specific sensitivity to UVR and grazing, resulting in interactions of these two factors. An increase in UV-B radiation may therefore affect the species composition (affecting some species more than others), with a consequent alteration of the trophic interactions in the benthic system in the Potter Cove.

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UV radiation- a threat to Antarctic benthic marine diatoms?

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Key words: Microalgae, microphytobenthos, P-I curve, optimum quantum yield, UV-B, UV-A

Abstract

In two different experiments the impact of ultraviolet radiation (UVR, 280-400 nm) on a semi-natural soft-bottom diatom community was investigated. Our study was motivated by the lack of UVR studies on Antarctic benthic marine microalgae. The objective was to estimate the impact of UV-B (280-315 nm) and UV-A (315-400 nm), on photosynthetic efficiency, species composition, cell density and specific growth rate. In both experiments, cell density increased over time. Most frequently observed species were *Navicula cancellata*, *Cylindrotheca closterium, Nitzschia* spp., and *Petroneis plagiostoma*. For both experiments, a shift in species composition and a decreased optimum quantum yield (F_v/F_m) over time, irrespective of treatment was observed. UVR significantly reduced F_v/F_m on days 3 and 10 (Expt 1), disappearing on the last sampling date. A similar trend was found in Expt 2. A significant UV effect on cell density was observed in Expt 1 (Day 10) but not in Expt 2. No treatment effects on species composition or specific growth rate were found. Thus, the UV effects were transient (photosynthetic efficiency and cell density) but the growth of the benthic diatoms was generally unaffected. Overall, according to our results UVR do not seem to be a threat to benthic marine Antarctic diatoms.

Introduction

The stratospheric ozone depletion over the Antarctic and the consequent increase of ultraviolet-B radiation (UV-B, 280-315 nm) is a well known fact and despite efforts to minimize the anthropogenic impact on ozone destruction, little improvement is expected for total column ozone in the Antarctic for the next several decades (Weatherhead & Andersen 2006). Ultraviolet-A radiation (UV-A, 315-400 nm) and photosynthetically active radiation (PAR, 400-700 nm) are involved in photoreactivation and photorepair of the DNA (Karentz 1994 and references therein). It is therefore of particular concern that ozone depletion results in increased harmful UV-B radiation without a proportional increase in UV-A and PAR. In the study area, UV radiation (UVR, 280-400 nm) can penetrate to considerable depth into the water column (19 m, corresponding to 1% of the irradiance at the water surface), and could thereby affect also subtidal organisms. Although the sediment has been considered to be a refuge to escape harmful radiation, UVR has been shown to penetrate ca 1 mm into a sandy sediment (Wulff *et al.* 1999).

In shallow water areas (estuaries) microphytobenthic communities can account for a substantial part (50%) of the total primary productivity (Underwood & Kromkamp 1999). Moreover, subtidal benthic microalgae on the continental shelves can account for 42% of the total areal primary productivity (Nelson *et al.* 1999). Also in polar areas, the marine microphytobenthos form an important food source for both benthic and pelagic heterotrophs. Particularly in Antarctic ecosystems, a poor development of pelagic microalgae (Schloss *et al.* 1998) but an important contribution of resuspended benthic diatoms to the phytoplankton has been suggested and/or observed (e.g. Ahn *et al.* 1994).

UV radiation has been shown to negatively affect benthic microalgae in various ways with a potential cascade effect on the whole ecosystem (Bothwell *et al.* 1994). For example, UVR reduced the photosynthetic performance of microphytobenthos, and damaged the DNA (reviewed in Franklin & Forster 1997). Primary targets are the D1/D2 protein complex in photosytem II (PS II), and the water-splitting complex (Franklin & Forster 1997 and references therein). However, the susceptibility of microphytobenthos towards UVR has mainly been studied on soft bottom communities. For example, ambient UV-B was proven to be a stress factor for sand-living microbenthic communities and a selective force during early growth and succession (Wulff *et al.* 2000). Important functional factors such as primary productivity and carbon allocation were strongly affected by ambient (Wulff *et al.* 1999) and enhanced levels of UV-B (Sundbäck *et al.* 1997; Wulff *et al.* 2000). Structural variables e.g. biomass and species composition were not affected by UV-B (Sundbäck *et al.* 1997; Wulff *et al.* 1999). However, responses of microalgal photosynthesis to ambient UV-B are not clear-cut and vary with substrate type and community density as well as irradiances applied (Franklin & Forster 1997, Villafañe *et al.* 2003).

There are basically two ways for the microalgae to react to UV-B radiation: adaptation or avoidance. Adaptation processes include protection and repair mechanisms. Mycosporine-like amino acids (MAAs) production has been found to be a protective mechanism in planktonic centric Antarctic diatoms (Hernando *et al.* 2002). However, benthic diatoms are mostly comprised of pennate diatoms producing very low, if any, amounts of UV absorbing compounds (Wulff *et al.* 1999, Roux *et al.* 2002). Avoidance of UV-B includes the ability to move away from the harmful radiation (Underwood *et al.* 1999, Wulff & Zacher in press) and, on community level, “self-shading” i.e. cells

deeper in the assemblages get protection through light absorption by cells at the surface (Blanchard & Cariou-Le Gall 1994).

Our study was motivated by the fact that very few studies deal with the response of Antarctic benthic marine microalgae to UVR. The objective of this study was to estimate the impact of UV-B and UV-A on benthic diatoms (photosynthetic efficiency, species composition, cell density and specific growth rate) over a 16 and 13 days period, respectively.

Material and Methods

The study was carried out in November and December 2003 at Dallmann Laboratory, Potter Cove, King George Island, Antarctica (62° 15´S, 58° 41´W). Fine grained sandy sediment was collected from 5 to 7 m water depth (SCUBA divers). The top layer (1 cm) was scraped off and the sediment was brought to the laboratory, gently shaken and sieved (mesh size 500 µm) using filtered surface seawater. The sediment was stirred and the overlying water containing suspended microalgae was transferred to a glass beaker gently bubbled with air and left to grow for ca 3 weeks under dim white light (ca 10 µmol photons m^{-2} s^{-1}). The overlying water was enriched once a week with nutrients corresponding to f/2 medium (Guillard 1975).

Experimental treatments

Two experiments were carried out (A1 and A2, Table 1) in a temperature controlled laboratory container at 2-4 °C. Suspended microalgae were transferred to a plastic tray with 32 Petri dishes (55 mm, n = 4) for each experiment. Each Petri dish contained a ca

0.5 mm layer of acid-cleaned sand (5 g). After 12 hours the Petri dishes were transferred to the experimental aquarium (55*45*20 cm). To increase the light reflection, the bottom of the aquarium was covered with aluminium foil and a flow-through system was installed to ensure nutrient exchange between the sediment and the overlying water. In addition, every third day, 1 litre (5% of the total water volume) of the water in the aquarium was replaced with new 0.2 μm filtered surface seawater. The water depth in the aquarium was 8 cm and the Petri dishes were placed to get an even light intensity between replicate treatments. Salinity was 37 PSU and the pH 6-6.5.

Microalgae were repeatedly exposed to PAR+UV-B+UV-A (PAB) and PAR+UV-A (PA, Experiment A1), and PAB and PAR (P, Experiment A2) for 16 and 13 days, respectively. The light:dark cycle was 15:9 h, while UV-B radiation was applied for 6 h daily (Table 1). The PA treatments were covered by 0.13 mm transparent polyester film (Folanorm-SF/AS, Folex GmbH, Cologne, Germany), radiation <320 nm was blocked. The P treatment was covered by Ultraphan URUV Farblos (Digefra GmbH, Munich, Germany). Before the treatments started, 8 Petri dishes were sampled for initial values, while the remaining 24 Petri dishes were placed in the aquarium and given their respective treatment. Each Petri dish was randomly assigned a treatment and a sampling date. After 3, 10 and 16 days (A1) and 3, 10 and 13 days (A2), 4 replicates of each treatment were sampled for species identification, cell counts and to measure photosynthetic efficiency (see below for details). To allow for a maximal UV-B effect, samples were taken just before the UV-B lamps were turned off.

Light treatments

Light was provided by white fluorescent lamps (Osram GmbH, L65 Watt/25S, Munich, Germany), emitting background PAR and UV lamps (Q-Panel UV-A-340, 40 Watt, Cleveland, USA), emitting a spectrum qualitatively similar to solar radiation in the range of 295 to 340 nm. Light intensity in the laboratory was measured using a Solar Light PMA 2100 radiometer (Solar Light, Philadelphia, USA) equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar light, Philadelphia, USA). PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1400 datalogger (LI-COR Bioscience, Lincoln, USA) and a spherical microquantum sensor (US-SQ S/W, Water-PAM, Walz GmbH, Effeltrich, Germany). Light intensities and doses under the different treatments are shown in Table 1. Mean daily UV-A and UV-B doses in the air for November/December 2003 (n = 45) were 514 (±203) and 18 (±7) kJ m^{-2}, respectively, measured with a 32-channel single-quantum counting spectroradiometer (Isitec, Bremerhaven, Germany)

Microalgal photosynthesis

To characterise the adaptation of the diatom suspension, photosynthesis (in terms of relative electron transport rate, rETR = PFR (photon fluence rate) x ΔF/Fm' (effective quantum yield) versus irradiance curves (P-I curves) were measured as described by Bischof *et al.* (1998). Measurements were performed for diatom suspensions exposed to dim white light from the fluorescent tubes described above (<10 µmol photons m^{-2} s^{-1}), to one hour of 90 and for 2 hours of 223 µmol photons m^{-2} s^{-1} (n = 3). The hyperbolic tangent model of Jassby & Platt (1976) was used to estimate P-I curve parameters described as:

$rETR = rETR_{max} * \tan h (\alpha * I_{PAR} * rETR_{max}^{-1})$

where $rETR_{max}$ is the maximum relative electron transport rate, tan h is the hyperbolic tangent function, α is the initial slope in the light limited part of the P-I curve (as a measure for the electron transport efficiency) and *I* is the photon fluence rate of PAR. The saturation irradiance for electron transport (I_k) was calculated as the intercept between α and the ETR_{max} values. Curve fit was calculated with the Solver module of MS Excel using the least square method comparing differences between measured and calculated data.

The effects of UV radiation on photosynthetic efficiency of the microalgal suspension was determined by measuring the variable chlorophyll fluorescence of PS II by use of a pulse-amplitude modulated fluorometer (Water-PAM, connected to a PC with WIN CONTROL Software, Walz GmbH, Effeltrich, Germany). The content of the whole Petri dish was sampled in a 20 ml vial, the bottle was shaken 30 sec, the sand grains left to settle for 30 sec, and 4 ml of the microalgal suspension was filled into 5 ml quartz cuvettes for measurements in the Water-PAM. Optimum quantum yield (F_v/F_m) was measured after 5 min dark adaptation to determine changes in the photosynthetic efficiency. A 5 min dark adaptation was checked to be sufficient. Prior to the dark adaptation, the samples were exposed for 5 s weak far-red light (intensity 6, Water PAM). Two measurements were performed in each sample and the average values were used for further calculations of treatment effects.

Microalgal density and identification

After the measurements of photosynthetic efficiency, microalgal cell density was estimated. The sample was vigorously shaken by hand for 30 seconds and after ca 30

seconds (to allow sand grains to settle), two individual subsamples (40 µl) of the algal suspension were pipetted onto a light microscope slide (20 x, Zeiss, Axiolab, Germany) and cells with and without intact chloroplasts were counted. Specific growth rate day^{-1} was calculated for the different treatments. In Expt A2, no cell counts were done the last sampling date (Day 13). Naphrax mounted slides were prepared for diatom species identification. Samples were washed with distilled water to remove the salts and then boiled with 30% H_2O_2 to remove organic matter. 1-2 drops of 50 % HCl were added to remove carbonates and to eliminate H_2O_2. After washing, diatom suspensions were allowed to settle on a cover slip and left to dry before mounted. For species identification, differential interference contrast and phase contrast microscopy (100 x magnification) were used (Axioplan 2 imaging, Zeiss, Germany). Diatoms were identified following Hustedt (1927-1966), Krammer & Lange-Bertalot (1986-1991) Hendey (1952; 1964) and Witkowski *et al.* (2000). The nomenclature was updated with the help of Round *et al.* (1990).

Statistical analyses

A one-way ANOVA was used to test for the effects of UVR on photosynthetic efficiency for each exposure time ($p < 0.05$). Prior to analysis, data were tested for homogeneity of variances (Cochran´s test). Statistical analysis were done using Statistica™ 6.0 software package or MS Excel. Species composition of communities was compared by ANOSIM (for treatment and time effects, respectively), and in case of significance, followed by SIMPER to quantify the relative contribution of species to observed dissimilarities (PRIMER ™ 5 software package, Plymouth Marine Laboratory). ANOSIM used a Bray-Curtis similarity matrix based on square root transformed data (cell number m^{-2}).

Results

General photosynthetic performance

The P-I curves shown in Fig. 1a-c reveal the differences in the photosynthetic performance of the diatom suspension after different light exposures. The values for α (an index of light-harvesting efficiency) varied between 0.161 and 0.086 (Fig. 1a-c). Photosynthetic efficiency was clearly highest during the weak light exposure and decreased after high light exposure (233 µmol photons m^{-2} s^{-1}). Photosynthetic capacity ($rETR_{max}$) was higher in the initials ($rETR_{max}$ = 23.4) and after an exposure to 1 h of 90 µmol photons m^{-2} s^{-1} ($rETR_{max}$ = 23.5) in comparison to the exposure to 2 h of 233 µmol photons m^{-2} s^{-1} ($rETR_{max}$ = 15.8). At photon fluence rates >600 µmol photons m^{-2} s^{-1}, the photosynthetic capacity slightly decreased (data not shown). The I_k values increased from145.4 µmol photons m^{-2} s^{-1} in dim light to 184.4 µmol photons m^{-2} s^{-1} after exposure to 233 µmol photons m^{-2} s^{-1} (Fig. 1a-c).

General observations and community succession in the various treatments

At all sampling occasions the sediment surface was brown with air bubbles. The cell numbers increased over time in both experiments. Most frequently observed genera or species in experiment A1 (PAB exposure) were *Navicula cancellata* Donkin, *Cylindrotheca closterium* (Ehr.) Lewin & Reimann, *Nitzschia* spp., and *Petroneis plagiostoma* (Grun.) Mann. In Expt A2 (PA exposure), similar genera /species were observed with addition of *Navicula* spp. (Fig 2a-b). A general shift in genera / species composition of the microalgal community was observed for both experiments (ANOSIM: A1 Global R = 0.928, $p < 0.001$ and A2 Global R = 0.984, $p < 0.001$) and was not due to the respective light treatments. The changes were mainly caused by an

increase in the cell number of *Cylindrotheca closterium*, *Navicula cancellata* and cells of the genus *Nitzschia*. In Expt A1, percentage of cells without intact chloroplasts was initially around 25 % ± 1.4 and decreased with time in both treatments (Day 3: 12 % ± 0.6, Day 10: 11 % ± 1.0). The percentage of cells without chloroplasts in experiment A2, was initially around 29 % ± 3.9, decreased on Day 3 (Day 3: 9 % ± 0.6) and increased again on Day 10, both treatments (Day 10: 25 % ± 2.4). For A1, Day 0 to Day 16, the specific growth rates day^{-1} were 0.15 (PAB and PA). In A2, the growth rates were higher with values of 0.33 (PAB) and 0.34 (P) (Day 0 to Day 10).

UV treatment effects on photosynthesis

In both A1 and A2 the optimum quantum yield (F_v/F_m) decreased over time irrespective of treatment (Fig. 3a-b). In experiment A1, there was a significant reduced F_v/F_m in the PAB treatment on Day 3 (ANOVA, $F_{1,6} = 6.87$, $p = 0.040$), and on Day 10 (ANOVA, $F_{1,6} = 18.57$, $p = 0.005$). On the last sampling date, however, this treatment effect had disappeared although a tendency was still visible. A similar trend was found in experiment A2, where a significant reduction in F_v/F_m for the PAB treatment was observed on both Day 3 (ANOVA, $F_{1,6} = 37.63$, $p < 0.001$) and Day 10 (ANOVA, $F_{1,6} = 9.48$, $p = 0.022$), disappearing on Day 13.

No significant treatment effects on species composition were found throughout experiment A1. In the second experiment (A2), again no significant UV effects on species composition were observed (but Day 10; ANOSIM, Global R = 0.292, p = 0.057). Some species for example *Nitzschia* spp. and *Navicula cancellata* were negatively affected (abundance) by UVR, whereas others were more frequently

observed under the PAB treatment in comparison with the P treatment (*Navicula* spp. and *Cylindrotheca closterium*), although not statistically significant.

In Expt A1, a significant UV effect on total cell numbers was found on Day 10 (ANOVA, $F_{1,6} = 11.38$, $p = 0.015$, Fig 2a). In Expt A2, no significant UV effects were found on cell numbers for any sampling date (Fig 2b). No significant treatment effects were found for specific growth rate in any of the two experiments.

Discussion

In the study area, ca 20% of the incoming UV-B radiation reached 5 m water depth and the daily dose reaching the subtidal microalgal community was 3.6 kJ (Richter *et al.* accepted). In our experiment, the microalgae were exposed for 4.7 kJ per day, which is 23% higher than the actual UV-B dose received at 5 m depth. The applied UV-A intensity, however, could penetrate down to 10 m in the water column. The daily UV-A dose in our experiment was 45% higher than the actual UV-A dose received at 5 m depth. Together with the low PAR dose, the ratio between PAR/UV-A/UV-B differed from natural conditions. Thus, the study was not designed to perfectly mimic natural conditions and should be considered mechanistic.

The general decrease in optimum quantum yield (Fig. 3, Day 13 and 16) may indicate a possible nutrient limitation (Geider *et al.* 1993, Underwood *et al.* 1999). Although F_v/F_m as an indicator of nutrient stress is questioned (Kruskopf & Flynn 2005), in our case (under conditions with constant irradiance) a decreasing F_v/F_m over time likely reflects a

nutrient starvation. Furthermore, the high cell numbers on Day 16 (Expt A1) could indicate an incipient nutrient limitation. Self-shading as a possible explanation can be excluded, although high cell numbers at the end of the experimental period (A1, A2) may point to it. Self-shading means light limitation, and under this condition F_v/F_m generally increases. In addition, the P-I curves showed, that the diatoms could be considered low light adapted. At irradiances < 10 µmol photons m^{-2} s^{-1} the photosynthetic efficiency was highest and with increasing light levels the photosynthetic capacity decreased, indicating an arising high light stress rather than a high light acclimation. The maximum I_k value (ca 184 µmol photons m^{-2} s^{-1}) was within values observed by others. For intertidal benthic diatoms the I_k varied between 258 and 411 µmol photons m^{-2} s^{-1} (Blanchard & Cariou-Le Gall 1994) and Barranguet & Kromkamp (2000) observed I_k (E_k) values of 400-1000 µmol photons m^{-2} s^{-1}. In these studies, the microphytobenthos were very seldom photoinhibited under *in situ* conditions. Benthic diatoms from 5 m depth at the Swedish west coast showed typical I_k values of 185 µmol photons m^{-2} s^{-1} (Diving-PAM, Walz, Germany) and when treated with light intensities of 6 µmol photons m^{-2} s^{-1} for 2 hours the I_k values decreased to 166 µmol photons m^{-2} s^{-1} (Engelsen unpublished report). In our study, however, the decrease in the P-I curves (α and ETR_{max}) with increasing irradiance indicated a photoinhibition (especially at 233 µmol photons m^{-2} s^{-1}).

The initial species/group composition differed between the experiments possibly due to a different light history or a later stage of succession. The microalgae used in the two experiments were sampled at the same site but at different dates (ca 3 weeks in between). Therefore, a direct comparison of the results from the two experiments might be difficult to interpret. Moreover, a shift in species composition regardless of treatment

was observed over time. An enclosure of a microalgal community will naturally influence the species composition simply due to changed environmental conditions (cf Wängberg & Selmer 1997). Some species grow better under culture-like conditions than others, in our study, for example, *Cylindrotheca closterium* was found to completely dominate the microalgal community after 3 to 10 days.

UV treatment effects

The exposure of benthic Antarctic diatoms to UVR resulted in a decreased optimum quantum yield on Day 3 and 10 compared with the control treatments (PA and P). The significant decrease was caused by the UV-B part of the spectrum. Furthermore, the cell density was lower in the UV-B treatment after 10 days (A1). However, these treatment effects diminished after 13 and 16 days, respectively. The microalgae seemed to have a capability to acclimate to the repeated UV exposure with time (see also Waring *et al.* 2006); similar effects have also been reported for macroalgae (Bischof et al. 1999).

UV effects on microalgae are often species-specific (e.g. Karentz *et al*. 1991). For example, UVR can affect the photosynthetic efficiency of some species more negatively than others (Waring *et al.* 2006). Over a longer experimental time (e.g like in our study) this could lead to a shift in the microalgal composition towards more UV resistant species (Worrest *et al.* 1981, Wängberg & Selmer 1997, Vinebrooke & Leavitt 1999). This was observed also in our experiments, although no significant shifts in species composition between the treatments could be detected.

One of the dominating species in later successional stages of our experiments was the diatom *Cylindrotheca closterium*. The photosynthetic efficiency of *C. closterium* has

earlier been found to be negatively affected by UV-B (Waring *et al.* 2006). However, according to our observations this diatom was in our study not negatively affected by UVR (in terms of growth). The UV-B intensities applied by Waring *et al.* (2006) were ca 3 times higher than in the present study. Our results were in agreement with Wängberg *et al.* (1999) where *C. closterium* remained unaffected by UV-B over a 7 day microcosm study.

Many of the species in our mixed diatom community presumably have a good ability to recover from UV-B induced stress, later confirmed by further experiments performed in the study area (Wulff *et al.* unpublished). In these experiments, the diatom photosynthesis recovered within 10 min (20% recovery) after exposure to UV radiation. Furthermore, two 3.5 months UV field experiments in the same area (intertidal and subtidal), showed no impact of UVR on the diatom assemblages studied (Campana *et al.* accepted, Zacher *et al.* submitted)

Vertical migration has been suggested to be a key mechanism for epipelic benthic diatoms to avoid UV-B radiation (Underwood *et al.* 1999). In the study by Underwood *et al.* (1999), the epipelic diatom *Gyrosigma balticum* (Ehr.) Rabenhorst responded to UV-B by vertical migration, but a significant damage to PSII was still apparent after 5 days of repeated UV-B exposure. UV-B radiation has been shown to penetrate down to 0.6 mm sediment depth (Wulff *et al.* 1999), and we cannot exclude that the diatoms in our experiment escaped the UV-B. In a short-term experiment (6 h UV exposure), an indication of UV-induced vertical migration was found (Wulff & Zacher in press). Other UV protective mechanisms are the production of photoprotective carotenoids, such as beta-carotene. Furthermore, de-epoxidation of diadinoxanthin to diatoxanthin is

known to occur in excessive light (PAR) as a protection against photooxidation (Arsalane *et al.* 1994), but the effect of UV-B on the de-epoxidation process is not clear. DNA repair has been proposed as an UV protective mechanism (Buma *et al.* 2001), but no DNA damage could be found in benthic Antarctic diatoms from the same study area (Wulff *et al.* unpublished).

In conclusion, laboratory experiments should never be extrapolated to determine community responses but they still give valuable information of underlying mechanistic processes. In our study, the UV effects found were transient (photosynthetic efficiency and cell density but the growth of the benthic diatoms was generally unaffected). Thus, according to our results UVR do not seem to be a threat to benthic marine Antarctic diatoms. From an evolutionary perspective, it might be that these species have a capacity to endure UVR. During the course of evolution, they have been exposed to high irradiance levels and UVR exerted a selective pressure. Therefore, it is plausible that the cause of this "endurance" is due to not yet established key mechanisms. However, determinations of UV effects on natural Antarctic microphytobenthos require more *in situ* measurements of the photosynthetic activity and productivity.

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Figure legends

Figure 1a-c. Photosynthetic performance (P-I curves, n = 3) of diatom suspensions exposed to (a) dim white light (<10 µmol photons m^{-2} s^{-1}), (b) 1 h of 90 µmol photons m^{-2} s^{-1}, and (c) 2 h of 233 µmol photons m^{-2} s^{-1}. PFR is the respective photon fluence rate of actinic white light and rETR is the relative electron transport rate

Figure 2a-b. Diatom cell numbers of the most frequently observed groups after exposure to different light treatments. Experiment A1 (a), and A2 (b) (n = 4) without UV-B or UVR, respectively. (*) marks significant differences in total cell number (one-way ANOVA).

Figure 3a-b. Experiment A1 (a), and A2 (b) (n = 4). Optimum quantum yield of microalgae after exposure to different light treatments (± SE), without UV-B or UVR, respectively. (*) marks significant differences within the respective light treatments (one-way ANOVA). Dark grey = PAB (PAR + UV-A + UV-B), light grey = PA (PAR + UV-A) and white = P (PAR) treatment.

Table 1: Treatment, exposure time and radiation conditions of the two experiments. PAR and UV-A was applied for 15 h daily, UV-B for 6 h daily.

Exp.	**Treatment**	**PAR µmol photons m^{-2} s^{-1}**		**PAR kJ/d**	**UV-A W m^{-2}**	**UV-A kJ/d**	**UV-B W m^{-2}**	**UV-B kJ/d**
		flathead	sphere					
A1	PAB	32 (ca 6.88 W m^{-2})	75	372	2.75	146	0.22	4.7
A1	PA	32	75	372	2.75	146	0	0
A2	PAB	32	75	372	2.75	146	0.22	4.7
A2	P	32	75	372	0	0	0	0

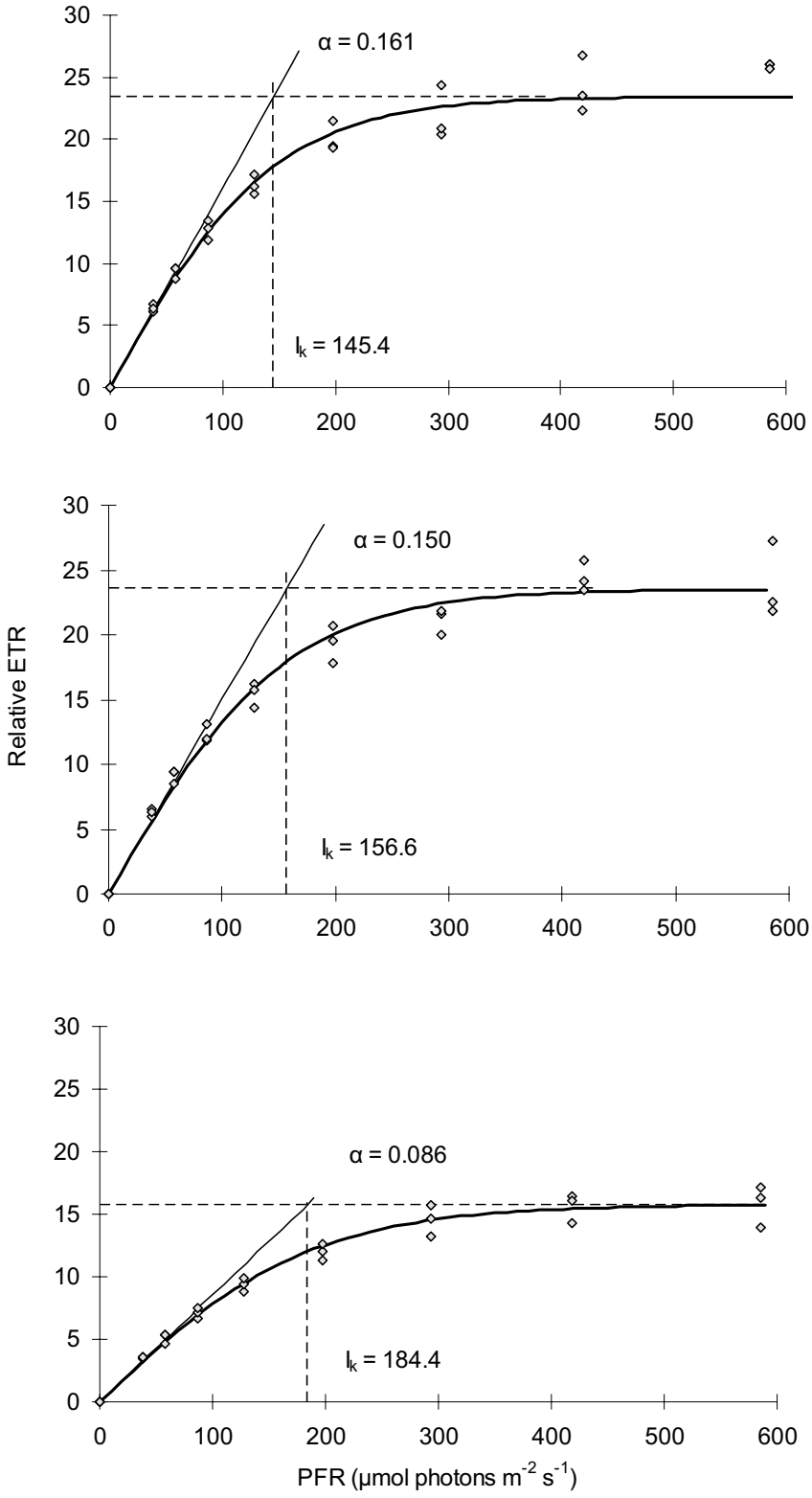
α = 0.161
I_k = 145.4
α = 0.150
I_k = 156.6
α = 0.086
I_k = 184.4
Relative ETR
PFR (µmol photons m^{-2} s^{-1})

Fig. 1

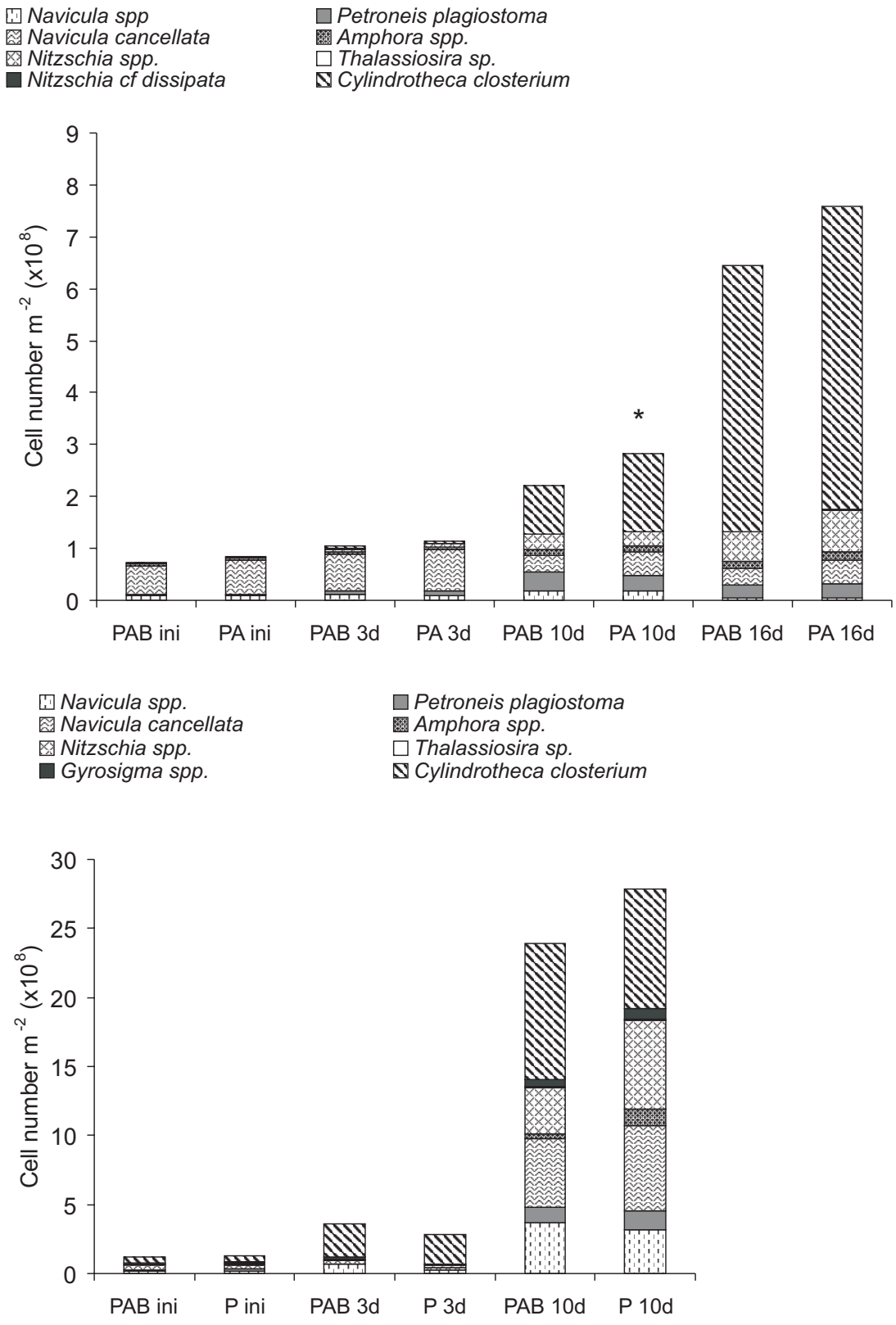
Navicula spp
Navicula cancellata
Nitzschia spp.
Nitzschia cf dissipata
Petroneis plagiostoma
Amphora spp.
Thalassiosira sp.
Cylindrotheca closterium
Cell number m-2 (x10^8)
0
1
2
3
4
5
6
7
8
9
*
PAB ini
PA ini
PAB 3d
PA 3d
PAB 10d
PA 10d
PAB 16d
PA 16d
Navicula spp.
Navicula cancellata
Nitzschia spp.
Gyrosigma spp.
Petroneis plagiostoma
Amphora spp.
Thalassiosira sp.
Cylindrotheca closterium
Cell number m-2 (x10^8)
0
5
10
15
20
25
30
PAB ini
P ini
PAB 3d
P 3d
PAB 10d
P 10d

Fig. 2

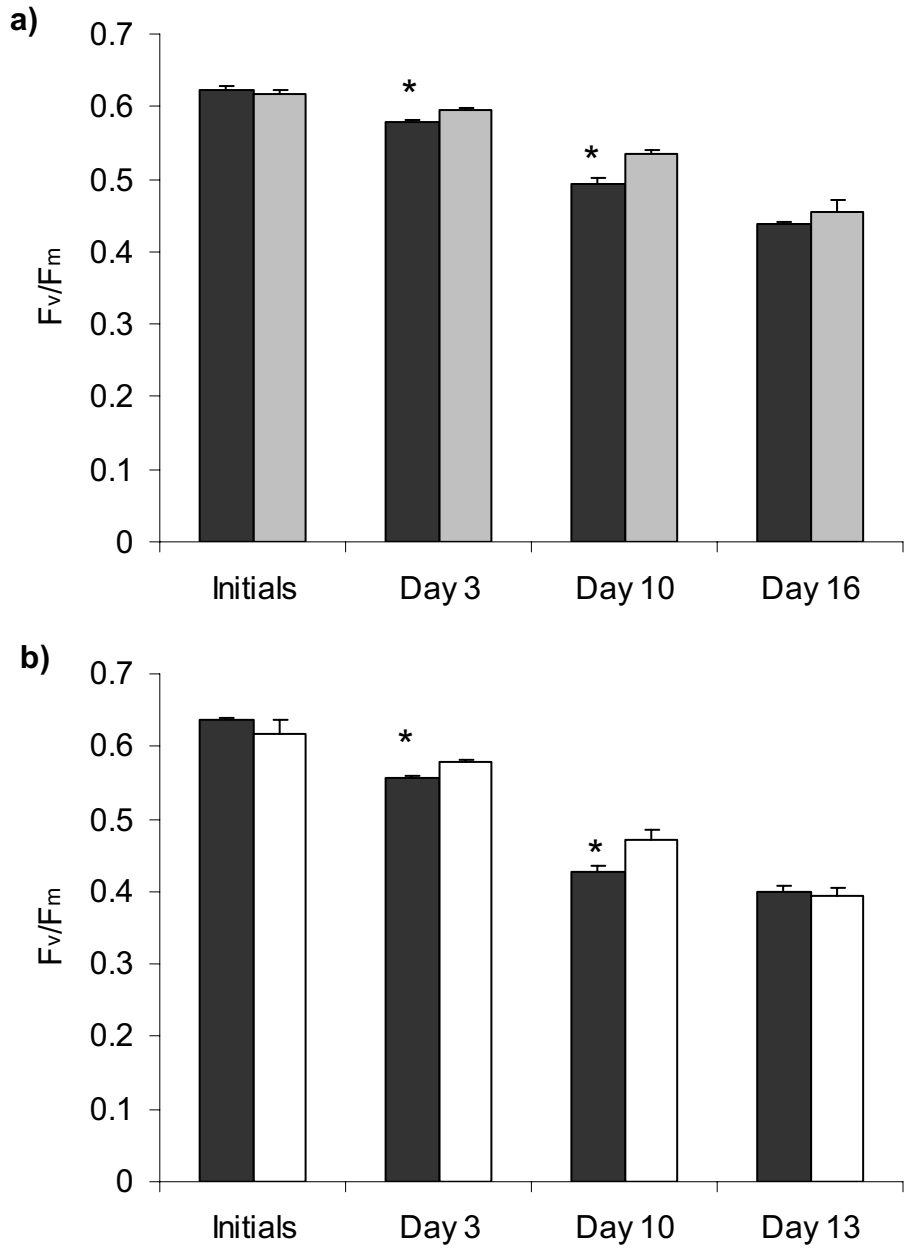
a)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fv/Fm
*
*
Initials
Day 3
Day 10
Day 16
b)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fv/Fm
*
*
Initials
Day 3
Day 10
Day 13

Fig. 3

Short-term UV effects on the photosynthesis of Antarctic benthic diatoms

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Introduction

The seasonal depletion of the stratospheric ozone layer and the resulting increase in solar ultraviolet-B radiation (UV-B; 280-315 nm) reaching the Earth's surface, particularly over the Antarctic region, is a potential threat to all organisms including marine microalgae. In the area studied (Potter Cove, King George Island), UV-B could penetrate down to 16 m and UV-A (315-400 nm) down to 19 m water depth (1% of the air measurements, see also Richter et al. this issue), thus, also benthic subtidal diatoms may be affected by ultraviolet radiation (UVR).

Marine microalgae constitute the basis of the marine food web and are responsible for 40% of the global primary productivity (Field et al. 1998). In Potter Cove, benthic macro- and microalgae are important primary producers. On the soft-bottoms, the microalgae are of particular interest because the phytoplankton biomass is not sufficient to explain the benthic consumer abundance and it has been hypothesized that microbenthic algae account for the nutrition of the local fauna (Schloss et al. 1998).

In some studies on benthic microalgae, UV radiation has been shown to damage the photosynthetic apparatus, the photosynthetic pigments, the DNA and, in addition, to decrease growth and primary productivity (reviewed in Villafañe et al. 2003). Ultraviolet-A radiation (UV-A; 315-400 nm) and photosynthetically active radiation (PAR; 400-700 nm) are involved in photoreactivation and photorepair of the DNA (Karentz 1994 and references therein). And therefore of particular concern is that ozone depletion results in increased harmful UV-B radiation without a proportional increase in UV-A and PAR.

The study was motivated by the fact that there are very few studies dealing with the response of Antarctic benthic microalgae to UVR, particularly marine benthic microalgae. The objective of this study was to estimate the short-term impact of UV-B and UV-A radiation, respectively, on the photosynthetic efficiency of a benthic microalgal community.

Materials and methods

The study was carried out in December 2003 at Dallmann Laboratory, Potter Cove, King George Island, Antarctica (62° 15´S, 58° 41´W). Fine grained sandy sediment was collected from 5-7 m water depth. The top layer (1 cm) was

scraped off and the sediment was brought to the laboratory, gently shaken and sieved (mesh size 500 µm) using filtered surface seawater. The sediment was stirred and the overlying water containing suspended microalgae (diatoms) was left to grow and develop a diatom mat under dim white radiation (ca 10 mol photons m^{-2} s^{-1}).

Experimental set-up

Four short-term experiments (B1, B2, C1 and C2) took place in a temperature (2-4 °C) and radiation controlled chamber. Different radiation treatments applied were PAR+UV-B+UV-A (PAB), PAR+UV-A (PA), and PAR (P). The PA treatments were covered by 0.13 mm transparent polyester film (Folanorm-SF/AS, Folex GmbH, Cologne, Germany). The P treatment was covered by Ultraphan URUV Farblos (Digefra GmbH, Munich, Germany). The experimental treatments are shown in Table 1 and 2.

Prior to experiment B1 and B2, 24 pieces (ca 1 cm^2) of the mat dominated by the benthic diatoms *Cylindrotheca closterium* and *Gyrosigma fasciola* were transferred to a 24 well microtiter plate. For B2, the semi-natural community was kept in darkness (4°C) ca 48 h before the experiment started. The cell number in the respective wells varied between 0.7 and 1.2 *10^6 cells L^{-1} (no significant differences between cell numbers in the wells, P = 0.88). The microalgae were left under PAR for 5 min before the treatments started. The diatoms were exposed to UV radiation for 6 h followed by recovery radiation for 20 h and a period of darkness (<1 µmol photons m^{-2} s^{-1}) for another 20 h (Table 1).

Table 1. Treatments and exposure time of the two short term experiment with microalgal mats (n = 12). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR
PAR = 212 (180-226) µmol m^{-2} s^{-1}, UV-A = 6.85 (5.5-8.2) W m^{-2}, UV-B = 0.575 (0.5-0.7) W m^{-2}.

expt	treatments	exposure time	"recovery"	darkness <1 µmol s^{-1} m^{-2}
			without UVB	
B1	PAB and PA	0, 2, 4, 6 h	1, 3, 5, 10, 20 h	4, 7, 10, 20 h
			without UV	
B2	PAB and P	0, 2, 4, 6 h	1, 3, 5, 10, 20 h	4, 7, 10, 20 h

Table 2. Treatments and exposure time of the two short term experiments with microalgal suspensions (n = 3). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR.
PAR = 107 (80-136) µmol m^{-2} s^{-1}, UV-A = 4.0 (3.5-4.5) W m^{-2}, UV-B = 0.4 (0.32-0.46) W m^{-2}.

expt	treatments	exposure time	"recovery"	darkness <1 µmol s^{-1} m^{-2}
			without UVB	
C1	PAB and PA	0, 6 h	6 h	12 h
			without UV	
C2	PAB and P	0, 6 h	6 h	12 h

Because benthic diatoms can migrate down into the sediment to reach more favourable radiation conditions, another set of experiments were run but instead of using the intact mat diatom suspensions were used (C1, C2). The diatom mat was diluted in seawater and 2 ml of the suspension was put in each of the 24 wells. The cell numbers in the wells varied between 0.4 * 10^5 and 2.1 * 10^5 cells

L^{-1}. No significant differences between cell numbers in the wells were found (*P* = 0.88). The microalgae were exposed for PAR for 15 min before the treatments started. The diatom suspensions were exposed to UV radiation for 6 h followed by recovery radiation for 6 h and a period of darkness (<1 μmol photons m^{-2} s^{-1}) for another 12 h (Table 2). In experiment C2, after 3 h radiation treatment the lamp had to be changed and the microalgae were left in the chamber for 25 min before the radiation treatment could continue.

Radiation treatments
Radiation was provided by a 400 W Metallogen lamp (Philips MSR 400 HR). Irradiation in the chamber was measured using a Solar Light PMA 2100 radiometer (Solar Light, Philadelphia, USA) equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar Light, Philadelphia, USA). PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1000 datalogger (LI-COR Bioscience, Lincoln, USA, Table 1 and 2).

Diatom photosynthesis
The effects of UV radiation on photosynthetic efficiency of the diatom mat and suspension, respectively, were determined by measuring the emission of variable chlorophyll fluorescence of PS II by use of a pulse-amplitude modulated fluorometer (B1 and B2 = PAM 2000, C1 and C2 = Water-PAM, connected to a PC with WIN CONTROL Software, Walz GmbH, Effeltrich, Germany). For B1 and B2, the whole mat was used for repeated (non-destructive) measurements of effective quantum yield of photosynthesis $\Delta F/F_m'$ directly under the respective treatment (n = 12, Table 1). For C1 and C2, the content of the well was sampled immediately after the radiation treatment (n = 3, Table 2). The cell suspension was filled into a 5 ml Quartz cuvette equipped with an automatic stirrer. The sample was stirred during the last minute of a 5 min dark adaptation. The last 10 seconds of the dark adaptation the stirrer was turned off to let larger grains settle. Optimum quantum yield of photosynthesis (F_v/F_m) was measured. Prior to the dark adaptation, the samples were exposed for 5 s of far-red light.

Results

In the experiments with the intact microalgal mats (B1 and B2), initial values of effective quantum yield were 0.601 to 0.585 and 0.472 to 0.478, respectively (Fig. 1a-b). In both experiments, effective quantum yield decreased significantly after 2, 4 and 6 hours exposure to UVR or UV-A, respectively (Fig. 1a-b).

During “recovery” under radiation, in experiment B1, the PAB treatment differed significantly from the PA treatment after 1, 3, 5 and 10 hours of exposure to PAR + UV-A (no UV-B). The non-significant difference after 20 hours was the result of a steady decline of the effective quantum yield of the PA treatment rather than a recovery of the PAB treatment (Fig. 1a). Effective quantum yield of both treatments (PAB and PA) was reduced to 70 and 77 % (after 20 h recovery) in comparison with the initial values, respectively. In the following exposure to “darkness”, both treatments recovered to initial values.

During “recovery” under radiation, in experiment B2, the PAB treatment differed significantly from the P treatment after 1, 3 and 5 hours of exposure to PAR (no UVR). The non-significant difference after 10 and 20 hours resulted in a steady decline of the effective quantum yield of the P treatment rather than in a recovery of the PAB treatment (Fig. 1b). Effective quantum yield of both treatments (PAB and P) was reduced to 69 and 77 % (after 20 h recovery) in comparison with the initial values, respectively. A partly recovery was observed after exposure to “darkness”, being faster in the P treatment than in the PAB treatment (Fig. 1b). However, the effective quantum yield didn’t recover completely to initial values.

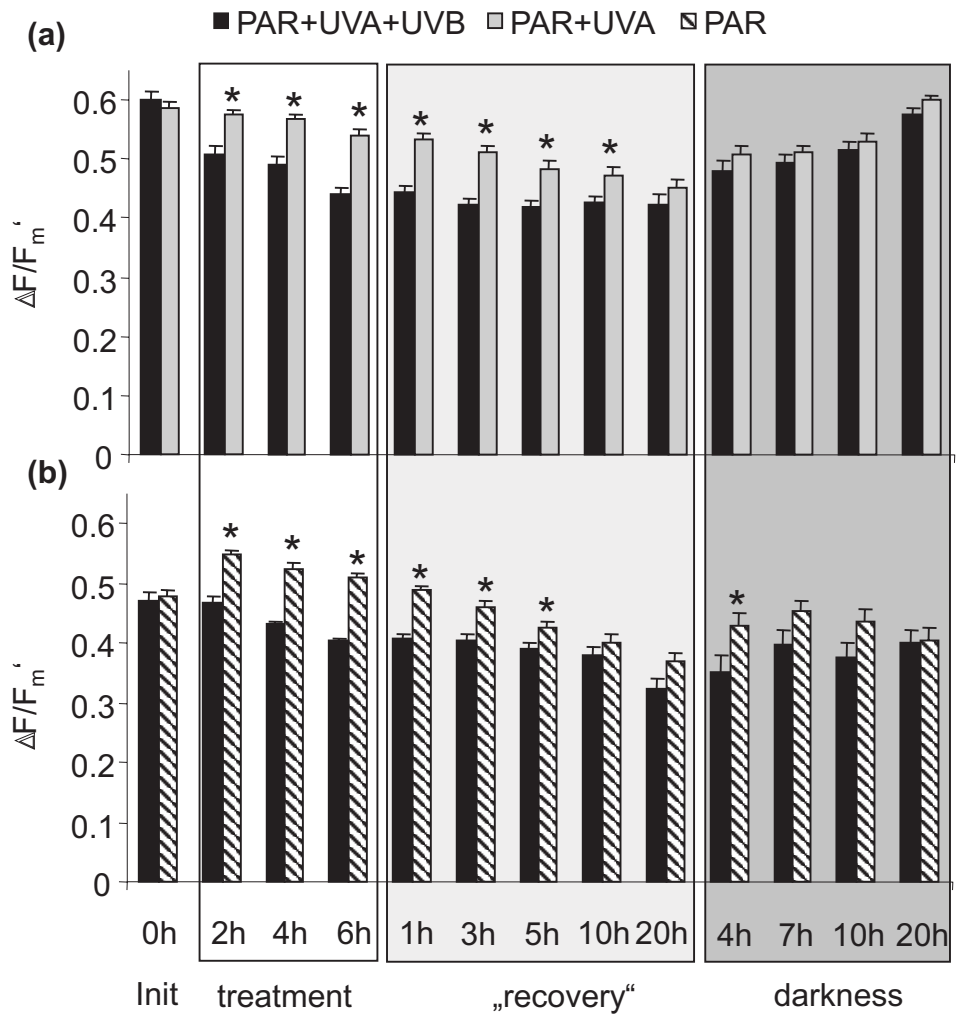

Fig. 1a-b. Experiment B1 **(a)** and B2 **(b)** (n = 12). Effective quantum yield of microalgal mats after exposure to different radiation treatments (± SE), without UV-B or UVR, respectively (“recovery”) and in darkness (<1 μmol photons m^{-2} s^{-1}). (*) marks significant differences within the respective radiation treatments (one-way ANOVA). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR

In the experiments with the microalgal suspension (C1 and C2), initial values of optimum quantum yield (F_v/F_m) were 0.570 to 0.557 and 0.530 to 0.573 (Fig 2a-b), respectively. In both experiments, exposure to UVR or UV-A didn’t lead to significant differences in optimum quantum yield (Fig. 2a-b). However, the PAB values were always lower than F_v/F_m from PA or P treatments, although not statistically significant.

In experiment C1, both treatments recovered completely after 12 hours in "darkness" (Fig. 2a). In experiment C2, the PAB treatment did not recover to initial values, resulting in a significant difference after 12 hours of exposure to darkness (Fig. 2b).

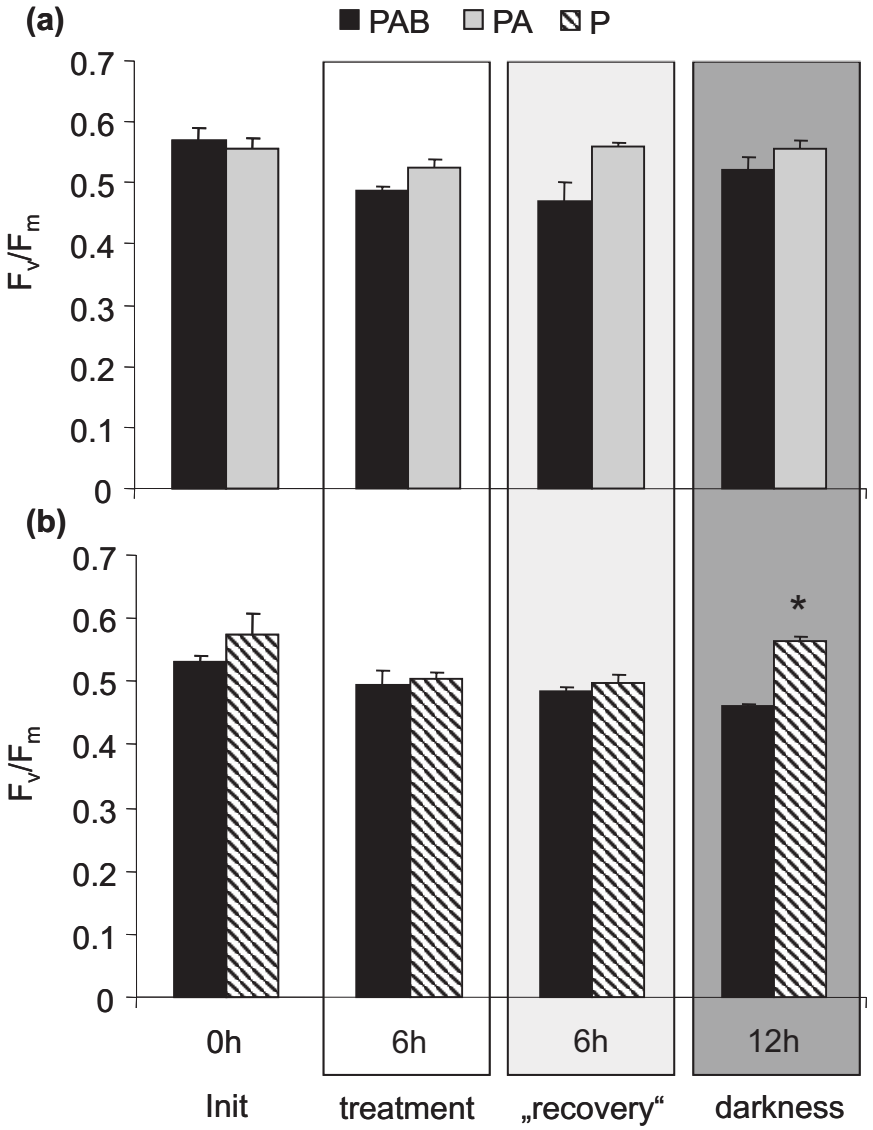

Fig. 2a-b. Experiment C1 **(a)** and C2 **(b)** (n = 3). Optimum quantum yield of microalgal suspension after exposure to different radiation treatments (± SE), without UV-B or UVR, respectively ("recovery") and in darkness (<1 μmol photons m^{-2} s^{-1}). (*) marks significant differences within the respective radiation treatments (one-way ANOVA). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR

Discussion

Decreased photosynthetic rate appear to be the most frequently observed short-term effect for benthic microalgae (Villafañe et al. 2003). The results are, however, ecologically relevant only when realistic, moderate increases of UV levels are used (Villafañe et al. 2003). In our experiments, the diatoms were exposed to a UV-B intensity of ca 0.4-0.6 W m^{-2}, an intensity they will probably

never experience at 5 m water depth at the study site. We can thus conclude that due to the high UV-B intensities applied, the effectiveness of UV-B was overestimated compared with field conditions and our experimental approach was thus mechanistic. Nevertheless, in all our experiments the diatom photosynthesis recovered from the UV treatment effects.

The reduced photosynthetic capacity observed in the first set of experiments (B1, B2) was due to the UV-B part of the spectrum because there was no apparent difference between the P and PA treatments.

Vertical migration has been suggested to be a key mechanism for epipelic benthic diatoms to avoid UV-B radiation (Underwood et al. 1999). To detect a possible impact of a downward migration we used two different experimental approaches. First we used an intact diatom mat and a non-destructive probe for measuring effective quantum yield of photosynthesis (expts B1, B2). We thus measured the upper layer of the most exposed cells. Lower $\Delta F/F_m'$ in the UV-B treatment could be caused by photoinhibition and/or photodamage of PSII (Hanelt et al. 1997) but it could also be due to downward migration of the cells (Underwood et al. 1999). In the study by Underwood et al (1999), the epipelic diatom *Gyrosigma balticum* responded to UV-B by vertical migration, but a significant damage to PSII was still apparent after 5 days of repeated UV-B exposure. Although UV-B has been shown to penetrate down to 0.6 mm sediment depth (Wulff et al. 1999), we cannot exclude that the diatoms in our experiment escaped the UV-B radiation. In the second set of experiments (C1, C2) we used a diatom suspension and we measured the optimum quantum yield of photosynthesis (dark adapted cells). Experiment C1 and C2 confirmed the hypothesis of vertical migration because no UV-B (or UV) effects were observed although *all* cells and not only cells of the upper layer were measured. We want to point out, however, that the UV-B intensity differed between the first and second experimental set-ups (0.6 and 0.4 W m^{-2}).

If only the UV-B or UV part of the radiation spectrum was removed, no recovery was observed. Under “darkness”, however, a recovery in all treatments formerly exposed to PAR and UV-A could be observed (B1, C2). When shielded from UVR, no increase in photosynthetic capacity was detected (B2, C2). UV-A has been suggested to counteract UV-B effects in phytoplankton (Smith et al. 1992) and cyanobacteria (Quesada et al. 1995). The reduction in UV-B damage has been attributed to a UV-A and blue-light mediated repair of the DNA and a stimulation of photosynthesis (Franklin and Forster 1997 and references therein). In experiment B2, however, the P treatment did not recover to initial values and no treatment effects could be observed. The diatom assemblage in experiment B2 seemed to be stressed (photoinhibited) already at the beginning of the experiment possibly influencing the outcome of the experiment because no recovery to initial values was found over time.

Self shading has been proposed as a mechanism to avoid harmful UVR (Garcia-Pichel et al. 1996). However, due to the low cell numbers this was not the case in our experiment. If all cells lie flat on the sediment they could still not cover the sediment surface

In earlier field experiments regarding UV effects on marine microbenthic communities the benthic diatoms generally seemed to be very tolerant to UV-B radiation (Wulff 1999 and references therein). Our study confirms these earlier results. However, we believe that determinations of UV-B effects on natural microphytobenthos require *in situ* measurements of the photosynthetic activity and productivity.

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ORIGINAL ARTICLE

UV effects on photosynthesis and DNA in propagules of three Antarctic seaweeds (*Adenocystis utricularis*, *Monostroma hariotii* and *Porphyra endiviifolium*)

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Abstract Ozone depletion is highest during spring and summer in Antarctica, coinciding with the seasonal reproduction of most macroalgae. Propagules are the life-stage of an alga most susceptible to environmental perturbations therefore, reproductive cells of three intertidal macroalgal species *Adenocystis utricularis* (Bory) Skottsberg, *Monostroma hariotii* Gain, and *Porphyra endiviifolium* (A and E Gepp) Chamberlain were exposed to photosynthetically active radiation (PAR), PAR + UV-A and PAR + UV-A + UV-B radiation in the laboratory. During 1, 2, 4, and 8 h of exposure and after 48 h of recovery, photosynthetic efficiency, and DNA damage were determined. Saturation irradiance of freshly released propagules varied between 33 and 83 μmol photons m^{-2} s^{-1} with lowest values in *P. endiviifolium* and highest values in *M. hariotii*. Exposure to 22 μmol photons m^{-2} s^{-1} PAR significantly reduced photosynthetic efficiency in *P. endiviifolium* and *M. hariotii*, but not in *A. utricularis*. UV radiation (UVR) further decreased the photosynthetic efficiency in all species but all propagules recovered completely after 48 h. DNA damage was minimal or not existing. Repeated exposure of *A. utricularis* spores to 4 h of UVR daily did not show any acclimation of photosynthesis to UVR but fully recovered after 20 h. UVR effects on photosynthesis are shown to be species-specific. Among the tested species, *A. utricularis* propagules were the most light adapted. Propagules obviously possess good repair and protective mechanisms. Our study indicates that the applied UV dose has no long-lasting negative effects on the propagules, a precondition for the ecological success of macroalgal species in the intertidal.

Keywords Antarctica · DNA damage · Photosynthetic efficiency · *P–I* curve · Propagules · UV radiation

Abbreviations

PAR	Photosynthetically active radiation
UV-A	Ultraviolet-A
UV-B	Ultraviolet-B
UVR	UV radiation
P	PAR
PA	PAR + UV-A
PAB	PAR + UV-A + UV-B
CPDs	Cyclobutane pyrimidine dimers
F_v/F_m	Optimum quantum yield
I_k	Saturation irradiance
PFD	Photon flux density
P–I curves	Photosynthesis irradiance curves
rETR	Relative electron transport rate

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Introduction

Seaweeds are the most important primary producers in coastal waters contributing 3.2% to the global aquatic primary production (Mann 1973). In contrast to pelagic primary producers, macroalgae have complex life cycles including unicellular reproductive cells and microbenthic stages, apart from the macrobenthic thallus. Especially the early developmental stages are highly susceptible to a variety of stresses (Coelho et al. 2000). Therefore, the survival of the early phases of marine macroalgae is critical to the successful establishment of benthic populations (Vadas Sr et al. 1992).

Propagules can be exposed to high photosynthetically active radiation (PAR = 400–700 nm) and ultraviolet radiation (UVR = 280–400 nm) after release during their planktonic phase. For example kelp spores can be transported at least several kilometers in the water column and thereby be especially exposed to UVR because no protective shading by canopy algae occurs (Reed et al. 1988). The negative effects to UV exposure on cellular level include, e.g., photoinhibition and/or photodamage (Hanelt et al. 1997), protein breakdown (Lao and Glazer 1996), the production of reactive oxygen species (Rijstenbil et al. 2000) as well as damage to the DNA (van de Poll et al. 2001, 2002) and other biomolecules through the direct absorption of UVR (Vass 1997). These impacts can result in low growth rates (Roleda et al. 2006b). Moreover diversity and species richness of algal communities can be negatively affected due to UVR (K. Zacher unpublished data; Dobretsov et al. 2005).

UVR effects on macroalgae are species-specific. Different acclimation and repair mechanisms exist in species most tolerant to UV stress coming from shallow waters (Larkum and Wood 1993). Photosynthesis is a dynamic process and excessively absorbed energy, which is not utilized in photochemistry, can be converted into harmless thermal radiation until a certain point (Hanelt 1996). Maximum quantum yield of photosynthesis of, e.g., the intertidal brown alga *Alaria esculenta* can acclimate to enhanced levels of UVR within a few days (Bischof et al. 1999). Recovery after photodamage of the D1 protein of photosystem II is reflected by the new synthesis of this protein (Bischof et al. 1998). Other strategies can be avoidance of UVR or the production of screening compounds (reviewed in Franklin and Forster 1997; Bischof et al. 2006). Furthermore, DNA damage can be repaired enzymatically by light-dependent photolyases and light-independent nucleotide excision (van de Poll et al. 2002).

Although the unicellular propagules are clearly the stages most susceptible to UVR (Wiencke et al. 2006b) most UV studies have been carried out on the adult macrothalli. Some studies exist on the UV impact on spores of Arctic and temperate Laminariales and Gigartinales, showing that their sensitivity is related to their depth distribution and, hence influencing recruitment of the species in the eulittoral zone (Roleda et al. 2004b; Wiencke et al. 2006b). Antarctic intertidal algae are particularly suffering from elevated UV-B radiation (280–315 nm) during the last two decades due to stratospheric ozone depletion (>50% over this area; WMO 2003). UV-B and UV-A radiation (315–400 nm) can reach intensities of more than two and 40 W m^{-2} in spring in the studied area (King George Island, Antarctica), respectively. Furthermore, 1% of the surface irradiance of UV-B radiation can still be measured in a depth of about 15 m at clear water conditions. However, UV experiments with Antarctic macroalgae are scarce. To our knowledge these experiments are the first testing the UV sensitivity of reproductive cells from Antarctic field material.

In laboratory experiments propagules of three intertidal Antarctic macroalgal species were exposed to different light treatments to measure photosynthetic performance and DNA damage. The ability of these early developmental stages to recover from UV induced damage was also studied. The study gives valuable insights in the ecological success of Antarctic intertidal algae growing under a highly variable light regime including high UV values during spring and summer. It is hypothesized that propagules from Antarctic intertidal macroalgae can better cope with high UV levels in comparison with algae from Arctic or temperate regions.

Materials and methods

Algal material

Fertile specimen of the brown alga *Adenocystis utricularis* (Bory) Skottsberg, the green alga *Monostroma hariotii* Gain and the red alga *Porphyra endiviifolium* (A and E Gepp) Chamberlain were collected between January and March 2005 at Peñon Uno (Dallmann Laboratory, King George Island, South Shetland Islands, 62°14.80′S, 58°41.26′W). *A. utricularis* and *M. hariotii* were collected from the eulittoral were they occur together, whereas *P. endiviifolium* grows on rocks in the upper eulittoral. After collection the specimen were brought immediately to the nearby laboratory and put into filtered seawater (2°C under low light conditions) until further processing.

Spore release

Numerous individuals of each species were cleaned with tissue paper, divided randomly in five replicates and prepared for spore release in a temperature controlled room (2 ± 1.5°C). *P. endiviifolium* was put into Petri dishes with seawater for collection of monospores from the asexual thallus. Individuals of *A. utricularis* and *M. hariotii* were put in a wet chamber and left overnight under dim light. Propagules release was obtained by flooding the algae with filtered seawater in Petri dishes. Spore suspension was adjusted for *A. utricularis* spores (zoospore length around 4 µm) to ~7.1×10^4, for *M. hariotii* gametes (length around 7 µm) to ~1.57×10^4 spores ml^{-1} and for *P. endiviifolium* monospores (mean diameter 15 µm ± 2. 4 SD, $n = 32$) to ~1.12×10^4 spores ml^{-1} after counting (Sedgewick-Rafter Cell S50 spore counter, Graticules Ltd., Tonbridge, UK) to obtain the desired background fluorescence for photosynthetic measurements.

Experimental treatments

Light was provided by white fluorescent lamps (Osram GmbH, L65 Watt/25S, Munich, Germany), emitting background PAR of 400–700 nm and UV lamps (Q-Panel UV-A-340, 40 W, Cleveland, USA), emitting a spectrum qualitatively similar to solar radiation in the range of 295–340 nm. Three kinds of filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent lamps: (1) Ultraphan transparent (Digefra GmbH, Munich, Germany), (2) Folanorm 320 (Folex GmbH, Cologne, Germany), and (3) Ultraphan URUV farblos (Digefra), corresponding to the PAR + UV-A + UV-B (PAB, 280–700 nm), PAR + UV-A (PA, 320–700 nm) and PAR (P, 400–700 nm) treatments, respectively. The available filters cut off wavelengths were slightly differing from the definition of CIE (Commission Internationale De l'Éclairage, UV-B = 280–315 nm, UV-A = 315–400nm).

Irradiance measurements

Irradiation in the laboratory was measured below the cut-off filters using a Solar Light PMA 2100 radiometer (Solar Light, Philadelphia, PA, USA) equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar light). As the spectral range of the UV-A sensor extends into the UV-B region of the spectrum, UV-A radiation measurements were always made using a Schott WG320 filter (Schott, Mainz, Germany) to exclude wavelengths below 320 nm. The UV-B measurements recorded were obtained by subtracting the reading with the WG320 filter from the reading without the filter. PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1400 datalogger (LI-COR Bioscience, Lincoln, NE, USA). Irradiance under the different treatments is shown in Table 1. Furthermore, ambient UV-A and UV-B radiation in the air was measured permanently with a 32-channel single-photon counting spectroradiometer (Isitec, Bremerhaven, Germany; Hanken and Tüg 2002) at the Dallmann Laboratory.

Spore photosynthesis

Photosynthetic efficiency of reproductive cells measured as variable fluorescence of photosystem II (PSII), was determined using a Water Pulse Amplitude Modulation fluorometer (Water-PAM) connected to a PC with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). Immediately after adjustment of cell density (not exceeding 1 h after spore release), spore suspension was filled into 5 ml Quartz cuvettes. Optimum quantum yield (F_v/F_m) was measured after 3 min dark adaptation to determine initial photosynthetic efficiency at time zero ($n = 5$) as described by Roleda (2006a), designated as control. After that, the controls were maintained under dim white light (4 µmol photons m^{-2} s^{-1}) for 2 days before the final measurement. Photosynthesis (in terms of relative electron transport rate, rETR = PFR × $\Delta F/F_{m'}$) versus irradiance curves (*P–I* curves) were also measured in the time zero control ($n = 3$, chosen at random from the five replicates) as described by Bischof et al. (1998). The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate *P–I* curve parameters described as: $rETR = rETR_{max} \times \tanh(\alpha \times I_{PAR} \times rETR_{max}^{-1})$, where $rETR_{max}$ is the maximum relative electron transport rate, tanh is the hyperbolic tangent function, α is the initial slope in the light limited part of the *P–I* curve (as a measure for the electron transport efficiency) and *I* is the photon fluence rate of PAR. The saturation irradiance for

Table 1 Irradiance under the different experimental treatments

Treatments	PAR (W m^{-2})	UV-A (W m^{-2})	UV-B (W m^{-2})
PAB (PAR + UV-A + UV-B)	4.73	4.34	0.35
PA (PAR + UV-A)	4.73	4.05	0.07
P (PAR)	4.73	0.06	0.00

Under the recovery shelf PAR irradiance was 0.86 W m^{-2} (4 µmol m^{-2} s^{-1})

electron transport (I_k) was calculated as the intercept between α and the $rETR_{max}$ values. Curve fit was calculated with the Solver module of MS-Excel using the least square method comparing differences between measured and calculated data.

To evaluate the effect of different radiation and exposure time treatments, 5 ml spore suspension were filled into 35 × 10 mm cell culture dish ($n = 5$) and exposed to the three radiation conditions for 1, 2, 4, and 8 h at 2 ± 1.5°C. Spores from *A. utricularis* were exposed in another experiment for 2, 8, and 16 h (Table 2). After F_v/F_m measurements, the spore suspension was returned to their respective culture dishes and allowed to recover for 2 days under dim white light (4 μmol photons m^{-2} s^{-1}) condition. Furthermore, a time series experiment was performed exposing *A. utricularis* spores repeatedly to PAB, PA and P for 4 h daily followed by 20 h under dim white light (4 μmol photons m^{-2} s^{-1}) over a period of 5 days. Photosynthetic efficiency was measured directly after the treatment and after recovery (Table 2).

Spore DNA damage and repair

DNA damage and subsequent repair of this damage was determined after 1, 2, 4, and 8 h exposure to UV-B radiation. From the working spore suspension, 40 ml was used for each experimental unit. For each treatment, six experimental units were prepared. After the irradiation treatment, three experimental units (as replicates) were processed immediately while the other three were allowed to recover for 2 days in low white light before processing. The spore samples were filtered through 44 mm diameter 1.0–2.0 μm pore size Nuclepore® polycarbonate membrane filters (Whatman, London, UK) and frozen at −80°C in 2-ml Eppendorf tubes for further DNA extraction and analysis of cyclobutane pyrimidine dimers (CPDs).

DNA was extracted using CTAB and quantified as described by Roleda et al. (2004b). The accumulation of CPDs was determined following a two step antibody assay using anti-thymine dimer H3 (Affitech, Oslo, Norway) and rabbit anti-mouse immunoglobulins (conjugated with horseradish peroxidase, DakoCytomation, Glostrup, Denmark). Chemiluminescent detection was done using ECL Western blotting detection reagent (Amersham, Buckinghamshire, UK; Roleda et al. 2005). Developed films (using X-ray film developer) were scanned using Bio-Rad imaging densitometer (Model GS-700, Bio-Rad Laboratories, Hercules, CA, USA) and gray scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's Image Analysis Systems). A calibration series of UV irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 μg ml^{-1} DNA for each calibration point. The UV irradiated DNA (45 min exposure to 2 TL 20W/12 lamps, Philips, Eindhoven, The Netherlands) was previously calibrated against UV irradiated Hela DNA with known amounts of CPDs. CPDs were quantified by comparing the gray scales within the linear range of the film.

Data analysis

A one-way ANOVA was used to test for the effects of UVR on photosynthetic efficiency and DNA damage separately for each species and each exposure time ($P < 0.05$). Prior to analysis data were tested for homogeneity of variances (Cochran´s test). Post-hoc comparisons were performed with Newman–Keuls test. Statistical analysis were done using Statistica™ 6.0 software package.

Results

UV irradiance in the field and in the laboratory

Mean daily doses of UV-A and UV-B radiation in the field (air measurements from January to February 2005)

Table 2 Different treatments for the performed experiments including measured parameters (optimum quantum yield = F_v/F_m and DNA damage = CPDs)

Species	Parameter	Treatment	Exposure	Recovery
Monostroma hariotii	F_v/F_m	PAB + PA + P	1, 2, 4, 8 h	48 h
	CPDs	PAB	1, 2, 4, 8 h	48 h
Porphyra endiviifolium	F_v/F_m	PAB + PA + P	1, 2, 4, 8 h	48 h
	CPDs	PAB	1, 2, 4, 8 h	48 h
Adenocystis utricularis	F_v/F_m	PAB + PA + P	1, 2, 4, 8 h	48 h
	CPDs	PAB	1, 2, 4, 8 h	48 h
	F_v/F_m	PAB + PA + P	2, 8, 16 h	24, 48 h
	F_v/F_m	PAB + PA + P	4 h/days over 5 days	20 h/days over 5 days

Exposure and recovery time is the duration of the treatments PAB (PAR + UV-A + UV-B), PA (PAR + UV-A) and P (PAR) and recovery under dim white light, respectively. During recovery spores were exposed to a PAR of 4 μmol m^{-2} s^{-1}

are shown in Table 3 in comparison to our treatments. Exposure to artificial UV-A radiation was lower in our experiments even after 16 h of irradiance than daily doses in the field. In contrast UV-B radiation in the 8 h treatment was similar to the daily doses measured in the field (Table 3).

Photosynthesis: Irradiance curves

The *P–I* curves shown in Fig. 1a–c reveal the differences in the photosynthetic performance of spores of the three species directly after spore release. The values for α (an index of light-harvesting system efficiency) varied between 0.065 and 0.139 (Fig. 1a–c). A similar steep slope was found in *A. utricularis* and *P. endiviifolium*, whereas *M. hariotii* showed the lowest α value. Highest saturating irradiance (I_k) was measured for reproductive cells of *M. hariotii* (83 µmol photons m^{-2} s^{-1}), followed by *A. utricularis* (64 µmol photons m^{-2} s^{-1}) and *P. endiviifolium* (33 µmol photons m^{-2} s^{-1}; Fig. 1a–c). At photon fluence rates >300 µmol photons m^{-2} s^{-1}, $rETR_{max}$ slightly decreased in *A. utricularis* and *M. hariotii* (Fig. 1a, b) whereas in *P. endiviifolium* $rETR_{max}$ decreased strongly after exceeding the actinic light level of 300 µmol photons m^{-2} s^{-1}. $rETR_{max}$ was higher in *A. utricularis* ($rETR_{max}$ = 9) in comparison to *M. hariotii* ($rETR_{max}$ = 5) and *P. endiviifolium* ($rETR_{max}$ = 4).

Photosynthetic efficiency after short term exposure to UV radiation

Photosynthetic performance of the three species was affected differently by PAR, UV-A and UV-B radiation, respectively. Initial measurements of the controls showed that *P. endiviifolium* monospores had a slightly higher mean optimum quantum yield (F_v/F_m = 0.488 ± 0.04) than *A. utricularis* spores (F_v/F_m = 0.462 ± 0.11) and both had a much higher optimum quantum yield compared with *M. hariotii* gametes (F_v/F_m = 0.288 ± 0.04, Fig. 2). The changes in optimum quantum yield during treatments are shown in Fig. 2. After 1 h treatment with PAR (*P*; 22 µmol photons m^{-2} s^{-1}) the optimum quantum yield (F_v/F_m) was not affected in *A. utricularis* whereas in *M. hariotii* and *P. endiviifolium* it was already reduced to 62 and 81% (expressed as the percentage of control), respectively (Fig. 2a, c, e). Increasing exposure time did not further affect F_v/F_m in *A. utricularis* which remained still high after 8 h exposure. In *M. hariotii* highest inhibition was found after 1 h and did not significantly change with further exposure. However, in *P. endiviifolium* increasing exposure time further decreased the F_v/F_m. PAR supplemented with UV-A (PA treatment) decreased photosynthetic efficiency significantly compared to the *P* treatment in all three species during exposure. Two exceptions were the 8 h treatment of *M. hariotii* and *P. endiviifolium* where no significant UV effect compared to the 8 h exposure to higher PAR was detected. Additional UV-B (PAB treatment) generally did not reveal a further significant decrease of optimum quantum yield. Interestingly, F_v/F_m of the PAB treatment in *A. utricularis* increased again after 8 h exposure (reduction after 1 h to 37%, after 8 h to 57% of the control measurement). In all treatments photosynthetic efficiency was reduced by 55–82% due to UVR in comparison to the respective *P* treatments.

However, all species showed a complete recovery of photosynthesis after 2 days under dim white light compared to the controls (ANOVA, $P > 0.05$) and no differences between treatments were detected. In *A. utricularis* F_v/F_m increased in the controls from 0.462 ± 0.114 before treatment to 0.601 ± 0.044 (mean ± SD) after 2 days recovery and in *M. hariotii* from 0.288 ± 0.040 to 0.400 ± 0.149 (mean ± SD), respectively. However, in *P. endiviifolium* F_v/F_m decreased in the controls from 0.488 ± 0.040 at the beginning of the experiment to 0.249 ± 0.023 (mean ± SD) after 2 days (Fig. 2a–f).

A second experiment with *A. utricularis* conducted with a longer exposure time (see also light doses in Table 3) and recovery measured after 24 and 48 h followed the same pattern as described above. UVR significantly reduced optimum quantum yield of spores in comparison to the *P* treatment (Fig. 3). After 8 and 16 h additional UV-B radiation reduced F_v/F_m significantly more than UV-A alone (Newman–Keuls, $P < 0.05$ between PAB and PA). An incomplete recovery occurred after 24 h in the 16 h exposure treatments of PA and PAB (ANOVA, $F_{2,12} = 15.03$, $P = 0.0005$). However, all samples recovered in all treatments after 48 h (Fig. 3).

Table 3 UV-A and UV-B doses in the PAB (PAR + UV-A + UV-B) treatment for different exposure times and in the field (n = 50) as means ± SD of daily doses measured in January and February

	UV-A (kJ m^{-2})	UV-B (kJ m^{-2})
Laboratory		
1 h	15.62	1.26
2 h	31.25	2.52
4 h	62.50	5.04
8 h	124.99	10.08
16 h	249.98	20.17
Field daily	318.06 ± 122.39	11.20 ± 4.33

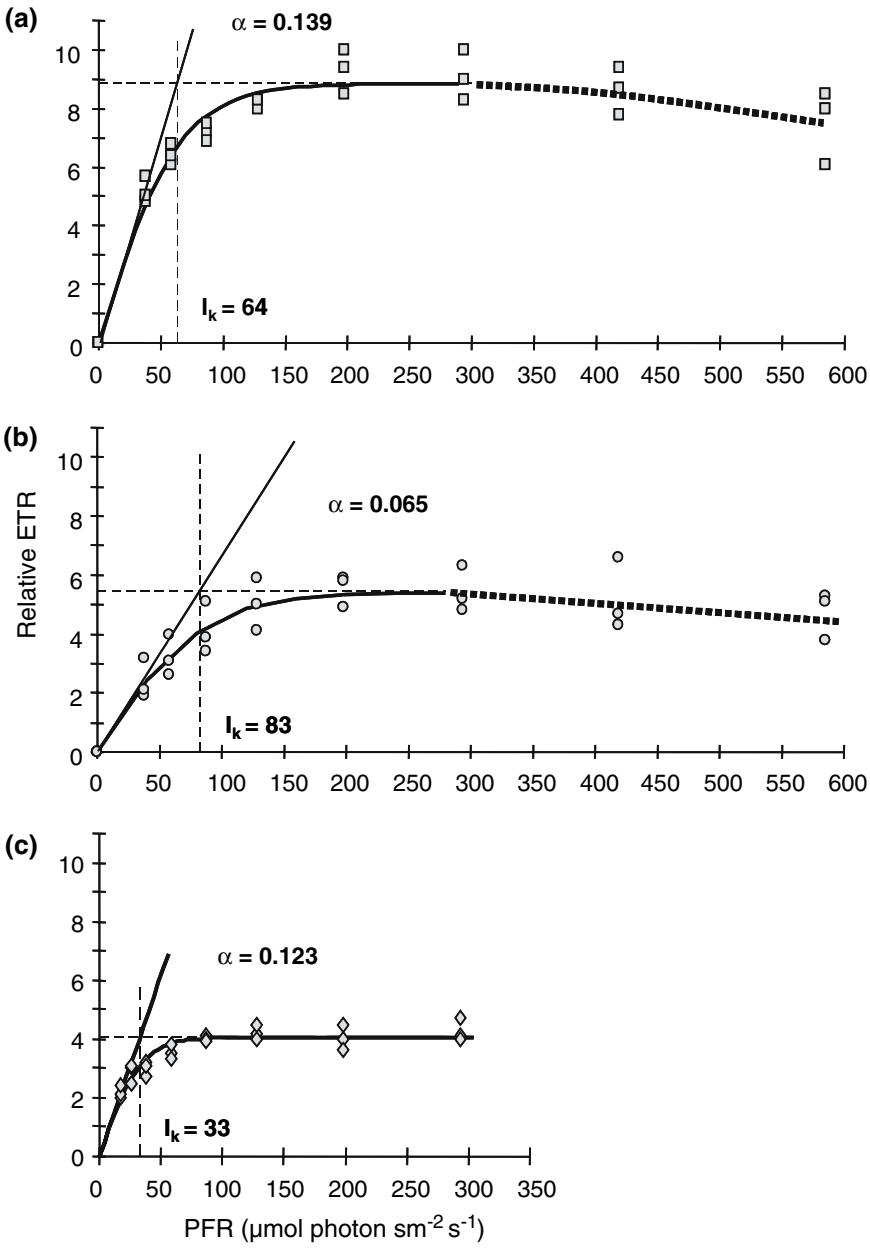

Fig. 1 Photosynthetic performance (*P–I* curves, $n = 3$) of spores of *Adenocystis utricularis* (**a**), *Monostroma hariotii* (**b**) and *Porphyra endiviifolium* (**c**) after spore release. PFR is the respective photon fluence rate of actinic white light and rETR is the relative electron transport rate

Time series of repeated UVR irradiance on *A. utricularis* spores

Repeated measurements of the same samples did not significantly affect the optimum quantum yields, as shown with the comparison of undisturbed control and disturbed control (measured at the beginning and the end of the experiment, Table 4, $P > 0.005$).

The time series measurements over a 5 days period did not show significant differences between the optimum quantum yield of the controls (maintained under 4 µmol photons m^{-2} s^{-1}) and the *P* treatments under higher PAR (22 µmol photons m^{-2} s^{-1}, $P > 0.05$). However, additional UV-A and the combination from UV-A and UV-B radiation led to a significant decrease in F_v/F_m after each of the 4 h treatments (Fig. 4). The decrease over time in the PA treatment did not significantly change (ANOVA, $F_{4, 20} = 0.91$, $P = 0.477$) during the 5 days and ranged from 48 to 56%. On the other hand, the effects of the PAB treatment changed over time (ANOVA, $F_{4, 20} = 5.36$, $P = 0.004$). After a significant increase in F_v/F_m from days 1 to 2 (Newman–Keuls, $P = 0.020$), F_v/F_m dropped significantly from days 2 to 5 (Newman–Keuls, $P = 0.003$) after 4 h exposure. Optimum quantum yield was reduced to 41% (on day 2) and to 28% (on day 5) in comparison to the control.

A complete recovery was measured after 20 h under dim white light with one exception on day 4 (PAB sig-

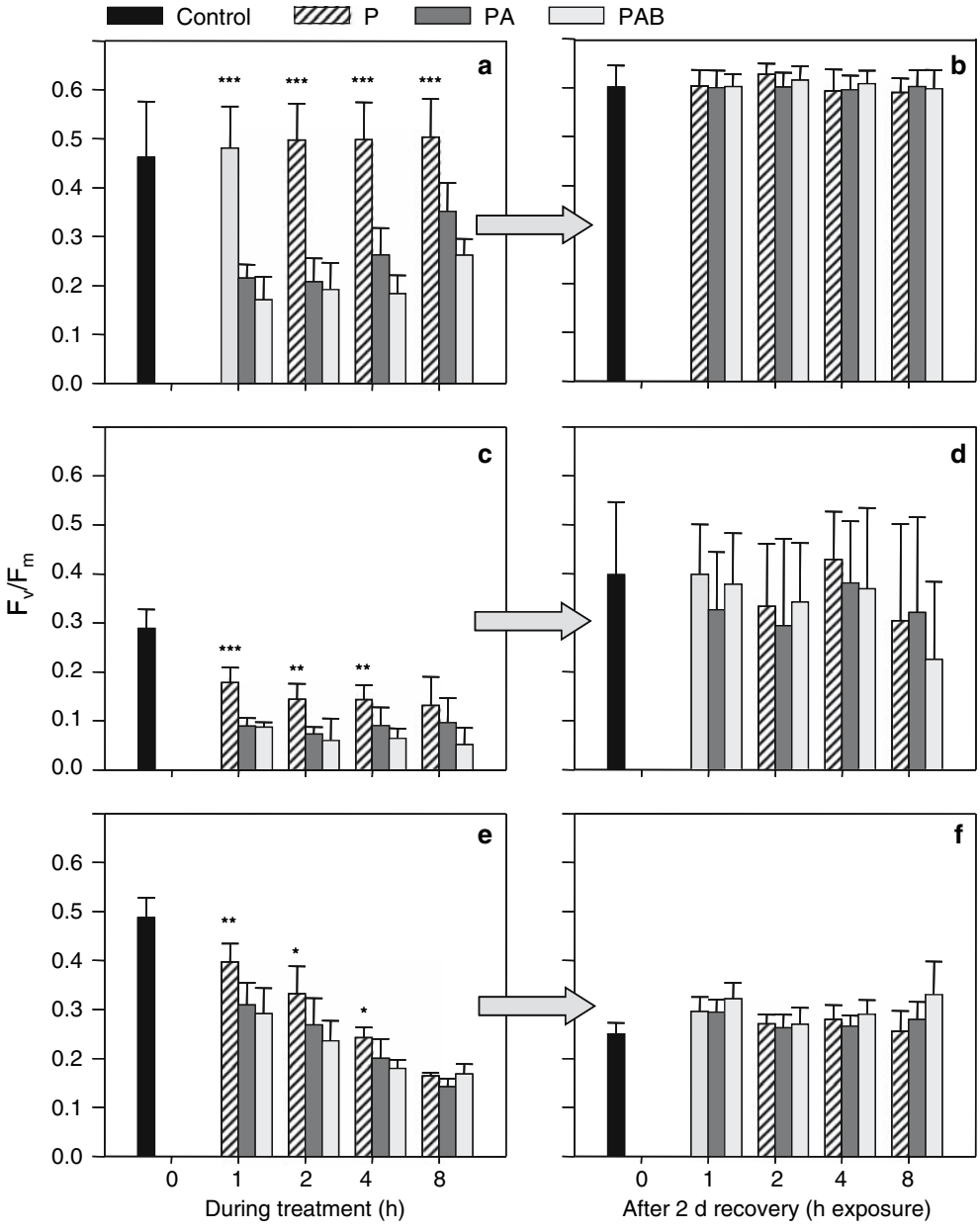

Fig. 2 Mean optimum quantum yield (F_v/F_m) ± SD ($n = 5$) of reproductive cells of *Adenocystis utricularis* (**a, b**) *Monostroma hariotii* (**c, d**) and *Porphyra endiviifolium* (**e, f**) after exposure to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) and after 2 days of recovery, respectively. Control (C) is without treatment and continuously maintained under 4 μmol photons m^{-2} s^{-1} white light. *Asterisks* indicate significant differences between the *P* and PA and/or PAB treatment. Significance levels were defined as follows: ***$P < 0.001$, **$P = 0.001$–0.01, *$P = 0.01$–0.05

nificantly different from *P*, ANOVA, $F_{2,\ 12} = 5.00$, $P = 0.026$; Fig. 4).

DNA damage and repair

No detectable DNA damage (measured as CPD concentrations per million nucleotides, CPD Mbp^{-1}) was found in *P. endiviifolium* monospores and only minimal DNA damage in propagules of *A. utricularis* (2, 4, and 8 h treatment) and *M. hariotii* (4 and 8 h treatment; Fig. 5) after exposure to PAB. CPD induction significantly increased in both species from 2 (4) to 8 h PAB exposure time (ANOVA, $F_{1,6} = 11.95$, $P = 0.008$ and $F_{1,4} = 7.85$, $P = 0.049$ for *A. utricularis* and *M. hariotii*, respectively). After 2 days recovery under dim white light all species were able to repair the DNA damage.

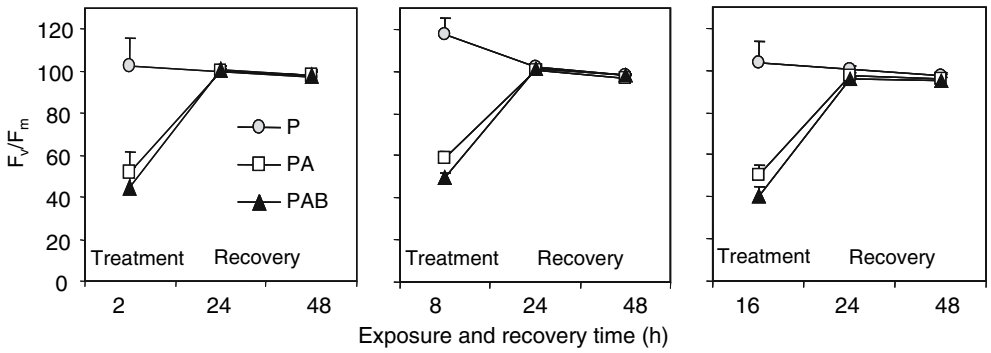

Fig. 3 Mean optimum quantum yield (F_v/F_m) ± SD ($n = 5$) expressed as percentage of the respective control of *Adenocystis utricularis* spores after exposure (treatment 2, 8, and 16 h) to PAR (*P*), PAR + UV-A (*PA*) and PAR + UV-A + UV-B (*PAB*) and after 24 and 48 h of recovery, respectively. F_v/F_m of controls were 0.4574 ± 0.0541 (*treatment*), 0.6072 ± 0.0209 (24-h recovery) and 0.6408 ± 0.0113 (48-h recovery), respectively

Table 4 Mean optimum quantum yield (F_v/F_m ± SD) of untreated zoospores of *Adenocystis utricularis* (controls) after release and at different time series intervals (see Fig. 4 for treatment effects)

Day	Hours	Disturbed control	Undisturbed control
0 initial	0	0.579 ± 0.031	0.571 ± 0.028
1	4	0.598 ± 0.020	–
	24	0.642 ± 0.014	–
2	28	0.631 ± 0.012	–
	48	0.643 ± 0.011	–
3	52	0.640 ± 0.012	–
	72	0.649 ± 0.006	–
4	76	0.652 ± 0.005	–
	96	0.658 ± 0.007	–
5	100	0.653 ± 0.007	–
	120	0.665 ± 0.007	0.669 ± 0.005

Disturbed control is without treatment, continuously maintained under 4 µmol photons m^{-2} s^{-1} white light and measured at the same time intervals as the treated samples. Undisturbed control was measured once before the start and at the end of the experiment to determine weather samples get disturbed due to the measurements. In the meantime it was continuously maintained under 4 µmol photons m^{-2} s^{-1}

Discussion

Our experiments showed that propagules from the Antarctic intertidal are well fitted to survive in their extreme habitat, although this life stage is the most susceptible to environmental stress factors. This study is among the first testing UVR effects on intertidal propagules of seaweeds and the first with Antarctic species.

In the laboratory experiments a UV-A:UV-B ratio of 12:1 is emitted by the lamps, whereas in the field the proportion of UV-A is more than two times higher. Even stronger is the difference in the ratio between PAR:UV-A:UV-B which was ~790:19:1 ($n = 112$) in the field in air (data not shown) and only 13.5:12.4:1 in the laboratory. The lower doses in the laboratory in relation to field air measurements were chosen to take the absorption by the water column into account. For example only 55% of UV-A and 60% of UV-B radiation reached the sample area in 10 cm water depth. Therefore, the 8-h treatment reflects the most natural situation in terms of daily UV doses for *A. utricularis* and *M. hariotii* whereas *P. endiviifolium* is exposed to higher doses due to its occurrence at the high tide level. However, maximal irradiances and doses in the intertidal are generally highly variable, depending, e.g., on tide level, water turbidity and weather conditions.

The I_k of *P. endiviifolium* is much lower than the I_k values of *A. utricularis* and *M. hariotii*. Low light adapted macroalgae have a saturation point ranging between 14 and 52 µmol m^{2-} s^{-1} (Hanelt et al. 2003) characterizing spores of *P. endiviifolium* as strongly shade adapted. In contrast propagules of *A. utricularis* and *M. hariotii* seem to be less strongly shade adapted.

These results are in agreement with measured I_k values for the adult thalli. Weykam et al. (1996) showed that the I_k values of adult Antarctic Rhodophyta are low compared to Chlorophyta or Phaeophyta. Results on zoospores of Arctic Laminariales generally showed a high shade adaption (I_k between 13 and 18 µmol m^{-2} s^{-1}, Roleda et al. 2006a). In contrast, I_k values of zoospores of cold temperate *Laminaria* species range between 20 and 40 µmol m^{-2} s^{-1} (Roleda et al. 2005) while kelp zoospores from the warm temperate regions have higher I_k ranging from 41 to 77 µmol m^{-2} s^{-1} (Amsler and Neushul 1991). The geographical trend with low values in the polar and higher ones in warmer regions corresponding to the increasing solar irradiance from the poles to the equator (Roleda et al. 2006a) could not be confirmed for Antarctic propagules with saturating irradiances within the range of temperate species. However, I_k values are also

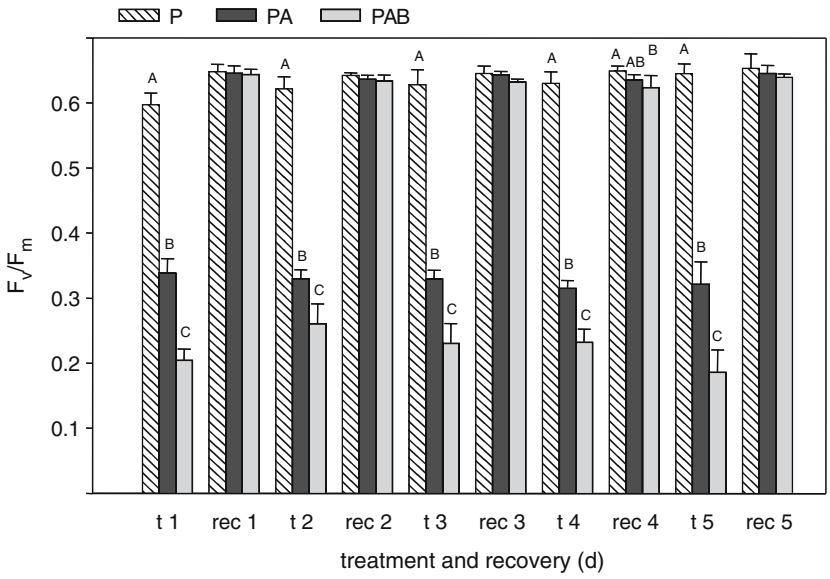

Fig. 4 Time series of repeated UV irradiation on spores of *Adenocystis utricularis*. Mean optimum quantum yield (F_v/F_m) ± 1 SD (n = 5) of spores after 4 h exposure (t 1 to t 5) to PAR (*P*), PAR +UV-A (*PA*) and PAR + UV-A + UV-B (*PAB*) and after subsequent 20 h of recovery (rec 1–5) repeated over 5 days, respectively. *Different letters* indicate significant differences between the treatments

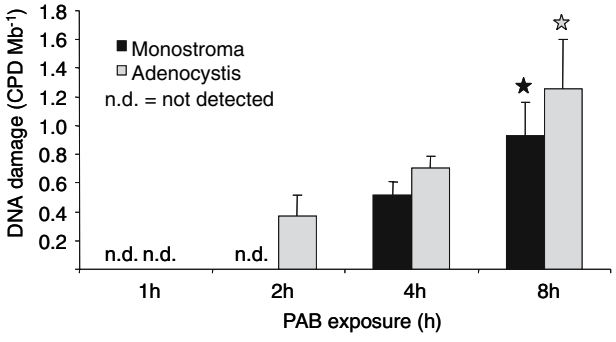

Fig. 5 UV-B induced DNA damage (mean ± SD, n = 3, induced CPD concentrations per million nucleotides) in *Adenocystis utricularis* spores and *Monostroma hariotii* gametes after exposure to different doses of PAB (PAR + UV-A + UV-B, 1, 2, 4, and 8 h). CPDs were not detected in *Porphyra endiviifolium* and in all species after 2 days recovery under dim white light (4 µmol photons m^{-2} s^{-1}) no more CPDs were detected. Significant differences among the different exposure times for each species are marked with *asterisk*

dependent on algal zonation on the shore with higher values measured for macroalgae from shallower water depth (Roleda et al. 2006a). In our study only intertidal species were tested explaining the relatively high I_k values. $rETR_{max}$ was not inhibited by actinic light <300 µmol m^{-2} s^{-1} showing good adaptations to higher PAR levels according to the occurrence of the adult thalli in the upper eulittoral. Eulittoral algae are periodically exposed to air where they experience a variety of stressful environmental conditions, e.g., very high light intensities (Davison and Pearson 1996). The potential for acclimation and recovery of the photosynthetic apparatus to high or damaging radiation conditions is therefore an important pre-requisite for the recruitment and ecological success of algae growing in the intertidal (Roleda et al. 2006a).

In general, photosynthetic efficiency (F_v/F_m) of freshly released propagules was lower compared to young or adult macrothalli as also shown in other studies (Wiencke et al. 2000; Roleda et al. 2004b). This can be attributed to the development stage of the chloroplast in different life stages. Laminariales zoospores with thin plasmalemma and one chloroplast per cell, e.g., are more sensitive to light stress (Roleda et al. 2006a). In contrast to the other two species optimum quantum yield of *Porphyra* spores decreased during the experiment in the controls maybe due to non-optimal cultivation conditions for this species.

Reaction to P, PA and PAB exposure in propagules was species-specific, indicating a higher light sensitivity of the green algae *M. hariotii* and the red algae *P. endiviifolium*. High light conditions during e.g., low tide or high water transparency might therefore influence their propagules survival more negatively than in *A. utricularis*. However, the reaction of propagules to high light stress remains to be tested.

Reduction of photosynthetic efficiency while exposed to high PAR is a protective mechanism to dissipate energy absorbed by PSII as heat via the xanthophylls cycle to avoid photodamage (dynamic photoinhibition; Osmond 1994). UVR exhibited an additional effect in the reduction of F_v/F_m in all species. The measurable effects of both PAR and UVR in the reduction of photosynthetic efficiency are similar but the mechanisms behind PAR and UVR induced inhibition of photosynthesis are different (Hanelt et al. 2003). UVR exhibits adverse effects on photosynthesis causing a direct molecular damage due to the absorp-

tion by biomolecules (Vass 1997). Depression of photosynthetic performance by UVR is, e.g., implicated to the damage of the oxidizing site of the reaction center of photosystem II (Franklin et al. 2003).

In contrast to eulittoral *A. utricularis*, spores of sublittoral Arctic Laminariales reacted already with a strong depression of F_v/F_m under PAR of 22 µmol photon m^{-2} s^{-1} (Roleda et al. 2006a). Furthermore Laminariales zoospores from Helgoland (Germany) were not able to recover after 8 h exposure to PAB in a comparable experiment (Roleda et al. 2005) indicating that the Antarctic intertidal *Adenocystis* is better acclimated to PAR and UVR. Results from irradiance experiments with intertidal carpospores from the red algae *Chondrus crispus* and *Mastocarpus stellatus* from the North Sea (Helgoland, Germany) showed that spores of these species react more sensitive to PAR and UVR than *P. endiviifolium* monospores (Roleda et al. 2004b). This difference is surprising as at least *M. stellatus* is able to grow in the upper eulittoral zone as well (Roleda et al. 2004b).

The monostromatic thallus of *Monostroma arcticum* from the Arctic is, in comparison to other investigated Arctic Chlorophyta and eulittoral Phaeophyta quite light sensitive, fast photoinhibited but recovering slowly, an indication of chronic photoinhibiton (Hanelt 1998). The monospores from *M. hariotii* exibit a similar behavior, as they were more light sensitive than the brown alga *A. utricularis*, but less sensitive than the red alga *P. endiviifolium*.

Short time experiments (8-h exposure) gave evidence of a possible acclimation of the photosynthetic apparatus of *A. utricularis* to UV-A and UV-B radiation as found in other studies with brown algae (Bischof et al. 1999; Roleda et al. 2004a). However, no such effect was observed after exposure to 16 h and after repeated exposure over a period of 5 days. In contrast to the earlier experiments additional UV-B significantly decreased photosynthetic efficiency further and inhibition was highest after 5 days suggesting a higher degree of damage due to repeated exposures. Apparently spores of *A. utricularis* lack acclimation abilities and are not able to diminish the inhibition caused by repeated UV exposure. After 20 h under dim white light, however, F_v/F_m recovered completely.

The non-detectable DNA damage in *P. endiviifolium* spores and minimal CPD formation in *A. utricularis* and *M. hariotii* propagules indicate effective shielding of the DNA and/or fast repair mechanism in the Antarctic intertidal propagules. The degree of damage due to UVR was observed to be related to cell size as DNA damage was observed to decrease in species (*Adenocystis*, *Monostroma*, *Porphyra*) with increasing cell size (4, 7, and 15 µm, respectively). This might be attributed to the increasing pathway for UV-B penetration through the cytoplasm (filtering effect; Swanson and Druehl 2000). In other studies, UV induced damage was related to thallus thickness (Franklin and Forster 1997; Johansson and Snoeijs 2002), e.g., thinner and relatively translucent species showed more DNA damage than thicker ones. An effective DNA repair mechanism was also observed in spores of Arctic and temperate Laminariales and Gigartinales but initial CPD formation was much higher (Roleda et al. 2004b, 2005).

DNA damage can be repaired through photolyase enzyme (light-dependent), nucleotide excision and recombination repair (light-independent; van de Poll et al. 2002). *M. arcticum* from Spitsbergen was not able to repair UV-B induced CPD formation probably due to low photolyase activity which has an important role in removing the majority of CPDs (van de Poll et al. 2002). The small amount of DNA damage in the tested Antarctic species might therefore be related to high photolyase activity. Another possibility is shielding due to UV absorbing compounds. However, whether the tested propagules are able to produce some kind of UV protective substance or have a high photolyase activity remains to be studied. Anyway, the ability of the propagules to cope with UV-B induced DNA damage seems to be crucial for the vertical zonation of the macrothalli at the coastline. If not repaired, DNA lesions can disrupt metabolism, cell division and impair growth and germination. Most macroalgae in Antarctica occur only in the subtidal (Wiencke et al. 2006a) and few are able to recruit in the intertidal partly due to their capacity to successfully repair DNA damage.

In general, exposure to the UV doses used in our laboratory experiment should not affect the survival and success of the investigated intertidal algae on short term view as all species recovered effectively from UV induced damage. However, in the field, maximal light intensities can be much higher especially when low tide coincides with noon and cloudless weather conditions. Longer exposure to ambient radiation over more than 8 h can take place and PAR would be much higher when cells are suspended within the euphotic layer of the water column. Therefore, field experiments on propagules are of great importance also taking into account parameters like germination and growth as integrative parameters of all physiological processes. Nevertheless, laboratory experiments give valuable insights in physiological mechanisms and common adaptations. Another important point is that these experiments were performed with field grown material as Swanson and Druehl (2000) hypothesized that kelp

spores might be pre-adapted to the UV conditions of their parent plants. If so, experiments with cultured material would not reflect the actual situation in the field and might overestimate UV effects because culturing usually takes place under PAR light only. Generally, the propagules studied here seem to be better adapted to UVR than temperate or Arctic ones. On the other hand, most previous studies were performed with subtidal species, mostly Laminariales which makes a direct comparison difficult. More comparative studies on related species and their reproductive cells respectively, from different geographical regions but similar zonation would improve our knowledge about the species-specific reactions and adaptations to (elevated) UVR.

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Photosynthetic performance, DNA damage and repair in gametes of the endemic Antarctic brown alga *Ascoseira mirabilis* exposed to ultraviolet radiation

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Running Title: UVR effects on gametes of *Ascoseira*

Abstract

Stress physiology on the reproductive cells of Antarctic macroalgae remained unstudied. *Ascoseira mirabilis* is endemic to the Antarctic region, an isolated ecosystem exposed to extreme environmental condition. Moreover, stratospheric ozone depletion leads to increasing UV radiation (UVR, 280-400nm) at the earth surface, thus it is necessary to investigate the capacity of reproductive cells to cope with different UV irradiances. This study is aimed to investigate the impact of exposure to different spectral irradiance on the photosynthetic performance, DNA damage and gamete morphology of the species. Gametangia, gametes and zygotes of the upper sublittoral brown alga *A. mirabilis* were exposed to photosynthetically active radiation (PAR= P; 400-700 nm), P + UV-A radiation (PA; UV-A, 320-400 nm) and P + UVA + UV-B radiation (PAB; UV-B, 280-320 nm). Rapid P-I curves of freshly released propagules were measured. Photosynthetic efficiencies and DNA damage (in terms of cyclobutane-pyrimidine dimers, CPD) were determined after 1, 2, 4 and 8 hours exposure as well as after 2 days of recovery in dim white light. Saturation irradiance (I_k) in freshly released propagules was 52 µmol photon m^{-2} s^{-1}. Exposure for 1 hour under 22 µmol photon m^{-2} s^{-1} of PAR significantly reduced the optimum quantum yield (F_v/F_m) suggesting that propagules are low light adapted. Furthermore, UVR significantly contributed to the photoinhibition of photosynthesis. Increasing dose as a function of exposure time additionally exacerbates the effects of different light treatments. Recovery of F_v/F_m in gametes and developing embryos was observed after two days post cultivation in all treatments but was found to be dependent on the fitness of individual *A. mirabilis* parental sporophytes. The amount of DNA damage increased with the UV-B dose but an efficient repair mechanism was observed in gametes pre-exposed to a dose lower than 5.8 x 10^3 J m^{-2} of UV-B. The results of this study demonstrate the negative impact

of UV-B radiation. However, gametes of *A. mirabilis* are capable of photosynthetic recovery and DNA repair when the stress factor is removed. This capacity was observed to be dependent on the fitness of the parental sporophyte.

INTRODUCTION

Ascoseira mirabilis Skottsberg is a perennial brown macroalga endemic to the Antarctic region. The high level of endemism in Antarctica is attributed to the surrounding deep ocean basins, the circumpolar current and the Polar Frontal Zone that have isolated the continent and its organisms for approximately 30 million years (Peck *et al*. 2006). During that time, atmospheric oxygen (O_2) and therefore, stratospheric ozone (O_3) were probably lower than at present exposing the earth to very high doses of UV-radiation (Rozema *et al*. 1997). Marine macroalgae have also been subjected to higher mean sea surface temperatures over most of their evolutionary history, than prevailing today (Raven *et al*. 2002). The lowering of sea surface temperatures at southern high latitudes occurred during the late Miocene (Crame 1993). The capacity of Antarctic macroalgae to adapt to ice, low temperatures, and seasonal light fluctuations is an important determinant of their biological fitness.

The marine benthic flora of Antarctica is predominantly sublittoral. Higher number of species and dense macroalgal assemblages occur at depths in the sublittoral called the "bare zone" while eulittoral floras is less well developed where inconspicuous algae occur only on short stretches of rocky coastline that remain ice-free for a few months in summer (Clayton 1994). *Ascoseira mirabilis* grows in the upper subtidal zone (Klöser *et al*. 1996; Quartino *et al*. 2005) while other large brown algae of the order Desmarestiales grow most abundantly at greater depths (Wiencke *et al*. 2006a).

The *Ascoseira mirabilis* thallus is diploid and monoecious. Sexual reproduction is isogamous. Conceptacles are scattered all over the blades and containing chains of gametangia surrounding a central hair. Gametangia are eight-chambered and following the segregation of a vestigial nucleus, each chamber releases one gamete (Clayton 1987; Müller *et al*. 1990). Gametes are heterokont and zygote formation follows immediately after fusion of gametes.

Advances in Antarctic macroalgal research are constrained with logistic difficulties. Aside from the studies on the life history of *Ascoseira*, only limited studies on its anatomy (Clayton & Ashburner 1990) and basic physiology (Gómez *et al*. 1995a, 1995b, 1996) are available. Concerns on global environmental changes, especially on the increase in ultraviolet radiation due to ozone depletion, make it necessary to study stress physiology on primary producers. In Antarctica, research efforts to evaluate the impact of this phenomenon have mostly focused on phytoplankton (Meador *et al*. 2002; Nunez *et al*. 2006). However, phytoplankton does not represent the only significant photoautotroph component of the aquatic environment susceptible to elevated UV-B. Reviews on Antarctic macroalgal research show the lack of information on the effect of UVR on seaweeds (Wiencke 1996; Wiencke *et al*. 2006a). This is in contrast to Arctic species where recent studies have shown that early life stages of macroalgae are most sensitive to ultraviolet radiation and their sensitivity is related to the depth distribution pattern of the adult sporophytes (Roleda *et al*. 2006a; Wiencke *et al*. 2006b).

Total ozone varies strongly with latitude over the globe, with highest concentration in middle and high latitudes. Before the stratosphere at polar latitudes was affected by anthropogenic chlorine and bromine, the naturally occurring springtime ozone levels over Antarctica were about 25% lower than springtime ozone levels over the Arctic (Fahey 2003). This natural difference between Antarctic and Arctic conditions exists

due to the exceptionally cold temperatures and different winter wind patterns within the Antarctic stratosphere as compared to the Arctic. Since the detection of stratospheric ozone depletion over Antarctica in the early 1980s, a yearly net springtime loss of 60-70% has been a recurring phenomenon that intensifies ambient UV-B radiation on the biosphere (Crutzen 1992; Herman *et al*. 1996). Moreover, the area affected by ozone depletion has expanded to 5 fold over the past decades in the continental Antarctica. The negative impact of exposure to UVR on reproductive cells of macroalgae includes (1) inhibition of photosynthesis and eventual photodamage to the photosynthetic apparatus (Roleda *et al*. 2006a); (2) damage to microtubules causing inhibition of nuclear division (Huovinen *et al*. 2000); (3) formation of cyclobutane pyrimidine dimers (CPDs) in the DNA (Roleda *et al*. 2004, 2005, 2006b); and (4) production of reactive oxygen species responsible for oxidative damage within the cell (Rijstenbil *et al*. 2000).

To our knowledge, no physiological study has been carried out on the effects of ultraviolet radiation on the reproductive cells of Antarctic macroalgae, which are the life history stage considered to be most susceptible to environmental stress. This study is aimed to investigate the impact of exposure to different spectral irradiance on the photosynthetic performance, DNA damage and gamete morphology of *Ascoseira mirabilis*. We further investigated the capacity of gametes to recover and repair UVR-induced damage when UVR stress was removed. The present study is of interest to account the first result on the deleterious effect of enhanced UV-B radiation due to stratospheric ozone depletion on the gametes of endemic Antarctic macroalgae.

MATERIALS AND METHOD

Algal material

Fertile sporophytes of *Ascoseira mirabilis* were collected in October 2004 during low tide in the upper sublittoral flat of Peñon Uno, King George Island (Antarctica, 62° 14.82'S, 58° 41.05'W). Mature blades with conceptacles containing gametangia were excised from five different sporophytes, cleaned of epiphytes, blotted with tissue paper and kept in darkness in a moist chamber at 0 °C overnight. Blades were then immersed in 5-10 ml filtered (0.2 µm pore size) seawater at 2 ± 1.5 °C and exposed to light (10 µmol photon m^{-2} s^{-1}) to induce release of the reproductive cells. Freshly released gametangia were maintained under low light condition (1-2 µmol photon m^{-2} s^{-1}). The initial gametangium density was counted by use of a Sedgewick-Rafter Cell S50 spore counter (Graticules Ltd., Tonbridge, England). Stock suspensions were diluted with filtered seawater to give densities between 3 x 10^3 – 4 x 10^3 gametangia ml^{-1} among the five replicates. During the course of the experiments, gametes were released and zygotes were formed.

Irradiation treatments

Photosynthetically active radiation (PAR) was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany). Ultraviolet radiation (UVR) was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland,OH, USA). Cell culture dishes were covered with one of the following filters to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes: Ultraphan transparent (Digefra GmbH, Germany), Folanorm (Folex GmbH, Germany) or Ultraphan URUV farblos corresponding to the PAR + UV-A + UV-B (PAB), PAR + UV-A (PA) and PAR (P)

treatments, respectively. Ultraviolet radiation was measured using a Solar Light PMA 2100 radiometer equipped with the UV-A sensor PMA 2110 and the UV-B Sensor PMA 2106 (Solar light, Philadelphia, USA). Ultraviolet radiation below the UV-transparent filter was 4.34 W m^{-2} UV-A and 0.40 W m^{-2} UV-B. Photosynthetically active radiation (PAR) was measured using a cosine quantum sensor attached to a LI-COR data logger (LI-1000, LI-COR Biosciences, Lincoln, Nebraska, USA) to be 22 µmol photon m^{-2} s^{-1} (~ 4.73 W m^{-2}).

Light microscopy

The structure of released reproductive cells was observed by light microscopy (Zeiss Axiolab, Germany). Structural changes after exposure to different light treatments and 2 days post cultivation in low white light were documented. Microscopic pictures were taken with a digital camera (Canon PowerShot A80, Switzerland).

Chlorophyll fluorescence measurements

Photosynthetic efficiency was measured as variable fluorescence of photosystem II (PSII) using a Water Pulse Amplitude Modulation fluorometer (Water-PAM) consisting of Emitter-Detector Unit Water-ED and PAM-Control Universal Control Unit connected to a PC operated with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). Immediately after adjustment of gametangium density, the gametangium suspension was filled into 5 ml Quartz cuvettes and the maximum quantum yield (F_v/F_m) was measured inside the Emitter-Detector Unit at time zero (n=5). After 3 min dark incubation, F_o was measured with a red measuring light pulse (~ 0.3 µmol photon m^{-2} s^{-1}, 650 nm), and F_m was determined with a 600 ms completely saturating white light pulse (~275 µmol photon m^{-2} s^{-1}). Photosynthesis (in terms of relative electron

transport rate, rETR = PFR x ΔF/Fm') versus irradiance curves (P-I curve) was measured two times in every replicate of the time zero control (using fresh suspension every measurement; n=3, chosen at random from the 5 replicates) using low and high actinic light intensities making up 10 points (17, 26, 38, 58, 87, 128, 198, 294, 419, 585 µmol photons m^{-2} s^{-1}). The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate P-I curve parameters described as:

$rETR = rETR_{max} * \tanh (\alpha * I_{PAR} * rETR_{max}^{-1})$

where $rETR_{max}$ is the maximum relative electron transport rate, tanh is the hyperbolic tangent function, α is the electron transport efficiency and I is the photon fluence rate of PAR. The saturation irradiance for electron transport (I_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote ($rETR_{max}$). Curve fit was calculated with the Solver Module of MS-Excel using the least squares method comparing differences between measured and calculated data.

Controls measured at time zero were filled into corresponding culture dishes (35mm x 10mm; CorningTM, Corning Inc., NY, USA). To evaluate the effect of different radiation treatments (3 levels: P, PA and PAB) and exposure times (4 levels: 1, 2, 4 and 8 hrs), samples of fresh gametangium suspension (not exceeding 1 hour after release) were filled into each culture dishes (total experimental units = 60). Samples corresponding to the 5 replicates were exposed to each treatment combination of radiation and exposure time at 2 ± 1.5 °C. After treatments, F_v/F_m was determined and the suspension was returned to the same culture dish and cultivated under dim white light (4 ± 1 µmol photons m^{-2} s^{-1}) at the same temperature for recovery. Time zero control was also maintained at the same condition. Measurements of photosynthetic recovery were made after 48 hours in dim white light condition. Settled and germinating

zygotes were slowly resuspended by sucking and jetting the medium against the bottom of the culture dish using Eppendorf pipettes.

DNA damage and repair

DNA damage and its subsequent repair were determined after exposure to the whole light spectrum at different exposure times of 1, 2, 4, and 8 hours. From the gametangia suspension ($3 \times 10^3 - 4 \times 10^3$ ml^{-1}), 40 ml was used for each experimental unit. For each treatment, 6 experimental units were prepared. After the irradiation treatment, 3 experimental units (as replicates) were processed immediately while the other 3 were allowed to recover for 2 days in low white light (4 ± 1 μmol photons m^{-2} s^{-1}) before processing. Reproductive cells were resuspended from the bottom of the Petri dishes by jetting pressurized seawater from a wash bottle. The suspensions were filtered through 44 mm diameter 1.0 μm pore size Nuclepore® polycarbonate membrane (Whatman, UK). Filters were individually put into 2 ml Eppendorf tubes and frozen at –80°C. DNA was extracted using 2 % CTAB extraction buffer and quantified fluorometrically using the PicoGreen assay (Molecular Probes, Eugene, OR) and a Cary Eclipse Fluorescence Spectrophotometer (Variance Scientific Instrument, CA) (Roleda *et al*. 2004). The accumulation of cyclobutane pyrimidine dimers (CPDs) was determined following a two-step antibody assay using anti-thymine dimer H3 (Affitech, Oslo, Norway) and rabbit anti-mouse immunoglobulins (conjugated with horseradish peroxidase, DakoCytomation, Glostrup, Denmark). Chemiluminescent detection was subsequently done using ECL Western blotting detection reagent (Amersham Buckinghamshire, UK) (Roleda *et al*. 2005). Developed films (using x-ray film developer) were scanned using Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and gray scale values were quantified using Multi-Analyst (Macintosh Software for Bio-Rad's

Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 μg ml^{-1} DNA for each calibration point. The UV-irradiated DNA (45 min exposure to 2 TL 20W/12 lamps, Philips, Eindhoven, Netherlands) was previously calibrated against UV-irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the gray scales within the linear range of the film.

Statistical analysis

Data were tested for homogeneity (Levene Statistics) of variance. Corresponding transformations were made to heteroskedastic data. Photosynthetic response of each sporophyte (as random variable) to varying irradiance, exposure time (as fixed variables) and their interaction effect were tested using analyses of covariance (ANCOVA, $P < 0.05$). When interaction effect was observed, significant difference between subgroups was determined using post hoc multiple comparison test. DNA damage and repair were tested using one-way analysis of variance (ANOVA, $P < 0.05$), followed by Duncan's multiple range test (DMRT, $P < 0.05$). Statistical analyses were made using SPSS software (Chicago, IL, USA).

RESULTS

Upon immersion of fertile thallus in seawater, masses of gametangia (8- 10 μm length) were released through the ostioles of the conceptacles (Fig. 1a). Each gametangium contains eight gametes (2.5- 3.0 μm, Fig. 1b). Gamete release and fertilization were observed in all light treatments but retarded in PAB treatments. Under highest exposure

time of 8h PAB, 80- 90% of the viable gametes remained inside the gametangium (Fig. 1f).

The steep initial slope, alpha (α= 0.095, R^2= 0.98), in the photosynthesis-irradiance curve of freshly released gametes showed efficient use of photon fluence in the reproductive cells of *Ascoseira*. A minimal relative electron transport rate ($rETR_{max}$ = 5) was observed and photosynthesis was saturated already at 52 µmol photon m^{-2} s^{-1} of actinic light. The $rETR_{max}$ of *Ascoseira* was already photoinhibited above 300 µmol photon m^{-2} s^{-1} (Fig. 2).

Initial mean optimum quantum yield (F_v/F_m) of low light adapted gametes was 0.400 ± 0.06. Exposure to 1 hour of 22 µmol photon m^{-2} s^{-1} of PAR decreased the F_v/F_m by 62%. Additional 20% and 25% reduction in F_v/F_m was observed when light was supplemented with UV-A and UV-A + UV-B, respectively (Fig. 3a). Increasing exposure time to 2, 4 and 8 hours further resulted in a decrease of the F_v/F_m in all treatments indicating that propagules are low light adapted and susceptible to UVR.

After post cultivation for 2 days in dim white light, the F_v/F_m of untreated gametes (0.446 ± 0.05) increased slightly. An efficient recovery was observed under all light and exposure time treatments (Fig. 3b). Photosynthetic recovery was, however, found to be dependent on the fitness of the parental sporophyte (Table 1). Optimum quantum yields of propagules obtained from plant 1 in all treatments, except 8 hours pre-exposure to PA and PAB, recovered to the control value (Table 1). Propagules obtained from two sporophytes (designated as plant 2 and 3) were moderately fit. Photosynthetic efficiency of propagules in the other 2 sporophytes (plant 4 and 5) did not fully recover after pre-exposure to treatments longer than 2 h PA and 1 h PAB equivalent to doses higher than 6.8 x 10^4 J m^{-2} of PAR, 3.1 x 10^4 J m^{-2} of UV-A and 1.4 x 10^3 J m^{-2} of UV-B. Analysis of covariance (ANCOVA, $P < 0.01$) showed that gametes obtained from individual

sporophytes exclusively differ in their response to different treatments. Significant effects of spectral irradiance and exposure time were also observed in the optimum quantum yields of propagules after treatment exposure and after recovery (Table 2). Significant interactive effect between irradiance and exposure time was observed in the F_v/F_m of propagules after exposure but not after recovery. *Post hoc* multiple comparison test showed several significantly different subgroups in F_v/F_m after exposure treatment. Groups ranking showed that photosynthetic efficiency was lowest in 8h PAB followed by a subgroup consisting of 2h, 4h and 8h PA and 2h and 4h PAB. Photosynthetic efficiency was highest in the subgroup consisting of 1h and 2h P treatment, and followed by the subgroup consisting of 1h PA, 1h PAB, and 4h and 8h P treatments.

Formation of cyclobutane pyrimidine dimers was observed in propagules after 1 hour exposure to PAB treatment. DNA damage increased with increasing UV-B dose to a maximum of 37.55 ± 2.3 CPD Mb^{-1} after 8 hours exposure. Analysis of variance (ANOVA, $P < 0.01$) showed significant effect of UV-B dose on CPD formation. Duncan's multiple range test (DMRT, $P < 0.05$) revealed three different subsets (Fig. 4). Effective DNA repair was found after 2 days post-cultivation in low white light. No detectable CPD was observed in gametes pre-exposed to dose lower than 5.8×10^3 J m^{-2} of UV-B. The remaining DNA damage in spores pre-exposed to higher UV-B dose was minimal (0.84- 2.32 CPD Mb^{-1}).

DISCUSSION

This study shows for the first time the UVR-sensitivity of the reproductive cells of an endemic Antarctic macroalga. Propagules of *Ascoseira mirabilis* are low light adapted and strongly photoinhibited by PAR. UVR further contributes to the photoinhibition of

photosynthesis. UV-B-induces DNA damage but an effective repair mechanism is observed. The gametangium walls are assumed to offer UV-protection to gametes inside the gametangia released from the conceptacles.

Ascoseira mirabilis is the only species in the order Ascoseirales. Its growth form is reminiscent of the Laminariales while their life history follows a fucalean pattern but sexual reproduction in this species is isogamous in contrast to the Fucales (Moe & Henry 1982, Clayton 1987). In Laminariales, zoospores are directly liberated from the sorus. Release of whole sporangia is sometimes observed under unfavourable condition. In Ascoseirales, the extrusion of gametangial masses through the ostioles of their conceptacles precedes the release of gametes from the gametangia (Müller *et al*. 1990). The sustained release of gametes takes several hours until gametangia become empty. Under PAB treatment, however, most gametes were not released after 2 days from the gametangia. They were observed to remain enclosed inside the gametangium walls and look healthy and bigger in size. Relative to P- treatment, the retention of gametes inside the gametangium wall seems to be a reproductive strategy to protect sensitive cells from environmental stress. Whether viable gametes will be eventually released from the gametangia remains to be studied.

The I_k estimates (52 µmol photon m^{-2} s^{-1}) derived from $rETR_{max}$ as minimum saturating irradiance is higher compared to temperate (20 - 40 µmol photon m^{-2} s^{-1}) and Arctic (13- 18 µmol photon m^{-2} s^{-1}) Laminariales (Roleda *et al*. 2005, 2006a) and rather comparable to subtropical/warm temperate (41- 77 µmol photon m^{-2} s^{-1}) kelp species (Amsler & Neushul 1991). No study on P-I curve estimates on fucalean gametes or zygotes is available for comparison.

Differences in P, PA and PAB sensitivity of the optimum quantum yield in gametes are presumably caused by the degree of damage to PSII components or due to the

xanthophyll cycle mediated down regulation of PSII (Gevaert *et al*. 2003). The interactive effects of radiation and exposure time treatments, however, showed that the measurable effects of different doses of PAR and UVR in the reduction of photosynthetic efficiency can be similar but the mechanisms behind PAR- and UVR-induced inhibition of photosynthesis are different (Franklin *et al*. 2003). Photosynthetic performance may be additionally depressed in light treatments supplemented with UVR by possible damage to the oxidizing site and reaction center of PS II (Grzymski *et al*. 2001; Turcsányi & Vass 2002). Part of the D1/D2 heterodimer, the major structural complex within PSII, can also be degraded by UV-B (Richter *et al*. 1990). Inactivation of oxygen-evolving complex is moreover induced by blue light as well UV radiation while red light inactivates the photochemical reaction center (Ohnishi *et al*. 2005). UV-A radiation, on the other hand, was found to be damaging for PSII by decreasing the electron flow from reaction centers to plastoquinone (Grzymski *et al*. 2001) affecting electron transport both at the water oxidizing complex and the binding site of the Q_B quinine electron acceptor (Turcsányi & Vass 2002).

Exposure under comparable UV-B dose, induction and accumulation of UV-B-induced CPDs in *A. mirabilis* gametes is higher compared to zoospores of upper sublittoral Laminariales species from Helgoland (*Laminaria digitata*, Roleda *et al*. 2005) and Spitsbergen (*Saccorhiza dermatodea*, Roleda *et al*. 2006b). However, an efficient DNA damage repair mechanism was observed in gametes of *A. mirabilis*. The repair rate was relatively more efficient compared to the reproductive cells of Laminariales and Gigartinales species from the northern Hemisphere (Roleda *et al*. 2004, 2005, 2006b). UV-B-induced DNA damage is generally repaired through photo-reactivating light (van de Poll *et al*. 2002). Other repair mechanisms include nucleotide and base excision repair, and recombination repair (Roy 2000).

Impact of UVR can be counterbalanced by protection strategies such as avoidance, screening, photochemical quenching and repair (Vincent & Neale 2000). The occurrence of phenolic compounds in reproductive cells of brown algae plays an important role in UV protection. In zoospores of Laminariales, increase in number and size of phlorotannin-containing physodes after exposure to UVR was invoked to contribute protection against cellular damage and enhanced germination rate (Wiencke *et al*. 2004). Induction of phlorotannin synthesis is, however, species specific and is not only inducible by UV-B radiation but also by PAR and UV-A (Roleda *et al*. 2006b, 2006c). Mass release of spores can screen each other serving as 'biofilter' and protect the lower layer of spores from excessive radiation (Roleda *et al*. 2006c).

*A*scoseira *mirabilis* is endemic to Antarctica and has a distinct life history and reproductive biology apparently without any close affinities to any extant Phaeophyceae (Clayton 1987). In this species, gametes are enclosed in an extracellular sheath, the gametangium wall, which may function as UV-filter. Extracellular sheaths covering unicellular alga and cyanobacteria were found to contain scytonemin and sporopollenin-like material that offer protection against UV-C and UV-B radiation respectively (Morrill & Loeblich 1981; Dillon & Castenholz 1999; Hagen *et al*., 2002). The UV-absorbing phlorotannins are common compounds in brown algal cell walls (Schoenwaelder & Clayton 1999). Whether this compound is present in the gametangium wall remain to be studied which may, if present, give some protection against UV radiation to the gametes.

Gametangial masses are first released from the conceptacle before the release of gametes, a strategy different from Laminariales where zoospores are immediately released from the sporangium-containing sorus when environmental condition is optimal. Furthermore, gametes of *Ascoseira* were retained inside the gametangium in

PAB-treated samples compared to P-treated samples where syngamy followed immediately after gamete release. The Antarctic marine environment is characterized by extreme physical characteristics, such as low sea and air temperatures, annual extremes in light regime, wind speeds, disturbance and isolation (Peck *et al*. 2006) as well as naturally lower net spring time ozone levels (Fahey 2003). These environmental constraints might have exerted an evolutionary pressure to development a unique life history and reproductive biology in *A. mirabilis* compared to other Phaeophyceae.

Despite the artificial laboratory irradiance in the absence of high PAR, the UV irradiances comparable to that encountered in the field was observed to have a negative impact on the ecophysiology and biochemistry of *A*. *mirabilis*. Future study on the kinetics of photosynthetic recovery of reproductive cells (e.g. Roleda *et al*. 2006a) as well as the presence of UV-screening compounds (e.g. Wiencke *et al*. 2004; Roleda *et al*. 2006c) and *in situ* germination capacity (e.g. Wiencke *et al*. 2006b) could further enhance our understanding on the adaptive characteristics of this endemic Antarctic macroalga subjected to climate change due to depletion of stratospheric ozone layer and accumulation of greenhouse gases (Braathen 2005; GAW Report 165). More studies on other Antarctic species with different zonation pattern are also necessary to determine how widespread the adaptive UV tolerance demonstrated here is.

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Tables

Table 1. Individual sporophyte-specific vitality in *Ascoseira mirabilis* impacts the photosynthetic recovery (optimum quantum yield, F_v/F_m) of their respective reproductive cells after exposure to different spectral irradiance. Gametes were released from five individual sporophytes designated as source plant. Photosynthetic recovery of gametes and embryos were initiated in dim white light of 4 µmol photon m^{-2} s^{-1} after treatment.

	Exposure treatments (h)											
	PAR				PAR+UV-A				PAR+UV-A+UV-B			
	1	2	4	8	1	2	4	8	1	2	4	8
Source plant	Optimum quantum yield (F_v/F_m) of gametes, 48 h recovery after exposure											
1	0.558	0.536	0.559	0.521	0.549	0.542	0.533	0.487	0.542	0.534	0.519	0.461
2	0.459	0.468	0.459	0.411	0.481	0.442	0.426	0.353	0.476	0.451	0.400	0.320
3	0.405	0.411	0.481	0.374	0.428	0.395	0.418	0.295	0.426	0.413	0.361	0.290
4	0.378	0.354	0.246	0.152	0.337	0.285	0.204	0.073	0.334	0.246	0.157	0.040
5	0.394	0.387	0.266	0.170	0.368	0.309	0.197	0.058	0.367	0.266	0.152	0.034

Mean of untreated control after 48 h post-cultivation is 0.446 ± 0.054

Table 2. Analysis of covariance and significance values for the photosynthetic efficiency of *Ascoseira mirabilis* gametes exposed to different irradiance treatments and after recovery (* significant; ns, not significant)

Dependent variables	Independent variables		df	*F*- value	*P*- value
	Covariate	Fixed factors			
F_v/F_m	Sporophyte		1	6.858	0.012*
After exposure		Spectral irradiance (A)	2	188.091	<0.001*
		Exposure time (B)	3	119.250	<0.001*
		A * B	6	3.858	0.003*
F_v/F_m	Sporophyte		1	192.612	<0.001*
After recovery		Spectral irradiance (A)	2	6.994	0.002*
		Exposure time (B)	3	22.334	<0.001*
		A * B	6	0.846	0.541ns

Figure Legends

Figure 1. *Ascoseira mirabilis* sporophyte with conceptacles containing gametangia (a), releasing masses of gametangia containing 8 gametes (b) upon re-immersion in seawater. Gamete release and syngamy were observed in controls (c) and in 8 hours P- and PA-exposed gametangial suspensions (d and e respectively). Viable gametes remained inside the gametangial complex among 8 hours PAB-exposed samples (f). Micrographs (scale = 5 μm) were taken after exposure treatments and 2 days recovery in low white light.

Figure 2. Photosynthetic performance (P-I curve) of gametangial masses and gametes from *Ascoseira mirabilis* (n=3) immediately after release from the conceptacle. PFR is the respective photon fluence rate of actinic white light and rETR is the relative transport rate. Saturating irradiance (I_k) is estimated as the point at which the initial slope (α= 0.095, R^2= 0.98) crosses the maximum photosynthesis ($rETR_{max}$) using a hyperbolic tangent model.

Figure 3. Mean optimum quantum yield (F_v/F_m) of propagules (gametangia, gametes and zygotes) during treatment (a) of photosynthetically active radiation, PAR = P; PAR + UV-A = PA; PA + UV-B = PAB and exposure time. Corresponding photosynthetic recovery (b) after 48 h post culture in low white light (4 μmol photon m^{-2} s^{-1}). Control (= 0) is without treatment and continuously maintained at 10 μmol photon m^{-2} s^{-1}. Vertical bars are standard deviations (SD), n=5. Corresponding statistical analysis is presented in Table 2.

Figure 4. UV-B-induced DNA damage (cyclobutane pyrimidine dimers per million nucleotides) in propagules of *Ascoseira mirabilis* after exposure to varying dose of UV-B radiation and remaining DNA damage after 2 days recovery in low white light (4 μmol photon m^{-2} s^{-1}). Vertical bars are standard deviations (SD), n=3. Analysis of variance (ANOVA, $p < 0.01$) shows significant difference between treatments. Letters and number on graph show result of Duncan multiple range's test (DMRT, $p < 0.05$); different letters and numbers refer to significant differences between mean values.

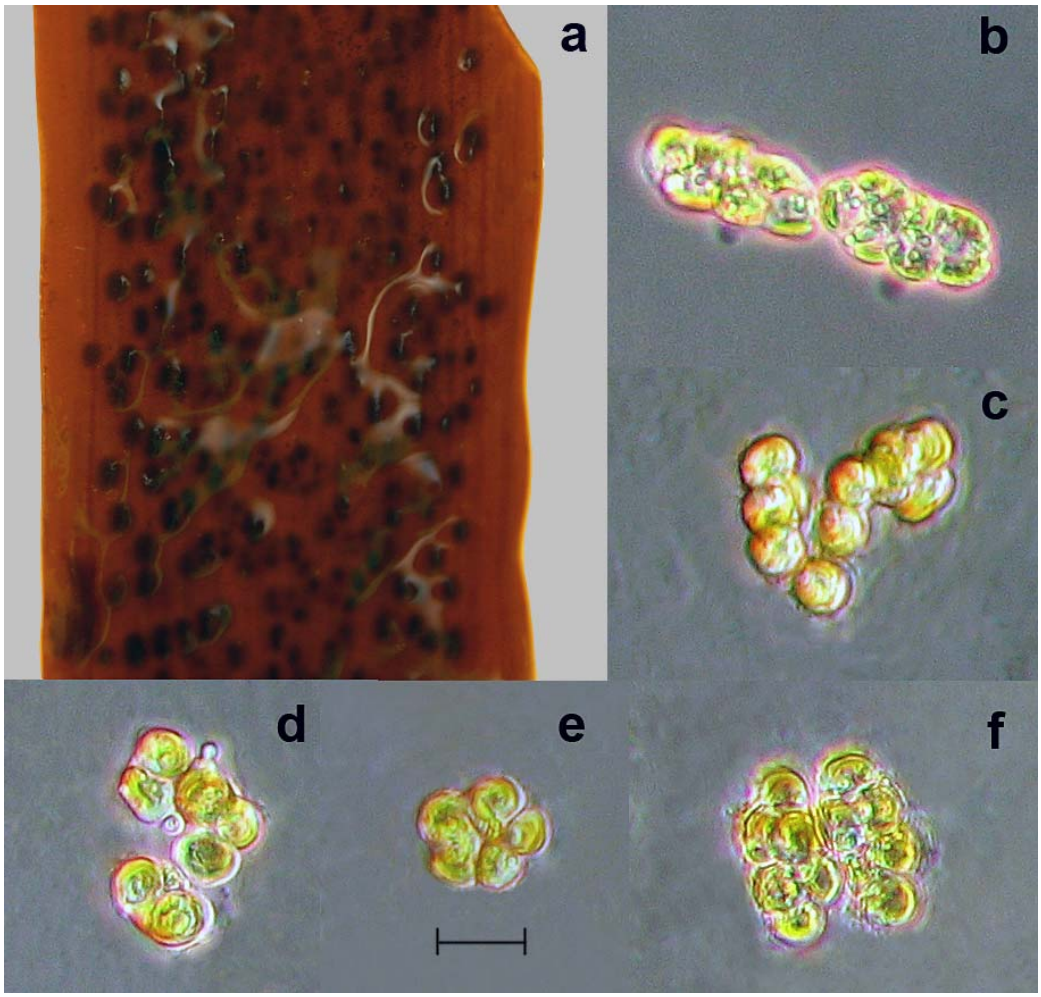

Figure 1.

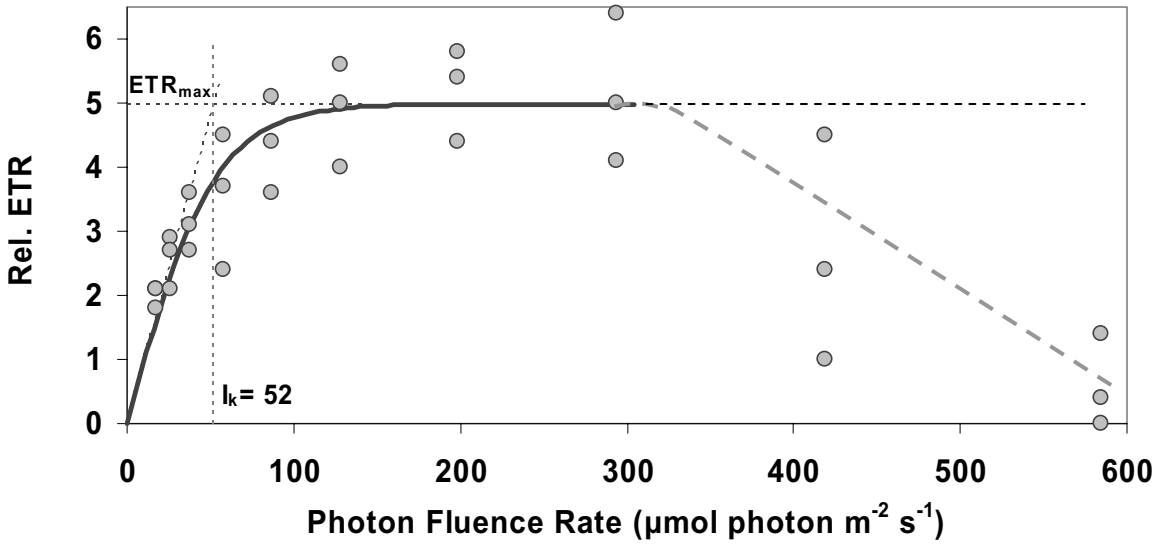
Rel. ETR
ETRmax
Ik= 52
0
1
2
3
4
5
6
0
100
200
300
400
500
600
Photon Fluence Rate (µmol photon m-2 s-1)

Figure 2.

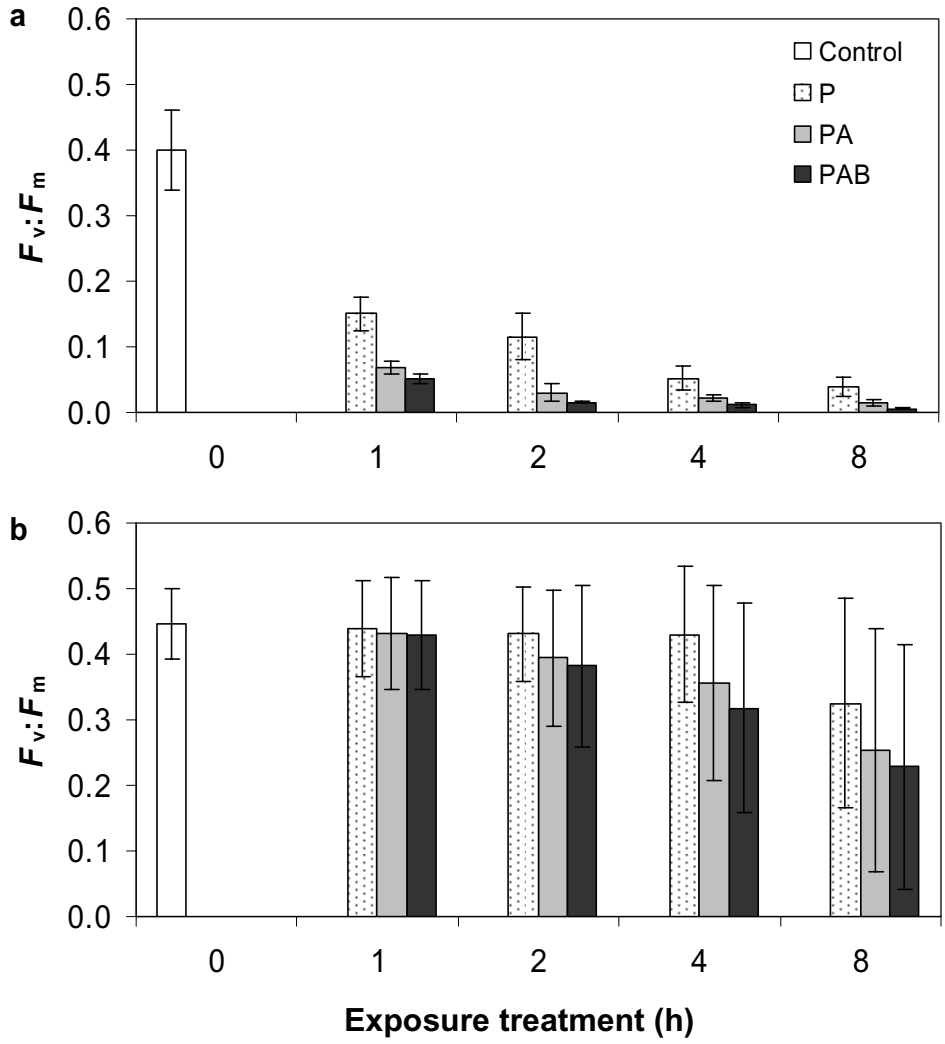
a
b
Control
P
PA
PAB
$F_v:F_m$
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
1
2
4
8
Exposure treatment (h)

Figure 3.

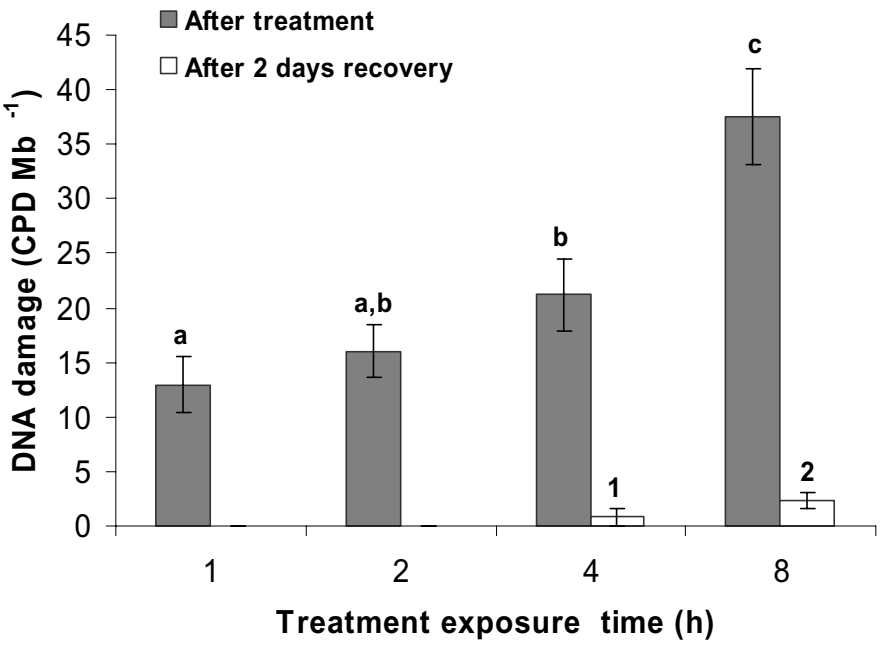
After treatment
After 2 days recovery
DNA damage (CPD Mb -1)
45
40
35
30
25
20
15
10
5
0
a
a,b
b
c
1
2
1
2
4
8
Treatment exposure time (h)

Figure 4.

Physiological and biochemical responses of Antarctic *Iridaea cordata* (Rhodophyta) tetraspores exposed to ultraviolet radiation

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Abstract

Anthropogenic ozone depletion is highest during spring and summer in Antarctica, coinciding with the seasonal reproduction of most macroalgae. Propagules are the life-stage of an alga most susceptible to environmental perturbations. In our study fertile *Iridaea cordata* Turner (Bory) (Rhodophyta) from the intertidal and the subtidal were collected in the field. Freshly released tetraspores were exposed to PAR (photosynthetically active radiation, P, 400-700 nm), PAR + UV-A (PA, 320-700 nm) and PAR + UV-A + UV-B (PAB, 280-700 nm) radiation in the laboratory. During 1, 2, 4 and 8 h of exposure and after 48 h of recovery, photosynthetic efficiency was measured. Mycosporine-like amino acids (MAAs) concentrations and DNA damage were determined in intertidal tetraspores. Saturating irradiance of freshly released tetraspores was higher in intertidal (57 µmol photons m^{-2} s^{-1}) and lower in subtidal *I. cordata* (31 µmol photons m^{-2} s^{-1}). Photosynthetic capacity of intertidal *I. cordata* (ETR_{max} = 6.9) was significantly higher compared to the subtidal ones (ETR_{max} = 2.1). Exposure to 22 µmol photons m^{-2} s^{-1} PAR significantly reduced photosynthetic efficiency of tetraspores isolated from algae collected from different tidal zone. UV radiation (UVR) further decreased the photosynthetic efficiencies of tetraspores. Spores of intertidal tetrasporophytes were, however, able to completely recover their photosynthetic efficiency 48 h after exposure to PAR+UV-A+UV-B in contrast to the ones from the subtidal. DNA damage was minimal and DNA lesions were repaired during 48 h post-cultivation under dim white light. Concentrations of the MAAs shinorine and palythine were higher in tetraspores treated with UVR than in spores only exposed to PAR. Generally, tetraspores from the intertidal had a better photoprotection compared to its subtidal counterpart and showed an effective DNA repair mechanism and shielding due to UV absorbing compounds. Adaptation to the extreme environmental condition in the intertidal might be responsible for the broader vertical distribution pattern (from the eulittoral to sublittoral zone) of this species compared to other Gigartinales only growing in the subtidal.

Key words: Antarctica, DNA damage, MAAs, photosynthetic efficiency, P-I curve, tetraspores, UV radiation

Introduction

Iridaea cordata Turner (Bory) is a cold adapted red algae occurring in West and East Antarctica, the Ross Sea and in cold waters of the sub-Antarctic islands. *Iridaea* is growing from the upper intertidal to depth of 30 m. It is a pseudoperennial species with a triphasic life cycle, reproducing and growing in summer, blades can reach up to 1 m in diameter (Wiencke and Clayton 2002). In the studied area, on King George Island, *I. cordata* is commonly found both, in the eulittoral and in the sublittoral zone.

The stratospheric ozone depletion over the Antarctic (WMO 2003) and the consequent increase of ultraviolet-B radiation (UV-B, 280-320 nm) makes it necessary to study the impact of UV radiation (UVR, 280-400 nm) on important primary producers in Antarctica. Despite efforts to minimize the anthropogenic impact on ozone destruction, little improvement is expected for total column ozone in the Antarctic for the next several decades and it is still uncertain if ozone levels will ever recover to pre-1980s values (Weatherhead and Andersen 2006). Consequently, organisms are and will be exposed to elevated UV-B radiation. In the study area, UVR can further penetrate to considerable depth into the water column (UV-A 19 m and UV-B 16 m, 1% of the surface values, Zacher et al. submitted), and can thereby affect also subtidal organisms.

UVR effects on macroalgae were shown to be species-specific. The negative effects to UV-exposure on cellular level include e.g. photoinhibition and/or photodamage (Hanelt et al. 1997), protein breakdown (Lao and Glazer 1996), the production of reactive oxygen species (Rijstenbil et al. 2000) as well as damage to the DNA (van de Poll et al. 2001; 2002; Roleda et al. 2004a) and other biomolecules through the direct absorption of UVR (Vass 1997). These impacts can result in lower growth rates, tissue necrosis and morphological deformation (Roleda et al. 2004b, 2006a, 2006b) and affect negatively diversity and species richness of algal communities (Lotze et al. 2002; Dobretsov et al. 2005; Zacher et al. 2006). On the other hand macroalgae have developed a number of different recovery strategies, such as avoidance (growing in deeper waters), DNA repair mechanisms or the production of sunscreen compounds (reviewed in Bischof et al. 2006). Some red algae can produce mycosporine-like amino acids (MAAs) small, colorless, water-soluble molecules with a maximal absorbance of the wavelength between 309 and 360 nm (reviewed in Karentz 2001). Adult *I. cordata*

are able to synthesize the MAAs shinorine and palythine and their concentrations were found to decrease with increasing depths (Hoyer et al. 2001). Little is know, however, about MAA concentrations in young developmental stages e.g. tetraspores.

Especially these unicellular propagules of macroalgae were shown to be the life-stage most susceptible to anthropogenic stresses, e.g. increased UVR (Coelho et al. 2000; Roleda et al. 2004a; 2005; Wiencke et al. 2006). The survival of the early stages of marine macroalgae is therefore critical to the successful establishment of the benthic algal population (Vadas Sr. et al. 1992). During their planktonic phase they can be exposed to particularly high solar irradiance within the water column which might affect their survival negatively. Despite this knowledge, most UVR studies on the physiology of algae have been carried out on the adult macrothalli. Some studies exist on the UVR impact on spores of Arctic and temperate Laminariales and Gigartinales, showing that their sensitivity is related to their depth distribution and, hence influencing recruitment of the species in the eulittoral zone (Roleda et al. 2004a; 2005; Wiencke et al. 2006). Only recently few studies on the impact of UVR on reproductive cells of Antarctic macroalgae have been published indicating good recovery and repair mechanisms in propagules from the intertidal (Zacher et al. 2006) and upper subtidal (Roleda et al. 2007) with UVR effects being related to the zonation pattern of the macrothalli. Strongest impairment of the propagules of e.g. photosynthesis was found in species from the subtidal in comparison with intertidal species. However, little is still known about the reaction to UVR of the same species growing at different water depth.

In laboratory experiments tetraspores of intertidal and subtidal *Iridaea cordata* were exposed to different light treatments to measure photosynthetic performance, cell structure, MAAs concentrations and DNA damage. The ability of these early developmental stages to recover from UV-induced damage was also studied. *Iridaea* inhibits a broad vertical zone therefore this species need to have the ability to acclimate to a variety of stress factors from e.g. excessive light to low light conditions. We hypothesize that the possibility to acclimate photosynthesis to the different radiations and further protective mechanisms of intertidal species against high UVR as an important pre-requisite for the survival of this species in the inter-and subtidal zone and should be greater than in reproductive cells from species exclusively growing in the subtidal.

Material and Methods

Algal material. Fertile specimen of the red alga *Iridaea cordata* were collected in October 2004 in the upper intertidal (Peñón Uno, King George Island, South Shetland Islands, Antarctica, 62°14.80´S, 58°41.26´W) and in March 2005 in the subtidal at around 4 m depth (Peñón de Pesca, 62°14.41´S, 58°42.91´W). After collection the specimen were brought immediately to the nearby laboratory and put into filtered seawater (2°C under low light conditions) until further processing.

Spore release. Numerous individuals were cleaned with tissue paper, divided randomly in 5 replicates and prepared for spore release in a temperature-controlled room (2 ± 1.5°C). The individuals were cut into pieces and put into Petri dishes with seawater for collection of the tetraspores (mean diameter 20 μm ± 2.4). Spore suspensions were adjusted to 4 x 10^3 to 1 x 10^4 spores ml^{-1} after counting to obtain the desired fluorescence signal for photosynthetic measurements (intertidal and subtidal, respectively).

Experimental treatments. Light was provided by white fluorescent lamps (Osram, L65 Watt/25S, Germany), emitting background photosynthetically active radiation (PAR) of 400 to 700 nm and UV lamps (Q-Panel UV-A-340, 40 Watt, Cleveland, USA), emitting a spectrum qualitatively similar to solar radiation in the range of 295 to 340 nm. Three kinds of filter foils were used to cut off different wavelength ranges from the spectrum emitted by the fluorescent lamps: (i) Ultraphan transparent (Digefra GmbH, Germany), (ii) Folanorm 320 (Folex GmbH, Germany), and (iii) Ultraphan URUV farblos, corresponding to the PAR+UV-A+UV-B (PAB, 280 to 700 nm), PAR+UV-A (PA, 320 to 700 nm) and PAR (P, 400 to 700 nm) treatments, respectively.

Irradiance measurements. Irradiation in the laboratory was measured below the cut-off filters using a Solar Light PMA 2100 radiometer equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar light, Philadelphia, USA). PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1400 datalogger (Lincoln, USA).
UVR below the cut-off filters was 4.34 W m^{-2} UV-A and 0.40 W m^{-2} UV-B and PAR was 22 μmol photons m^{-2} s^{-1} (~ 4.73 W m^{-2}).

Spore photosynthesis. From the different spore suspensions, 5 ml were put into each 35 x 10 mm cell culture dish (n = 5). To evaluate the effect of different radiation and exposure time treatments, tetraspores from the intertidal and subtidal were exposed to the three radiation conditions for 1, 2, 4 and 8 h at 2 ± 1.5°C. After measurements, the culture dishes were exposed for 2 d under dim white light (4 µmol photons m^{-2} s^{-1}) to recover.

Photosynthetic efficiency of reproductive cells measured as variable fluorescence of photosystem II (PSII), was determined using a Water Pulse Amplitude Modulation fluorometer (Water-PAM) connected to a PC with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). Immediately after adjustment of cell density (not exceeding 1 hour after spore release), spore suspension was filled into 5 ml Quartz cuvettes and the optimum quantum yield (F_v/F_m) was measured after 3 min dark adaptation to determine initial photosynthetic efficiency at time zero (n = 5) as described by Roleda et al. (2006c), designated as control. After that, the controls were maintained under dim white light (4 µmol photons m^{-2} s^{-1}) for 2 d before the final measurement. Photosynthesis (in terms of relative electron transport rate, rETR = PFR x $\Delta F/F_{m'}$) versus irradiance curves (P-I curve) were also measured in the time zero control (n = 3, chosen at random from the 5 replicates) as described by Roleda et al. (2006c). The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate P-I curve parameters described as:

$rETR = rETR_{max} * \tan h (\alpha * I_{PAR} * rETR_{max}^{-1})$

where $rETR_{max}$ is the maximum relative electron transport rate, tan h is the hyperbolic tangent function, α is the electron transport efficiency and I is the photon fluence rate of PAR. The saturation irradiance for electron transport (I_k) was calculated as the intercept between α and the ETR_{max} values. Curve fit was calculated with the Solver module of MS-Excel using the least square method comparing differences between measured and calculated data.

Light microscopy. Cell structure of intertidal tetraspores was observed under light microscope (Zeiss Axiolab, Germany). Gross structural morphology was observed after exposure to different light treatments and 2 days post cultivation under dim white light. Microscopic pictures were taken using a digital camera (Canon A70, Switzerland).

Spore DNA damage and repair. DNA damage and subsequent repair of DNA lesions were determined in tetraspores of intertidal *I. cordata* after 1, 2, 4 and 8 h exposure to PAR+UV-A+UV-B. From the working spore suspension, 40 ml was used for each experimental unit. For each treatment, six experimental units were prepared. After the irradiation treatment, three experimental units (as replicates) were processed immediately while the other three were allowed to recover for 2 d in low white light before processing. The spore samples were filtered through 44 mm diameter 1.0 to 2.0 μm pore size Nuclepore® polycarbonate membrane filters (Whatman, UK) and frozen at –80°C in 2 ml Eppendorf tubes for further DNA extraction and analysis of CPDs.

DNA extraction. DNA was isolated following the CTAB extraction procedure (van de Poll et al. 2001). After DNA extraction, the pellet was dissolved in 0.2 ml TE buffer (10 mM Tris, 1mM EDTA, pH 8.0), treated with RNAase (5 μl 10 mg ml–1, 30 min, 37°C; Sigma, MO, USA) and stored at –20°C. The DNA concentration was quantified fluorometrically using PicoGreen assay (Molecular Probes, Eugene, OR, USA) and a Cary Eclipse Fluorescence spectrophotometer (Variance Scientific Instrument, CA, USA). A dilution series with a known amount of DNA (Serva, Heidelberg, Germany) was included for calibration purposes.

Assay for CPD detection. The immunoassay for CPDs was modified after Vink et al. (1994) and van de Poll et al. (2001). Heat denatured samples containing 50 ng DNA were transferred to a nitrocellulose membrane (Protran BA 79, pore size 0.1 μm, Schleicher & Schuell, Keene, NH, USA) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell). After a two step anti-body assay, the membrane was treated with ECL Western blotting detection reagent (Amersham, UK) and sealed in a transparent vinyl plastic folder (Leitz, Stuttgart, Germany). This was subsequently exposed to photosensitive ECL films (Amersham) for different exposure times. The films were developed using X-ray film developer. Developed films were scanned using Biorad imaging densitometer (Model GS-700, Bio-Rad Laboratories, USA) and grey-scale values were quantified using Multi-Analyst (Macintosh Software for Bio- Rad's Image Analysis Systems). A calibration series of UV-irradiated calf thymus DNA (Serva) supplemented with unexposed DNA was included giving 1 μg ml^{-1} DNA for each calibration point. The UV-irradiated DNA was previously calibrated against UV-

irradiated Hela DNA with known amounts of CPDs (kindly provided by A. Vink). CPDs were quantified by comparing the grey scales within the linear range of the film.

MAAs. MAAs concentration was determined in tetraspores of intertidal *I. cordata* in the initials (controls) and after 8 h exposure to PAR+UV-A+UV-B. Samples were filtered through a GF/F filter. The MAAs were extracted in 2 ml 25% MeOH in a water bath (45°C) for 2 hours. After extraction, the spore solution was centrifuged and the supernatant was analyzed. The HPLC analysis continued as described in Karsten et al. (1998); it consisted of an isocratic reverse-phased analysis using a C8 column (4 µm, Jones Genesis, 250 × 4.6 mm) equipped with a C8 guard column (SecurityGuard Phenomenex, 4 × 3 mm). The mobile phase was 25% aqueous methanol (v/v) plus 0.1% acetic acid (v/v). The flow rate was 0.7 ml min^{-1}. The detector was an absorbance diode-array detector (Spectraphysics UV6000LP), and the peaks were identified by on-line recording of absorbance spectra (280 – 400 nm). Due to the lack of commercially available standards, the MAAs concentrations are expressed as percentage relative to the control treatment.

Data analysis. A one-way ANOVA was used to test for the effects of UVR on photosynthetic efficiency; MAAs and DNA damage ($p = 0.05$). Prior to data analysis, data sets were tested for homogeneity of variances (Cochran´s test) and in case of heterogeneity data were analyzed using the non-parametric Kruskal-Wallis test. Post-hoc comparisons were performed with Newman-Keuls test. Statistical analysis were done using Statistica ™ 6.0 software package.

Results

Photosynthesis - Irradiance (P-I) curves

The P-I curves in Fig. 1 show the photosynthetic performance of *Iridaea cordata* tetraspores collected in the intertidal and the subtidal. The values for α (an index of light-harvesting system efficiency) varied between 0.121 and 0.068 for the intertidal and the subtidal spores, respectively (Fig. 1a, b). The saturating irradiance (I_k) was higher for tetraspores coming from the intertidal than from the subtidal (57 and 31 µmol photons m^{-2} s^{-1}, Fig. 1a, b). Photosynthetic capacity ($rETR_{max}$) was higher in intertidal

spores ($rETR_{max}$ = 6.9) in comparison with the subtidal ones ($rETR_{max}$ = 2.1). At photon fluence rates around 200 µmol photons m^{-2} s^{-1}, the $rETR_{max}$ decreased in intertidal and subtidal tetraspores (Fig. 1) indicating photoinhibition. The decrease was faster in the subtidal tetraspores than in the ones form the intertidal.

Photosynthetic efficiency after short term exposure to UV radiation

Initial measurements of the controls showed that *I. cordata* tetraspores from the intertidal and the subtidal had a similar mean optimum quantum yield (F_v/F_m intertidal = 0.476 ± 0.04; subtidal = 0.445 ± 0.04, Fig. 2a, b). The changes in optimum quantum yield after treatments are shown in Fig. 2. After exposure to 1, 2, 4 and 8 h PAR (P; 22 µmol photons m^{-2} s^{-1}) the optimum quantum yield (F_v/F_m) of both, *I. cordata* tetraspores from the intertidal and the subtidal decreased steadily (Fig. 2a, b). The decline in tetraspores from the subtidal is, however, higher than in spores from the intertidal (e.g. after 8 h in the intertidal tetraspores 40% and in the subtidal tetraspores 28% of the control measurements).

In both experiments treatment effects due to UV exposure were similar. PAR supplemented with UV-A (PA treatment) decreased photosynthetic efficiency significantly compared to the P treatment in both experiments during exposure. Additional UV-B (PAB treatment) generally did not reveal in a further significant decrease of optimum quantum yield (only after 1 h of exposure, Fig. 2a, b). Increasing exposure time to 2, 4 and 8 h further decreased the F_v/F_m in all treatments.

However, tetraspores from *Iridaea cordata* collected in the intertidal showed a complete recovery of photosynthesis after 2 d under dim white light and no differences between treatments were detected (Fig. 2c). This is in contrast to the tetraspores from the subtidal *I. cordata* which generally showed no complete recovery of the spores exposed to UV-B after 2 d under dim white light (64 and 50% of control for the 4 and 8 h treatment, respectively; Fig. 2 d).

In *I. cordata* from the intertidal F_v/F_m of the controls did not change significantly during the experimental time, in *I. cordata* from the subtidal F_v/F_m increased slightly from

0.445 ± 0.04 (initials) to 0.523 ± 0.02 (mean ± SD) after 2 d under dim white light, respectively.

Cell structure in intertidal tetraspores

No visible negative impact on cell structure of intertidal tetraspores was observed after UVR exposure and 2 days recovery (Fig. 3). Minimal number of dead cells were observed (< 5%). Tetraspores germinated even after 8h exposure to PAR + UV-A (PA, Fig. 3d) and looked vital after exposure to PAB (Fig. 3e).

DNA damage and repair

Minimal DNA damage (measured as CPD concentrations per million nucleotides, CPD Mbp^{-1}) was measured after exposure to PAB treatment (Fig. 4). The amount of CPDs increases with increasing UV-B dose. After 2 d of recovery under dim white light, effective DNA damage repair was observed with 37-60% repair of DNA lesions. CPD repair, however, was significantly lower after 8 h exposure to PAB than in the shorter exposure times.

Mycosporine-like amino acids (MAAs)

Two peaks corresponding to different MAAs were observed in tetraspores of *Iridaea cordata*. They were identified as shinorine (λ_{max} = 334 nm) and palythine (λ_{max} = 320 nm). Higher concentrations of palythine were measured compared to shinorine (Fig. 5). Relative higher amounts of different MAAs were detected in spores immediately after release (control) from the macrothalli of *Iridaea* collected in the field in comparison with the treatments after 8 h exposure. Under experimental laboratory irradiance condition, MAAs concentrations were lower compared to the control. Among experimentally treated spores, total MAAs are significantly higher under the PAB treatment in comparison with the PA and the P treatments.

Discussion

Former results generally showed a species-specific reaction of propagules to UVR (Wiencke et al. 2006; Roleda et al. 2006c; Zacher et al. 2006). Our study showed that furthermore spores from the same species but growing at different water depth can react

differently to UVR. In Antarctica this is of particular interest because the UV-B penetration into the water column can be quite high and reaches almost the lower depth distribution of the studied species.

Reproductive cells of various algal species were shown to be low light adapted with I_k values ranging between 13 (zoospores from Arctic Laminariales, Roleda et al. 2005) to 77 μmol m^{-2} s^{-1} (Amsler and Neushul 1991; Zacher et al. 2006) characterizing *Iridaea cordata* tetraspores as well as shade adapted. These results are in agreement with measured I_k values for the adult thalli (Weykam et al. 1996).

P-I curve measurements show that the saturating irradiance I_k of tetraspores from the intertidal were higher than from the subtidal spores, hence, photosynthesis became saturated at a higher irradiance in the intertidal spores, indicating that subtidal spores were more photoinhibited. Photosynthetic performance ($rETR_{max}$) and photosynthetic efficiency (α) are lower in spores from the subtidal. Lower activity of Calvin cycle enzymes or a smaller number of active reaction centres would cause a lower $rETR_{max}$, whereas a lower slope (α) indicates a smaller light harvesting complex (LHC) or less efficient energy transfer from the antenna to the reaction centre (Prézelin 1981; Raven and Geider 2003). The former is indicative for a shade acclimation whereas the later is rather indicative for high light acclimation. The spores from the subtidal tend to be shade adapted, however, the lower slope of the P-I curve also means that light is less efficiently used for the electron transport as in the spores from the intertidal, which is to a certain degree contradictive. It is needed to point out that the time of collection of intertidal and subtidal algae was different. Intertidal specimens were collected in austral spring (October) and subtidal algae in late summer (March). In October irradiance suddenly rises after sea ice break-up and algae have to acclimate to this new light condition, whereas in March, when subtidal *I. cordata* was sampled an acclimation to higher PAR levels could have taken place during summer. PAR values at the sampling depth (around 4 m) in the study area could reach values between 40 and 270 μmol m^{-2} s^{-1}.

Macrothalli of *I. cordata* have an optimal quantum yield of about 0.622 ± 0.09 (n = 5) typically for red algae. This means that the lower yield of the tetraspores (F_v/F_m = 0.448) might be caused by a still incomplete developed photosynthetic apparatus. If

macrothalli were cultured at 10, respectively, 100 µmol m^{-2} s^{-1} no difference in the yield or in the sensitivity for photoinhibition occurred (Hanelt et al. 1997). A fast recovery after photoinhibition under high light conditions (2h, 500 µmol m^{-2} s^{-1} PAR) showed that macrothalli of *I. cordata* are well adapted to cope with PAR conditions of the intertidal/upper subtidal zone (Hanelt et al. 1997).

Tetraspores exposed to PAR showed a decline in optimum quantum yield with a higher inhibition in tetraspores from the subtidal. UVR exhibited an additional effect in the reduction of F_v/F_m. The measurable effects of both PAR and UVR in the reduction of photosynthetic efficiency are similar but the mechanisms behind PAR and UVR induced inhibition of photosynthesis are different (Hanelt et al. 2003). Reduction of photosynthetic efficiency while exposed to high PAR is a protective mechanism to dissipate energy absorbed by PSII as heat via the xanthophylls cycle to avoid photoinactivation (chronic photoinhibition; Franklin and Forster 1997; Hanelt et al. 2003). Specimen from the sublittoral were shown to have a lower ability to downregulate their photosynthesis through photoprotection (Franklin et al. 2003). An inactivation due to impairment of the D1 protein of the PSII reaction center can cause photoinactivation (Aro et al. 1993). UVR exhibits additional effects on photosynthesis causing a direct molecular damage due to the absorption by biomolecules (Vass 1997). Depression of photosynthetic performance by UVR is e.g. implicated to the damage of the oxidizing site of the reaction center of photosystem II (Franklin et al. 2003).

UV effects on specimens of both tidal zones were similar. While the reduction of photosynthetic efficiency was mainly cause by UV-A, differences, however, were measured in the recovery phase were tetraspores from the subtidal did not recover completely after 2 d under dim white light after exposure to PAB in contrast to spores from the intertidal. The impact of the UV-B part of the spectrum lies therefore more in the incomplete recovery in tetraspores from the subtidal. Intertidal tetraspores process apparently better recovery and/or protective mechanisms than the subtidal ones. In contrast to algae from the subtidal which are exposed to a more stable radiation regime *Iridaea cordata* form the upper intertidal is frequently exposed to high PAR and UVR. Maximal values of PAR, UV-A and UV-B in the air reached e.g. 2130 µmol m^{-2} s^{-1}, 53 W m^{-2} and 2.56 W m^{-2}, whereas in the subtidal at 4 m depth maximal values measured were 270 µmol m^{-2} s^{-1}, 7.9 W m^{-2} and 0.24 W m^{-2} during the study period, respectively.

Different acclimation and repair mechanisms can be observed in macroalgae. Species most tolerant to UV stress were coming from shallow waters (Larkum and Wood 1993; Bischof et al. 1998). Photosynthesis is a dynamic process and excessively absorbed energy, which is not utilized in photochemistry, can be converted into harmless thermal radiation until a certain point (Hanelt 1996). Recovery after photodamage of the D1 protein of photosystem II is reflected by the new synthesis of this protein (Bischof et al. 1998). In our study it seems that in the tetraspores from the subtidal the UV-B part of the spectrum has caused a photodamage which could not be repaired within the recovery phase in contrast to the spores from the intertidal. These points to photoinactivation and photorepair of PS II which is especially characterized by a slow kinetics of the recovery process (Hanelt 1998).

DNA damage was generally low in relation to Laminariales zoospores from other climatic regions and Gigartinales from the North Sea (Roleda et al. 2004a; 2005). The minimal CPD formation indicates effective shielding of the DNA and/or fast repair mechanism in *Iridaea* tetraspores. Similar low or no DNA damage was found in spores from the red algae *P. endiviifolium* and other propagules from eulittoral species from the study area (Zacher et al. 2006). According to the few studies existing on DNA damages of macroalgal propagules, red algae spores seem to be less affected by DNA damage than e.g. kelp spores (e.g. Roleda et al. 2004a; 2005; Zacher et al. 2006). This could be due to a bigger cell size of the tetra- and carpospores in relation to the Laminariales zoospores as observed before (Swanson and Druehl 2000). The ability of the propagules to cope with UV-B induced DNA damage as well as the capacity to repair seems to be related to the macroalgal zonation on the coastline. If not repaired, DNA lesions can disrupt metabolism, cell division and impair growth and germination.

DNA damage can be repaired through photolyase enzyme (light-dependent), nucleotide excision and recombination repair (light-independent; van de Poll et al. 2002). The small amount of DNA damage in *Iridaea* tetraspores observed after the recovery phase might therefore be related to e.g. photolyase activity. However, whether the tested tetraspores have a high photolyase activity remains to be studied. A protective mechanisms is further shielding due to UV absorbing compounds.

In our experiment tetraspores of *I. cordata* were shown to have MAAs which might help them to survive the planktonic phase where they can be exposed to high PAR and UVR. In adult thalli from *I. cordata* two different MAAs; shinorine and palythine, were found in high concentrations. Shinorine was much more abundant than palythine (Hoyer et al. 2001; Karsten et al. in press), in contrast to the spore experiment. The algae were able to synthesize and accumulate MAAs under the PAB treatment in relation to the control treatment. Also in our study a higher amount of MAAs under the PAB treatment was observed.

The higher amount of total MAAs in the control might be attributed to the higher *in situ* incident light, when macrothalli were collected in the field compared to the artificial laboratory irradiance. Under natural solar radiation, the UV-A wavelengths exhibits the highest quantum efficiency on the synthesis of shinorine and and palythine (Kräbs et al. 2002). The high UV-B:UV-A:PAR ratio of the light treatment in the laboratory could be responsible for the decrease in the MAAs concentrations. UV-B radiation had been reported to exhibit negative effects on the accumulation of the major MAA shinorine and palythine (Kräbs et al. 2002). The relatively higher MAAs under PAB treatment compared to PA treatment is inconsistent to the wavelength-dependence of the induction of MAAs but could partly be attributed to the cut-off filters used. The filter foils used in this experiment does not exactly cut-off between the UV-A and UV-B wavelength range compared to the glass filters used by Kräbs and co-workers (2002). However, more experiments have to be performed regarding MAAs concentrations in spores under different light conditions in order to get a better quantification.

Generally, photosynthesis of intertidal and subtidal tetraspores of Antarctic *Iridaea cordata* did not react differently to UV exposure but showed significant differences in the recovery phase after exposure. The difference lies most likely in better repair and protective mechanisms of the intertidal tetraspores. For the future this could mean that more sensitive species but also more UV-sensitive populations of the same species growing in the subtidal shift to even greater water depth. On the other hand, the experiments showed that an intraspecific adaptation and/or acclimation to UVR is possible. However, nothing is known about the time spans of this acclimation or adaptation phase. Our study shows further that working with material grown only under culture conditions can often lead to an overestimation of UV effects because culturing usually takes place under white light only and propagules seem to be already pre-

adapted when leaving the adult thallus, shown e.g. by the presence of MAAs in freshly released intertidal tetraspores.

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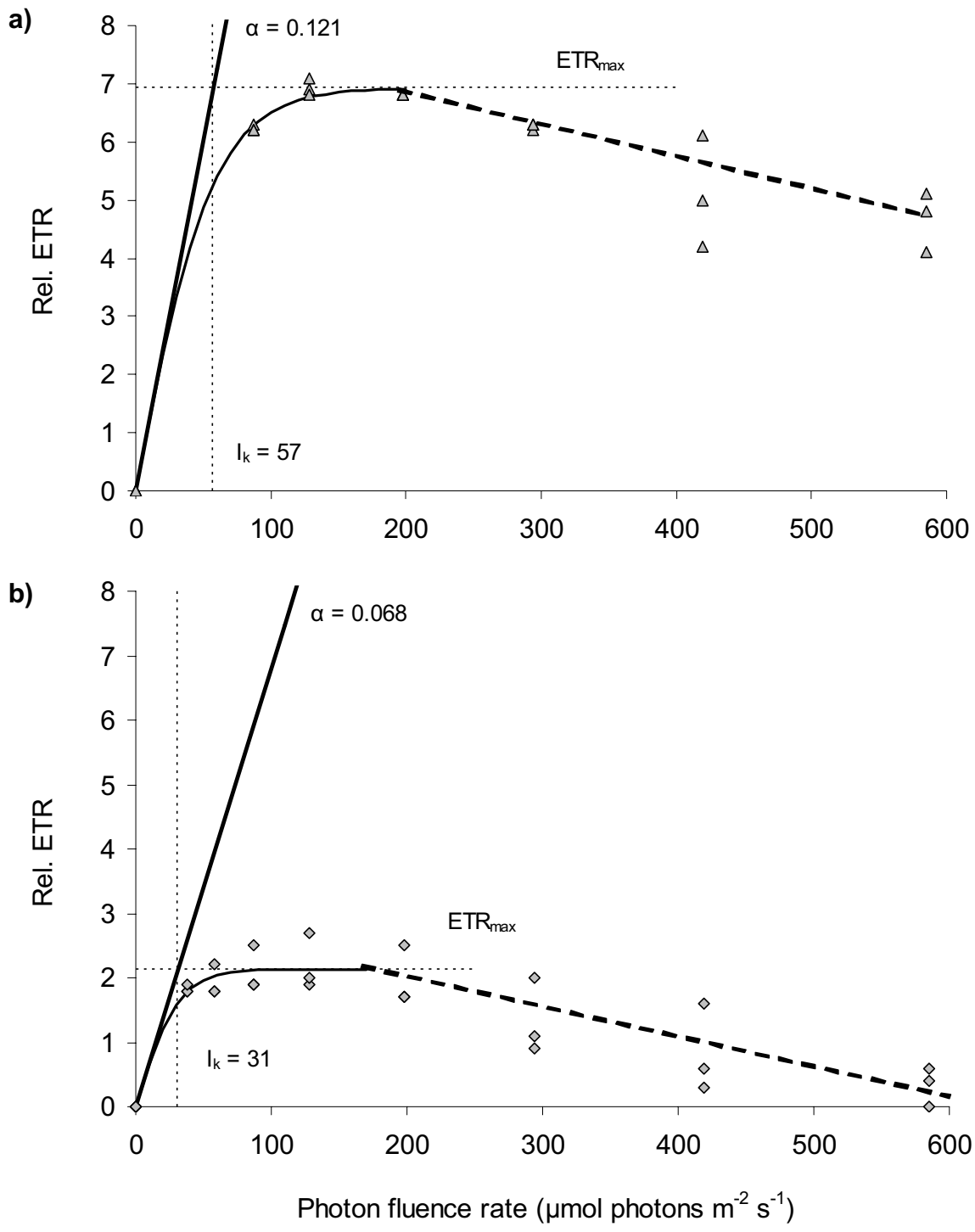

Fig. 1a, b. Photosynthetic performance (P-I curve) of tetraspores from *Iridaea cordata*, a = intertidal, b = subtidal (n = 3) shortly after release. PFR is the respective photon fluence rate of actinic white light and rETR is the relative transport rate. Saturating irradiance (I_k) is estimated as the point at which the initial slope crosses the maximum photosynthesis ($rETR_{max}$) using a hyperbolic tangent model.

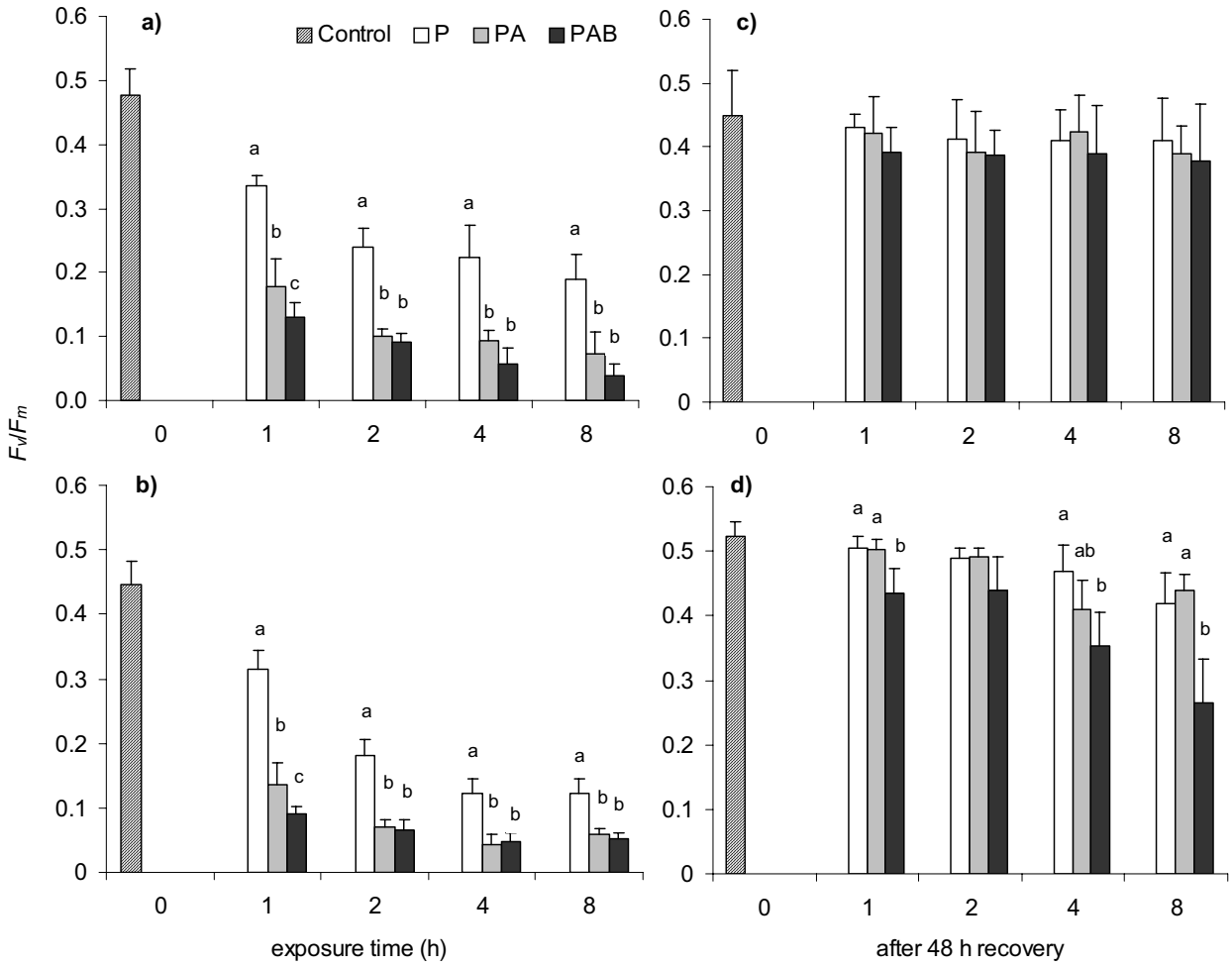

Fig. 2a-d. Mean optimum quantum yield (F_v/F_m) ± SD (n = 5) of tetraspores of *Iridaea cordata* (**a, c** = intertidal**, b, d =** subtidal) after exposure to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) and after 2 d of recovery, respectively. Control is without treatment and continuously maintained under 4 µmol photons m^{-2} s^{-1} white light. Different letters indicate significant differences between the treatments at one exposure time.

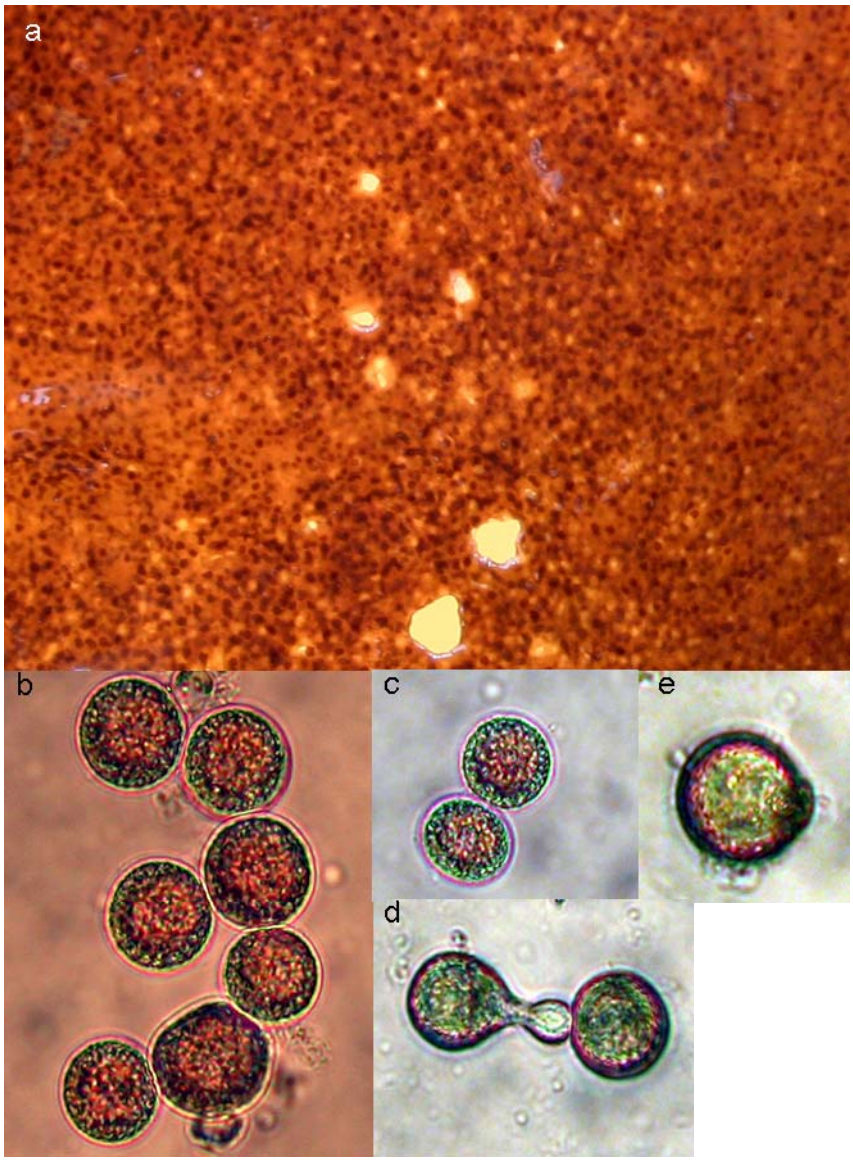

Fig. 3a-e. *Iridaea cordata* tetrasporophyte with tetraspores (**a**). Tetraspores before starting the treatment (**b** and **c**), after 8 h exposure to PAR + UV-A (**d**) and 2 h of PAR + UV-A + UV-B (**e**). Pictures were taken after exposure treatments.

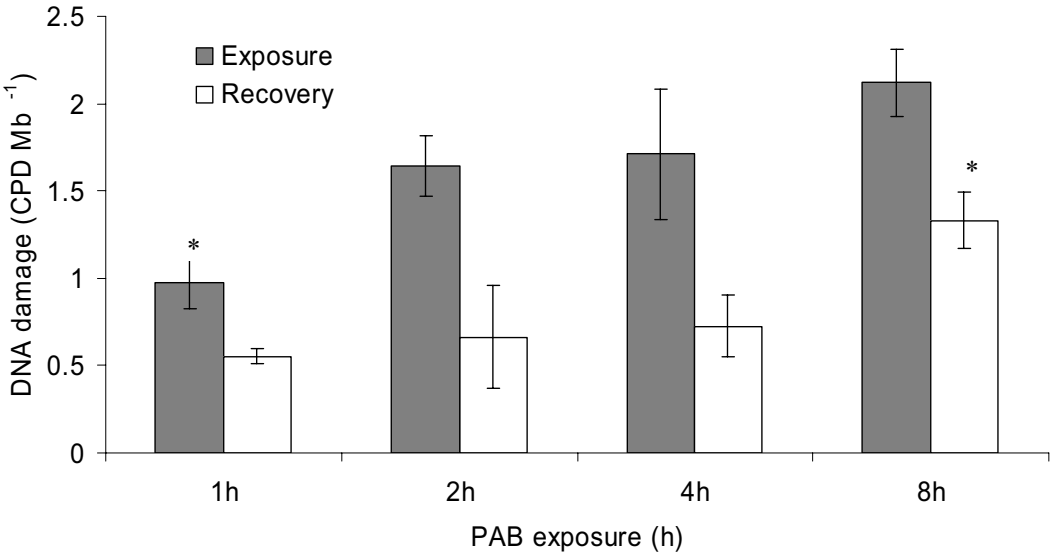

Fig. 4. UV-B induced DNA damage (mean ± SD, n = 3, induced CPD concentration per million nucleotides) In Iridaea cordata tetraspores collected in the intertidal after exposure to different doses of PAB (PAR + UV-A + UV-B). The white bar shows the CPDs after 48 h recovery under dim white light. Asterix (*) marks significant differences between the treatments

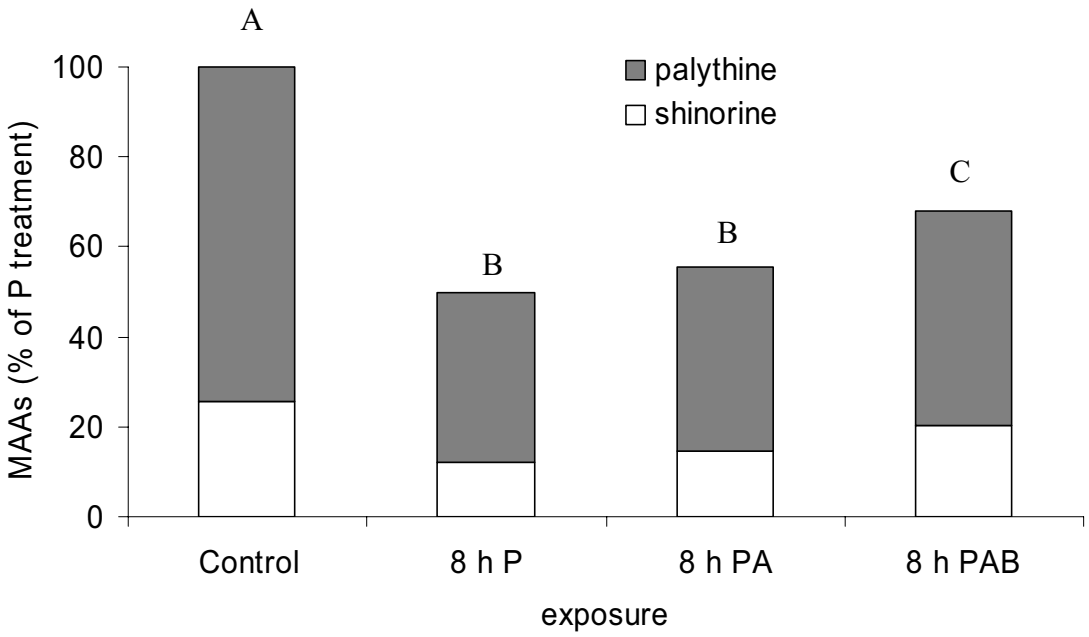

Fig. 5. Mean MAA content (n = 3) ± SD of *Iridaea cordata* tetraspores at the beginning of the experiments (controls) and after 8 h of exposure to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB). Different letters indicate significant differences between the treatments and control.

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VIEW PAPER

Ultraviolet radiation shapes seaweed communities

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Abstract Stratospheric ozone depletion and the concomitant increase in irradiance of ultraviolet-B radiation (UVB) at the earth's surface represent major threats to terrestrial and aquatic ecosystems. In costal rocky shore environments, seaweeds constitute a group of organisms of particular significance to ecosystem function. Thus, impairment of seaweed performance by UVB-exposure may result in severe changes in the functioning of coastal ecosystems. Here we present our view on how UVB radiation affects seaweed physiology and ecology and, thus, shapes the coastal environment by affecting the spatial, species and functional structure of seaweed communities.

Keywords Acclimation · Photosynthesis · Seaweeds · Ultraviolet radiation · Vertical zonation

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Introduction to seaweed communities

Seaweeds (also referred to as ''marine macroalgae'') represent key components within coastal ecosystems (Lüning 1990). As primary producers these green, brown or red coloured marine plants serve a multitude of ecosystem functions. They may grow from just a few millimetres in size up to 60 m or even more. At their growth site they often form submersible forests (i.e., ''kelp forests'' in cold-temperate oceans) characterized by high primary productivity. For instance, along the coasts of cold-temperate regions, the communities dominated by the brown algal genus *Laminaria* have annual productivity rates of about 2 kg carbon per m^2 (Thomas 2002). Seaweeds are globally distributed from the Tropics to the Polar Regions and primarily settle on hard bottom substrate, such as rocky shorelines. Here, seaweed communities typically show distinct vertical zonation patterns exhibiting a characteristic sequence of

species with increasing depth (Stephenson and Stephenson 1972). Along with the depth gradient, the environmental parameters seaweeds are exposed to change drastically. Species settling in the supralittoral zone (the fringe above the high tide level) are exposed to drought, high solar radiation and also atmospheric changes in temperature. The intertidal fringe (the eulittoral zone) is moreover characterised by regular and extreme changes in abiotic conditions, based on tidal influence (Davison and Pearson 1996). During low tide, organisms are exposed to high solar irradiance, drought, atmospheric temperatures, and, depending on current weather conditions, sometimes extreme changes in salinity (Schonbeck and Norton 1978; Davison and Pearson 1996). Furthermore, mechanical stress due to tidal currents and wave exposure has a strong impact on intertidal communities. Underneath, in the sublittoral zone extending below the low tide level, seaweeds usually encounter a more stable habitat, as the water column above buffers against strong changes in abiotic parameters. As outlined below, it is also the respective characteristics of the water column, which determines exposure of sublittoral organisms towards UVB exposure.

Seaweeds interact with and play an existential role for many marine animals (Lubchenco and Gaines 1981; Hay and Fenical 1992). The most important functions of seaweeds in this context are the supply of food and habitat to and their effects on the recruitment and dispersal of animals. Being at the basis of marine food webs, seaweeds are directly consumed by a diversified suite of micro- and macrograzers, which in turn can alter structure and species composition of seaweed communities (Duffy and Hay 2000). In addition, algal exudates might fuel the microbial loop if they are used by free-living and alga-associated bacteria. However, algae can also adversely affect animals. For instance, decomposing algal mats produce anoxic conditions affecting survival of covered animals.

Seaweeds serve many species as habitat, to which sessile forms attach directly, and may host motile animals by provision of shelter from predators (Lippert et al. 2001). Open space is a key resource for sessile marine species and in this way, seaweeds ameliorate competition for space. Seaweeds are also among the first multi-cellular colonizers and thus precondition the substratum for later successional recruits. Subsequent settlement of spores and larvae may be facilitated, tolerated, or inhibited in dependency of the already existing seaweed community (Sousa and Connell 1992). Furthermore, larger seaweeds serve as egg-deposition sites of predatory fin and shell fish, thereby supporting indirectly top-down control in coastal habitats. Furthermore, dislodged seaweeds serve as dispersal vectors for species, accelerating the transport of invasive species and the recolonisation of defaunated habitats (Thiel and Gutow 2005).

Due to their enormous importance within coastal ecosystems a decrease in seaweed abundance related to environmental change e.g., under increased UVB irradiance will thus have dramatic consequences for the sum of organisms associated. Even under the present radiation conditions, UVB represents a crucial environmental factor organisms have to deal with. The knowledge on the physiological and ecological effects of present UVB irradiances is a precondition in order to be able to estimate future consequences of ozone depletion and increased UVB levels. Thus, in the following we present our view on how UVB radiation may shape seaweed dominated coastal ecosystems under the present radiation conditions and how the spatial and functional structure of seaweed communities might be affected in the future.

Enhanced UVB radiation

Solar radiation is the most important prerequisite for life on earth. In the process of photosynthesis, photoautotrophic organisms (like seaweeds) convert light energy into chemically bound energy, which is used for biomass production; as a side effect, molecular oxygen is generated as a basis for all heterotrophic organisms. Changes in irradiance and light quality can either promote photosynthesis, but can also inhibit many biological processes if radiation becomes excessive (Barber and Andersson 1992), or if short wavelength radiation with high energy content, such as UVB radiation, is absorbed by

biomolecules (Vass 1997). Consequently, damage to important components in plant metabolism results in reduced photosynthetic and general metabolic activity and, hence, lead to a decrease in biomass production.

Ever since the discovery of stratospheric ozone depletion in the Antarctic in the 1970s (Farman et al. 1985), serious concerns have arisen about the impacts of increasing UVB radiation on the biosphere (Madronich et al. 1998; Björn et al. 1999). Ozone is predominantly generated in the low latitudes, by photolysis of molecular oxygen. In the stratosphere, ozone molecules are subject to UV-mediated photolysis and may also be degraded due to the reaction within catalytic cycles with NO, Cl or Br serving as catalysts (Lary 1997; Langer 1999). The concentration of these compounds in the atmosphere increases mainly due to anthropogenic emissions, thus leading to ozone depletion.

Ultraviolet radiation includes the wavelengths below those visible to the human eye. This spectral range is according to the CIE definition (Commission Internationale de l'Eclairage 1935) divided into three wavebands: 315–400 nm UVA, 280–315 nm UVB, and 190–280 nm UVC, which does not reach the earth's surface, as it is completely absorbed on its way through the atmosphere. Due to the optical characteristics of ozone, it is the UVB range, which is likely to increase at the earth's surface, as a consequence of a decrease in stratospheric ozone concentration. Calculations based on the absorption characteristics of O_3 indicate that a 10% decline in column ozone would result in an approx. 5% increase of surface irradiance at 320 nm while the same decline would be accompanied by a 100% increase at 300 nm (Frederick et al. 1989).

UVB in the aquatic environment

The effects of UVB on aquatic ecosystems are strongly dependent on the optical properties of the water body (Holm-Hansen et al. 1993; Hanelt et al. 2001). Therefore, it is necessary to estimate the penetration of UV radiation into the water column. The UV irradiance reaching the water surface is influenced by various *atmospheric* factors, such as latitude and altitude, elevation of the sun coinciding to the season and the time of day, weather conditions (clouds and fog), ozone and aerosol concentrations. The *underwater* light field is even more variable: In coastal sea waters, UV radiation and blue light are strongly attenuated due to dissolved organic matter (DOM; Kirk 1994), and this depends largely on the input of this material from the terrestrial ecosystem. According to Kirk (1994), the diffuse vertical attenuation coefficient of downward irradiance (K_d) in the water column is determined by the following formula:

$$K_d = \ln(\mathrm{Ed}_{(z_2)}/\mathrm{Ed}_{(z_1)})^{*}(z_1 - z_2)^{-1}$$

with $\mathrm{Ed}_{(z_1)}$ and $\mathrm{Ed}_{(z_2)}$ as the respective irradiance in depth z_1 and z_2. The value of the K_d in the UV-waveband is naturally much higher than for the PAR range (Jerlov 1976) and typically increases tremendously during the summer season in polar coastal waters (Hanelt et al. 2001). The reason is that the turbidity of the coastal water in polar regions rises due to rainfall or melt water from snow layers and glaciers. A high discharge of turbid fresh water into the coastal zone carries fine terrigenous sediments into the seawater (Svendsen et al. 2002). In the Arctic the variation in K_d-values showed that from middle of June, UVB transparency decreased strongly due to the input of turbid melt waters on Spitsbergen (Hanelt et al. 2001) or in the Hudson bay (Vincent and Belzile 2003). While the UVB transmittance decreased only by about 22% per meter in clear waters during spring, the attenuation increased to about 53% per meter in summer, so that UVB was almost fully absorbed within the first 3 m of the water body at Spitsbergen. In spring and late autumn at low air temperatures the water conditions are relatively clear. Then, UV radiation penetrates deeply into the water column and the threshold irradiance of UVB with the potential to affect primary plant productivity negatively is still at about 5–6 m depth. In contrast to the situation in sheltered bays and fjords, at open coastlines with strong current, melt water can be replaced much faster with clearer oceanic water, which diminishes the observed turbidity effects on light penetration. Climate-induced changes in planktonic or

allochthonous sources of DOM, either through changes in vegetation cover, decomposition, or glacial meltwater may have a higher impact on the underwater UVB regime than ozone depletion would produce (Franklin et al. 2003). Vincent and Belzile (2003) found a close correlation between UV attenuation and seston concentration in the Antarctic region. In the south western Ross Sea a high particulate absorption in the UVB waveband was observed during a phytoplankton bloom which was rather caused by the absorption of chromophoric dissolved organic matter (Arrigo and Brown 1996). Arctic and Antarctic Oceans both experience increased biological UV exposure resulting from stratospheric ozone depletion. However, climate related sea ice melting in the Arctic may potentially result in greater change in underwater UVB exposure than the increase caused by recent ozone depletion in Antarctica (Vincent and Belzile 2003). In summary, the respective optical characteristics of the water column determine the under water light climate and thus also UVB absorption in the aquatic environment. As UVB exposure is decreasing with increasing depth, the attenuation of UV radiation in the water column represents a major structuring frame for seaweed communities.

Biological effects of UVB

The effects of UVB exposure on biological systems are manifold, and reach from the molecular to the organism level, thereby affecting growth and production, and, consequently, ecosystem structure and function. A prerequisite for UVB induced damage is the absorption by biomolecules. Potential UV-chromophores in plants mainly include nucleic acids (such as DNA, RNA) and proteins (Vass 1997). DNA is one of the most UV-sensitive molecules and UV-induced damage occurs directly by the absorption of UVB quanta by aromatic residues. The results are direct structural alterations such as formation of cyclobutane dimers (Lois and Buchanan 1994), but can also be indirectly mediated due to the presence of free oxygen radicals, generated by the electron transfer from chromophore molecules, excited by UV absorption (Mitchell and Karentz 1993). UV-induced damage to the DNA represents a serious effect, as photoproducts can inhibit replication or even cause mutations, thereby affecting gene expression. UVB absorbing aromatic residues are also present in certain amino acids (e.g., tyrosine, phenylalanine, tryptophan) and, therefore, in proteins. Consequently, damage to protein molecules is a major effect of UVB in organisms. Furthermore, disulphide bonds between cysteine residues in the protein can be cleaved by UVB radiation (Vass 1997). These bonds have an important role in protein folding, and thus, are essential for proper functioning of the protein. Lipids, a major compound in all biological membranes, may be destroyed by UVB in the presence of oxygen. This peroxidation of unsaturated fatty acids has a direct effect on membrane structure and the generation of lipid peroxy radicals can induce further damage by participating in free radical cascades (Murphy 1983). In plants, pigments of the photosynthetic apparatus can also be destroyed by UV exposure (Strid et al. 1990), with the phycobilins being the most sensitive, and carotenoids generally being less affected than the chlorophylls (Teramura 1983; Häder and Häder 1989). As a consequence of a number of molecular effects, several physiological processes are impaired, such as photosynthesis (Bornman 1989; Strid et al. 1990; Nogues and Baker 1995; Allen et al. 1997), and nutrient uptake (Döhler 1985, 1992; Flores-Moya et al. 1998; Gómez et al. 1998), while others, e.g., respiration, appear to be less affected (Larkum and Wood 1993; Aguilera et al. 1999).

Photosynthesis is probably the most intensively studied process in plant biology. Due to its central role in plant metabolism, as well as its importance for all oxygen dependent life on earth, studies on adverse effects on photosynthesis, in the context of a globally changing environment are of particular interest. Due to numerous effects of UVB radiation to the respective molecules involved in photosynthesis, the effects of UV-exposure are also multiple (see Vass 1997 for review). The common consequences on photosynthetic function are decreased CO_2-fixation and oxygen evolution (Renger et al. 1986; Allen et al. 1997). This could be caused by several molecular events: While most studies have found that photosystem I (PS I) is only

minimally affected by UVB (by inhibiting PS I-mediated cyclic photophosphorylation; Iwanzik et al. 1983; Renger et al. 1986), photosystem II (PS II) seems to be a more important target (Bornman 1989). It is likely that UVB causes an inhibition of energy transfer within the PS II reaction centre by blocking electron flow. Furthermore, the function of the D_1 protein may be impaired by the UVB induced fragmentation of the protein (Renger et al. 1986; Vass 1997). On the oxidising side of PS II, the oxygen evolving system (water splitting complex) is another sensitive target of UVB (Renger et al. 1986). Furthermore, it has been suggested that UVB may affect the light-harvesting complex (LHC) by its functional disconnection from the photosystem, resulting in an impairment of energy transfer to the reaction centre (Renger et al. 1986; Lorenz et al. 1997). A decrease in photosynthetic activity may also be due to the photodestruction of pigments; within the chlorophylls, Chl *a* has been observed to be more affected than Chl *b* (Teramura 1983; Strid et al. 1990).

The CO_2 fixing enzyme RubisCO has been shown to be another critical component in UVB-induced inhibition of photosynthesis. The UVB-induced decline in its activity is related to the decreasing amount of both subunits as well as the corresponding mRNA levels (Strid et al. 1990; Jordan et al. 1992; Bischof et al. 2000a, 2002a). Another effect of UVB on reactions related to photosynthesis represents the inactivation of chloroplast ATPase (Strid et al. 1990). Impairment of any of the components mentioned above contributes to lower the photosynthetic activity during and following UV-exposure.

The physiological effects are also reflected on the ultrastructural level. UVB radiation can lead to dramatic changes of the fine structure of chloroplasts and mitochondria. Mild UV stress leads to a wrinkled appearance of the thylakoids, lumen dilatations and damage of the outer membranes. In the mitochondria a swelling of the cristae is often observed (Poppe et al. 2003; Holzinger et al. 2004). After strong UVB exposure the formation of 'inside-out' vesicles from thylakoids was demonstrated in four red algal species. In *Palmaria decipiens* the fine structural changes are reversible indicating acclimation to UV stress (Poppe et al. 2002, 2003).

On the organism level, the effects mentioned above can result in reduced growth and production, as shown in higher plants, seaweeds, phytoplankton and ice algae (Caldwell 1971; Worrest 1983; Ekelund 1990; Karentz et al. 1991a, b; Holm-Hansen 1993; McMinn et al. 1999, Han 1996a, b; Makarov 1999; Aguilera et al. 2000; Altamirano et al. 2000a, b). Other effects include the impairment of reproductive success or may even bear lethal consequences. Consequently, all aspects mentioned may also affect ecosystem structures (Holm-Hansen et al. 1993; Johanson et al. 1995; Caldwell et al. 1998).

Seaweed responses to UVB

Seaweeds became a prominent group of organisms in UVB research for two reasons. Firstly, seaweeds represent a crucial component for coastal ecosystems. Thus, UVB related damage to these organisms might have drastic consequences to the entire ecosystem. Secondly, in a range of field and laboratory studies, seaweeds were proven to be in general rather sensitive to UVB exposure (at least compared to terrestrial plants) but also it was shown in some species growing over a wide depth or even geographical range, that seaweeds do apply a vast variety of acclimation mechanisms. Thus they became very suitable model systems also in basic stress physiology.

Adaptation versus acclimation

The respective reaction of a species towards UVB exposure is determined by the interplay of genetically fixed adaptation and physiological acclimation. Generally spoken, adaptation is setting the frame in which acclimation to changing environmental conditions might occur.

In a laboratory study on Antarctic seaweeds, which were isolated in the field decades ago and subsequently kept in stock cultures it was shown that these specimens still exhibit distinct species-specific differences in UV-tolerance once they are grown to macrothalli and exposed to identical culture and experimental UV conditions (Bischof et al. 1998a). Due to the cultivation of sporophytes under low-light conditions and UV-exclusion

no acclimation to UV radiation had occurred prior to the experiments. The two shallow-water green algae *Enteromorpha bulbosa* and *Acrosiphonia arcta* were least affected by UVB radiation. Photosynthesis in the brown algae *Desmarestia antarctica* and *D. anceps* and the red alga *Gymnogongrus antarcticus*, inhabiting slightly deeper waters, was inhibited to a similar and intermediate extent. However, two other red algal species from the lower subtidal, *Phycodrys austrogeorgica* and *Delesseria lancifolia*, responded extremely sensitively towards UVB-exposure. In the case of *Delesseria sanguinea*, a deep sublittoral species, growth can be also strongly impaired when the alga is exposed to surface solar radiation (Pang et al. 2001) indicating these plants may lack all protecting mechanisms against excessive radiation. In the field protective mechanisms against UV radiation might not be necessary because they live in the shade of the canopy algae and/or in great depths. Similarly, zoospores of the deep-water species *L. saccharina* and *L. hyperborea* are more strongly photoinhibited after exposure to UV radiation than zoospores from the shallow water species *L. digitata* (Roleda et al. 2005a). In addition, recovery of PS II activity is high in *L. digitata*, low in *L. saccharina* and lowest in *L. hyperborea*. Spores of the eulittoral *M. stellatus* and *C. crispus* are photoinhibited after UV exposure but recover quickly after exposure to dim white light (Bischof et al. 2000b; Roleda et al. 2004). The first attempt to study kinetics of photoinhibition and recovery in zoospores of Arctic Laminariales showed that zoospores of the lower sublittoral *L. saccharina* were more sensitive to PAR- and UV-induced photoinhibition than upper- to midsublittoral *S. dermatodea, A. esculenta* and *L. digitata*. Kinetics of recovery in zoospores showed a fast phase in *S. dermatodea* which indicates a reduction of the photoprotective process while a slow phase in *L. saccharina* indicates recovery from severe photodamage (Roleda et al. 2006d). These experiments were focussed on short-term effects, thus the preadaptive setting of species could be revealed, but not the respective potential of acclimation, also determined by the genetic features of species.

In another study, six different red algal species from cold-temperate regions and with different zonation patterns were cultivated under identical culture conditions and exposed to similar irradiance of UVB (van de Poll et al. 2001). The inhibition of growth became stronger in accordance with the position on the shore these algae usually take in the field. The two species from the upper sublittoral or even lower eulittoral zone (*Palmaria palmata* and *Chondrus crispus*) did hardly exhibit inhibition in growth, whereas growth in the species from the middle sublittoral zone (*Phyllophora pseudoceranoides* and *Rhodymenia pseudopalmata*) was inhibited up to 50%. In the deep-water algae *Phycodrys rubens* and *Polyneura hilliae*, growth was inhibited almost completely. Curiously, pronounced accumulation of damaged DNA, expressed as thymine dimer formation, was only found in these two species. Apparently these true deep-water algae do lack mechanisms to shield the DNA from UVB exposure or to repair already damaged DNA e.g., by the activity of repair-enzymes (as e.g., DNA phytolyase). This study illustrates how genetic pre-adaptation is setting the frame in which acclimation may occur. The fact that not any acclimation to UVB was shown by these species points to a strong degree of adaptation to low irradiance environments.

Modulation of ecophysiological reactions towards variation in abiotic factors is conditioned by genetic adaptation. This is also visible in two red algal species from Spitsbergen with slightly different vertical zonation preferences (Karsten et al. 1999). *Devaleraea ramentacea* as a species from shallow waters is permanently equipped with high activities of reactive oxygen scavenging superoxide dismutase (SOD). This high but static activity is reasonable for a species from shallow waters, where usually strong variation in abiotic conditions, and thus the onset of stressfull conditions to photosynthesis resulting in increased ROS production, is more likely than in more stable deeper waters. However, maintaining a protective systems on such a high level throughout the year is probably energetically costly. Thus, species which are not permanently exposed to stressfull conditions, e.g., in deeper waters, may favour the

strategy to respond to abiotic stress and to increase protective strategies, like SOD activity, only when they are needed during times of e.g. high UV irradiance. *Palmaria palmata* inhabiting slightly deeper waters than *D. ramentacea* is applying this strategy (Karsten et al. 1999). In the case of the estuarine red alga *Gracilaria chilensis*, which is subject to extremely changing light conditions both during tidal cycles and seasonally, exposures to surface UVB irradiances induce marked reductions in photosynthesis (Gómez et al. 2005a). Although the species can display rapid acclimation mechanisms, the constitutively high pigment contents and low concentrations of sunscreen substances (e.g., MAAs), clearly suggest that the *Gracilaria* retains its shade adapted characteristics, probably as a consequence of the normally turbid waters at the estuary.

Acclimation of photosynthesis to UV radiation

In seaweed species inhabiting a flexible environment, i.e., the shallow water zones down to approx. 15 m depth, acclimation to changing abiotic conditions is important, to adjust photosynthetic performance in order to maintain energy supply for growth, but also to prevail under periods of stressfull conditions. The ability for fast acclimation to increased UV irradiance has been demonstrated in the Arctic/cold-temperate kelp *Alaria esculenta* from Spitsbergen (Bischof et al. 1999). It was shown that its macrothalli are able to adjust photosynthetic performance to changes in irradiance at their respective growth site. This capability may represent one prerequisite for this species to establish over a wide depth range and also to endure the seasonal variation of radiation conditions (Chapman and Lindley 1980; Falkowski and LaRoche 1991; Klöser et al. 1996; Bischof et al. 1998b, 1999).

Within the brown algae studied so far, two different responses were observed in the process of acclimation of photosynthetic activity to changing radiation conditions. Firstly, the rate of recovery from UV-induced photoinhibition increases. Secondly, the degree of inhibition becomes smaller (Bischof et al. 1998b, 1999). Increases in the rate of recovery may result from an activation of different repair mechanisms, counteracting the impact of UV-exposure by a faster replacement of damaged molecules. The molecular mechanism responsible may be the same as discovered in the cyanobacteria *Synechocystis* sp. and *Synechococcus* sp. In both species, it was found that exposure to moderate doses of UVB results in an increased turnover rate of the D_1 and D_2 reaction centre subunits of PS II, thus, rapidly replacing damaged protein by newly synthesised polypeptides (Campbell et al. 1998; Máté et al. 1998). The latter authors found that UVB induces the transcription of psbA genes, which encode the D_1 reaction centre protein of PS II. Although comparable studies are lacking for macroalgae, it may be that a similar response provides an explanation for the increasing rate of recovery in the studied brown algal species. However, this mechanism may only be successful as long as UVB exposure does not induce stronger damage to DNA, thus impairing gene expression. Results also showed, that in algae previously acclimated to high PAR, additional UV-exposure rather results in a delay of the recovery process than in a further inhibition of photosynthesis (Bischof et al. 1999). These findings support data from field experiments on *Fucus distichus* from Spitsbergen, indicating that at their natural growth site in the eulittoral zone photoinhibition is mainly caused by high irradiances of PAR and natural UVB causing a delay in recovery (Hanelt et al. 1997a). The observed delay in recovery is indicative for damage to the D_1 protein (Aro et al. 1993). Under UVB exclusion the rate of D_1 degradation mediated by solar radiation was found to be as much as 30% slower than under full sunlight (Greenberg et al. 1989), thus supporting those results for high light acclimated algae.

In contrast to subtidal species, intertidal brown algae, have to cope with highly changing solar radiation scenarios on a short term basis. In this sense, high PAR irradiances become as ecological important as UV radiation. Flores-Moya et al. (1999) observed a significant delay in recovery from photoinhibition in the brown alga *Dictyota dichotoma* from Southern Spain, when samples were exposed to solar radiation depleted from the UVB range and subsequently transferred to dim light conditions. Recovery in samples receiving

either the whole solar spectrum or PAR only, recovered at the same rate. This indicates the presence of complex synergistic effects involved in the inhibition of photosynthesis in the field, which need to be studied further. On the other hand, it must be emphasized that brown algae include many large, perennial species, which exhibit complex responses to UV-exposure. For example, in the southern kelp *Lessonia nigrescens*, the photosynthetic responses to seasonally changing UV conditions form part of the suite of adaptations along with its ontogenic development, i.e., the alga has a complex UVB exposure history characterized by high levels of UVB in summer and low levels in winter (Huovinen et al. 2006). Moreover, gradients in UV tolerance have been reported along of the massive thallus (Gómez et al. 2005b). Thus, the morpho-functional factors involved in UV photobiology of brown algae are important and have to be considered in further studies in order to evaluate more accurately the effects of enhanced UVB on coastal primary productivity.

A common response observed in the brown algal species during acclimation to UV radiation is the reduction in the degree of photoinhibition. This effect may be explained either by the activation of the antioxidative response, increased activity of repair and recovery mechanisms counteracting the inhibitory effects (see above), or by the formation of UV-screening compounds (Lesser 1996).

Mycosporine-like amino acids (MAA)

One of the most important physiochemical acclimation mechanism against biologically harmful UV radiation involves the biosynthesis and accumulation of UV-screening substances. Typically absorbing in the UVA and UVB range, these biomolecules were invoked to function as passive shielding solutes by dissipating the absorbed short wavelength radiation energy in form of harmless heat without generating photochemical reactions (Bandaranayake 1998). The most common photoprotective sunscreens in Antarctic macroalgae are the mycosporine-like amino acids (MAAs), a suite of chemically closely related, colourless, water-soluble, polar and at cellular pH uncharged or zwitterionic amino acid derivatives. MAAs exhibit a high molar absorptivity for UVA and UVB, and have been reported as photochemically stable molecules, which are prerequisites for their sunscreen function (Conde et al. 2000). While MAAs have been mainly observed in numerous Antarctic (Karentz et al. 1991a; Hoyer et al. 2001) and cold-temperate Rhodophyta (Huovinen et al. 2004), Phaeophyta and most Chlorophyta typically lack these compounds, except the green alga *Prasiola crispa* ssp. *antarctica* which contains high concentrations of an unique MAA with an absorption maximum at 324 nm (Hoyer et al. 2001; Karsten et al. 2005). Many Phaeophyta synthesise photoprotective phlorotannins under UV exposure (Pavia et al. 1997; Schoenwaelder 2002b; Schoenwaelder et al. 2003), this strategy will be reviewed in detail below.

The function of MAAs as intracellular screening agents has been inferred from a decrease in concentration with increasing depth (Hoyer et al. 2001, 2003). Supra- and eulittoral Antarctic red algal species experience the strongest insolation, and consequently synthesise and accumulate very high MAA contents, which generally are positively correlated with the natural UV doses (Karsten et al. 1998a; Huovinen et al. 2004). In contrast, many taxa growing in the sublittoral are physiologically not capable to produce MAAs, which well explains their strong sensitivity, for example, of photosynthesis against ambient solar radiation. These Rhodophyta avoid any UV exposure, and, hence, there is no physiological need to synthesise and accumulate metabolically expensive nitrogen-containing MAAs. This in turn would save energy to better support other essential pathways such as light-harvesting phycobilisomes to guarantee sufficient PAR absorption under the prevailing low-light conditions.

While juvenile lateral fronds of the red alga *Palmaria decipiens* collected in Antarctic winter contained low concentrations of UV-absorbing compounds, mature plants in late spring and summer exhibited significantly higher values indicating strong seasonal effects (Post and Larkum 1993), which may be related to the changing daylengths and radiation conditions. Based on the MAA concentrations and the

induction patterns after exposure to different radiation conditions Antarctic Rhodophyta can be physiologically classified in 3 categories (Hoyer et al. 2001): Type I—no MAAs at all; Type II—MAAs inducible in variable concentrations, and Type III—permanently high MAA values. While Type I represents deep-water red algae, Type II and III species are growing in the supra- and eulittoral zone. Experiments with Antarctic Rhodophyta under defined radiation sources indicate that the induction, biosynthesis and accumulation of MAAs is a very flexible and species-specific process. While some taxa synthesise MAAs particularly under UVB, others prefer UVA or higher PAR only (Hoyer et al. 2003). Although experimental evidence for a particular trigger mechanism as well as details for the biosynthetic pathway for individual MAAs are still missing, it is reasonable to assume that a signal transduction pathway must be involved in the overall process leading to high MAA concentrations. Due to the different types of MAA induction patterns the presence of various photoreceptors, most probably between the blue light and UVB wavelengths, have to be taken into consideration (Kräbs et al. 2002).

Not the whole red algal thallus is uniformly responding to the ambient solar conditions, but especially young apical or marginal zones, i.e., growing cells synthesise and accumulate MAAs leading to cross sectional and longitudinal concentration gradients (Hoyer et al. 2001). Older tissue regions exhibit thicker cell walls and a leathery texture, and are therefore optically well protected. In contrast, higher MAA concentrations in the most exposed outer cortex are essential to guarantee protection of the delicate meristematic cells.

Besides the stimulating effect of increasing solar radiation on the biosynthesis and accumulation of MAAs in macroalgae other environmental factors may also act as controlling parameter. Particularly lower temperatures have been experimentally proven to stimulate the MAA concentration of Antarctic Rhodophyta (Hoyer, unpublished data). Nutrient availability may also affect the MAA contents (Korbee et al. 2005). Some MAAs also exhibit antioxidative activity (Dunlap and Yamamoto 1995). However, further functional abilities of MAAs are unexplored in macroalgae.

Also in this example, the genetically determined ability to synthesise UV-screening MAAs in the different species of seaweeds is closely related to the spatial structure of the algal community in the field. In summary, the comparison of the species-dependent ability to form UV-screening compounds under laboratory and field conditions provide strong indications for differential genetic preadaptations to the potentially harmful radiation at the natural growth site.

Phenolic compounds

A special class of polyphenolic compounds are phlorotannins, which are exclusively found in brown seaweeds (Ragan and Glombitza 1986). Phlorotannins are secondary metabolites and occur in tissue concentration of up to 20% of the algal dry weight. Several functions are commonly accepted, including a role in adhesion and a strengthening role in cell walls (Schoenwaelder 2002b). Phlorotannins absorb UV radiation, mainly UVC and partly UVB, with maxima at 195 nm and 265 nm (Ragan and Glombitza 1986; Pavia et al. 1997; Henry and Van Alstyne 2004). As tannins of higher plants, phlorotannins possess a high antioxidant activity. Thus, phlorotannins are important for scavenging cell toxic reactive oxygen species (ROS), such as superoxide anion radicals produced by harmful UVB radiation. Therefore, phlorotannins are proposed to function in protecting against excess irradiance, in particular ultraviolet radiation, by screening UV radiation and/or by being an antioxidant.

We suggest four strategies to consider phlorotannins as UV-protecting compounds (1) a generally high tissue concentration of phlorotannins that absorb harmful radiation and prevent cell damages, (2) an induction of phlorotannins stimulated by harmful radiation, (3) an exudation of phlorotannins in the surrounding medium shielding harmful radiation, or (4) an excess inclusion of phlorotannins in cell walls shielding harmful radiation.

High concentrations of phlorotannins in the outer cell layers protect *Hormosira banksii* from sunburn during Australian summer

(Schoenwaelder 2002a). While the outer cell layers are damaged and disrupted by sunlight, phlorotannins are released from the phlorotannin containing vesicles, the physodes, into the cytoplasma where they cause oxidative burn. Oxidised phlorotannins become brownish and form a dark brownish protective cell layer for the underlying photosynthetic tissue. Similarly, the high density of physodes at the periphery of egg and zygote cells of *Fucus spiralis* is most probably responsible for the high tolerance to UV-exposure, in contrast to *F. serratus*, in which physodes are less abundant (Schoenwaelder and Wiencke 2000; Schoenwaelder et al. 2003).

An induction of phlorotannins after a 2-week exposure of artificial UVB radiation was first described in *Ascophyllum nodosum* (Pavia et al. 1997). A weaker response was found in *A. nodosum* when exposed to natural UVB radiation (Pavia and Brock 2000). In that study significant differences in the phlorotannin content of UVB treated and untreated individuals were found after 7 weeks of exposure to natural UVB radiation, while only slightly increased phlorotannin concentrations were measured in the UVB treated individuals at week 2 and week 4. An induction of phlorotannins due to UVB and UVA radiation was also described for *Macrocycstis integrifolia* (Swanson and Druehl 2002). In contrast, no induction of phlorotannins was found in juveniles and embryos of *Fucus gardneri* after 3-week exposure to UV radiation, while growth of embryos was inhibited and growth of juveniles was not affected by UVB radiation (Henry and Van Alstyne 2004). An increase in the size of physodes was observed in various Laminariales from Spitsbergen after UVB exposure indicating an induction of phlorotannin synthesis (Wiencke et al. 2004a). This has recently been verified in the UV tolerant species *Alaria esculenta* and *Saccorhiza dermatodea*. The absorbance of zoospore suspensions from these species increased considerably after UVB exposure, whereas the absorbance of spore suspensions of the UVB sensitive species *L. digitata* did not change at all (Roleda et al. 2006c).

An exudation of phlorotannins as response to artificial UVB radiation was observed in *Macrocyctis integrifolia* (Swanson and Druehl 2002). As a result UVB transmission through the water column was reduced, thereby protecting germinating meiospores of *Laminaria groenlandica* against harmful UVB radiation. Similarly, biofilters containing phloroglucinol (the monomer of phlorotannins) mitigated the harmful effect of UV-exposure on developing zygotes and embryos of *Fucus serratus* (Schoenwaelder et al. 2003). Biofilters made of UV transparent acrylic sheet, filled with zoospore suspensions of *S. dermatodea*, *A. esculenta*, *L. digitata* or phloroglucinol showed a varying capacity to protect zoospore cultures from the lethal effects of UVB (Roleda et al. 2006c). Generally, high phlorotannin content and high exudation rates might reflect an adaptation of seaweeds to enhanced UV radiation. Induction and variable exudation rates of phlorotannins reflect the acclimation potential to environmental changes.

Do phlorotannins play a role in the determination of depth zonation of brown algae? Evidence was found in zygotes and embryos of *Fucus* species (Schoenwaelder and Wiencke 2000; Schoenwaelder et al. 2003). *F. spiralis* from the high intertidal zone is less sensitive to UV-exposure and contains much more physodes than *F. serratus*, which is found further down the shore. Similarly, zoospores of *Laminaria digitata*, growing on Helgoland in the upper sublittoral, exhibit a strong absorbance below 300 nm, indicative of phlorotannins, compared to zoospores of the mid and lower sublittoral *Laminaria* species (Roleda et al. 2005a). Phlorotannins may also play a role for the UV-sensitivity of zoospores of Arctic Laminariales from different depths (Wiencke et al. 2004a). Similarly, higher phlorotannin concentrations were found in *Desmarestia anceps* collected in shallow waters compared to deep water samples, which might indicate an induction of phlorotannins due to higher irradiances and/or UV exposure (Fairhead et al. 2005). Conversely, *D. menziesii* shows no differences in phlorotannin concentrations in samples from different collecting depth and even exhibits lower concentrations than *D. anceps*, which usually occurs in deeper waters (Fairhead et al. 2005).

In contrast to the data sets available on potential UV-screens in red and brown algal species information on specific UV-absorbing

compounds in marine green algae is still very limited. Very recent studies demonstrated the solely UVB-induced increase in thallus absorption in *Ulva pertusa* with a maximum at 295 nm (Han and Han 2005), however, the responsible screening compound is still unidentified.

DNA damage and repair

As outlined above, a particularly hazardous event of UV-exposure is the induction of DNA damage. Thus strategies in order to prevent damage or to efficiently repair existing damage represent acclimation mechanisms of crucial importance. Differential induction and accumulation of UVB-induced cyclobutane-pyrimidine dimers (CPD) were measured in several red macroalgae from Brittany and Spitsbergen (van de Poll et al. 2001, 2002). In the course of 15 days repeated exposure to artificial UV radiation, no accumulation of DNA damage but rather a decrease in CPD concentration was observed in the temperate littoral species *Palmaria palmata* and *Chondrus crispus*. Conversely, an approximately 6-fold increase in the amount of CPD was observed in sublittoral species *Phycodrys rubens* and *Polyneura hilliae* (van de Poll et al. 2001). In Arctic red macrophytes, the amount of solar radiation-induced CPD concentrations in *Devaleraea ramentacea*, *Palmaria palmata*, *Odonthalia dentata*, *Coccotylus truncatus* and *Phycodrys rubens* (van de Poll et al. 2002) is related to their upper depth distribution limit in Kongsfjorden described by Wiencke et al. (2004b). In shallow coastal waters, blooms of floating *Ulva* are exposed to the full solar radiation. The amount of thallus DNA damage is, however, relatively low ranging from 1.0 to 1.88 CPD Mb^{-6} depending on solar exposure of the investigated canopy layer (Bischof et al. 2002b) compared to those reported in red macrophytes (van de Poll et al. 2001, 2002). These data suggest that the low sublittoral habitat of the Ceramiales (*P. hilliae*, *C. truncatus* and *P. rubens*) is primarily due to their lack of tolerance to UV radiation and that UV protection mechanisms are not sufficient to prevent the accumulation of DNA-damage in these species.

Obviously, UV-susceptibility of DNA damage is highly depending on the respective developmental stage of the species under investigation. The impact of UVB-induced DNA damage on the early life stages of macroalgae is important in shaping up community structure and zonation pattern. DNA damage in carpospores of eulittoral *Mastocarpus stellatus* and *Chondrus crispus* (Roleda et al. 2004) was lower compared to zoospores of the sublittoral Laminariales in Helgoland (*Laminaria digitata*, *L. saccharina* and *L. hyperborea*; Roleda et al. 2005a) and Spitsbergen (*Saccorhiza dermatodea*, *Alaria esculenta* and *L. digitata*; Roleda et al. unpublished data). The UV-sensitivity of the carpospores and zoospores were related to the depth distribution of the foliose gametophyte and adult sporophytes, respectively. In the subsequent life history stage investigated, young gametophytes and sporophytes were less susceptible to UVB-induced DNA damage compared to spores. No detectable CPDs were observed in the young foliose gametophyte stages of the eulittoral *M. stellatus* and *C. crispus* (Roleda et al. 2004). Conversely, the remaining tissue DNA damage among juvenile Laminariales sporophytes was observed to be dependent on the thallus thickness and optical property (Roleda et al. 2005b, 2006a, 2006b). Increasing thallus thickness and opacity (in relation to available cell-bound UV-absorbing compounds) minimise UV-effects where outer phlorotannin-rich cortical layers can selectively filter short UV-wavelength from reaching the UV-sensitive targets (i.e., chloroplasts).

Less genetic damage was incurred in diploid carpospores compared to haploid zoospores (Roleda 2006). Zoospores were, however, found to be more efficient in DNA damage repair. In a sexual organism, the advantage of both ploidy states can be combined by spending much of the life cycle in the haploid state, then fusing to become diploid. During the diploid state DNA damage can be repaired, since there are two copies of the gene in the cell and one copy is presumed to be undamaged (Long and Michod 1995). In the life history of Laminariales, haploid zoospores were more sensitive to DNA damage compared to the diploid young sporophytes.

Between the investigated Gigartinales, the lower DNA damage and effective DNA damage repair mechanism in carpospores of *M. stellatus* is responsible to its recruitment success and colonization of the upper eulittoral zone effectively changing the rocky intertidal biotope of Helgoland (Roleda et al. 2004).

Acclimation via morphogenetic variation

Several morphogenetic effects have been described for higher plants grown under UVB irradiation. Compared to white light, UVB exposed plants exhibit reduced leaf area and stem growth, but increased leaf thickness (Tevini and Teramura 1989; Mepsted et al. 1996). Information on UV-induced morphogenetic effects on the thalli of marine macroalgae is very limited. Studies on the brown alga *Alaria esculenta* have shown that UVB radiation results in reduced growth in length and a significant increase in fresh weight, indicative for increasing thallus thickness (Michler et al. 2002). In previous studies on various Laminariales and seagrasses it was shown that thicker thalli generally exhibit a higher UV-tolerance than thin thalli (Dawson and Dennison 1996; Dring et al. 1996a; Hanelt et al. 1997b). On the other hand, blades of the southern kelp *Lessonia nigrescens* are more UV sensitive than stipes and holdfasts, which has been though as a strategy to minimise mortality. In this species, the higher UV damage is concentrated in the phylloids, which are transient and with high turnover rate, whereas the massive stipes and holdfast, which are supporting structures, are less exposed to UV radiation (Gómez et al. 2005b). In general, the individual effects of UVB radiation on algal thalli with differences in morphological features are largely unexplored, thus we lack information on how UVB might influence the individual thallus structure of the individual seaweed.

Vertical versus latitudinal distribution

Depth zonation

Ecophysiological studies indicate a general correlation between stress tolerance and vertical distribution of seaweeds (Davison and Pearson 1996; Hanelt 1998). Hitherto, there is agreement that the species sensitivity to solar radiation stress is a function of depth distribution (Dring et al. 1996b; Larkum and Wood 1993; Hanelt et al. 1997a, c; Hanelt 1998; Bischof et al. 1998a; Yakovleva et al. 1998). Moreover, some authors regard solar UVB as one of the most important factors controlling the upper distribution limit of seaweeds in the field (Maegawa et al. 1993). Therefore, it is reasonable to assume that increased UVB, penetrating deeper into the water column, may result in a shift of the upper distribution limit of single species to greater water depths.

Larkum and Wood (1993) were the first who have stressed the correlation between UV-tolerance and the depth-zonation of marine macroalgae. For the vertical distribution of tropical seagrasses UV radiation was also proven to be an important factor (Dawson and Dennison 1996). Dring et al. (1996b) showed that sensitivity to UV in red algae growing around the island of Helgoland (Germany) varies with species and depth of collection. As for UVB radiation, investigations on the photoinhibition induced by high levels of PAR also shows a correlation between depth-zonation and the ability for dynamic photoinhibition of macroalgae both from sublittoral (Hanelt 1992, 1998; Hanelt et al. 1994, 1997a) and intertidal populations (Gómez et al. 2004)

In support of this concept the green algae *Enteromorpha bulbosa* and *Acrosiphonia arcta* which occur in the middle and lower eulittoral at the Antarctic Peninsula (Wiencke and Clayton 2002) do almost show no negative UV-effects on photosynthesis and are able to acclimate to even further elevated UV-exposure within hours or days (Bischof et al. 1998a). In contrast, the brown algae *Desmarestia antarctica* and *D. anceps* are described for the middle sublittoral zone off the Antarctic Peninsula (Klöser et al. 1996) but grow mostly in greater depths and occur only occasionally in depths shallower than 17 m. In these depths biologically relevant doses of UVB radiation occur only in very transparent waters, under clear skies and at a high solar declination (Karentz 1989). This might explain why *D. anceps* is quite sensitive to UVB radiation. The red alga

Gymnogongrus antarcticus occurs from the upper sublittoral zone down to 20 m (Klöser et al. 1996), i.e., the upper distribution limit is similar to that of the brown algae *D. anceps* and *Himantothallus grandifolius* and upon UVB exposure these three species show a similar inhibition rate of photosynthesis. *H. grandifolius* is found at 5 m (Lamb and Zimmermann 1977) with the lowest depth at 90 m (Zielinski 1990). This zonation pattern is in line with its high sensitivity. Finally, the red algae *Phycodrys austrogeorgica* and *Delesseria lancifolia* are described for the middle sublittoral zone (Delaca and Lipps 1976; Zielinski 1990; Klöser et al. 1996), but they grow under canopy plants such as *D. anceps* and *H. grandifolius*. This explains their extreme sensitivity to UV radiation. These plants may lack all protecting mechanisms against excessive radiation, because recovery from UV-exposure is poor in both species. The observed differences in species dependent UV-sensitivity are as outlined above genetically determined.

The decisive role of UVB radiation in determining vertical zonation patterns in seaweeds was recently evidenced in studies on the UV effects on the particularly sensitive developmental and reproductive stages. Only few studies were conducted using the unicellular propagation units of seaweeds, although it is widely recognised that the early developmental stages are the most susceptible to a variety of anthropogenic stresses (Coelho et al. 2000).

Seaweed propagules, spores, gametes and zygotes, are the unicellular products of asexual and sexual reproduction and have essential functions in the life-history of seaweeds with respect to dispersal, settlement, attachment, survival and recruitment (Clayton 1992; Norton 1992). They are naked cells, bounded by a plasma membrane, and in some species covered by a layer of mucilage. Their size ranges from 2 μm to >250 μm in diameter and determines the sinking rate in the water column (Okuda and Neushul 1981). Brown and green algal spores and gametes are flagellated and usually small, between 3 μm and 10 μm in diameter (Henry and Cole 1982; Clayton 1992). Zygotes of the brown algal order Fucales have diameters between 60 μm and 250 μm (Clayton 1992; Schoenwaelder et al. 2003). Spores of red algae are between about 10–100 μm in diameter (Clayton 1992). A positive phototaxis is found in spores of the brown algal genus *Ectocarpus* (Müller 1977) and the green algal genus *Ulva* (Evans and Cristie 1970), a negative phototaxis is found in some primitive Laminariales including *Saccorhiza dermatodea*. However, the capability for active swimming over long distances is rather limited. Even motile propagules have little control over their fate. The range of dispersal is at least 200 m and is driven mainly by currents and water motion (Norton 1992; Frederiksen et al. 1995). The number of chloroplasts per propagule can be very low. The zoospores e.g. of the more advanced families of the Laminariales, the Alariaceae, Laminariaceae and Lessoniaceae only contain one small chloroplast (Henry and Cole 1982), with comparatively low photosynthetic activity (Amsler and Neushul 1991; Wiencke et al. 2000). Storage lipids are certainly the main energy source of spores, supporting swimming and potentially germination processes (Brezezinski et al. 1993; Reed et al. 1999).

Among the different stages in the life-cycle of seaweeds the unicellular propagules are clearly the stages most susceptible to UV radiation. Germination of *Ectocarpus rhodochondroides* spores is inhibited by UVB, while adult specimens survive (Santas et al. 1998). Similarly, the photosynthetic efficiencies (F_v/F_m) of sporophytes and zoospores of *Laminaria digitata* differ strongly when exposed to PAR, PAR + UVA or PAR + UVA + UVB (Wiencke et al. 2000). Irrespective of the radiation treatment large sporophytes show always considerably higher F_v/F_m values compared to zoospores. Motile zoospores of *Lessonia nigrescens* and *L. trabeculata* from Chile are more UV susceptible than settled spores, gametophytes and young sporophytes (Véliz et al. 2006). The macrothalli of red algae *Mastocarpus stellatus* and *Chondrus crispus* are relatively UV tolerant whereas their carpospores are not (Bischof et al. 2000b; Roleda et al. 2004). Similarly, photosynthetic efficiency of zoospores of the green alga *Ulva intestinalis* exhibits an up to 6-fold higher UVB sensitivity compared to the mature thalli (Cordi et al. 2001). Interestingly, the UV susceptibility of the gametes is even greater.

The UV susceptibility of the photosynthesis and of the DNA in spores is unequivocally related to the depth distribution of the macrothalli as outlined above (see chapters 5.1, 5.1.1.3; Bischof et al. 2000a; Wiencke et al. 2000; Roleda et al. 2004, 2005a). Other negative effects of UV radiation especially on macroalgal spores presumably important for determination of the upper distribution limit concern the cytoskeleton. The phototactic response of zoids of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia* is negatively influenced by UVB (Flores-Moya et al. 2002). Moreover, the motility of zoospores of *L. saccharina* is affected by UVB depending on the actual UV doses (Makarov and Voskoboinikov 2001). This might be related to the observation by Huovinen et al. (2000) who reported that the nuclear division of spores of *Macrocystis pyrifera* is inhibited after UVB exposure. Both nuclear division and the activity of the flagellar apparatus depend on a functional microtubular system, which might be damaged by UV. This would explain also the drastic affects on zygotes observed in *Fucus serratus* and *F. distichus* (Schoenwaelder et al. 2003). No polarisation has been observed in UV exposed zygotes, rather, they remain spherical and there is no further development. Similarly, amphibian and fish zygotes remain undifferentiated and this has been related to UV effects on the microtubuli (Scharf and Gerhard 1983; Strahle and Jesuthasan 1993). The actin cytoskeleton may be affected as well as studies on other fucoids suggest. Actin inhibitors prevent polarisation, cross wall formation and vesicle movement (Schoenwaelder and Clayton 1999), the same effect as after UV treatment of zygotes of the two *Fucus* species (Schoenwaelder et al. 2003).

In spores the balance between the damaging effects of UV radiation and the various repair and protective mechanisms is indicated by the integrative parameter germination. If germination is not inhibited after UV exposure the repair and protective mechanisms are strong enough to outweigh the damaging effects of UV. Spore vitality after UV exposure in *M. stellatus* is higher compared to *C. crispus*, probably due to the higher capability for DNA repair in *Mastocarpus* (Roleda et al. 2004). In this context the size of the algal propagules may be of importance as the UV susceptibility of zoospores of various Laminariales from the eastern Pacific depends on the size of the spores (Swanson and Druehl 2000). The spores most tolerant to UV stress come from shallow water species, whereas the progeny of kelps occupying low-level UV-environments exhibit a lower germination capacity after UV stress. These results are similar to studies of phytoplankton species, in which larger organisms are more tolerant to UV exposure (Karentz et al. 1991b).

A clear dependence of the UV susceptibility of germination and growth of sporelings of coralline algae on the radiation conditions in the habitat of the various species has been demonstrated by Bañares et al. (2002). Spores from species in sun-exposed habitats in the eulittoral were more UV tolerant than spores from a species growing in shaded crevices. Similarly, zygotes of *F. serratus* from the mid eulittoral on Helgoland show an abnormal development after UV exposure, whereas zygotes of *F. spiralis*, a species from the upper eulittoral much stronger exposed to UV, is not affected by the same UV treatment (Schoenwaelder et al. 2003). A clear dependence of the upper depth distribution limit of seaweeds on the UV susceptibility of their spores has also been proven for the three *Laminaria* species from Helgoland mainly due to the different DNA repair capacities and the different content of UV absorbing compounds (Roleda et al. 2005a). Similarly, the UV tolerance of different brown algae from Spitsbergen determines the upper depth distribution limit as indicated by experiments in the laboratory (Wiencke et al. 2000, 2004) and in the field (Wiencke et al. 2006).

Microscale gradients in UVB exposure

Pronounced acclimation of photosynthesis to UV exposure does also take place along microscale gradients as for example in algal mats: Green algal mats do frequently occur as a result of eutrophication in sheltered coastal lagoons. Within these mats usually a steep gradient of solar radiation occurs (Vergara et al. 1997): Top layers are exposed to high surface irradiance, whereas bottom layers are permanently exposed

to very low light conditions or even stay in darkness. It has been show for the two bloom-forming green algal species *Ulva rotundata* and *Chaetomorpha linum* that UVB irradiance substantially contributes in structuring these mat-like canopies (Bischof et al. 2002b, 2003, 2006). Inside the mats, strong gradients are visible for solar (UVB) radiation, but also for physiological responses: loss in photosynthetic pigments and proteins is strongly pronounced in top layers and is diminished with increasing depth inside the mat. Photosynthetic activity is inhibited in the top layers, performs at maximum rates in intermediate thallus layers and, due to light limitation, is again strongly reduced in bottom layers (Krause-Jensen et al. 1996; Vergara et al. 1997; Bischof et al. 2002b). A specific response to the degree of UVB exposure inside these mat-like structures is the specific activity of superoxide dismutase (SOD), an enzyme responsible for the scavenging of superoxide anions generated in photosynthesis under stress full conditions (Bischof et al. 2003, 2006). Depending on the degree of UVB exposure and inhibition of photosynthesis along the depth gradient, the activity of SOD becomes stimulated. Two mechanisms might be involved in this response: either the enzyme becomes activated due to a light effect (due to exposure to short wavelength radiation) or it is the presence of previously generated oxygen radicals (ROS) which act as trigger for increasing SOD activity (Bischof et al. 2006). For higher plants, it was shown that ROS play an important signalling role in signal transduction pathways, in order to respond to UVB exposure (Mackerness et al. 1999).

From the tropics to the poles

As the problem of ozone depletion is rather restricted to the polar and temperate regions, organisms inhabiting the (shallow water zone of) tropical regions are unlikely to be affected by seasonally *enhanced* UVB irradiance, but are of course permanently exposed to much higher UV levels than encountered in polar regions (even under ozone depleted conditions). Thus, tropical species must have developed particularly effective protection strategies in response to high UVB irradiance. Unfortunately, data on UVB protection in tropical species as well as comparative studies on related species from different geographical regions are extremely scarce and the limited information available is not conclusive. In a study on different species of the green alga genus *Cladophora* no hints were found for a particular higher UVB tolerance of a true tropical species (*C. zolingerii* from the Philippines) compared to its cold-temperate congener *C. rupestris* (Bischof and van de Poll unpublished data). Preliminary data on UVB absorption characteristics within the cell wall of both species did not provide any hint for a more effective UVB shielding in the tropical species. Moreover, also physiological parameters tested did not point to a particularly higher UVB tolerance of the tropical species compared to the congener from temperate waters.

Like at the species level, studies on latitudinal UV-patterns at the community level are extremely scarce. To our knowledge, only the study by Wahl et al. (2004) investigated UV-effects on shallow-water macro-epibenthic assemblages at different bio-geographical regions. Their study revealed a consistent pattern of UV-effects at both hemispheres. Species richness and community biomass were negatively affected by UV-treatments. Surprisingly, effects by UVA were more detrimental than those of UVB. UV-effects were transient, disappearing at 80% of all sites within 2–3 months, but persisted at one polar (Norway) and one tropical (China) site, suggesting lack of latitudinal patterns of UV-effects at the community level.

Ecological implications—UVB and the structure of seaweed communities

The consequences of enhanced UVB exposure to ecosystem function are still largely unexplored and hypothetical. However, based on the studies accomplished so far the following assumptions can be made.

Potential effects on primary productivity

Throughout the previous studies, it is shown that all species, which have to withstand UVB in the

field (i.e., the species inhabiting the intertidal and the upper sublittoral zone) possess different and largely efficient mechanisms for acclimation to respond to changes in light climate. However, formation of screening compounds as well as the development of further protective mechanisms require additional energy costs, which may result in reduced growth and primary productivity (Roleda et al. 2006a). This problem is still largely unaddressed but an important field of future investigation. As long as the energy costs for applying protective mechanisms remain unknown a reduction of seaweed productivity in response to increased UVB levels in future cannot be excluded.

Reduced reproductive success and shifts in age structure

The information available on UV-susceptibility of different developmental stages indicates the unicellular spores and zygotes as being most sensitive. Other information on how UV-exposure may affect reproduction and the timing of developmental cycles is hardly available. It is obvious that increased UV-induced spore mortality will result in impaired reproductive success, but may also affect the age structure of seaweed populations (Wiencke et al. 2000, 2004a). Due to high interannual variations in light climate particularly in polar areas (Hanelt et al. 2001) high UV-irradiance may affect zoospore survival in shallow waters in 1 year, as in another year it may not. Thus new recruits are only likely to develop to adult sporophytes if they are protected from high UVB exposure. In certain years with particularly high fluences of UVB reaching the benthic communities a new generation of recruits might therefore fail to develop, while larger age and size classes of sporophytes were rather unaffected due to the already accomplished acclimation on a physiological and morphological base (Altamirano et al. 2003a, b).

Downward shifts in depth distribution

As outlined above there is now evidence, that the species sensitivity to solar radiation stress is a function of depth distribution (Larkum and Wood 1993; Dring et al. 1996b; Hanelt et al. 1997a, c; Hanelt 1998; Bischof et al. 1998a; Wiencke et al. 2006). For several physiological parameters a strong correlation of UVB sensitivity and vertical position on the shore was shown for numerous species (van de Poll et al. 2001, 2002; Bischof et al. 1998a, Gómez et al. 2004, Wiencke et al. 2006). Therefore, it is reasonable to argue that increased UVB, penetrating deeper into the water column, results in a shift of the upper distribution limit of single species to greater water depths. However, at least in the macrothalli several acclimation processes can counteract radiation stress, but the particular sensitivity of spores may be the decisive aspect in this scenario, thus preventing recruitment in shallow waters with high UVB irradiances. Through this process, elevated UVB may result in a shift of seaweed communities to deeper waters (Wiencke et al. 2006).

Succession

Species do not exist in isolation. Rather, multiple species form complex, interacting habitat-specific communities. Results from physiological studies at the species level might not reflect biological responses when repeated at the community level due to e.g., indirect or synergistic effects. Consequently, it seems reasonable that an ultimate assessment of UV-effects should be inferred at the community level (Bothwell et al. 1994). Open space represents a key resource for many species of seaweeds and its colonisation is characterised by a series of species replacements (Sousa and Connell 1992). Three alternative successional models have been proposed by Connell and Slatyer (1977). The facilitation model suggests that initially the propagules of a few species will recruit on open space and their existence will modify the substratum for the settlement of other species. The tolerance model predicts a neutral effect of early on later colonizers with the latter replacing early colonizers due to more efficient resource use by competitive exclusion. Under the inhibition model, early settlers pre-empt the substratum, hindering invasion of subsequent species. The predictions of these models may be altered by overlaying patterns of climatic or

ecological factors (e.g., grazing Farrell 1991; Sousa and Connell 1992).

Only a few studies assessed UV-effects during species succession of shallow-water seaweed-dominated communities (Santas et al. 1998; Lotze et al. 2002; Molis et al. 2003; Molis and Wahl 2004; Wahl et al. 2004; Dobretsov et al. 2005). Surprisingly in all but one study (Dobretsov et al. 2005), UV-effects were only apparent during the early phase of succession. In the study by Dobretsov et al. (2005) it seemed very unlikely that later successional species could cope with UV-effects by acclimatizing at the same time to the UV-regime, e.g., by induction of UV-screening substances. Alternatively and more likely, transient UV-effects during community assemblage might result from intra- and interspecific differences in the susceptibility to UV-exposure for which experimental evidence exists. For instance, differential UV-sensitivities of carpospores influenced recruitment success of two competing Gigartinales-species, partly explaining vertical zonation patterns of both species (Roleda et al. 2004). Similarly, spores from deeper dwelling species exhibit higher mortality rates compared to spores from shallow water species (Wiencke et al. 2000, 2004a, 2006). Moreover, sorus of *Laminaria digitata* is completely opaque, resulting in a sudden and drastic change in UV-exposure ('UV-shock') for released zoospores (Gruber, pers. communication). Finally, several seaweed species, e.g. green filamentous forms, seem particularly well adapted to recruit at UV-exposed sites in the upper eulittoral, i.e., where UV-irradiance is strongest on the shore, and it was experimentally demonstrated that their abundance correlates positively with UV-exposure time (Molis et al. 2003). The invasion of sites high on the intertidal shore might be a selective advantage, as this guarantees a relative shorter exposure to grazers compared to sites lower on the shore. Thus, macroalgal propagules may show species-specific differential UV-sensitivities, which favour initial colonisation of empty space by UV-resistant species. Wahl et al. (2004) suggested that these early colonizers facilitate the recruitment of later successional seaweed and invertebrate species at the majority of their study sites by amelioration of UV-regimes due to protective shading. Similarly Vinebrooke and Leavitt (1999) concluded that diatom mats can precondition the substratum for macroalgal spores by provision of UV-free micro-climates.

The effect of UVB exposure on the early succession of macroalgae was also studied at a rocky intertidal platform at King George Island, Antarctica, revealing a significant reduction of species diversity based on the effects of UVB (Zacher et al. unpublished data). Species recruitment and dry weight was monitored over a period of 15 weeks on artificial substrates exposed in the intertidal zone. Natural UV-exposure was found to affect the density of the green alga *Monostroma hariotii* in the first 10 weeks of the experiment, whereas the density of red algal recruits decreased significantly due to UV after 8 and 15 weeks, respectively. Shannon diversity *H'* dropped as succession proceeded in the PAR + UVA + UVB treatment, increased slightly in the PAR + UVA treatment, and increased in the PAR treatment from the beginning until the end of the experiment. Furthermore, the treatment with PAR alone resulted in a significantly higher diversity at the end of the experiment than the treatment including the total UV-range. After 15 weeks the community excluded from UV radiation showed a significantly higher diversity, evenness and number of red algal germlings and species than communities exposed to the whole solar spectrum. This led to a significant dissimilarity in species composition between these two communities. Diversity was negatively influenced by both UVA and UVB radiation. The results show that Antarctic macroalgal recruits are particularly sensitive to UV-exposure during their first month of development, but that effects change during succession.

Competition

Very few examples of UV-mediated changes in competitive abilities of macroalgae exist (Bischof et al. 2000b; Roleda et al. 2004). However, UV-exposure may quite commonly affect the competitive ability of a macroalgae, if UV-induced changes, e.g., production of MAAs, represent a metabolic cost for the alga. Moreover, the competitive ability of seaweeds may be negatively

affected, if UVB has detrimental effects on growth. As a result, the algae will be shaded by more UV-resistant species and experience further reduced growth due to limited PAR-regimes, similar to what has been predicted for terrestrial plants (Caldwell et al. 1989).

Several species of algae are known to affect the community structure of nearby developing benthic assemblages (Wahl 2001). One possible mechanism of this biogenic neighbourhood effect is the exudation of metabolites that influence settlement. Little is known about UV-induced changes in chemical composition of macroalgal exudates and the possible indirect effects of UV-exposure on the structure and species composition of communities that establish in the vicinity of macroalgae. For example, tri-hydroxycoumarins (phenolics) excreted by the green alga *Dasycladus vermicularis* in response to enhanced radiation conditions protects other macroalgal species from UV-exposure (Pérez-Rodriguez 2000). UV-resistant seaweeds may have a selective advantage over UV-sensitive species by indirectly reducing herbivory. Given that two species of macroalgae have identical physiological properties, except with respect to UV-susceptibility, one would expect the less sensitive species to occur higher on the shore, i.e., where UV-regimes are more severe than lower on the shore, than the UV-sensitive counterpart. Consequently, the UV-sensitive species would be exposed over longer periods to motile consumers, e.g., isopods, and other important herbivores, e.g. sea urchins.

Alga-herbivore interactions

The consumption of plant biomass represents the most fundamental plant-animal interaction, affecting biomass accrual and community composition of photoautotrophs (Sousa and Connell 1992; Duffy and Hay 2000). Consequently, herbivory is one key factor controlling the central ecosystem services of primary producers. Grazing intensity is considered to be lower on terrestrial plants than on aquatic macrophytes (Cyr and Pace 1993), due to a higher availability of seaweed biomass to herbivore attacks, the larger proportion of digestible algal biomass (Hay 1991), and higher mass-specific consumption rates of aquatic herbivores (Cyr and Pace 1993). As a result, aquatic plants may be more strongly top-down controlled than terrestrial counterparts and, thus, trophic interactions in aquatic habitats may be of stronger ecological outreach, e.g., in driving algal recruitment (Diaz-Pulido and McCook 2003), community succession (Farrell 1991) or mediating stability-diversity-productivity relationships of communities (Worm and Duffy 2003).

Seaweeds actively participate in the interaction with herbivores by tolerating consumption with compensatory growth (Cronin 2001), escape from herbivory (Hay et al. 1988), or the defence of grazing (e.g., Cronin 2001). Anti-herbivory defences in seaweeds may either be permanently expressed (constitutive) or induced in response to herbivory (Amsler and Fairhead 2006). Experimental evidence suggests that the induction of anti-herbivory defences is of selective advantage in variable grazing regimes (Karban and Nagasaka 2004), while constitutive defences are more beneficial to algae under a constant herbivory load (Karban et al. 1999). Induced chemical anti-herbivory defences can result in reduced consumption of previously attacked tissues (Ceh et al. 2005), increased feeding dispersal with a concomitant higher risk of herbivores becoming visible to predators (Borell et al. 2004) or both (Borell et al. 2004). The induction of anti-herbivory defences in seaweeds is known to be tissue- and (Sotka et al. 2002; Taylor et al. 2002) grazer-specific (Pavia and Toth 2000; Molis et al. 2006) and that at least some of this specificity is seasonally variable (Molis et al. 2006). Thus, phenotypic plasticity of algal responses to herbivory adds complexity to the trophic interactions between algae and their consumers. Furthermore, seasonality in inducible defences suggests that top-down forces may vary under variable environmental conditions, e.g., UV-regimes.

Cronin and Hay (1996) reported that UV-exposure reduced chemical defences in *Dictyota ciliolata*, making this brown seaweed more palatable to sea urchins and to a lesser extend also to amphipods. The selected UV-exposure time represents common emergence periods for intertidal algae. Consequently, UV-exposure seems to have

the potential to increase algal susceptibility to consumers, especially during summer, i.e., at maximum UV-levels. However, algae form multi-layered piles during low tide, suggesting that only top-layered individuals are fully affected by UV, while specimens positioned deeper in the pile are protected to some extend from UV-exposure. Similar indirect UV-protective effects are known from cyanobacterial mats (Karsten et al. 1998b) and macroalgal dominated subtidal macrobenthic communities (Wahl et al. 2004). Furthermore, UV-effects on algae-animal interactions were absent in the study of Macaya et al. (2005) who did not detect any changes in response patterns between UV-exposed and -shielded *Macrocystis* individuals. This suggests that UV-exposure did not alter the ability to induce anti-herbivory defences in this brown macroalga, at least in the case of some invertebrates. Lotze and Worm (2002) investigated the interactive effects of UV-exposure and grazing on early life stages of a green alga. Their study revealed that the influence of ecological controls, i.e., grazers, on *Enteromorpha*-recruitment were stronger than climatic controls, i.e., UV and temperature, and of opposite sign.

Linking UV-effects on macroalgae with trophic interactions seems to be an interesting, but as yet not fully accounted venue for further exploiting the effects of climatic and ecological drivers on the performance and fitness of seaweeds and the species composition and productivity of seaweed communities. Presently, too few studies tested the interactive effects of UV-exposure and herbivory on alga-consumer interactions to draw general conclusions about the influence of UV-radiation on herbivory. It seems likely that UV-induced chemicals may indirectly change the susceptibility of algae to consumers by altering the function of existing chemicals, which could in- or decrease algal palatability. Alternatively, UV-induced compounds may have multiple functions. Schmitt et al. (1995) demonstrated that herbivore-deterrent chemicals displayed also anti-fouling activities. To our knowledge, only the study by Pavia et al. (1997) suggests multiple functions in UV-induced phlorotannins. The concentration of phlorotannins was lower in controls than in UV-exposed *Ascophyllum nodosum*, with the latter stimulating *Idotea* grazing. As UV-exposure is known to induce the production of many secondary metabolites (e.g., MAAs) it would be interesting to study the direct and indirect effects of these compounds on algae-herbivore interactions. For instance, UV-induced secondary metabolites may affect the anti-fouling ability of a macroalgae and altered epibiontic communities may change algal susceptibility to consumers, resulting in shared doom scenarios or associational resistance (*sensu* Wahl and Hay 1995).

Besides UV-induced effects on macroalgae, patterns of herbivory can also be altered by UV-effects on consumers. McNamara and Hill (1999) showed differential susceptibility of consumers to UVB, suggesting shifts in grazing regimes at sites of high UVB-exposure. Experimental evidence comes from studies on periphyton communities. DeNicola and Hoagland (1996) showed that herbivore density under UV-exposure was on average 50% reduced compared to PAR-treatments at the end of a 28 d long experiment. Bothwell et al. (1994) reported that, in the presence of herbivores, biomass accrual in UVB-exposed periphyton communities was higher than in PAR-irradiated communities. This suggests stronger negative effects at the consumer than at the producer level and, consequently, an indirect positive UVB-effect on periphytic biomass production. Future studies should focus on UV-effects on herbivores to elicit the relative harmfulness of UV-exposure on seaweeds and their consumers.

Synthesis

Under present radiation conditions, UVB radiation has already to be regarded as a common abiotic factor, which influences the physiology of individual seaweeds but does also contribute in structuring seaweed communities in various ways. However, its potential threat to biologic processes is counteracted by several adaptive strategies activating different protective and repair mechanisms. These mechanisms (e.g., formation of screening compounds, antioxidative systems, regulation of enzyme activity and gene expression, DNA repair mechanisms) serve as a physiological filter to reduce the adverse effect of the impinging

solar UVB, in addition canopy algae also protect more susceptible subcanopy organisms from solar exposure. Based on the differential adaptation and acclimation capabilities realised in the different species UVB radiation may, even under non-depleted ozone conditions, substantially affect the structure of seaweed communities. As outlined above, UVB exposure may modulate productivity, reproduction, vertical distribution, species diversity and succession, competition and alga-herbivore interactions. However, the effect extent of each of the aspects under ozone-depleted conditions is largely unknown. To improve our knowledge on how UVB may shape seaweed communities and thus rocky coastal ecosystems in the future, further research should predominantly focus on the following three directions: (1.) UVB is not the only factor potentially causing abiotic stress to seaweed photosynthesis. Particularly the intertidal zone is characterized by large variation of abiotic parameters, furthermore anthropogenically caused increases in seawater temperature and nutrient loads also affect seaweed performance in the field. Physiological studies on the interaction of a multitude of abiotic (stress) factors are indispensable. (2.) The molecular mechanisms triggering acclimation strategies are largely unknown. The application of molecular tools also in seaweed physiology will be a major step forward in this respect. (3.) Certainly, the most serious lack of information is still present regarding the ecological interactions modulated by UVB exposure. As outlined above, only fragmentary data sets are available so far addressing how UVB is presently interfering with seaweed succession or in interactions with seaweed-associated consumers. Field studies on a large spatial and time scale would be required including the generation of UV-free, ambient UV and increased UV irradiation treatments. Such as kind of experiment is logistically ambitious but would substantially broaden our knowledge on how UVB will alter the structure of seaweed communities in the future.

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Two years of *in situ* UV measurements at Dallmann Laboratory/ Jubany Base

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1. Introduction

Solar radiation measured at the Earth's surface is subject to atmospheric absorption and scattering by air molecules, trace gases, aerosols and clouds. Of great importance for life on Earth is the photosynthetically active radiation (PAR, wavelengths: 400-700 nm) and ultraviolet (UV) radiation. UV radiation is sub-divided into UVC (<280 nm), UVB (280-320 nm) and UVA (320-400 nm). While the UVC radiation is completely absorbed in the atmosphere, some UVB can reach the Earth surface. With the stratospheric ozone layer at an altitude of 15 to 30 km thinning (e.g. Nardi et al. 1999), more of the energetic and biologically effective UVB radiation can reach the Earth surface and can damage living organisms (Environmental Effects Assessment Panel, 2006). Towards shorter wavelengths spectral UVB irradiance measured at the Earth's surface shows a steep decrease over six orders of magnitude (see Figure 1).

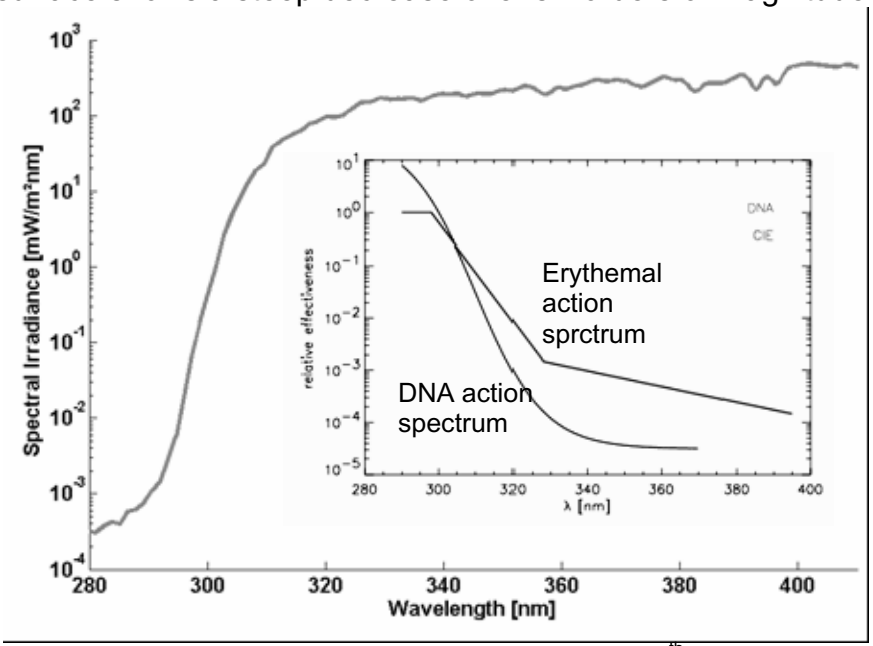

Figure 1: Spectrum of UV irradiance measured on 15th October 2005 aboard the research vessel *Polarstern*. The smaller picture shows the DNA and erythemal action spectrum. To obtain the biologically effective irradiance, the UV spectrum needs to be convoluted with the action spectrum.

Particularly organisms in Antarctica are exposed to enhanced UV irradiance every spring due to the transient loss of 70 to 80% of the stratospheric ozone, the so-called "ozone hole" (WMO, 2006). Enhanced UV radiation can penetrate deeper into the water column, thereby affecting marine organisms. Therefore it is of great importance to study the attenuation of UV in the water body. All kinds of particles in the water modify the radiation by scattering and absorption

processes. Due to refraction at the water surface, the apparent solar zenith angle under water differs from the one in the atmosphere.
At Dallmann Laboratory/Jubany Base (King George Island, Antarctic Peninsula), UV measurements have been carried out with broadband instruments since the 1990s (Gómez *et al.* 1997). A ground based UV spectroradiometer is in use during the summer seasons since 2002.

Here, we report UV and PAR measurements with broadband instruments on ground and under water for the summer season 2004/05 as well as ground based spectral measurements for 2004. Additionally, first measurements with a submergible multichannel UV spectroradiometer have been performed during two summers to characterize the seasonal variation of PAR and UV transmittance in the water column. We give a brief description of the instruments used, the measurement techniques, data quality control, and present results obtained during the austral summers 2003/04 and 2004/05.

2. Radiation detectors

Broadband sensors measure the radiation over a wide spectral range. Some instruments have a response similar to the erythemal action spectrum (McKinley and Diffey, 1987), others integrate the incident radiation without weighting. Broadband instruments are comparably cheap and easy to handle.
The advantage of spectrally resolved measurements of UV irradiance is that any type of action spectrum can be applied to the data. However, these instruments need careful calibration in the laboratory and in the field. Two major challenges have to be tackled when measuring spectral UV irradiance, which result from the properties of the solar spectrum: First, the wavelength calibration has to be very precise. According to Bernhard and Seckmeyer (1999) the misalignment of 0.1 nm in the UVB regime implies an error of up to 3.5% for the DNA weighted UV irradiance. UV data have to be corrected for possible wavelength shifts. A widely used method is the comparison of the UV spectra to an extraterrestrial solar spectrum (Slaper *et al.* 1995). Second, UV sensors have to be able to record the incident irradiance over six orders of magnitude. This is due to the strong ozone absorption of UVB irradiance in the atmosphere (see Figure 1). Thus, UV spectroradiometers have to be capable of detecting high intensities without saturating the detector and of suppressing noise and stray light well enough to give reliable data for the shorter wavelengths down to the detection limit.

2.1 Broadband sensors

A LiCor data logger (LI-1400, Li-Cor, USA) equipped with a flat-head cosine corrected PAR quantum sensor for air and underwater measurements (LICOR 190 SA and LI-192, respectively) was used to record PAR values at the surface (5 min intervals in summer 2004/2005) and under water. For the underwater measurements two sensors were used, one fixed at 1 m depth and the other lowered in 1 m steps. A Solar Light (PMA2100, Solar Light Co. Inc., USA) with a UVB (PMA2106-UW) and a UVA (PMA2110-UW) radiation broadband sensor was used for weekly radiation measurements, both at the surface and at certain depths under water in 2004/05.

2.2 UV spectroradiometers

The land based UV spectroradiometer designed by ISITEC GmbH consists of a separate UVA and UVB sensor. It was installed on the roof of the Dallmann Laboratory. As the solar UVA spectrum does not cover such a big range of orders of magnitude, it is sufficient to use a single monochromator for the UVA spectroradiometers. The detector consists of a 256 channel diode array. The UVB sensor is equipped with a Bentham DM 150 double monochromator and a 32 channel photomultiplier plate. The signals of all channels are recorded simultaneously, and 5-minute means were stored. For a complete description of the instrument we refer to Hanken and Tüg (2002).

The underwater measurements have been carried out with an underwater spectroradiometer, similar to the ground based instrument, mounted in a waterproof housing. The UVA sensor covers also the PAR region and was developed by Kruse. The diode array detector (MOS Linear Image Sensor S3901-265Q, Hamamatsu, Japan) is composed of 265 pixels. The spectral distance between two neighbouring channels is 1.6 nm, resulting in a wavelength range of 300-700 nm. The UVB sensor measures irradiance from 280 to 323 nm. The spectral distance between two channels is 1.35 nm. The power supply during field campaigns was realised by storage batteries. The underwater instruments were deployed from a small zodiac. For taking a vertical profile of UV irradiance in the water column, the sensor was usually put into the water in steps of 1 m to record data for one minute.

3. Calibration and correction of the spectral data

In contrast to the ground based spectroradiometer, the underwater ones are not temperature stabilized. Dark current and sensitivity of the instruments strongly depend upon temperature. Reliable absolute irradiance values can only be obtained by calibrating the instruments at the ambient water temperature. Otherwise only relative data can be calculated. The water temperature does not change much with depth (compare with Hanelt *et al.* 2004), so sensitivity and dark current are expected not to vary during the measurement time. The dark current of the instruments is determined before and after each field measurement by closing the optics with a black cover.

The spectral instruments provide raw data S in counts per second for each channel. The sensitivity R of each channel is determined in the calibration process, where a lamp with a known emittance E is measured. With correction for dark current DC we obtain $R=(S-DC)/E$ (1). With known sensitivity R, equation 1 can be rearranged to calculate the spectral irradiance $E = (S-DC)/R$ (2).

The underwater spectra obtained during the field campaigns 2003/04 and 2004/05 were corrected in the following way: Their wavelength shift was corrected by comparing with the Kurucz extraterrestrial solar spectrum (Kurucz, solar flux atlas). It was found to be generally less than 1 nm. The data cannot be given in absolute values, due to missing calibration at the corresponding temperature, but after dark current correction they were normalized to the surface value. The UV irradiance at different depths is hence given in per cent of the surface value. In the following examples, the UV irradiance was integrated over wavelength for the different parts of the spectrum, UVB, UVA and PAR.

During the measurement time required to complete a profile, atmospheric conditions may change resulting in enhanced or decreased values of UV at greater depths. In this case parallel ground based UV irradiance measurements can be used to correct for this effect. Even on almost cloud free days, which are quite rare at Antarctic Peninsula, the changes of radiation due to thin, barely visible cirrus clouds are not negligible.

4. Area of investigation

Land based measurements were performed directly at Dallmann Laboratory/Jubany Base, King George Island, Antarctica, during the austral summer seasons 2003/2004 and 2004/2005. Three locations were chosen to perform underwater measurements (see map in Zacher & Campana, this issue): (i) Peñón de Pesca (62°14'S 58°43'W; max. measured depth 14 m), (ii) Potter Cove (62°13'S 58° 40'W; max. measured depth 20 m) and (iii) Peñón Uno (62°14'S 58°41'W; max. measured depth 7 m). Potter Cove is a bay opening towards the west. Due to a cyclonic circulation pattern, fresh water from the open ocean enters the cove via the measuring point Peñón de Pesca. Then the water passes the area of a glacier in the east of the bay (measurement point Potter Cove), and finally passes Peñón Uno (see also Roese and Drabble, 1998).

5. UV field measurements

5.1 Land-based UV irradiance

Highest daily UVA and UVB doses were observed in December whereas in the austral winter UV radiation does not reach the surface (Figure 2). A high seasonal variation and day to day variability depending on cloud cover was observed.

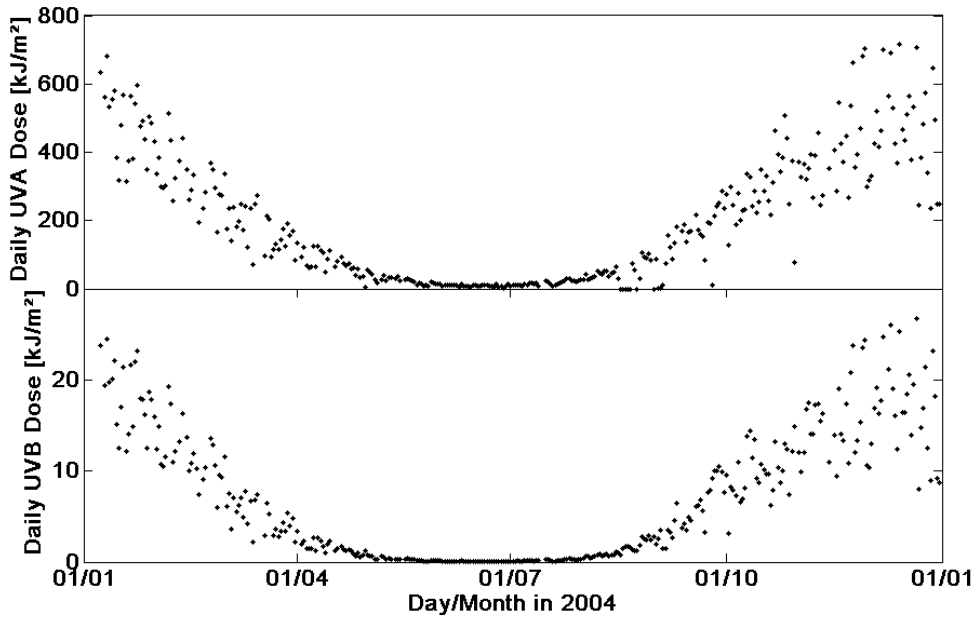

Figure 2: Daily dose of UVA (upper panel) and UVB (lower panel) irradiance measured with the ground based spectroradiometer at Dallmann Laboratory/Jubany Base in 2004.

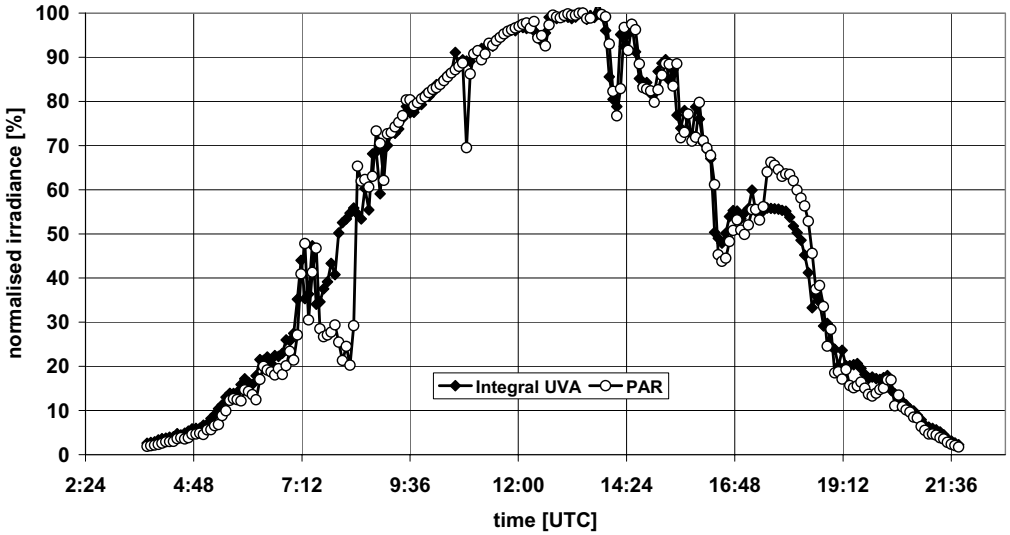

Figure 3: PAR and UVA measured at Dallmann Laboratory/Jubany Base on 14th December 2004 on a mostly sunny day. The values of each data set are divided by their maximum value to better illustrate the differences. Without clouds, both curves show the same behaviour. When clouds were passing, high differences in radiation (up to 35%) were detected by the two systems.

Also the PAR measurements (2004/2005) showed a high variability, both from day to day as within one day (Figure 3).

Table 1: 10% depth and k_d value of UVB, UVA and PAR ± S.D. at the three different sampling areas measured with broadband and spectral instruments.

Mean 10% depth [m], k_d	**Peñón de Pesca**			**Potter Cove**			**Peñón Uno**		
Broadband 10% depth	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Nov/Dec 04	2.7 (±2.3)	5.5 (±4.9)	-	1.7 (±1.0)	2.6 (±2.1)	-	1.9 (±0.2)	3.1 (±2.0)	-
Jan/Feb 2005	4.3 (±1.9)	7.1 (±1.6)	10	1.7	0.8	-	2.1 (±0.8)	3.9 (±1.7)	3.5
Spectral 10% depth	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Nov/Dec 2003	10	3.5	12.2	6.6 (± 1.3)	5.8	8.1 (±0.1)	4.5 (±0.1)	3.6	4.5
Jan/Feb 2004	4.9 (±3.9)	-	-	-	-	-	3.2 (±1.9)	-	-
Nov/Dec 2004	-	6.9	7.1	0.8	4.5	6.0	-	5.0	5.5
Jan/Feb 2005	-	11.1	14.0	-	-	-	-	4.2	5.5
Broadband k_d	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Nov/Dec 2004	1.0 (±0.7)	0.7 (±0.4)	-	1.4 (±0.6)	1.0 (±0.4)	-	1.5 (±0.9)	1.2 (±1.1)	-
Jan/Feb 2005	0.6 (±0.2)	0.5 (±0.1)	0.4	2.4	2.3	-	1.0 (±0.4)	0.7 (±0.3)	0.8
Spectral k_d	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Dec 2003	0.3	0.4	0.2	0.4 (±0.1)	0.4 (±0.2)	0.3 (±0.2)	0.5 (±0.2)	0.6	0.5
Jan 2004	0.7 (±0.2)	-	-	-	-	-	0.8 (±0.2)	-	-
Nov/Dec 04	-	0.4	0.4	0.6	0.5	0.4	-	0.6	0.8
Jan/Feb 2005	-	0.3	0.3	-	-	-	-	0.6	0.5

5.2 Underwater UV irradiance

As examples for the results of radiation profile measurements, the 10% penetration depth of radiation (± Standard Deviation; S.D.) as well as the diffuse vertical attenuation coefficient of downward irradiance (k_d, definition after Kirk 1994) are reported. Low k_d values describe transparent water with little attenuation of radiation, whereas high k_d values mean turbulent water with a high extinction. Table 1 shows the mean 10% depth (± S.D.) at the three different study sites measured with the broadband and spectral instruments and the mean k_d value (± S.D.), respectively.

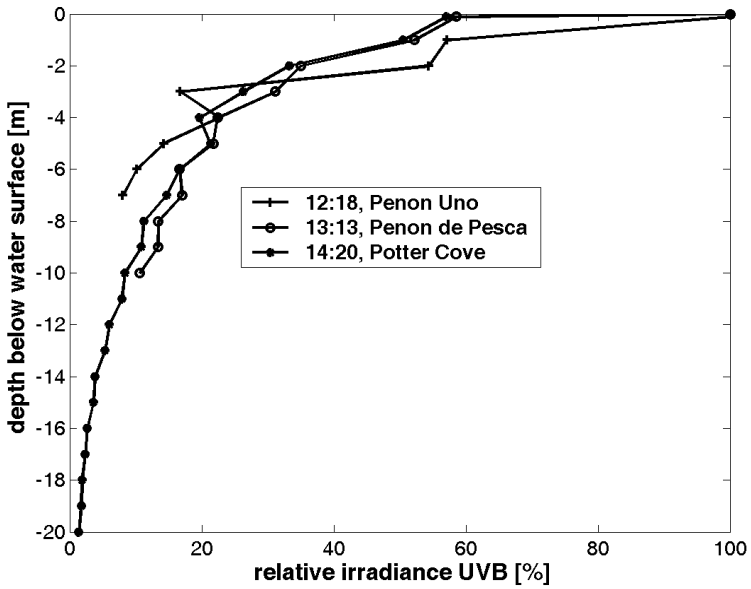

Figure 4: UVB irradiance profiles measured at 3 different sites on 29th December 2003, before the onset of glacier melting.

Figure 4 shows UVB profiles, measured at the three different sites on the same day, 29th December 2003, under clear water conditions. The profiles of Peñón de Pesca and Potter Cove show the same attenuation of radiation, the profile of Peñón Uno is characterised by variations due to changing radiation conditions. Additionally, the profiles of relative PAR radiation were measured at Peñón de Pesca and Peñón Uno with two LiCor sensors on January 2nd 2005 (Figure 5). PAR is much more attenuated at Peñón Uno compared to Peñón de Pesca.

6. Discussion and recommendations for future measurements

As shown in Section 5.1, UV radiation values measured in the Antarctic during the existence of the ozone hole have a high variability both from day to day and in the time scale of minutes. Figure 4 illustrates that even instruments that are operated at very close distance and averaged over the same time interval do not necessarily measure the same changes in radiation caused by fast moving clouds.

It is a difficult task to measure underwater UV profiles. Only on days with very low wind speeds (<10 m/s) it was possible to perform successful measurements. The measurements have shown that the penetration depth into the water is also subject to high variations (see Section 5.2). Those facts are

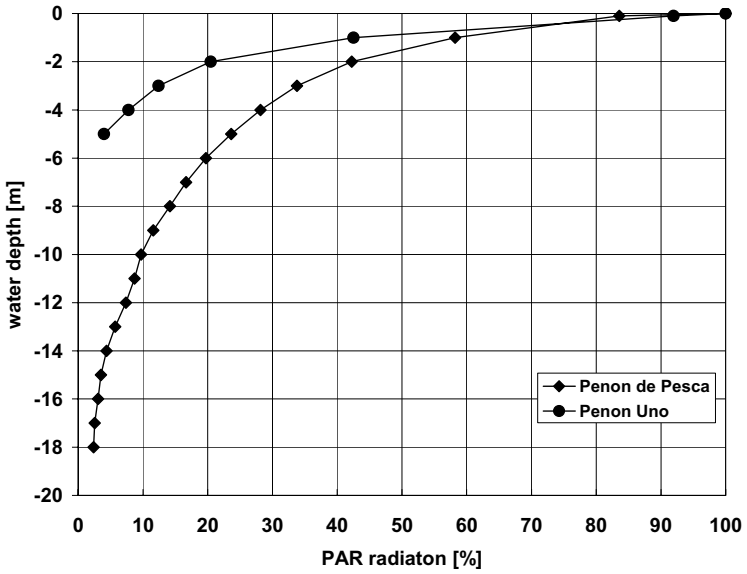

Figure 5: PAR profiles measured with two instruments at Peñón Uno and Peñón de Pesca on the same day, 2nd January 2005, after the onset of glacier melting.

important for biological studies of the effects of UV radiation on e.g. algae (Gomez *et al.* 1998, Zacher *et al.* unpublished).

The profile measurements performed at the three sites in spring before the melting process enriches the water with sediment showed a similar behaviour for both seasons, for UVA and UVB. The penetration of radiation into the water body was in the same order of magnitude with high uncertainties. In spring, before the end of December, the water was very clear at all places. With the onset of melting and the glacier bringing sediments into the water, it became more opaque during summer, especially at Potter Cove, near the source of the sediments, and at Peñón Uno, where the turbid water passes on the way out of the cove, whereas Peñón de Pesca was little or not affected by the meltwater. The penetration depth of radiation at Potter Cove was much less than at Peñón Uno for some days and the UV radiation at Potter Cove was already completely absorbed at 2 m depth in summer. The variability of UV profiles generally depends on water turbidity, mixing of the water with sediments from the melting and calving glacier as well as algae blooms (Vasilkov *et al.* 2005, Piazini *et al.* 2001). The 10% penetration depth of UVB and UVA in spring at our measuring sites were similar to other measurements in Antarctic waters reviewed by Tedetti and Sempéré (2006), and clearly higher than generally for coastal waters. The k_d values were comparable to values for clear waters reported by Smith and Baker (1981). This means that subtidal organisms in this area can be especially affected due to coinciding enhanced UVB radiation and very clear water conditions during spring.

The high uncertainties, sometimes up to the same order of magnitude as the measured value, are caused by the highly variable radiation conditions with time and the ratio of two values measured at different times. This effect can only be corrected successfully with a second sensor that is not subject to underwater changes, recording the radiation with the same time resolution at the surface. The need for such an instrument is demonstrated in Figure 3. A first example with two PAR sensors is shown in Figure 5.

Another problem for the spectroradiometers is the change in water temperature. In laboratory studies it was found that a change of the surrounding temperature by 1°C results in changes in the sensitivity of up to 7%, with differences for each channel. The large standard deviation is due to the few measurements and their high variability. To obtain statistically significant underwater UV irradiance data, more measurements are needed.

For future measurements, it is crucial to pay attention to the characterization of the spectral instruments and quality control of the measured radiation data as described in Section 3 to obtain reliable absolute values. For recording vertical profiles in the water, a second instrument at constant depth or at the water surface, very close to the profiling site and with the same time resolution should be used to monitor atmospheric radiation conditions, as presented here with two PAR sensors.

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ANLAGE ZUR DISSERTATION

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Erklärung

Gem.§6 (5) Nr.1-3 PromO

Ich erkläre, dass ich KATHARINA ZACHER

1. **die Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,**
2. **keine anderen, als die von mir angegebenen Quellen und Hilfsmittel benutzt habe**
3. **die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.**

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(Unterschrift)