

Interactive abiotic stress effects on Arctic marine macroalgae
- Physiological responses of adult sporophytes

Jana Fredersdorf



University of Bremen, Germany
March 2009

University of Bremen
Alfred-Wegener-Institut for Polar and Marine Research

Interactive abiotic stress effects on Arctic marine macroalgae
- Physiological responses of adult sporophytes

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr.rer.nat.)
im Fachbereich Biologie/Chemie
der Universität Bremen

vorgelegt von

Jana Fredersdorf

Gutachter: 1. Prof. Dr. Kai Bischof
2. Prof. Dr. Christian Wiencke

Bremen, 2009

Contents

Summary

Zusammenfassung

1	Introduction	1
1.1	Environmental changes in the Arctic ecosystem: Influences of global warming and ozone depletion	1
1.2	Impacts of changes in abiotic factors on marine macroalgae, especially variations of UV-radiation, temperature and salinity	3
1.2.1	Effects of single stress factors	3
1.2.2	Interactive effects of combined factors	5
1.2.3	Protective mechanisms in macroalgae	7
1.3	Aims of the present thesis	8
2	Publications	10
	List of publications	
	Declaration of contributions to the publications	
3	Material and Methods	11
3.1	Study area and Algal material - an overview	11
3.2	Experimental set-up for multifactorial treatments	13
3.2.1	Field experiment under natural environmental conditions exposed to temperature und UV-radiation stress effects	13
3.2.2	Laboratory experiment with artificial defined stress conditions	14
3.2.2.1	Temperature combined with UV-radiation or salinity	14
3.2.2.2	Different PAR intensities under constant levels of UV-radiation	15
3.2.2.3	Three-factorial setup for impacts on germination success of zoospores	15

3.3	Analytical methods	16
3.3.1	Measurements of photosynthesis	16
3.3.2	Determination of pigment contents	16
3.3.3	Analysis of mycosporine-like amino acids (MAA)	17
3.3.4	Statistical analysis	17
4	Summary of Results	19
4.1	Photosynthetic responses to interactive effects of temperature and UV-radiation	19
4.2	Photosynthetic responses to interactive effects of temperature and salinity	20
4.3	Impacts of temperature, salinity and UV-radiation on different life history stages	22
4.4	Effects of different irradiance levels of PAR under constant UV-radiation	22
4.5	Impacts of abiotic factors on physiological protective mechanisms	23
4.5.1	Photosynthetic pigments	23
4.5.2	Mycosporine-like amino acids	24
5	Synopsis of Discussion	25
5.1	Interactive effects on photosynthesis of Arctic marine macroalgae	25
5.2	Predominant single effects on photosynthesis of Arctic marine macroalgae	27
5.3	Sensitivity of the zoospore stage of <i>Alaria esculenta</i> to abiotic stress	29
5.4	Influence of abiotic interactions on protective mechanisms	30
5.5	Importance of the ratios of PAR:UV in experimental studies	33
5.6	Ecological conclusion and future perspectives	35
	References	39
	Attached Publications I-V	46

Acknowledgements

Summary

In Arctic coastal ecosystems, marine macroalgae are exposed to a multitude of abiotic and biotic stressful conditions, including naturally seasonal variations and additionally potential effects of climate changes. Although the different abiotic variables are acting in combination and interdependently in the natural environment, the responses of macroalgae to interactive effects of multiple abiotic stressors are relatively unknown. UV-induced effects and growth temperatures are well studied on different macroalgal species, but mainly in single-factor experiments. Accordingly, the present thesis aims to detect interactive effects of combined abiotic environmental factors on Arctic marine macroalgae from the Kongsfjord (Spitsbergen) for a better understanding of their stress physiology and to estimate potential ecological implications. The study focused on physiological responses of different macroalgal species exposed to impacts of temperature stress in combination with radiation as well as salinity conditions.

In bi-factorial laboratory experiments, adult sporophytes of the three kelp species (Phaeophyceae) *Alaria esculenta*, *Saccharina latissima* and *Laminaria solidungula* were exposed to different temperatures between 0 and 21°C in combination with artificial radiation conditions (PAR= photosynthetically active radiation, UV= ultraviolet radiation: UV-A; UV-B) or with different artificially produced salinities (34, 28, 20) for a duration of six days. Additionally, the response of adult tetrasporophytes of the red alga *Devaleraea ramentacea* (Rhodophyta) was investigated under different temperatures between 4 and 17°C combined with natural ambient radiation conditions in a field experiment and with different diluted salinities in a laboratory experiment.

The photosynthetic response of these Arctic marine macroalgae reflected a wide tolerance of investigated abiotic stress factors. Accordingly, they turn out to be highly tolerant to both, changes of single as well as multiple and combined abiotic factors. Almost no interactive effects, neither of temperature in combination with radiation nor in combination with salinity were detected concerning photosynthetic performance of the macroalgae. Only the photosynthetic efficiency of the kelp *S. latissima* was affected by an inhibitory interaction of high temperature combined with photosynthetically active radiation on recovery. A possible reason for this incomplete recovery could be the absence of a potential supporting effect of short UV-radiation wavelengths during the recovery process.

Temperature as a single parameter was the factor causing most influences on the photosynthesis of all investigated Arctic marine macroalgae. The observed photosynthetic temperature tolerance showed species-dependent effects, agree basically with respective previous growth studies and correlates with the geographical distribution pattern of the species. In addition to pronounced temperature effects, significant UV-induced inhibitions were detected on photosynthetic performance of *A.esculenta* sporophytes exposed to the whole light spectrum under the lowest temperatures applied. This indicates that the sensitivity of algae to additional stress factors, e.g. to UV-radiation increases at temperatures lower than the optima, whereas increasing temperature seems to mitigate UV-induced effects. Overall, the results suggest a relatively high photosynthetic ability of the examined macroalgae to acclimate to a short-term stress caused by UV irradiation. Furthermore, all investigated macroalgae tolerated the tested salinity stress conditions ranging from fully marine to hyposaline conditions. The photosynthetic responses of the macroalgal sporophytes showed only few species-dependent individual effects of salinity. The Arctic endemic, deep-water species *L.solidungula* seems to have a more restricted acclimation potential to salinity changes.

For comparison, two different life history stages of the kelp *Alaria esculenta* were examined to detect stage-specific interactive effects of temperature, radiation and salinity. The response to abiotic stress of the different life stages was diversely. In the bi-factorial laboratory experiments using diploid adult sporophytes, photosynthetic efficiency mainly showed individual temperature effects. In contrast, the germination capacity of haploid *Alaria* zoospores was affected by temperature and salinity changes, and additionally by interactions of both. In the three-factorial experiment, germination rates of zoospores were strongly affected by the interaction of low salinity combined with a relatively high temperature. Microscopic zoospores were proved to be more sensitive than adult macroscopic sporophytes.

Multiple strategies as protection against mainly high radiation conditions exist in marine macroalgae, but diverse abiotic stressors or their interactions can also influence those. Findings of the present study could provide also indications of potential additional protective mechanisms occurring in the investigated marine macroalgae.

The amount of the photosynthetic pigments fucoxanthin in the kelp *L.solidungula* was affected by salinity changes after exposure to combined temperature and salinity stress. In contrast to an unaffected chlorophyll *a* content, a higher fucoxanthin concentration was measured at ambient fjord salinity, but no temperature effects were detected. Changes of the

total content of mycosporine-like amino acids (MAAs) in the red alga *D.ramentacea* exposed at combined temperature and irradiation stress were also observed with a generally higher MAA concentration under UV-impact. The function of MAAs as natural UV-sunscreens was confirmed, a function as thermal protector can be excluded. However, interactive effects of radiation and temperature, especially UV-A radiation in combination with low temperature, affected the MAA accumulation in *D.ramentacea* and could be an indication for potential low temperature-induced MAAs in polar macroalgae as additional UV-protection. In the green alga *Ulva lactuca* (Chlorophyta), the conversion of violaxanthin within the xanthophyll cycle, a photoprotective mechanism seems to be decelerated due to the presence of UV-radiation. In addition, the concentration of the protective pigment lutein increased considerably in *U.lactuca* samples under radiation stress. This could be indicative for the existence of an additional light-protective mechanism, as e.g. the lutein-epoxid cycle in macroalgae.

In an ecological context, the respective ratio of photosynthetically active to ultraviolet radiation applied in laboratory experiments is of crucial importance in order to avoid overestimation of UV-induced effects. A study on photosynthetic response of the green macroalga *Ulva lactuca* exposed to constant UV-radiation at varied irradiances of PAR shows the importance of PAR/UV interactions. The results clearly demonstrate that the extent of UV-induced inhibition of photosynthesis is highly depending on the irradiance of background photosynthetically active radiation. With increasing PAR-intensity the share of UV-induced inhibition of photosynthetic efficiency is reduced: The higher the PAR-intensity the lower the UV-effects.

In conclusion, this thesis demonstrates the importance of research on physiological responses of macroalgae to interactions between two or more environmental stress factors. It suggests that multiple stress factors interact synergistically or that one factor prevails as a single effect. Temperature seems to be the ecological predominant and most influential environmental parameter for marine macroalgae. In an ecological context and in particular with regard to the forecasted environmental changes in the Arctic, the adult sporophytes of *D.ramentacea*, *S.latissima*, *A.esculenta*, *L.solidungula* proved to be highly tolerant and adaptable to increased temperature and UV-radiation and a decreased salinity. In comparison to the temperate kelp species, the Arctic endemic species *L.solidungula* seems to have a more limited ability to acclimate to its changing Arctic habitat. These detected tolerances are only valid up to a yet relatively unknown species-specific limit.

Zusammenfassung

Marine Makroalgen in arktischen Küstenökosystemen sind einer Vielzahl von abiotischen und biotischen Stressbedingungen ausgesetzt. Diese Bedingungen können sowohl durch natürliche saisonale Schwankungen, als auch durch mögliche Auswirkungen eines Klimawandels hervorgerufen sein. Die unterschiedlichen abiotischen Faktoren wirken dabei in ihrer natürlichen Umgebung zusammen und sind voneinander abhängig. Die Reaktion der Algen auf interaktive Einflüsse aus dem Zusammenwirken mehrerer Faktoren ist bisher relativ unbekannt. Im Rahmen von einfaktoriellen experimentellen Untersuchungen wurden in der Vergangenheit hauptsächlich die Einwirkungen von schädigender ultravioletter Strahlung auf Makroalgen untersucht sowie deren optimale Wachstumstemperaturen und jeweilige artspezifische Toleranzen und Anpassungsfähigkeiten bestimmt.

Vor diesem Hintergrund ist es das Ziel der vorliegenden Arbeit, interaktive Einflüsse kombinierter abiotischer Umweltfaktoren auf arktische marine Makroalgen aus dem Kongsfjord (Spitzbergen) zu untersuchen, um deren Stressphysiologie besser zu erfassen und mögliche Folgen für die Ökologie der Algen abschätzen zu können. Dabei konzentriert sich diese Studie auf die physiologischen Reaktionen unterschiedlicher Makroalgenarten auf die Einwirkungen von Temperaturstress in Kombination mit Strahlungs- beziehungsweise Salinitätsstress.

Zu diesem Zweck wurden zweifaktorielle Laborexperimente mit adulten Sporophyten der Braunalgen (Phaeophyceae) *Alaria esculenta*, *Saccharina latissima* and *Laminaria solidungula* durchgeführt. Dabei wurden die Algen für sechs Tage bei unterschiedlichen Temperaturen zwischen 0 und 21°C unter künstlichen Strahlungsbedingungen (PAR= photosynthetisch aktive Strahlung, UV= ultraviolette Strahlung: UV-A; UV-B) beziehungsweise in verschiedenen Salinitäten (34, 28, 20) exponiert. Darüber hinaus wurden adulte Sporophyten der Rotalge *Devaleraea ramentacea* (Rhodophyta) nach Exposition bei verschiedenen Temperaturen zwischen 4 und 17°C kombiniert mit natürlichen Strahlungsbedingungen im Freilandversuch, sowie mit verschiedenen Salinitäten im Laborversuch untersucht.

Die Reaktion der Photosynthese dieser arktischen marinen Makroalgen reflektiert eine hohe Toleranz gegenüber den untersuchten abiotischen Stressfaktoren. Die Algen erwiesen sich als sehr tolerant sowohl gegenüber einzelnen, als auch gegenüber kombinierten abiotischen Faktoren. Dabei wurden kaum interaktive Beeinflussungen der Photosynthese-Effizienz der

Algen, weder durch Temperatur in Kombination mit Strahlung noch mit Salinität beobachtet. Einzig die photosynthetische Effizienz von *S.latissima* war im Experiment nach der Erholungsphase durch das Zusammenwirken von hoher Temperatur mit photosynthetisch aktiver Strahlung ohne UV-Anteil beeinträchtigt. Ein möglicher Grund für diese unvollständige Erholung könnte im Fehlen eines potentiell fördernden Effekts kurzweiliger UV-Strahlung während des Erholungsprozesses liegen.

Temperatur als Einzeleffekt zeigte sich als der Faktor mit dem größten Einfluss auf die Photosynthese aller untersuchter marinen Makroalgen. Die beobachteten Temperaturtoleranzen in der Photosynthese-Effizienz zeigten artspezifische Effekte, die grundlegend mit den Ergebnissen bisheriger Wachstumsstudien der jeweiligen Arten übereinstimmen und mit ihren geografischen Verbreitungsmustern korrelieren. Zusätzlich zu den deutlichen Temperatureffekten wurde, allerdings ausschließlich in der photosynthetischen Effizienz von *A.esculenta*, eine signifikante, UV-induzierte Inhibition nach Exposition unter dem gesamten Lichtspektrum bei den niedrigsten Temperaturen festgestellt. Dies zeigt, dass die Empfindlichkeit von Algen gegenüber zusätzlichen Stressfaktoren, wie z.B. UV-Strahlung bei Temperaturen, die niedriger als das artspezifische Optimum sind, zunimmt, wobei höhere Temperaturen möglicherweise UV-induzierte Effekte abschwächen können. Insgesamt weisen die vorliegenden Ergebnisse auf eine relativ hohe Anpassungsfähigkeit der untersuchten Makroalgen an kurzzeitigen Stress durch UV-Strahlung hin. Überdies tolerierten alle untersuchten Algenarten die getesteten Salinitätsschwankungen von marinen bis hin zu hyposalinen Bedingungen. In den photosynthetischen Reaktionen der Algen zeigten sich nur einzelne, allerdings artspezifische Salinitätseffekte. Somit scheint die arktisch endemische Art *L.solidungula*, welche in größeren Wassertiefen vorkommt, ein eingeschränktes Anpassungspotential an Salzgehaltsschwankungen zu haben.

In einem weiteren Versuch wurden zwei unterschiedliche Lebensstadien der Braunalge *A.esculenta* untersucht, um mögliche spezifische interaktive Effekte von Temperatur-, Strahlungs- und Salinitätsstress festzustellen. Die Auswirkungen der untersuchten Stressfaktoren auf die zwei Lebensstadien war unterschiedlich: Die photosynthetische Effizienz der diploiden adulten Sporophyten zeigte in den zweifaktoriellen Laborversuchen vorrangig durch Temperaturstress induzierte Einflüsse. Dagegen beeinflussten Temperatur- und Salinitätsveränderungen sowie deren interaktiven Effekte die Keimungsfähigkeit der haploiden Zoosporen. In einem dreifaktoriellen Experiment zeigte sich die Keimungsrate der Zoosporen durch das Zusammenwirken von niedriger Salinität mit relativ hoher Temperatur

stark beeinflusst. Demnach reagieren die mikroskopisch kleinen Zoosporen scheinbar empfindlicher als die adulten makroskopischen Sporophyten auf abiotischen interaktiven Stress.

Als Schutz vor schädigender Strahlung haben marine Makroalgen unterschiedliche Strategien und Mechanismen entwickelt, aber auch diese können durch diverse abiotische Stressfaktoren oder deren Interaktionen beeinflusst werden. Die Ergebnisse dieser Arbeit geben auch Hinweise auf ein mögliches Vorkommen zusätzlicher Schutzmechanismen in den untersuchten arktischen Arten.

Nach Exposition unter kombiniertem Temperatur- und Salinitätsstress war zu beobachten, dass die Pigmentmenge von Fucoxanthin in der Braunalge *L.solidungula* durch verschiedene Salinitäten beeinflusst wurde. Im Gegensatz zum unveränderten Chlorophyll *a* Gehalt wurde eine erhöhte Fucoxanthin-Konzentration bei natürlicher Fjordsalinität ermittelt, Temperatureffekte wurden nicht entdeckt. Des Weiteren zeigten sich auch im Gesamtgehalt Mykospurin-ähnlicher Aminosäuren (MAAs) in der Rotalge *D.ramentacea* nach Exposition unter kombiniertem Temperatur- und Strahlungsstress Veränderungen, wobei es eine allgemein erhöhte MAA Konzentration unter UV-Einfluss gab. Damit kann die Funktion von MAAs als natürlicher Schutz vor UV-Strahlung bestätigt werden, eine zusätzliche Funktion als Schutz vor Temperaturstress kann hierbei ausgeschlossen werden. Allerdings beeinflussten interaktive Effekte von Strahlung und Temperatur, besonders UV-A Strahlung in Kombination mit niedriger Temperatur die MAA-Akkumulation in *D.ramentacea*, was wiederum ein Hinweis auf eine mögliche, durch niedrige Temperaturen induzierte MAA-Bildung als zusätzlicher UV-Schutz in polaren Algen sein könnte. In der Grünalge *Ulva lactuca* (Chlorophyta) scheint durch die Anwesenheit von UV-Strahlung die Umwandlung von Violaxanthin innerhalb des Xanthophyllzyklus, der als Lichtschutzmechanismus fungiert, verzögert zu sein. Zusätzlich stieg die Konzentration des Schutzpigmentes Lutein unter Strahlungsstress beträchtlich an. Das könnte auf ein mögliches Vorkommen eines weiteren Schutzmechanismus, den Lutein- Epoxidzyklus in Makroalgen hindeuten.

Vor allem bei Laborstudien zu Stresseffekten ist das jeweils verwendete Verhältnis von PAR zu UV-Strahlung von wesentlicher Bedeutung, besonders um im ökologischen Kontext eine Überschätzung von UV-induzierten Effekten zu vermeiden. Die Bedeutung von PAR/UV Wechselwirkungen zeigt sich in der Untersuchung der photosynthetischen Reaktion von *U.lactuca* nach Exposition unter verschiedenen PAR-Intensitäten bei gleich bleibender UV-Strahlung. Die Ergebnisse zeigen deutlich, dass das Ausmaß der Beeinträchtigung aufgrund

von UV-Strahlung stark von der jeweiligen PAR-Bestrahlungsstärke abhängig ist. Mit steigender PAR-Intensität wird der Anteil der UV-induzierten Inhibitionen der photosynthetischen Effizienz reduziert: Je höher die PAR-Intensität je niedriger die UV-Effekte.

Insgesamt zeigt diese Arbeit die Wichtigkeit von Studien zur physiologischen Reaktionen von Makroalgen auf Interaktionen zwischen zwei oder mehreren abiotischen Stressfaktoren. Es zeigt sich, dass mehrere Faktoren synergistisch zusammenwirken können aber auch, dass häufig der Einzeleffekt eines Faktors überwiegt. Die Temperatur scheint dabei der ökologisch vorherrschende und einflussreichste Umweltparameter für marine Makroalgen zu sein. Bei ökologischer Betrachtung der untersuchten Algen besonders in Bezug auf prognostizierte mögliche Umweltveränderungen in der Arktis, lässt sich schlussfolgern, dass adulte Sporophyten von *D.ramentacea*, *S.latissima*, *A.esculenta*, *L.solidungula* offensichtlich sehr tolerant und angepasst auf ansteigende Temperaturen und UV-Strahlung sowie auf sinkende Salinitäten reagieren. Im Vergleich zu den gemäßigten Arten, besitzt die arktisch endemische Art *L.solidungula* möglicherweise nur eine limitierte Fähigkeit zur Anpassung an Veränderungen ihres arktischen Lebensraums. Die ermittelten Toleranzen der einzelnen Arten sind allerdings nur bis zu einem bisher relativ unbekanntem, artspezifischen Limit gültig.

1 Introduction

1.1 Environmental changes in the Arctic ecosystem: Influences of global warming and ozone depletion

Past and Present. The global climate is currently subject to rapid changes, whereas the rate of change is expected to accelerate. The environmental conditions in the Arctic have already changed during the last decades. Records of increasing air temperature, reduction in the thickness of sea ice and a rising sea level provide strong evidence of recent warming in the Arctic (ACIA 2005). Even though regional variations exist, from the findings summarized in assessments conducted by the ACIA (Arctic Climate Impact Assessment, 2005) and the IPCC (Intergovernmental Panel on Climate Change, 2007) it can be said that there is a clear warming trend for the Arctic on the whole.

Numerous long-term changes in Arctic climate have been observed, from which temperature changes are one of the more obvious. Nevertheless the whole ecosystem is affected and changes in environmental conditions result from the interplay of various abiotic physical parameters. Widespread melting of glaciers resulting in a decrease of ocean salinity in the upper 500m, increased precipitation by about 8% over the past 100 years, changes in wind patterns, rising permafrost temperatures, declined sea-ice extent with an annual average loss of about 8% and increased ultraviolet (UV) radiation levels resulting from stratospheric ozone depletion present additional evidence of expected strong Arctic warming (ACIA, 2005).

Additionally, the Fourth Assessment Report of the IPCC 2007 shows that warming of the climate system is quite distinct and has occurred in both, the Northern and Southern Hemispheres and across the oceans. In general, land areas warm faster than the ocean water masses, but warming has occurred in both systems, whereas temperature in the Arctic increased nearly twice compared to the global average incline (ACIA, 2005). Oceans accumulate approximately 80% of the additional received sun energy and the oceanic warming could be measured in depths up to least 3000m (IPCC, 2007).

The most severe ozone depletion has been observed in the Arctic and Antarctic, whereas the springtime stratospheric ozone depletion in the Arctic was detected recently at a less severe loss of up to 20-25% (Dahlback 2002). In general, the stratospheric ozone layer protects all

living organisms from excess ultraviolet radiation. Ozone depletion leads to an increase of UV-B radiation (280-320nm) reaching the earth's surface. Although these wavelengths represent less than 1% of the total solar flux, they are biologically extremely harmful (Franklin and Forster 1997). There is also a high natural variability in ozone levels, which is directly affected by the temperature of the stratosphere. Over the past ten years, the total column ozone value for most of the world has levelled off or shows a slight increase, but it is difficult to predict trends (reviewed by Weatherhead and Andersen 2006).

Due to the fact that physiochemical changes happen most rapidly in Polar Regions, they are considered as a quite sensitive barometer for processing climate change. In order to assess whether recent changes in Arctic climate are unusual or in the range of natural variability, recent and past climate observations were compared. As a result this indicates that the amount, speed and pattern of warming seem to be indeed unusual and characteristic for a human-induced climate changes. A possible reason for this could be especially the tremendous increase of artificially produced greenhouse gases amplifying the natural variations of average climate conditions (ACIA, 2005).

Future. Different scenarios of future climate changes in both reports, ACIA (2005) and IPCC (2007) predict a further warming trend during the next two decades. “Even using the lowest emissions scenario, and the model that generates the least warming in responses to changes in atmospheric composition, leads to a projection that the earth will warm more than twice as much in this century as it warmed over the past century” (ACIA, 2005).

Generally, the climate change scenarios predict that the annual Arctic surface temperatures north of 60°N will increase by around 2-4°C by mid-century and by 4-7°C by the end of the 21st century. The predicted extreme increase of temperature will result in further subsequent changes, e.g. retreat of glaciers, decreased snow cover extent, sea level rise and an accumulation of extreme weather events. Additionally, an increase in precipitation of around 20% by 2100, mostly coming as rain is predicted for the Arctic. Consequently reduced salinity of ocean water in the North Atlantic could influence the global ocean circulation (ACIA 2005, IPCC 2007). Furthermore, an increase in total column ozone in the Arctic will partially depend on possible dynamical and also temperature changes in the coming decades and could result in either an expedited or delayed ozone increase. Total column ozone, carbon dioxide emissions, stratospheric temperatures and wind and water circulation patterns are

closely linked and changes in one of these variables can affect all the others (reviewed by Weatherhead and Andersen 2006).

These drastic changes of environmental conditions, especially in marine ecosystems and Arctic regions could most probably result in changes in abundance of species and their geographical distribution as well as in changes of community structures with a lot of ecological implications (IPCC, 2007). Meanwhile, climate change has already caused measurable temporary effects on kelps near thermal limit (Steneck et al. 2002).

1.2 Impacts of changes in abiotic factors on marine macroalgae, especially variations of UV-radiation, temperature and salinity

Within coastal ecosystems, marine macroalgae have been identified as a group of organisms of vital importance to ecosystem function (Lüning 1990). Light, temperature, nutrients, water movement and salinity primarily control the growth and distribution of marine algae (Kirst 1990). Natural seasonal variation, global warming and ozone layer thinning simultaneously expose Arctic marine macroalgae to multiple stressful conditions, including changes in temperature, salinity and UV-radiation in their natural environment. Against this background and due to their sensitivity to a broad range of environmental stressors and their rapid response to variations in environmental conditions algae can consequently act as indicator for changes in aquatic ecosystems (Mc Cormick and Cairns 1994).

1.2.1 Effects of single stress factors

Solar radiation is essential for life on earth. In the process of photosynthesis, photoautotrophic organisms like macroalgae absorb visible light energy and convert it to energy-rich organic compounds. Changes in irradiance and light quality can do both, promote photosynthesis or inhibit many biological processes, especially due to energy-rich short wavelengths in the range of UV-radiation (280-400nm). Effects of UV-radiation are manifold, reaching from the molecular and cellular up to the organism level. Especially the UV-B radiation (280-320nm) is deleterious for reproduction processes of macroalgae like germination and gametogenesis (reviewed by Franklin and Forster 1997, Adir et al. 2003, Franklin et al. 2003, Hanelt et al. 2003, Bischof et al. 2006, Roleda et al. 2007 and references therein). In comparison to adult stages, early life stages (spores, gametes, zygotes) of macroalgae are the most susceptible stages to UV-B radiation with regard to cyclobutane pyrimidine dimer (CPD) formation,

photosynthetic performance, germination and survival (Dring et al. 1996, Wiencke et al. 2000, Véliz et al. 2006, Roleda et al. 2007, Müller et al. 2008). Continuous records of ongoing climate change and stratospheric ozone depletion with the concomitant increase in solar UV-radiation have led to substantial UV-research during the last years, including also UV-studies on Arctic marine macroalgae from the Kongsfjord on Spitsbergen (Wiencke et al. 2004). Therefore, photosynthesis under UV-radiation stress with the respective adaptation mechanisms is probably the most intensively studied process in the Arctic adult macroalgae, mainly determined in single-factor experiments (see Franklin and Forster 1997, Hanelt et al. 2003, Bischof et al. 2006, Wiencke et al. 2007, Bartsch et al. 2008). The sensitivity of the photosynthetic process to UV-radiation is species-specific, dose dependent and indicates different genetically fixed adaptation and physiological acclimation ability of Arctic seaweeds. Macroalgal communities typically show distinct vertical zonation patterns (reviewed by Franklin and Forster 1997, Bischof et al. 2006, Wiencke et al. 2007). In particular, the vertical zonation pattern of macroalgal species in the Arctic Kongsfjord results from their responses and tolerance to a number of different environmental factors such as light penetration, wave exposure, competition and grazing. However, the main influence on depth distribution is the radiation intensity, due to the different tolerance and protection mechanisms of the algae against high PAR and UV-radiation and due to the limiting PAR quantity in the lower sublittoral (Gomez et al. 1997, Hanelt et al. 1997, Bischof et al. 1998, Aguilera et al. 1999, Wiencke et al. 2000, Roleda et al. 2007).

Exemplarily, the red alga *Devaleraea ramentacea* is a typical shallow-water species in the Arctic and hence often exposed to high solar radiation. This is reflected in a high UV-tolerance of these algae with only little seasonal variations and a high potential of acclimation (Karsten et al. 1999, Bischof et al. 2002). In contrast, a very high UV-sensitivity with strong inhibition of photosynthetic performance, oxygen production and growth was observed in the Arctic endemic deep-water species *Laminaria solidungula* (Bischof et al. 1998a, Aguilera et al. 1999, Bischof et al. 2000, 2002, Karsten et al. 2001, Michler et al. 2002).

Macroalgae of polar ecosystems seem to be more vulnerable to UV-radiation due to additionally inhibiting effects of low temperature on the repair of UV-damage on cellular level (Hoffman et al. 2003, Vincent et al. 2007). Temperature is probably the most important abiotic factor for the determination of growth, reproduction and biogeographical distribution of seaweeds (e.g. van den Hoek 1982, Breeman 1988, Lüning 1990). The southern and northern geographical distribution limits of Arctic and cold-temperate algae correspond to the

species-specific thermal requirements for growth and reproduction. The upper and lower thermal survival limits of these marine macroalgae are for gametophytes between -1.5-1°C and 20-23°C and for sporophytes between 0 and 20-21°C (Fortes and Lüning 1980, Bolton and Lüning 1982, Rueness and Tananger 1984, Breemann 1988, Lüning 1990, tom Dieck 1993, Wiencke 1994, Bischoff-Bäsmann 1997 and references therein).

Temperature and light conditions in an optimal range for marine macroalgae could induce a better tolerance to salinity changes (Kirst 1990). Salinity tolerance of algae is strongly related to their physiological capability of osmotic adjustment, whereas salinity stress can strongly affect their photosynthesis rate or pigment contents (Kirst 1990, Karsten 2007). Based on one of the few ecophysiological studies on salinity effects on Arctic seaweeds, the kelp species *Alaria esculenta*, *Saccharina latissima* and *Laminaria solidungula* were characterized as stenohaline macroalgae with strong inhibition of photosynthesis at hyposaline conditions. By contrast, the typically shallow-water species *Fucus distichus* with a broad salinity tolerance was characterized as euryhaline. The salinity tolerances of these macroalgal sporophytes correlate with the habitat and the species-specific acclimation potential (Karsten 2007).

Accordingly, not only high radiation and low temperature conditions are stressful in the Arctic, but a dark winter period also affects algal growth performance, whereby different growth strategies were developed by the different species. For example, the Arctic endemic kelp *Laminaria solidungula* grows predominantly in complete darkness during winter and is optimally adapted to low light conditions and long dark periods in the Arctic environment (Chapman and Lindley 1980, Dunton 1985, Dunton and Jodwalis 1988, Korb and Gerard 2000).

1.2.2 Interactive effects of combined factors

Under natural conditions, plants are subject to the influence of at least two different stress factors and have to respond to a multitude of abiotic and biotic factors (Alexieva et al. 2003). Stress factors usually not occur individually or independently, consequently spatial or temporal variations and interactions between them and co-variation of stresses are the norm in the natural environment (Vinebrooke et al. 2004, Jones et al. 2008). Stressors act synergistically when their combined effect on biological components is larger than the responses to each single stressor and antagonistically when the impact is smaller (Folt et al.

1999). It is also possible that plant organisms exposed to a single stress agent are capable of increasing their resistance to subsequent unfavourable impacts (cross-adaption, Alexieva et al. 2003).

Marine macroalgae inhabiting Arctic coastal ecosystems are exposed to different variations in environmental factors. While UV-damaging effects and growth temperatures on polar macroalgae in single-factor experiments are well-studied (as mentioned above), physiological responses of macroalgae to the impacts of multiple and combined abiotic factors are relatively unknown.

In particular, there are only very few ecophysiological studies on interactions of UV-radiation and/or temperature with salinity in polar seaweeds. In the study of Karsten et al. (2003) on interactive effects of UV-radiation and salinity on Arctic red macroalgae, *Devaleraea ramentacea* can be characterised as a euryhaline species acclimated well to UV-radiation and able to resist different environmental stress factors. But, *Palmaria palmata*, as a stenohaline plant, showed a limited ability to acclimate to changing PAR/UV radiation, which points at a relatively low physiological plasticity (Karsten et al. 2003).

There are a few studies on temperate macroalgae to effects of salinity and temperature, for example on the brown alga *Fucus vesiculosus*. Lower salinity had a negative effect on the Atlantic isolate of *F.vesiculosus* with a decreased photosynthetic maximum as well as a lower tolerance to UV-B in comparison to the isolate of *F.vesiculosus* from the northern Baltic Sea (Nygard and Ekelund 2006). Moreover, *F.vesiculosus* is evidently much more susceptible to salinity changes at extreme temperatures than to salinity changes within its limiting summer isotherms (Russell 1987). Based on these studies it seems that combined temperature and salinity limits affect the biogeographically pattern much more than the temperature limit alone.

Hoffman et al. (2003) supported the hypothesis that temperature mitigates the net biological effect of UV-radiation on macroalgae and *vice versa*. Correspondingly, the photosynthetic performance of the sporophytes of the Antarctic green alga *Ulva bulbosa*, the Subantarctic *Ulva clathrata* and the Arctic red algae *Palmaria palmata*, *Coccotylus truncates* and *Phycodrys rubens* was less impaired by UV-B at higher temperatures (van de Poll et al. 2002, Rautenberger & Bischof 2006). In the latter case of the three red algae, the recovery of photosynthesis after UV-B stress was likewise improved at higher temperatures, although the influence of UV-B radiation was predominant regarding the relative growth rate and the cyclobutane–pyrimidine dimer (CPD) accumulation indicative of DNA damage (van de Poll

et al. 2002). Also, the germination of highly UV-B vulnerable zoospores of the Arctic kelps *Saccharina latissima* and *Laminaria digitata* were more UV-B affected at unfavourable lower and higher temperatures rather than at the naturally ambient temperature of 7°C (Müller et al. 2008). For spores of the kelp *Alaria marginata*, 10°C was not a limiting temperature in the absence of UV-radiation, but under high levels of UV-radiation spores were unable to germinate at this temperature (Hoffman et al. 2003). Furthermore, Steinhoff et al. (2008) and Müller et al. (2008) illustrated that UV-B radiation and high temperatures detrimentally affected the physiology and ultrastructure of zoospores of temperate and Arctic Laminariales.

Nevertheless, there are numerous unexplained fundamental questions about the tolerance of macroalgae to stress caused by combined factors and besides more physiological and ecological studies on interactive effects are indispensable.

1.2.3 Protective mechanisms in marine macroalgae

Multiple protection strategies exist in marine macroalgae mainly against high radiation conditions. A crucial mechanism in Chlorophyta and Phaeophyceae for photoprotection from excess PAR irradiance is the xanthophyll cycle. During a rapid conversion of violaxanthin to zeaxanthin by the enzyme violaxanthin de-epoxidase, the excessively absorbed energy is dissipated as heat (Demmig-Adams 1990; Demmig-Adams and Adams 1992). Moreover, carotenoids act as light absorbing compounds within the reaction centre, but additionally they can serve as antioxidants or as screening pigments and passive UV-sunscreens (Franklin and Forster 1997, Karsten 2008).

Besides, the most Phaeophyceae have the ability to synthesize UV-absorbing phlorotannins (polyphenolic compounds) against physiological stress (Pavia et al. 1997, Bischof et al. 2006, Bartsch et al. 2008, Karsten 2008).

One of the most important UV-protection mechanisms represents the biosynthesis and accumulation of mycosporine-like amino acids (MAAs). MAAs are water-soluble, small molecules absorbing ultraviolet radiation. They act as natural UV-screening compounds and are widespread in nature with the highest diversity of them detected in Rhodophyta (reviewed by Bandaranayake 1998, Shick and Dunlap 2002). Phaeophyceae and most Chlorophyta typically lack these compounds, except the green alga genus *Prasiola* (Hoyer et al. 2001). The physiological function of MAAs as natural UV-sunscreens is well investigated, but in recent studies there is evidence for additional functions of MAAs such as antioxidants, nitrogen

reservoir or as protective compatible solutes against different stress conditions (reviewed by Oren and Gunde-Cimerman 2007). In macroalgae such additional functions of MAAs are not documented yet.

1.3 Aims of the present thesis

Marine macroalgae in their natural environment are exposed to a multitude of stressful conditions, which can be caused either due to natural seasonal variations or due to potential effects of climate change. However, the responses of marine macroalgae to simultaneous impacts of multiple abiotic stressors are relatively unknown. For that reason, the purpose of the present study is to explore the interactive effects of combined abiotic environmental factors on different key species of the Arctic coastal ecosystem in order to understand more about stress physiology of polar macroalgae and to estimate potential ecological implications.

Due to the fact that up to now mainly single-factor experiments were carried out and only very few studies on combined impacts of two or more abiotic factors on Arctic marine macroalgae are available, the present thesis was based on the following questions:

- (1) How does temperature stress in combination with different radiation conditions affect photosynthetic performance of Arctic marine macroalgae?
- (2) How does combined temperature and salinity stress affect photosynthetic performance of Arctic marine macroalgae?
- (3) Do different life history stages of an Arctic kelp species react diversely to interactive effects of abiotic factors?
- (4) Do combined abiotic stress factors effect the protective mechanisms of marine macroalgae- and if so, in what way?

Up to now, mainly the photosynthetic reaction of macroalgae to stress caused by single factors was investigated. Therefore, it is relatively unknown whether an interaction amplifies or mitigates the effects.

- (5) Are there specific dominant influences within the investigated stress combinations?

Furthermore, from different studies it is known that the respective ratio of photosynthetically active radiation to ultraviolet radiation applied in laboratory experiments is of crucial importance in order to avoid overestimation of UV-induced effects in an ecological context. Against this background the following question was also investigated in this study:

(6) Are there PAR/UV interactions on photosynthesis of a green macroalga exposed to constant UV-radiation at varied irradiances of PAR?

From an ecological point of view, the findings could lead to the overall question:

(7) Which ecological conclusions could result due to the physiological response of marine macroalgae to interactive environmental stress?

2 Publications

2.1 List of Publications

The present thesis is based on the following publications (see attachment), which are referred to in the text by their Roman numerals:

- I** **Fredersdorf J**, Karsten U, Bischof K (under review) Physiological responses of the Arctic red alga *Devaleraea ramentacea* to interactive environmental stress. Polar Biology

- II** **Fredersdorf J**, Müller R, Becker S, Wiencke C, Bischof K (in press) Interactive effects of radiation, temperature and salinity on different life history stages of Arctic kelp *Alaria esculenta* (Phaeophyceae). Oecologia

- III** **Fredersdorf J**, Bischof K (submitted) Impacts of combined abiotic stress factors on adult sporophytes of two Arctic kelp species *Saccharina latissima* and *Laminaria solidungula* (Phaeophyceae). Polar Research

- IV** **Fredersdorf J**, Bischof K (2007) Irradiance of photosynthetically active radiation determines UV-susceptibility of photosynthesis in *Ulva lactuca* L. (Chlorophyta). Phycological Research 55(4): 295–301

- V** Karsten U, Wulff A, Roleda M, Müller R, Steinhoff FS, **Fredersdorf J**, Wiencke C (under review) Physiological responses of polar benthic micro- and macroalgae to ultraviolet radiation. Botanica Marina (Special Issue)

2.2 Declaration of contributions to the publications

Publication I, III, IV and parts of publication II are based on my own laboratory and field experiments and were initiated, planned, analysed and written by myself in close cooperation with my supervisor Prof. Dr. K. Bischof. The first version of the manuscripts was written by myself and improved in collaboration with the co-authors. The study on *Alaria* sporophytes in publication II is based on my experiments and analyses together with data of the diploma thesis of S. Becker. The second author R. Müller has contributed the experimental work, data analysis and manuscript writing of the parts about *Alaria* zoospores to publication II. Technical assistants supported HPLC measurements of pigments and MAAs. The manuscript of the paragraph: *Interactive effects of UVR with other abiotic and biotic factors* (publication V, S. 20-23) in the review Karsten et al. was written by me in cooperation with R. Müller.

3 Material and Methods

This chapter provides a general overview on algal material and the different experimental set-ups used in order to study interactive effects on Arctic marine macroalgae. Furthermore, the mainly used methods for analysing the photosynthetic responses and changes in the content of pigments or mycosporine-like amino acids are described. More detailed information, especially on sampling, experimental conditions, experimental processing and analysis techniques are given in the respective publications (*Material and Methods* in publications I - IV).

3.1 Study area and Algal material - an overview

With the exception of the lab experiment on a stock culture of *Ulva lactuca*, all experimental studies were conducted on marine benthic macroalgae from the Kongsfjord, which is located at the north-western coast of Spitsbergen (Svalbard, Norway, 78°55.5'N; 11°56.0'E; Fig.1). The experiments were carried out either in the lab facilities of Ny Alesund or outdoors within the vicinity of the labs.

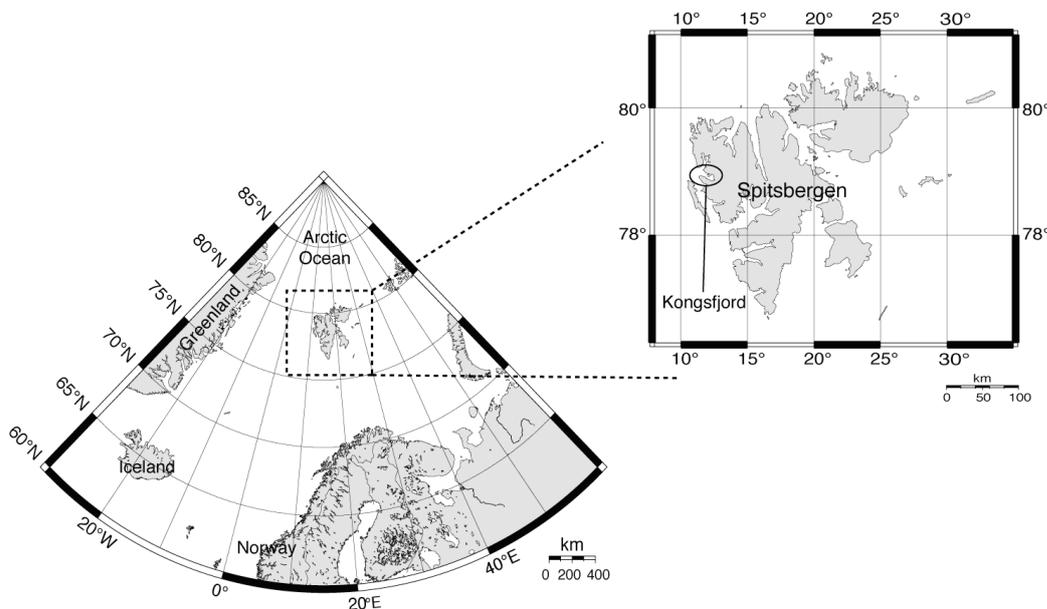


Figure 1 Map of the study site: the Kongsfjord on Spitsbergen (with: www.aquarius.geomar.de)

Natural abiotic environmental conditions of the study area

The light, temperature and salinity regimes of the Arctic Kongsfjord vary with the seasons (Hanelt et al. 2001, Svendsen et al. 2002). During spring and early summer, the Kongsfjord is generally free of ice, the water is clear, and thus, its transparency to UV radiation is very high. In summer (June/ July) under sunny conditions, the irradiances of PAR (400-700nm) at the surface can increase up to $1300\mu\text{mol m}^{-2} \text{ s}^{-1}$. In parallel, a maximum irradiance of about 19 Wm^{-2} in the UV-A range (320-400nm) and 1.2 W m^{-2} in the UV-B range (280-320nm) was recorded (Bischof et al. 1998, Hanelt et al. 2001). As an average maximum daily dose of UV-B irradiance 0.61 W m^{-2} was measured (Svendsen et al. 2002). The underwater radiation regime is subject to strong seasonal variations, sea ice as well as actual weather conditions and the turbidity of the water column (Hanelt et al. 2001, Svendsen et al. 2002). However, UV-B radiation can penetrate down to 6-10m depths in clear water conditions in the Kongsfjord, and consequently affects macroalgae inhabiting shallow waters. The 1% water depth for PAR is about 18m in spring and 7m in summer (Bischof et al 1998, Hanelt et al. 2001, Svendsen et al. 2002).

The above mentioned clear water conditions in spring are combined with relatively low air temperatures at that time (Hanelt et al. 2001, Svendsen et al. 2002). With increasing air temperature in summer, snow layers and glacier ice melt and cause a high discharge of turbid fresh water and sediments into the fjord. In general, the local water mass has a salinity of about 34.5psu (practical salinity units) in spring and drops below 28psu in the surface water near the glacier in summer, whereas the seawater temperature increases and varies by about 4°C during summer (Hanelt et al. 2001, Svendsen et al. 2002).

In recent years, the marine coastal ecosystem of the Arctic Kongsfjord was intensively studied and serves currently as a model ecosystem and monitoring site for effects of climate change in the Arctic (Svendsen et al. 2002, Wiencke 2004). The underwater flora is composed of at least 50 different macroalgal species and exhibits a zonation pattern of several main algal belts (Wiencke et al. 2004). The upper sublittoral (about 3-5m depth) is characterised by the brown algae *Fucus distichus*, some green macroalgae and the typical shallow water red algae *Devaleraea ramentacea*. In the mid sublittoral (3-15m depth), the brown algae *Laminaria digitata*, *Saccharina latissima* and *Alaria esculenta* are the key species, whereas the lower sublittoral down to about 30m depth is characterised, beside red macroalgae and crustose red algae, by the Arctic endemic, but rare species *Laminaria solidungula* (Wiencke et al. 2004).

Table 1 gives an overview on the investigated macroalgal species within this thesis with one species representative for each belt of the zonation. Their collecting depth, the respective life history stages that were exposed to various combinations of abiotic stress factors in different experiments and the investigated physiological parameters are summarized.

Table 1 Overview on macroalgal species exposed to different combinations of abiotic stress factors (UV=UV-radiation; T=temperature; S=salinity) and the respective measured parameters (photosynthetic efficiency, germination, content of pigments and mycosporine-like amino acids (MAA))

Algal species	<i>Ulva lactuca</i>	<i>Devaleraea ramentacea</i>	<i>Alaria esculenta</i>		<i>Saccharina latissima</i>	<i>Laminaria solidungula</i>
Taxonomy	Chlorophyta	Rhodophyta	Phaeophyceae	Phaeophyceae	Phaeophyceae	Phaeophyceae
Life history stage	Sporophyte	Tetrasporophyte	Sporophyte	Zoospore	Sporophyte	Sporophyte
Habitat	Disko Bay, Greenland	Kongsfjord, Spitsbergen, upper-sublittoral	Kongsfjord, Spitsbergen, mid-sublittoral	Kongsfjord, Spitsbergen, mid-sublittoral	Kongsfjord, Spitsbergen, mid-sublittoral	Kongsfjord, Spitsbergen, lower-sublittoral
Collecting water depth	Stock culture	3-5m	7-10m	4-8m	3-4m	14-18m
Field experiment: UV + T		Photosynthesis; MAA				
Laboratory experiments: UV + T			Photosynthesis		Photosynthesis	Photosynthesis
T + S		Photosynthesis	Photosynthesis			Photosynthesis; Pigments
UV + T + S				Germination		
UV + increasing PAR	Photosynthesis; Pigments					

3.2 Experimental set-up for multifactorial treatments

3.2.1 Field experiment under natural environmental conditions exposed to temperature und UV-radiation stress effects

For the bi-factorial field experiment, outdoors on the Old Pier of Ny Alesund, cleaned algal material was exposed to natural photosynthetically active radiation (PAR) and ultraviolet (UV) radiation at defined increasing temperatures (5-20°C) for a duration of six days. Small plastic baskets containing algal samples were placed in three large, temperature controlled water tanks (= mesocosm) supplied with flow-through running seawater causing water

circulation and permanent movement. Temperature in the three mesocosms was controlled by cryostats and monitored permanently. The natural solar radiation was measured close by on the roof of the NDSC observatory (Network for the Detection of Stratospheric Change) with a LI-190 Quantum Sensor connected to a LI-250 light meter (LI-COR, Lincoln, USA) and a UV-radiometer PMA2100 (Solar light, USA). In order to generate different radiation treatments with and without UV- radiation, the plastic baskets were shielded with different cut-off foils. Three baskets for each of the three radiation conditions (nine all in all) were randomly distributed in of the mesocosms, arranged as randomized block design. Foils for (1.) PAR treatments (=P) were transparent to wavelengths of 400–700 nm, for (2.) PAR+UVA treatments (=PA) transparent to wavelengths of 320–700 nm, whereas foils for (3.) PAR+UVA+UVB treatments (=PAB) were transparent to wavelengths of 295–700 nm. The respective transmission spectra of these cut-off foils are shown in Bischof et al. (2002). After six days of exposure, the mesocosms were covered by black net gauze and cryostats were switched off for a recovery period of two days at dim light and ambient fjord temperature.

3.2.2 Laboratory experiment with artificial defined stress conditions

3.2.2.1 Temperature combined with UV-radiation or salinity

For the inside bi-factorial laboratory experiments, algae were exposed to different temperatures (0-21°C) combined with artificial UV-radiation conditions or with different diluted salinities (34, 28, 20) for duration of 6 days. The basic experimental design was the same as in the field experiment, but with a smaller set-up and only artificial defined conditions. It was assured that the defined abiotic factors radiation, temperature and salinity were equably and steady allocated across the whole experimental setup and within all treatments.

Plastic beakers, containing the algal material were placed in small temperature controlled water tanks. Each beaker was equipped with a bubble stone connected to a self-priming pump in order to provide permanent water circulation and movement, which minimizes diffusive nutrient limitation. The temperature in each tank was controlled by cryostats and monitored. For each temperature applied, two of these tanks were established.

In the set-up for interactive effects of temperature and UV-radiation, in total 15 light-tubes provided permanent uniform irradiation of photosynthetically active radiation (PAR) and UV-radiation above the tanks. For the different radiation conditions (PAB, PA, P) beakers were covered with different cut-off foil. Three beakers for each of the three radiation conditions

(nine all in all) were randomly distributed in each temperature treatment (consisting of two tanks), arranged as split plot design. After six days of exposure, cryostats and light-tubes were switched off for a recovery period of two days at dim light and ambient fjord temperature.

In the experiment on combined temperature with salinity effects, only PAR-irradiance was provided above the tanks. The alga samples were exposed at different salinities inside the plastic beakers. These salinities were produced by mixing fjord water with MilliQ water and defined by a hand-held Refractometer. Three beakers for each of the three salinity conditions (nine all in all) were randomly distributed in each temperature treatment (consisting of two tanks), arranged as split plot design.

3.2.2.2 Different PAR intensities under constant levels of UV-radiation

In order to illustrate the importance of the respective ratio of PAR:UV, algal material of *Ulva lactuca* was exposed to constant irradiance of UV-radiation and at varying irradiances of background PAR. For acclimation to the experimental conditions, algae were cultivated in one temperature controlled climate chamber at 5° C and 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR for three days. After acclimation, the algae were directly transferred to the respective radiation conditions for six hours of exposure at 5°C in the second temperature controlled climate chamber. The emitted constant irradiance in the UV-range was adjusted to 1.0 (± 0.02) W m^{-2} UV-B and 10 (± 1.6) W m^{-2} UV-A. For each experiment the background irradiance of PAR was changed on the different days of exposure and set at 30, 100, 200 and 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$, respectively. A set of four different types of glass filters was used to generate different spectral radiation conditions: (1.) UVA+UVB (=AB) treatment without PAR; (2.) PAR+UVA+UVB (=PAB) treatment; (3.) PAR+UVA (=PA) treatment; (4.) PAR (=P) treatment. After exposure time, the algae were transferred back to the first climate chamber for two hours of recovery in dim light.

3.2.2.3 Three-factorial setup for impacts on germination success of zoospores

To study the germination success, separate zoospore suspensions obtained from five individual sporophylls were adjusted to three different salinities (34, 28, 20) artificially mixed with distilled water. The numbers of zoospores in suspensions were counted with a Neubauer chamber using an Axioplan microscope. Suspensions were allotted into culture dishes containing two cover slips each. Subsequently, zoospore suspensions with different salinities were exposed in culture dishes covered with cut-off foils to generate the different radiation

treatments (P, PA, PAB) in climate chambers, running at four different temperatures. After eight hours of UV exposure, six days of UVR recovery at dimmed PAR under constant temperatures and salinity, the percentage of germination was ascertained from 300 spores per replicate. Thereby, the germinated spores possessing a germination tube were distinguished from dead and living cells without germination tubes (for details see Müller et al. 2008).

3.3 Analysis methods

3.3.1 Measurements of photosynthesis

Photosynthetic performance of algal samples was determined by measuring in vivo chlorophyll-fluorescence of photosystem II (PSII) using a PAM 2100 chlorophyll fluorometer (Walz, Effeltrich, Germany) as described previously by Hanelt et al. (1997). The maximal quantum yield of PSII (Fv/Fm) of sporophytes indicating physiological performance (Schreiber et al. 1994 for details) was measured during the experiments. To estimate the photosynthetic capacity, photosynthesis vs. irradiance curves (PI-curves) were recorded by the PAM fluorometer (Schreiber et al. 1994) in order to reveal maximal relative electron transport rates (ETR_{max}) and initial light saturation points (I_k) of experimental specimens. The measurement of the maximum quantum yield on macroalgae is a widespread, fast and non-invasive method, which is suitable for implementation under extreme Arctic conditions, especially in field experiments. In an extensive review about chlorophyll fluorescence, Baker (2008) also concluded that Fv/Fm is a very useful relative measure of the maximum quantum yield of PSII photochemistry. Admittedly, due to the complex processes of photosynthesis Fv/Fm does not provide a rigorous quantitative value of this quantum yield and hence other processes such growth or protective mechanisms should be investigated as well.

3.3.2 Determination of pigment contents

The pigments chlorophyll *a* and *b*, lutein, viola- and zeaxanthin of the green algae *Ulva lactuca* were extracted and analysed by High Performance Liquid Chromatography (HPLC) according to the protocol of Bischof et al. (2002). In short, frozen samples were transferred to 2 ml of 100% N-N-dimethylformamide (DMF) and stored in darkness for approx. 18 h. Afterwards, the extracts were centrifuged at 5,000 g for 5 min at 4°C, the supernatant was subsequently mixed with diethylether and distilled water. Under a stream of gaseous nitrogen, pigments were vaporized and re-dissolved in 800µl of acetonitrile/methanol/tetrahydrofuran

(75:15:10). Sample volumes of 95 μl were injected into a PDA equipped HPLC system (Elite LaChrom, VWR-Merck, Darmstadt, Germany). For pigment identification, chromatograms and retention times of samples were compared to respective pigment standards obtained from DHI Water & Environment (Hørsholm, Denmark).

For extraction of chlorophyll *a* and fucoxanthin and the following HPLC analysis, a newly established protocol of Rautenberger (2008) was applied. In brief, frozen samples were extracted in 500 μl of 100% N-N-dimethylformamide (DMF) and incubated in a nitrogen atmosphere at darkness for 22 hours at 4°C. This step was repeated by using 800 μl of 100% DMF and afterwards the bleached thalli were washed with 500 μl of 100% DMF. To separate pigments extracted from the thalli, the samples were centrifuged, passed through a filter membrane and finally covered with nitrogen and stored at -20°C until the analysis. HPLC was performed with a Hitachi Elite LaChrom System (VWR International, Darmstadt, Germany), equipped with a temperature controlled auto sampler set to 5°C (L-2200) and a L-2450 photodiode array detector at 440 nm (for details see Rautenberger 2008). Each substance was identified due to the generated spectrum, the respective retention time, and co-chromatography of standard extracts from DHI Water & Environment (Hørsholm, Denmark).

3.3.3 Analysis of mycosporine-like amino acids (MAAs)

For extraction and HPLC analysis of the total content of mycosporin-like amino acids (MAAs) in the red macroalgae *Devaleraea ramentacea*, the procedure described by Karsten et al. (2003) was applied. In brief, freeze-dried samples (10-50mg) were extracted in 2ml of 25% methanol for 2.5h at 45°C. After centrifugation at 6000x g for 6min, 200 μl of supernatants were evaporated to dryness under vacuum and redissolved in 400 μl of 100% methanol. These samples were analysed with a Waters HPLC system. The mobile phase consisted of 2.5% methanol (v/v) and 0.1% acetic acid (v/v) in water run isocratically at a flow rate of 0.7ml min⁻¹. MAAs were detected online with a photodiode array detector at 330nm, and absorption spectra (290-400nm) were recorded from HPLC-separated peaks. Identification was done by spectra, retention time and co-chromatography with standard extracts.

3.3.4 Statistical analysis

All experiments on sporophytes were bi- factorial with repeated measures and a minimum of three replicates. Mean values and standard deviations were calculated from all replicates per

treatment. Results were analyzed using a two-way analysis of variance with repeated measures on the two factors and their interactions. Statistically significant differences and interactions of means were compared with the Post Hoc test Tukey's (HSD) at $p < 0.05$ (Sokal and Rohlf 1995). All analyses were performed using JMP version 5.1 or 6 (SAS Institute, Cary, NC, USA).

The statistical analysis on data from zoospores was conducted in accordance with Sokal & Rohlf (1995) by using the software Statistica Version 7 (StatSoft, Inc., USA). Percentage data were arcsine transformed and homogeneity of variances was tested with Cochran's test ($p < 0.01$) prior to testing data with a three-factorial ANOVA ($p < 0.05$) and Post-Hoc Tukey's test (HSD, $p < 0.05$).

4 Summary of Results

4.1 Photosynthetic responses to interactive effects of temperature and UV-radiation

In order to study the photosynthetic efficiency under multiple abiotic stresses, adult sporophytes of the three kelp species *Alaria esculenta*, *Saccharina latissima* and *Laminaria solidungula* were exposed to different artificial irradiation conditions (PAB, PA, P) at different temperatures (0-21°C) in laboratory experiments (publication II, III). By comparison, the response of *Devaleraea ramentacea* sporophytes at different temperatures (5-17°C) in combination with natural ambient radiation conditions was investigated in a field experiment (publication I).

The commonality of all studies is a significant individual temperature effect on the maximum quantum yields (Fv/Fm) of all four species. Representatively, the photosynthetic response of the brown algae *A. esculenta* is shown in Figure 2.

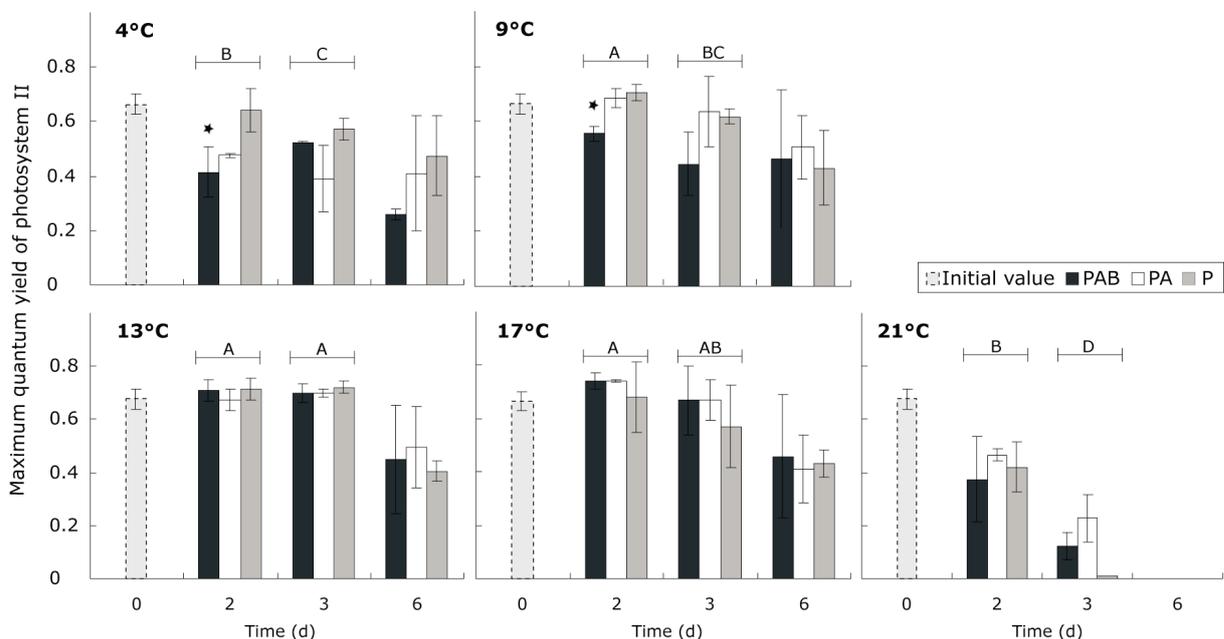


Figure 2 Maximum quantum yield of photosystem II (Fv/Fm) of *Alaria esculenta* sporophytes at five temperatures (4-21°C) and three radiation conditions (PAB = black bars, PA = white bars, P = grey bars), measured over six days of exposure under laboratory conditions. Standard deviations are represented by vertical bars (n=3). Different letters indicate significant temperature differences ($p < 0.05$) between treatments at the respective measuring date, and stars indicate significant radiation effects ($p < 0.05$) between treatments (publication II).

Maximum quantum yield of *A.esculenta* samples at higher temperatures of 13°C and 17°C was constant over three days of exposure and significant higher than that of the treatments at 4°C and 21°C (Fig. 2). The Fv/Fm of *S.latissima* and *L.solidungula* sporophytes was also relative constant over the experimental period with the exception of the 20°C treatments (Fig. 1 in publication III). Because of the exposure to the highest temperature of 20-21°C, Fv/Fm of specimens of *A.esculenta* as well as *L.solidungula* decreased rapidly, algal discs bleached, disintegrated and died. On the second and third day of exposure, the Fv/Fm of *S.latissima* samples exposed at 20°C was also significantly lower than that at 10°C at all radiation conditions, but samples recovered and survived. However, an interaction of high temperature combined with only PAR radiation affected the maximum quantum yield of *S.latissima* after recovery. There were no interactive effects caused by temperature and radiation on photosynthesis performance of *A.esculenta* and *L.solidungula*.

A significant decrease of Fv/Fm by radiation was only observed in *A.esculenta* samples exposed to the whole light spectrum (PAB) at lower temperatures of 4°C and 9°C on the second day of exposure (stars in Fig. 2). Additionally, it is noticeable that the Fv/Fm of both species *S.latissima* and *L.solidungula* exposed to the whole light spectrum (PAB) at 5°C showed a slight, but not significant downwards trend after six days of exposure.

In the field experiment, the maximum quantum yield of the red algae *D.ramentacea* decreased during the exposure time of six days with the strongest reduction at 17°C, but increased to the initial value after recovery. Only a significant individual effect of temperature were detected whereas Fv/Fm of *D.ramentacea* samples at 10°C was significantly higher than at 5°C and 17°C (Fig. 1 in publication I). However, there were no radiation effects and no interactions of temperature and radiation within this experiment.

4.2 Photosynthetic responses to interactive effects of temperature and salinity

The photosynthetic performance of the three species *D.ramentacea*, *A.esculenta* and *L.solidungula* exposed to three different artificially produced salinities (34, 28, 20) at defined temperatures (3/4, 8, 15°C) were studied (publication I, II, III). In figure 3 the photosynthetic response of the shallow-water red algae *D.ramentacea* compared to the deep-water brown algae *L.solidungula* is exemplarily shown.

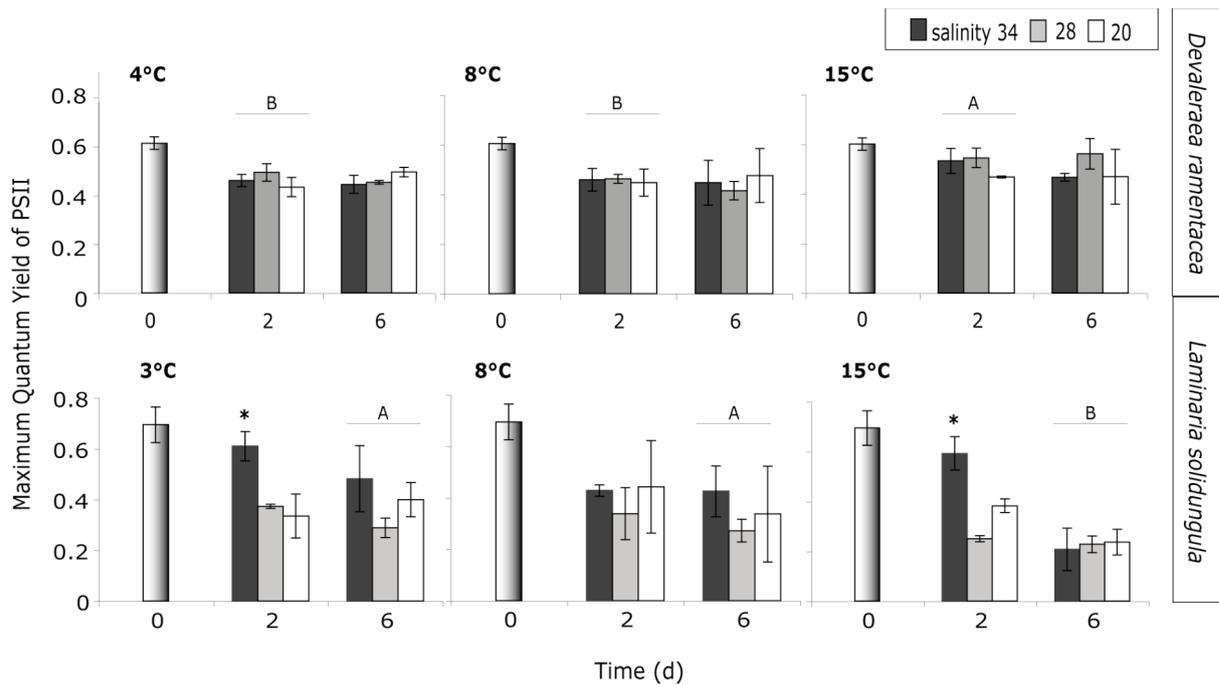


Figure 3 Maximum quantum yield of photosystem II (Fv/Fm) of *Devaleraea ramentacea* tetrasporophytes and *Laminaria solidungula* sporophytes at different temperatures (3/4, 8, 15°C) and salinities (initial value= shaded bars, salinity 34=black bars, 28=grey bars, 20=white bars), measured over six days of exposure under laboratory conditions. Standard deviations are represented by vertical bars (n=3). Stars indicate significant salinity effects; different letters indicate significant temperature differences ($p < 0.05$) between treatments at the respective measuring date (publication I, III).

In contrast to *D.ramentacea*, the maximum quantum yield of *A. esculenta* and *L.solidungula* sporophytes decreased during a period of six days of exposure. Significant individual effects of temperature were detected by Post-hoc analysis in all three species. In general, the Fv/Fm of the two algae *D.ramentacea* and *A.esculenta* at increased temperature of 15°C was significantly higher compared to 4 and 8°C. However, the Fv/Fm of *L.solidungula* at 3 and 8°C treatments was significantly higher than at 15°C (Fig. 3). Moreover, significant individual effects of salinity were found, but only in the both kelp species. The maximum quantum yield of *L.solidungula* samples in fjord salinity of 34 (3, 15°C) was significantly higher than at the diluted salinities of 28 and 20, whereas Fv/Fm of *A.esculenta* samples in the diluted salinity of 28 was significantly higher than at the fjord salinity. Nonetheless, interactive effects of temperature and salinity did not affect photosynthetic performances of all three species.

4.3 Impacts of temperature, salinity and UV-radiation on different life history stages

The response to abiotic stress factors of the different life stages of the kelp *Alaria esculenta* was diverse. In both experiments (above-mentioned) using diploid adult sporophytes of *Alaria*, photosynthetic efficiency mainly showed temperature effects and only very few radiation or salinity effects.

In contrast, the germination capacity of haploid *Alaria* zoospores was additionally affected by interaction of temperature and salinity (publication II). In the three-factorial experiment, germination rates of zoospores after eight hours of UV exposure and six days of post-culture varied with temperature and salinity conditions. Maximally 70-80% of zoospores of *A. esculenta* germinated at 7°C/ambient salinity of 34 and at 12°C/moderate salinity of 28 under all radiation conditions. The germination rates of zoospores at the temperature range of 2-12°C at all salinity (34, 28, 20) and radiation (P, PA, PAB) conditions were around 46-80% and relative minor impaired. However, germination of PA and PAB treated zoospores at diluted salinities of 28 and 20 was strongly inhibited by the high temperature of 16°C and decreased down to 38-43% (28) or 7-11% (20), respectively (Fig. 4 in publication II). Overall, germination of *A. esculenta* zoospores was strongly affected by the interaction of low salinity combined with a relatively high temperature. In this study interactive effects of UV-radiation and temperature on the germination of zoospores could not be observed.

4.4 Effects of different irradiance levels of PAR under constant UV-radiation

The effects on photosynthetic efficiency of *Ulva lactuca* exposed to different irradiances of PAR with constant levels of UV-radiation showed that the extent and the velocity of inhibition and recovery were dependent on irradiance and spectral quality (publication IV).

All different radiation treatments (AB, PAB, PA, P) resulted in a substantial reduction in Fv/Fm with the strongest decrease in algae exposed to the combination of high PAR intensity and UV-radiation (PAB, PA treatments, Fig. 2 in publication IV). Under these conditions, the maximum quantum yield decreased rapidly within the first hour of exposure and recovery was slowed down and not completed after two hours in dim white light. Differences in the degree of inhibition and recovery due to increasing PAR were minor in all treatments including UV-radiation (AB, PA, PAB). Most pronounced differences in the rate of inhibition were

observed in the PAR treatment (P) without UV. Photosynthetic measurements in samples exposed to PAR alone showed a marked dependence of the extent of photoinhibition on impinging irradiance (P treatment). However, the rate of recovery does not reflect these large differences under the different irradiances of PAR. In order to show the relative share of UV-radiation in total inhibition of maximal quantum yield of PSII, the Fv/Fm value of the PAR-treatment was subtracted from the respective values under the PAR-UV-treatment ($Fv/Fm_{PAB} - Fv/Fm_P$, Fig 3 in publication IV). From these calculations it can be seen that with increasing PAR-intensity the share of UV-induced inhibition of Fv/Fm is reduced.

4.5 Impacts of abiotic factors on physiological protective mechanisms

The investigated protective mechanisms, the accessory and xanthophyll cycle pigments as well as the UV-absorbing MAAs were influenced by diverse abiotic stress factors or their interactions.

4.5.1 Photosynthetic pigments

The concentration of the photosynthetic pigments violaxanthin and lutein of *Ulva lactuca* exposed to UV-radiation combined with a high PAR intensity was studied (publication IV). Within the first hour of exposure the content of the xanthophyll cycle pigment violaxanthin increased under all treatments containing UV-radiation, whereas under the PAR-treatment violaxanthin decreased significantly. After six hours of exposure, violaxanthin content was also diminished in the UV-exposed samples. The lutein content increased under all radiation-treatments during the course of the experiment, especially at PAR+UV-radiation (PAB) after one hour of exposure.

The total amount of the photosynthetic pigments chlorophyll *a* and fucoxanthin in the Arctic endemic kelp *Laminaria solidungula* was analysed after six days of exposure at combined temperatures (3, 8, 15°C) and salinities (34, 28, 20) conditions (publication III). In contrast to an unaffected total amount of chlorophyll *a*, increased fucoxanthin content was clearly recognizable under ambient fjord salinity. There was a significant effect of salinity and a tendency of a temperature and salinity interaction, whereas the total fucoxanthin concentration at salinity of 34 was significantly higher than at diluted salinities of 28 and 20.

However, the total content of fucoxanthin was not affected by temperature during this experiment.

4.5.2 Mycosporine-like amino acids

Changes of the total content of mycosporine-like amino acids (MAAs) in the red alga *Devaleraea ramentacea* under impact of combined temperature (5, 10, 17°C) and irradiation (PAB, PA, P) conditions were determined (publication I).

The total content of MAAs varied with the radiation conditions and showed interactive effects of radiation with temperature during the exposure time of six days. MAA-accumulation was clearly recognizable under UV-irradiation, especially at the lowest temperature. The highest amount of MAA was detected at 5°C and PAR+UV-A radiation (PA) and represents a 4-fold increase over the initial value. However, there were no temperature effects on the MAA synthesis within the experiment.

5 Synopsis of Discussion

This thesis demonstrates the importance of research on physiological responses of Arctic marine macroalgae with respect to interactions between two or more environmental stress factors. This is especially relevant to understand stress physiology of these algae with regard to ecological aspects and against the background of global climate change. There is evidence to suggest both, that multiple stress factors interact synergistically or that one factor prevails as a single effect.

5.1 Interactive effects on photosynthesis of Arctic marine macroalgae

Physiological studies on interactive effects of multiple abiotic or biotic stressors are scarce and the current state of knowledge is summarized in the respective chapter in publication V. Presently, there are only very few studies on interactions between temperature and UV-radiation on Arctic macroalgae available (see Rautenberger and Bischof 2006, Müller et al. 2008, Steinhoff et al. 2008). Except for the present study, interactive effects of salinity and temperature have never been evaluated in polar algae (Karsten 2007).

In the present study, no interactive effects, neither of temperature in combination with radiation nor in combination with salinity were detected concerning photosynthetic performance of the brown macroalgae *Alaria esculenta* and *Laminaria solidungula* as well as of the red alga *Devaleraea ramentacea*.

Only the maximum quantum yield of *Saccharina latissima* was inhibited by an interaction of high temperature combined with only photosynthetically active radiation during recovery (publication III). A possible reason for this incomplete recovery might be a potential supporting effect of the short UV-radiation wavelength range in the recovery process as hypothesised by Hanelt et al. (2006). The shallow-water aquatic macrophytes *Isoetes alpinus* and *Potamogeton cheesemanii* from New Zealand showed also the strongest photoinhibition and lowest recovery when UV-B was filtered out (Hanelt et al. 2006). Harmful effects of UV-B radiation were frequently reported, especially in UV-sensitive algae growing in deeper habitats with lower ambient irradiance (reviewed by Franklin and Forster 1997, Bischof et al. 2006, Wiencke et al. 2007). However, in high light adapted plants, a natural occurring UV-B

irradiation may function as a photoreceptor signal required for recovery or repair mechanisms (Hanelt et al. 2006, Hanelt and Roleda 2009). However, better recovery responses in the presence of UV-radiation were primarily observed in different tropical shallow-water marine macroalgae (Hanelt and Roleda 2009).

In some cases it was observed that plants exposed to a single stress agent were capable of increasing their resistance to subsequent unfavourable impacts (= cross-adaption, reviewed by Alexieva et al. 2003). A trend to cross-adaptation was detected e.g. in kelp sporophytes whereas increasing temperature reduced UV-effects. Significant UV-induced inhibitions were only detected in sporophytes of *A. esculenta* exposed to the whole light spectrum under the lowest temperatures applied (publication II). Furthermore, a possible tendency of an inhibitory UV-effect was observed on *S.latissima* with diminishing Fv/Fm values only in the PAB treatments at 5°C (publication III). The corresponding hypothesis that temperature mitigates the net biological effect of UV-radiation on macroalgae is supported by some studies (Gómez et al. 2001, van de Poll et al. 2002, Hoffman et al. 2003, Rautenberger and Bischof, 2006). Gómez et al. (2001) concluded from their study on *Gelidium pulchellum* that increasing growth temperature might stimulate repair processes. Due to increasing temperature, biochemical reactions like catalytic activities of enzymes are also increased which may result in a higher efficiency of photoprotective mechanisms (Raven and Geider 1988). Additionally, the UV-induced inhibition of photosynthesis at 0°C was much higher in *Ulva clathrata* from Chile than in *Ulva bulbosa* from Antarctica, whereas temperatures of 10°C compensated for UV- effects in both species (Rautenberger and Bischof 2006).

The above-mentioned findings indicates that the sensitivity of algae to additional stress factors, e.g. to UV-radiation increases at temperatures lower than the optimum growth temperatures. This simultaneous influence of several stress factors may elevate their deleterious effect, so the simple additive effect of their action alone is considerably exceeded (=cross-synergism, Alexieva et al. 2003). In general, interactions of UV-radiation and temperature represent a general phenomenon and have important implications for studies on climate change (Hoffman et al. 2003).

5.2 Predominant single effects on photosynthesis of Arctic marine macroalgae

Temperature effects

In the present study, temperature as a single parameter was the factor causing most influences on photosynthetic efficiency of Arctic marine macroalgae. There exist a few temperature studies on Arctic kelps, but mainly on growth at the upper or lower survival temperatures (Biebl 1970, Bolton and Lüning 1982, tom Dieck 1993, Wiencke et al. 1994, Bischoff-Bäsmann 1997). In these studies ecotypes, optimal growth conditions and the geographical boundaries are determined, which mostly define the latitudinal distribution of seaweeds.

The photosynthetic response of all four tested macroalgae from Spitsbergen to interactive stress showed species-dependent temperature effects, which correlate equally with the geographical distribution pattern of the species (publication I, II, III). The photosynthetic performance of the both temperate species *A. esculenta* and *S. latissima* reflects a wide range of geographical distribution (see Lüning 1990) and a broad temperature tolerance with an optimum above 10°C. An optimal temperature between 10-15°C is a typical range of cold-temperate North Atlantic species (Wiencke et al. 1994). *Devaleraea ramentacea* represents an almost endemic red alga species to the Arctic, because its geographical distribution is mainly in the Arctic and circumpolar, but reaches into cold-temperate regions as well. In *D. ramentacea* tetrasporophytes a temperature optimum for efficient photosynthesis was detected at about 10°C. However, the lowest temperature optimum of about 0-10°C was found in the Arctic endemic kelp *L. solidungula*. In general, endemic Arctic species grow at temperatures up to 10 or 15°C with growth optima between 5 and 10°C (Wiencke et al. 1994, Dunton and Dayton 1995). Arctic and cold temperate macroalgae from the Northern hemisphere are mostly eurythermic and differ from each other by variations of optimum growth temperatures (below or above 10°C) and upper survival temperatures in contrary to the generally stenothermal Antarctic macroalgae (Wiencke and tom Dieck 1989).

Measurements of photosynthetic efficiency of the four investigated species after exposure at around 20°C showed distinct inhibitory effects. This high water temperature is unnatural in Arctic waters, but by applying it we aimed for the physiological determination of the upper temperature limit. Temperatures around 20°C were lethal for sporophytes of *A. esculenta*, *D. ramentacea* and *L. solidungula*, as seen in decreased maximum quantum yields and bleached, disintegrated algal samples during the experiment. In contrast, photosynthetic

efficiency of *S.latissima* was also inhibited at 20°C, but the temperature was non-lethal for this temperate kelp. Consequently, *S.latissima* showed the broadest temperature tolerance of all examined four Arctic species, which also correlate with its most widespread geographical distribution up to the Portuguese coast (Lüning 1990).

The differences in temperature limits of photosynthetic performance agree basically with respective growth studies on Arctic sporophytes (Sundene 1962, Munda and Lüning 1977, Fortes and Lüning 1980, Rueness and Tananger 1984, Novaczek et al. 1990, Dunton and Dayton 1995) or with gametophyte survival studies (Bolton and Lüning 1982, tom Dieck 1993, Wiencke et al. 1994). Large-scale distribution patterns of seaweeds are determined by climate limits, whereas boundary populations would have either been adapted or became extinct. The southern and northern distribution limits of Arctic and cold-temperate macroalgae correspond to the species-specific thermal requirements for growth and reproduction (Breeman 1988, Gerard and Du Bois 1988, Wiencke et al. 1994). If the water temperatures - as an effect of climate change - increase drastically it can be generally predicted that existing patterns of species distribution will shift further northwards (Breeman 1988, IPCC 2007).

Radiation effects

In addition to pronounced temperature effects, inhibition of photosynthetic performance by UV-B radiation was only observed in *A.esculenta* sporophytes exposed to the whole light spectrum under the low temperatures of 4 and 9°C (publication II). The corresponding hypothesis that increasing temperatures reduces UV-effects was mentioned above. Photosynthesis of *D.ramentacea*, *S.latissima* and *L.solidungula* were unaffected by UV-radiation in the present study. However, the ratio of PAR:UV irradiance was unnatural in the laboratory set-ups, as the proportion of PAR was relatively low in comparison to the natural occurring UV irradiance. These particularly low PAR:UV ratios often applied in laboratory studies may result in a substantial overestimation of UV effects (discussed below).

In general, algae growing in shallow water like *D.ramentacea* and *S.latissima* are more resistant to PAR and UV-radiation than algae from deeper waters (Dring et al. 1996, Hanelt et al. 1997, Bischof et al. 1998). In contrast to these shallow-water species, the deep-water macroalga *L.solidungula* is normally not exposed to UV-radiation in its natural habitat. Overall, the results suggest a relatively high photosynthetic ability of the investigated adult sporophytes to acclimate to a short-term stress caused by UV irradiation.

Salinity effects

The three examined species *D.ramentacea*, *A.esculenta* and *L.solidungula* tolerated the tested salinities ranging from fully marine to hyposaline conditions. Photosynthetic efficiency of *D.ramentacea* sporophytes was unaffected whereas a few salinity effects were detected in the other both species. Significant higher maximum quantum yields were found in *A.esculenta* samples exposed at diluted salinity of 28 and in *L.solidungula* samples at fjord salinity of 34 (publication II, III). Due to the observed salinity tolerance in the present study, all three algal species seem to be well adapted to their Arctic habitat with salinity variations in spring and summer. By comparison, the Arctic endemic and deep-water species *L.solidungula* seems to have a more restricted acclimation potential to salinity changes.

Based on one of the few ecophysiological studies on salinity effects in polar seaweeds, the kelps *A.esculenta*, *S.latissima* and *L.solidungula* were characterized as stenohaline macroalgae (Karsten 2007). By contrast, the shallow-water species *D.ramentacea* with a broad salinity tolerance was identified as euryhaline. In summary, the photosynthetic responses of the Arctic macroalgae showed species-dependent salinity effects, which seem to correlate congruent to the depth zonation pattern of the species. There is the tendency that the inhibitory effects of salinity changes increase with the increasing habitat water depth. Moreover, Karsten (2007) hypothesized that acclimation responses to rapid salinity changes of cold temperate or Arctic species are usually slowed down due to temperature-limited physiological capacity in their natural Arctic habitat. Their temperature optima for photosynthesis or growth are generally higher compared to that of endemic Antarctic macroalgae.

5.3 Sensitivity of the zoospore stage of *Alaria esculenta* to abiotic stress

Interactions of temperature, radiation and salinity on two different life history stages of *Alaria esculenta* from the Kongsfjord were investigated for the first time within this study (publication II). Overall, *A.esculenta* exhibited a highly stage-specific susceptibility towards the experimental treatments. Photosynthetic activity of sporophytes showed individual temperature effects and only very few radiation and salinity effects, whereas germination capacity of zoospores was affected by temperature and salinity changes, and additionally by interactions of both. Microscopic haploid zoospores were proved to be more sensitive than

adult diploid macroscopic sporophytes as other studies showed before (Dring et al. 1996, Coelho et al. 2000, Wiencke et al. 2006, Roleda et al. 2007).

In comparison to *A. esculenta* sporophytes with a temperature optimum in the range between 13 and 17°C, germination of zoospores of *A. esculenta* exhibits a lower optimal temperature range between 2 and 12°C in the present study. However, in both investigated life stages of *A. esculenta*, an upper temperature limit close to 16 to 20°C was determined, which is considerably higher than the natural ambient temperature in the study area. Up to now there are no literature data available to compare thermal optimums of different life stages of Arctic kelp species. Moreover, there are only very few studies on interactions of different abiotic stress factors on early life stages of kelps presently available. Only Steinhoff et al. (2008) and Müller et al. (2008) illustrated that UV-B radiation and high temperatures detrimentally affected the physiology and ultrastructure of zoospores of temperate and Arctic Laminariales.

In the present study, UV-radiation and temperature caused no interactive effects on the germination of *A. esculenta* zoospores. However, zoospores were affected by the interaction of low salinity of 20 combined with the relative high temperature of 16°C. These damaging and increasing effects on germination of *A. esculenta* zoospores indicate a definite cross-synergism (see Alexieva et al. 2003), in which the simultaneous influence of several stress factors elevates their deleterious effect. Nevertheless, a temperature of 16°C is an unrealistic temperature scenario for Arctic seawater even under the forecasted global warming. In consequence both, zoospores and sporophytes of *A. esculenta* are relatively tolerant to changes of environmental conditions, but only up to a relatively unknown species-specific limit. Many more studies on interactive effects, especially on the microscopic, more sensitive developmental stages are required.

5.4 Influence of abiotic interactions on protective mechanisms

In the photosynthetic measurements, all treatments applied resulted in a reduction in Fv/Fm with different extent and velocity of inhibition and recovery. In general, this decrease in Fv/Fm is a response to diverse stress conditions on photosynthesis. Furthermore, such a decrease with an efficient recovery is indicative for ongoing dynamic photoinhibition. Dynamic photoinhibition is considered a photoprotective mechanism in higher plants and algae exposed to a high irradiance of PAR (Osmond 1994; Hanelt et al. 1998; Franklin et al.

2003). By rapid and reversible down-regulation of photosynthetic activity, algae protect themselves against excessive radiation and dissipate excessively-absorbed energy as physiologically harmless thermal radiation in order to prevent irreversible damage to photosynthetic or cellular components. Subsequently, recovery proceeds rapidly after stressful conditions have ceased. The mechanism behind, at least in green and brown algae, is likely to involve a rapid conversion of violaxanthin to zeaxanthin within the xanthophyll cycle, leading to the dissipation of excessively absorbed energy as heat (Demmig-Adams 1990; Demmig-Adams and Adams 1992, Vershinin and Kamnev 1996).

In the study on the green algae *Ulva lactuca* (publication IV), one hour of exposure to high PAR only resulted in an efficient conversion of violaxanthin within the xanthophyll cycle. In contrast, supplemental or exclusive UV-A and UV-B radiation resulted in a violaxanthin accumulation, which indicates a deceleration of violaxanthin conversion within the xanthophyll cycle due to the presence of UV-radiation. This finding is clearly supported by previous field studies conducted on green macroalgae (Bischof et al. 2002, 2006). It was assumed that the UV-induced deceleration of the xanthophyll cycle may be one important mechanism how UV-radiation may increase susceptibility of the photosynthetic machinery to high irradiances of PAR.

In addition, the concentration of the protective pigment lutein increased considerably in *Ulva lactuca* samples under radiation stress. This could be indicative for the existence of an additional light-protective mechanism, as e.g. the lutein-epoxid cycle as originally discovered in green tomato fruit by Rabinowitch et al. (1975). This cycle, described as a new xanthophyll cycle involves the de-epoxidation of lutein-5, 6-epoxid (Lx) to lutein by the xanthophyll cycle enzyme violaxanthin de-epoxidase (Bungard et al. 1999) in the light and the subsequent epoxidation in the dark. It was also demonstrated in the parasitic plant *Cuscuta reflexa* (Bungard et al. 1999, Snyder et al. 2005), in several tree species of *Quercus* (Garcia-Plazaola et al. 2002, 2003) and in the microalgae *Dunaliella tertiolecta* (Antia and Cheng 1983). Presently, our data and observation just allow hypothesizing, and hence more studies on the existence of the lutein-epoxid cycle in marine macroalgae have to be conducted.

Photosynthetic pigments, especially carotenoids as main accessory pigments are affected by a large variety of stresses (Rmiki et al. 1999). One of the most abundant xanthophyll in nature especially in brown algae is fucoxanthin. In the study conducted on the kelp species *Laminaria solidungula* (publication III), the amount of the pigment fucoxanthin was affected by the impact of salinity changes after exposure to combined temperature and salinity stress.

A higher fucoxanthin concentration was measured at ambient fjord salinity, but no temperature effects were detected. Fucoxanthin is a major light-harvesting pigment for photosynthesis and additional functions are unknown. In contrast to this, salinity or temperature changes did not influence the chlorophyll *a* content in *L.solidungula*. In the green microalgae *Dunaliella salina*, impacts of salinity stress on the content of photosynthetic pigments were detected as well (Borowitzka et al. 1990).

In the presence of UV radiation, a lot of red macroalgal species, also *Devaleraea ramentacea* are capable to synthesize and accumulate UV-absorbing mycosporine-like amino acids (MAAs), indicating their important role as sunscreen compounds (Bandaranayake 1998, Karsten et al. 1998, Hoyer et al. 2002, Shick and Dunlap 2002, Karsten et al. 2003). Seven different MAAs were identified yet in the red macroalga *D.ramentacea* from the Kongsfjord, additionally an unknown UV-absorbing compound was found (MAA 357-2, Karsten et al. 1998). In the present study (publication I), a generally higher MAA concentration was measured in the UV-treatments of *D.ramentacea* exposed to combined temperature and irradiation stress. This confirms their important function as natural UV-sunscreens, but recent studies show that these substances have also additional functions, such as antioxidants, nitrogen reservoir or as protective solutes against thermal stress or desiccation (reviewed by Oren and Gunde-Cimerman 2007). A function as osmolyte is negligibly, because salinity changes did not influence MAA accumulation in *D. ramentacea* (Karsten et al. 2003). Also no individual temperature effect on MAA synthesis was detected in the present study, so that a function as thermal protector can be excluded. However, interactive effects of radiation and temperature, especially UV-A radiation in combination with low temperature, affected the total intracellular MAA accumulation of *D. ramentacea*. This could be an indication for possible low temperature-induced MAAs in polar macroalgae as additional UV-protection. Hoyer et al. (2002, 2003) reported similar responses for the Antarctic red algae *Iridaea cordata* and *Palmaria decipiens*, i.e. under enhanced PAR and UV-radiation, the MAA concentrations were higher at 5 than at 10°C.

Furthermore, the total MAA concentration in Rhodophyta seems to correlate with both, the bio-geographic distribution and the ambient water depth (Karsten et al. 1998, 1999, Hoyer et al. 2001). Along the thalli of Rhodophyceae a clear uneven MAA distribution could be found (Hoyer et al. 2001, Karsten et al. 1999). Such distinct tissue gradient was detected in the filiform thallus of *D. ramentacea* as well. The exposed younger, often green apical tissues

contained a considerable higher MAA and chlorophyll *a* content compared to the older, always red basal region with phycobiliproteins (Karsten et al. 1999).

The present study was conducted with complete red thalli of *D. ramentacea*, collected under the sheltering cover of larger kelps. Nevertheless, the greening of tips with a reduced red coloured basis was also observed on samples from exposed places next to the kelps. Such algal samples were studied in a pre-test with the Imaging-PAM fluorometer (Walz, Effeltrich, Germany), which illustrates the photosynthetic activity of a complete algal thallus by different colours. A higher maximum quantum yield in the green tips compared to the red basal parts of the thalli could be observed (data not shown), which indicates a more effective photosynthetic activity of the modified green parts. Preliminary laboratory tests didn't show this greening presumably due to separate exposure to temperature or salinity stress. All these findings can be seen as indication that the greening of *D. ramentacea* is a specific photoacclimation mechanism by reducing phycobiliproteins with a simultaneous increase in the MAA and chlorophyll content as a protection against higher radiation stress at exposed locations. Nevertheless, further studies on the physiology of *D. ramentacea*, especially on the green parts are necessary.

5.5 Importance of the ratios of PAR:UV in experimental studies

The respective ratio of photosynthetically active to ultraviolet radiation is of crucial importance to results obtained in UV-research on photoautotrophic organisms. Exemplified by the green algae *Ulva lactuca* (publication IV), the results clearly show that the extent of UV-induced inhibition of photosynthesis to be found in UV-exposure experiments is highly depending on the irradiance of background photosynthetically active radiation. With increasing PAR-intensity the share of UV-induced inhibition of F_v/F_m is reduced: The higher the PAR-intensity the lower the UV-effects.

These findings correspond with data from experiments on higher plants (Teramura 1986, Fiscus and Booker 1995) in which significant interactions between UV-B radiation and PAR were observed with respect to photoinhibition of photosynthesis. In this study, the extent of UV-B effects in soybean depended on PAR-irradiance and, furthermore, adverse UV effects could be ameliorated under PAR-levels approaching normal natural conditions. These studies and present results from the irradiation-experiment with *Ulva lactuca* show the particular

importance of the ratios PAR:UV, especially for comparisons between laboratory and field experiments.

It is likely that the inconsistency of PAR:UV ratios applied in laboratory studies result in significant variations of UV effects observed in the different studies on algae conducted so far (see reviews by Fiscus and Booker 1995; Wängberg et al. 1996; Franklin and Forster 1997; Allen et al. 1998). Fiscus and Booker (1995) criticized unrealistic UV-B exposure levels applied in many experiments. They stated, that high UV-B exposures at very low PAR-levels are necessary to produce many of the described UV effects, resulting in a substantial overestimation of these UV-effects.

Artificial radiation conditions in laboratory studies are hardly able to mimic natural solar radiation. By natural high irradiances of UV-radiation do only occur in combination with high levels of PAR (Hanelt et al. 2003) and the natural solar spectrum exhibits ratios of energy distribution over the different wavelength ranges of UVB: UVA: PAR = 0.6:10:100 at the surface of the earth (Franklin and Forster 1997). In the laboratory set-ups during the present study, the ratio of PAR:UV irradiance was also unnatural. The proportion of PAR was relatively low in comparison to the UV irradiance, whereas UV-B radiance was rather similar to or somewhat higher than naturally occurring intensities in the ambient algal habitats in different water depths. UV-intensities were still lower, but more realistic than in many previous UV-studies in order to avoid an overestimation of UV-effects.

In the numerous studies on Arctic marine macroalgae, mainly in single-factor experiments to UV-effects the various ratios of PAR:UV irradiance resulted in differences in the degree of UV-inhibition and recovery (reviewed by Franklin and Forster 1997, Bischof et al. 2006, Roleda et al. 2007, Wiencke et al. 2007 and referees therein). Sometimes there were additionally stress parameters integrated accidentally in these previous studies, for example temperature below the optimum as a further stress factor. However, almost all stress responses of the Arctic macroalgae, including these of the present study agree in the following sensitivity tendencies: increased UV-sensitivity with increasing water depth, temperature tolerances depending on geographical distribution, higher sensibility of small developmental or younger stages.

The interpretation of the studies depends on the physiological or ecological questions applied. It is important to determine how the effects of the different abiotic stress factors interact on the physiological level and furthermore how effects can be distinguished. In an ecological context it is important to note that the proportion of UV-radiation in the inhibition of

photosynthesis is highly depending on the irradiance of PAR and also on interactions with other environmental factors, which is confirmed by this present study on interactive effects. However, the main part of this study focuses on the physiological responses, as photosynthesis or protection mechanisms of the Arctic marine macroalgae to multiple abiotic stress factors. Ecological implications of environmental changes were estimated with the respective caution.

5.6 Ecological conclusion and future perspectives

In summary, the photosynthetic response of adult sporophytes of Arctic marine macroalgae from Spitsbergen reflected a wide tolerance to investigated abiotic factors. Accordingly, they turn out to be highly tolerant to both, single as well as multiple and combined abiotic stress factors.

Temperature seems to be the predominant environmental parameter for all investigated macroalgal species, whereas the photosynthetic performance showed pronounced species-dependent optima or inhibitory effects. Temperature is not only responsible for the regulation of metabolism and reproduction but also for the range of distribution of macroalgae. The temperature tolerance of the investigated species correlates with the geographical distribution pattern. The tested (0-20°C) and tolerated temperature ranges are mainly considerably higher than the ambient temperature in situ in the study area. For comparison, the average summer seawater temperature in the Kongsfjord, the natural environment of the algae is around 4°C (Hanelt et al. 2001, Svendsen et al. 2002). Nonetheless, upper temperature limits below or near 20°C were determined in these macroalgae.

The investigated species tolerated also the tested salinities ranging from fully marine (34) to hyposaline (28, 20) conditions. In the study area, the local water mass of the Kongsfjord has a salinity of about 34.5psu in spring and drops below 28psu in the surface water near the glacier in summer (Hanelt et al. 2001, Svendsen et al. 2002). Therefore, *D.ramentacea* and *A.esculenta* seem to be well adapted to their Arctic habitat with the inflow of freshwater due to snow and glacier melts during spring and summer. However, the Arctic endemic species *L.solidungula* seems to have limited acclimation ability at diluted salinity conditions.

The underwater radiation regime of the Kongsfjord is also subject to strong seasonal variations, sea ice cover as well as actual weather conditions and the turbidity of the water column. UV-B radiation can penetrate down to 6-10m depths in clear waters conditions in the

Kongsfjord, and consequently affect macroalgae inhabiting shallow waters (Hanelt et al. 2001, Svendsen et al. 2002). Generally, algae growing in shallow water like *D.ramentacea* and *S.latissima* are more PAR and UV-radiation resistant (Hanelt et al. 1997, Bischof et al. 1998) than algae from deeper waters like *L.solidungula*, which, in their natural habitat, are normally not exposed to UV-radiation. Nevertheless, almost no impacts of UV-radiation on photosynthesis of the investigated adult sporophytes were detected, which suggested a relatively high photosynthetic ability to acclimate to short-term stress of UV irradiation. UV-induced inhibitions were only detected in sporophytes exposed to the whole light spectrum under the lowest temperatures applied. The UV-induced sensitivity of algae seems to increase under the very low temperatures like naturally occurring in their Arctic habitat.

Several protective mechanisms such as the xanthophyll cycle, protective pigments or MAAs were detected in macroalgae so far, but those can be also influenced by diverse abiotic stress factors or their interactions. However, the findings of this study could also provide indications of potential additional protective mechanisms occurring in the investigated marine macroalgae. The function of MAAs as natural UV-sunscreens in *D.ramentacea* was confirmed, but potentially with an accumulation of low temperature-induced MAAs in polar algae as possible additional UV-protection. The pigment analysis on *U.lactuca* indicated the potential existence of another light-protective mechanism, the lutein-epoxid cycle in macroalgae.

However, in the Arctic coastal ecosystem marine macroalgae are exposed to potential effects of climate changes. Widespread melting of glaciers and sea ice in correlation with a decreasing ocean salinity in the upper 500 m, increasing precipitation and changing wind pattern, warming of permafrost or increased UV-radiation resulting from stratospheric ozone depletion represent additional evidence of expected strong Arctic warming. The scenarios described in the IPCC report (2007) predict that the annual Arctic surface temperatures north of 60°N will increase by 2-4°C by mid-century and by 4-7°C compared to the present towards the end of the 21st century. Furthermore, the increased temperature of the Arctic Ocean, including Spitsbergen will lead to an earlier ice melt and later freezing within the yearly cycle and to a decrease in sea-ice coverage, especially during summer (ACIA 2005, IPCC 2007).

In an ecological context, and in particular with regard to the forecast possible environmental changes in the Arctic, the adult sporophytes of *D.ramentacea*, *S.latissima*, *A.esculenta*, *L.solidungula* proved to be highly tolerant and adaptable to increased temperature and UV-radiation and a decreased salinity. Microscopic stages of *A.esculenta* were shown to be more

sensitive than the adult macroscopic stages, since germination capacity of zoospores was additionally affected by interactions of temperature and salinity changes in the present study. These detected tolerances are only valid up to a yet relatively unknown species-specific limit. In comparison to the temperate kelp species, the Arctic endemic species *L.solidungula* seems to have a more limited ability to adapt to its changing Arctic habitat. However, based on the results of the present study it can be hypothesized that the macroscopic sporophytes will most probably be able to acclimate to global change scenarios in Arctic waters. Consequently, the relatively high tolerant adult stages of investigated Arctic marine macroalgae are only suitable to a limited extent for studies on impacts of climate change.

Future research on the basis of the present thesis should focus on further effects and interactions of diverse multiple abiotic and also biotic (stress) factors on marine macroalgae, especially on more algal species and on the probably more sensitive, yet often uninvestigated microscopic developmental stages as zoospores and gametes.

Many more studies, also with ecological background to interactive impacts on stress physiology of macroalgae and their acclimation mechanisms are required to make accurate predictions about the tolerance pattern of a complete species, its acclimation ability and specific limits of seaweeds. To clarify the existence of the lutein-epoxid cycle in macroalgae, further HPLC-analyses should be conducted with a potential new developed pigment standard for luteinepoxid. Additional functions of mycosporine-like amino acids and especially the occurrence of low temperature-induced MAAs in polar algae should be clarified. More studies should also concentrate on accumulated compounds or secondary metabolites in macroalgae, which have protective functions such as antioxidants (e.g. glutathione, superoxide dismutase), osmoprotectants (e.g. mannitol) or cryoprotectants.

Further laboratory studies should additionally focus on interactive effects at the molecular level of macroalgae, especially to DNA damages and changes in gene expressions. Especially field mesocosm studies with different species and life-stages under controlled, but natural environmental conditions are indispensably to analyse their complex interactions also on community level. This is essential to draw physiological and ecological conclusions about the development of macroalgal communities and potential impacts of global change in the future.

Several previous studies (Fiscus and Booker 1995; Allen et al. 1998; Han et al. 1998, Franklin et al. 2003, Bischof et al. 2006, Wiencke et al. 2007, Bartsch et al. 2008) as well as the present study suggest that future ecologically relevant experiments need a realistic range of PAR/UV irradiance in combination with other ecologically relevant abiotic and biotic factors, e.g. realistic nutrient concentrations, temperature or competitors. This is necessary if the results should be used in order to study not only physiological responses, but also be comparable with the natural environment and in relation to stratospheric ozone depletion and global warming.

References

- ACIA 2005, Arctic Council and International Arctic Science Committee (2005). Arctic climate impact assessment – Scientific report. Cambridge University Press, Cambridge, 1042 pp
- Adir N, Zer H, Shochat S, Ohad I (2003) Photoinhibition – a historical perspective. *Photosynth Res* 76(1-3):343–370
- Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Mar Ecol Prog Ser* 191:109–119
- Alexieva V, Ivanov S, Sergiev I, Karanov E (2003) Interaction between stresses. *Bulg J Plant Physiol (Special Issue)*:1–17
- Allen D, Nogues S, Baker NR (1998) Review article. Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *J Exp Bot* 49:1775–1788
- Antia NJ, Cheng JY (1983) Evidence for anomalous xanthophyll composition in a clone *Dunaliella tertiolecta* (Chlorophyceae). *Phycologia* 22(3):235–242
- Baker NR (2008) Chlorophyll Fluorescence: A probe of Photosynthesis in vivo. *Annu Rev Plant Biol* 59:89–113
- Bandaranayake WM (1998) Mycosporines: are they nature's sunscreens? *Nat Prod Rep* 15(2):159–172
- Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: recent insights and developments. *Eur J Phycol* 43(1):1–86
- Biebl R (1970) Vergleichende Untersuchungen zur Temperaturresistenz von Meeresalgen entlang der pazifischen Küste Nordamerikas. *Protoplasma* 69(1):61–83
- Bischof K, Gomez I, Molis M, Hanelt D, Karsten U, Lüder U, Roleda MY, Zacher K, Wiencke C (2006) Ultraviolet radiation shapes seaweed communities. *Rev Environ Sci Biotechnol* 5(2-3):141–166
- Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Mar Biol* 140(6):1097–1106
- Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol* 20(6):388–395
- Bischof K, Hanelt D, Wiencke C (1998) UV-radiation can affect depth-zonation of Antarctic macroalgae. *Mar Biol* 131(4):597–605
- Bischof K, Hanelt D, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biol* 1:435–444
- Bischof K, Hanelt D, Wiencke C (2000) Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* 211:555–562
- Bischof K, Kräbs G, Wiencke C, Hanelt D (2002) Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* 215(3):502–509
- Bischoff-Bäsmann B (1997) Temperature requirements and biogeography of marine macroalgae

- Adaptation of marine macroalgae to low temperatures. Rep Polar Res 245:134pp
- Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Mar Biol 66:89–94
- Borowitzka MA, Borowitzka LJ, Kessly D (1990) Effects of salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*. Journal of Applied Phycology 2(2):111–119
- Breeman AM (1988) Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: experimental and phenological evidence. Helgol Meeresunters 42:199–241
- Bungard RA, Ruban AV, Hibberd JM, Press MC, Horton P, Scholes JD (1999) Unusual carotenoid composition and a new type of xanthophyll cycle in plants. Proc Natl Acad Sci USA 96:1135–1139
- Chapman ARO, Lindley JE (1980) Seasonal growth of *Laminaria solidungula* in the Canadian High Arctic in relation to irradiance and dissolved nutrient concentrations. Mar Biol 57(1):1–5
- Coelho SM, Rijstenbil JW, Brown MT (2000) Impacts of anthropogenic stresses on the early development stages of seaweeds. J. Aquat. Ecosyst. Stress Recovery 7(4):317–333
- Dahlback A (2002) Recent changes in surface ultraviolet solar radiation and stratospheric ozone at a high Arctic site. In: Hessen DO (ed) UV radiation and Arctic ecosystems. Ecol Stud Anal Synth 153, pp 3–22
- Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochemica et Biophysica Acta, Bioenergetics 1020(1):1–24
- Demmig-Adams B, Adams III WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 43:599–626
- Dring MJ, Makarov V, Schoschina E, Lorenz M, Luening K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). Mar Biol 126(1):183–191
- Dunton KH (1985) Growth of dark-exposed *Laminaria saccharina* (L.) Lamour. and *Laminaria solidungula* J. Ag. (Laminariales: Phaeophyta) in the Alaskan Beaufort Sea. J Exp Mar Biol Ecol 94(1-3):181–189
- Dunton KH, Dayton PK (1995) The biology of high latitude kelp. In: Skjoldal HR, Hopkins C, Erikstad KE, Leinaas HP (eds) Ecology of Fjords and Coastal Waters: Proceedings of the mare Nor Symposium on the Ecology of Fjords and Coastal Waters, Tromsø, Norway, 5-9 December, 1994. Elsevier Science B.V., Amsterdam, pp 499–508
- Dunton KH, Jodwalis CM (1988) Photosynthetic performance of *Laminaria solidungula* measured *in situ* in the Alaskan High Arctic. Mar Biol 98(2):277–285
- Fiscus EL, Booker FL (1995) Is increased UV-B a threat to crop photosynthesis and productivity? Photosynth Res 43:81–92
- Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44(3 pt 2):864–877
- Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgol Meeresunters 34:15–29
- Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32(3):207–232
- Franklin LA, Osmond CB, Larkum AWD (2003) Photoinhibition, UV-B and Algal Photosynthesis. In: Larkum AWD, Douglas SE, Raven JA (eds) Photosynthesis in Algae. Kluwer Academic Publishers, Dordrecht, pp 351–384
- García-Plazaola JI, Hernández A, Errasti E, Becerril JM (2002) Occurrence and operation of the

- lutein epoxide cycle in *Quercus* species. *Funct Plant Biol* 29(9):1075–1080
- García-Plazaola JI, Hernández A, Olano JM, Becerril MB (2003) The operation of the lutein epoxide cycle correlates with energy dissipation. *Functional Plant Biology* 30(388):319–324
- Gomez I, Wiencke C (1997) Seasonal growth and photosynthetic performance of the Antarctic macroalga *Desmarestia menziesii* (Phaeophyceae) cultivated under fluctuating Antarctic daylengths. *Bot Acta* 110:25–31
- Gómez I, Figueroa FL, Sousa-Pinto I, Viñegla B, Pérez-Rodríguez E, Maestre C, Coelho S, Felga A, Pereira R (2001) Effects of UV radiation and temperature on photosynthesis as measured by PAM fluorescence in the red alga *Gelidium pulchellum* (Turner) Kützinger. *Bot Mar* 44(1):9–16
- Han T, Chung H, Kang S-H (1998) UV photobiology of marine macroalgae. *Korean J Polar Res* 9(1):37–46
- Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Mar Biol* 131(2):361–369
- Hanelt D, Roleda M (2009) UVB radiation may ameliorate photoinhibition in specific shallow-water tropical marine macrophytes. *Aquat Bot* in press
- Hanelt D, Wiencke C, Nultsch W (1997) Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *J Photochem Photobiol B: Biol* 38(1):40–47
- Hanelt D, Hawes I, Rae R (2006) Reduction of UV-B radiation causes an enhancement of photoinhibition in high light stressed aquatic plants from New Zealand lakes. *J. Photochem. Photobiol. B: Biol.* 84(2):89–102
- Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Mar Ecol Prog Ser* 149(1-3):255–266
- Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138(3):649–658
- Hanelt D, Wiencke C, Bischof K (2003) Photosynthesis in Marine Macroalgae. In: Larkum AW, Douglas SE, Raven JA (eds) *Photosynthesis in Algae*. *Advances in Photosynthesis and Respiration* 18, pp 413–435
- Hoffman JR, Hansen LJ, Klinger T (2003) Interactions between UV radiation and temperature limit inferences from single-factor experiments. *J Phycol* 39(2):268–272
- Hoyer K (2003) Occurrence, induction and physiological importance of UV-absorbing substances in polar macroalgae. Doctoral thesis. Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, *Rep Polar Mar Res* 440, 155 pp
- Hoyer K, Karsten U, Sawall T, Wiencke C (2001) Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. *Mar Ecol Prog Ser* 211:117–129
- Hoyer K, Karsten U, Wiencke C (2002) MAA synthesis and accumulation in polar macroalgae are controlled by abiotic factors. *J. Phycol.* 38(s1):17
- IPCC 2007, Intergovernmental Panel on Climate Change (2007). *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment. Cambridge University Press, Cambridge, 996 pp
- Jones HG, Flowers TJ, Jones MB (2008) *Plants under Stress, Biochemistry, Physiology and Ecology and their Application to Plant Improvement*. Cambridge University Press 1989
- Karsten U (2007) Research Note: Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55(4):257–262
- Karsten U (2008) Defense Strategies of Algae and Cyanobacteria Against Solar Ultraviolet

- Radiation. In: Amsler CD (ed) *Algal Chemical Ecology*. pp 273–296
- Karsten U, Bischof K, Hanelt D, Tüg H, Wiencke C (1999) The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). *Physiol Plant* 105:58–66
- Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters. *Oecologia* 127(1):11–20
- Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. *Polar Biol* 26(4):249–258
- Karsten U, Sawall T, Hanelt D, Bischof K, Figueroa FL, Flores-Moya A, Wiencke C (1998) An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Bot Mar* 41:443–453
- Kirst G (1990) Salinity Tolerance Of Eukaryotic Marine Algae. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:21–53
- Korb RE, Gerard VA (2000) Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments. *Mar Ecol Prog Ser* 198:83–92
- Lüning K (1990) *Seaweeds. Their environment, biogeography, and ecophysiology*. John Wiley & Sons, Inc, New York, 527 pp
- McCormick P, Cairns J (1994) Algae as indicators of environmental change. *Journal of Applied Phycology* 6(5):509–526
- Michler T, Aguilera J, Hanelt D, Bischof K, Wiencke C (2002) Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. *Mar Biol* 140(6):1117–1127
- Munda IM, Luening K (1977) Growth performance of *Alaria esculenta* off Helgoland. *Helgol. Wiss. Meeresunters* 29(3):311–314
- Müller R, Wiencke C, Bischof K (2008) Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Clim Res* 37(2-3):203–213
- Novaczek I, Lubbers G, Breeman A (1990) Thermal ecotypes of ampho-Atlantic algae. I. Algae of Arctic to cold-temperate distribution (*Chaetomorpha melagonium*, *Devaleraea ramentacea* and *Phycodryas rubens*). *Helgol Mar Res* 44(3):459–474
- Nygård C, Ekelund N (2006) Photosynthesis and UV-B Tolerance of the Marine Alga *Fucus vesiculosus* at Different Sea Water Salinities. *J Appl Phycol* 18(3):461–467
- Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiol. Lett.* 269(1):1–10
- Osmond CB (1994) What is photoinhibition? Insights from comparisons of shade and sun plants. In: Baker NR, Bowyer JR (eds) *Photoinhibition of Photosynthesis from molecular mechanisms to the field*. BIOS Scientific Publishers, Oxford, pp 1–24
- Rabinowitch HD, Budowski P, Kedar N (1975) Carotenoids and epoxide cycles in mature-green tomatoes. *Planta* 122(1):91–97
- Rautenberger R (2008) *Physiological reactions of marine macrophytes along abiotic stress gradients*. Doctoral thesis. University of Bremen, Bremen, Germany, 175pp
- Rautenberger R, Bischof K (2006) Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol* 29(11):988–996
- Raven JA, Geider RJ (1988) Temperature and algal growth. *New Phytol* 110:441–461
- Rmiki NE, Schoefs B, Lemoine Y (1999) Carotenoids and stress in higher plants and algae. In: M. Pessarakli (ed) *Handbook of Plant and Crop Stress*. CRC Press Inc., pp 465–482
- Roleda MY, Hanelt D, Wiencke C (2005) Growth kinetics related to physiological parameters in

- young *Saccorhiza* dermatodea and *Alaria* esculenta sporophytes exposed to UV radiation. *Polar Biol* 28:539–549
- Roleda MY, Wiencke C, Hanelt D, Bischof K (2007) Sensitivity of the Early Life Stages of Macroalgae from the Northern Hemisphere to Ultraviolet Radiation. *Photochemistry and Photobiology* 83(4):851–862
- Rueness J, Tananger T (1984) Growth in culture of four red algae from Norway with potential for mariculture. *Hydrobiologia* 116-117(1):303–307
- Russell G (1987) Spatial and environmental components of evolutionary change: interactive effects of salinity and temperature on *Fucus vesiculosus* as an example. *Helgol Mar Res* 41(3):371–376
- Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E-D, Caldwell MM (eds) *Ecophysiology of photosynthesis*. *Ecol Stud Anal Synth* 100, pp 49–70
- Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu Rev Physiol* 64:223–262
- Snyder AM, Clark BM, Bungard RA (2005) Light-dependent conversion of carotenoids in the parasitic angiosperm *Cuscuta reflexa* L. *Plant Cell Environ* 28:1326–1333
- Sokal R, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*. 887 pp
- Steinhoff FS, Wiencke C, Müller R, Bischof K (2008) Effects of ultraviolet radiation and temperature on the ultrastructure of zoospores of the brown macroalga *Laminaria hyperborea*. *Plant Biology* 10(3):388–397
- Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environ Conservat* 29(4):436–459
- Sundene O (1962) The implications of transplant and culture experiments on the growth and distribution of *Alaria esculenta*. *Nytt Magasin for Botanikk* 9:155–174
- Svendsen H, Beszczynska-Moeller A, Hagen JO, Lefauconnier B, Tverberg V, Gerland S, Oerbaeck JB, Bischof K, Papucci C, Zajaczkowski M, Attolini R, Bruland O, Wiencke C, Winther JG, Dallmann W (2002) The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 21(1):133–166
- Teramura AH (1986) Interaction between UV-B radiation and other stresses in plants. In: Worrest RC, Caldwell MM (eds) *Stratospheric Ozone Reduction / Solar Ultraviolet Radiation and Plant Life*. NATO ASI Ser, pp 327–343
- Thomas DN, Collins JC, Russell G (1988) Interactive effects of temperature and salinity upon net photosynthesis of *Cladophora glomerata* (L.) Kuetz. and *C. rupestris* (L.) Kuetz. *Bot.Mar.* 31(1):73–77
- tom Dieck(Bartsch) I (1993) Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *Mar Ecol Prog Ser* 100:253–264
- Van de Poll WH, Hanelt D, Hoyer K, Buma AGJ, Breeman AM (2002) Ultraviolet-B-induced Cyclobutane-pyrimidine Dimer Formation and repair in arctic Marine Macrophytes. *Photochem Photobiol* 76(5):493–500
- Véliz K, Edding M, Tala F, Gomez I (2006) Effects of ultraviolet radiation on different life cycle stages of the south Pacific kelps *Lessonia nigrescens* and *L. trabeculata* (Laminariales, Phaeophytaceae). *Mar Biol* 149(5):1015–1024
- Vershinin AO, Kamnev AN (1996) Xanthophyll Cycle in Marine Macroalgae. *Bot Mar*

- 39(5):421–425
- Vincent W, Rautio M, Pienitz R (2007) Climate control of biological UV exposure in polar and alpine aquatic ecosystems. *Arctic Alpine Ecosystems and People in a Changing Environment*. pp 227–249
- Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos* 104(451-457)
- Weatherhead EC, Andersen SB (2006) The search for signs of recovery of the ozone layer. *Nature* 441(7089):39–45
- Wiencke C (2004) The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991-2003. Alfred-Wegener Institut für Meeres- und Polarforschung, Bremerhaven, Rep Polar Mar Res 492
- Wiencke C, tom Dieck I (1989) Temperature requirements for growth and temperature tolerance of macroalgae endemic to the Antarctic region. *Mar Ecol Prog Ser* 54:189–197
- Wiencke C, Bartsch I, Bischoff B, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37(3):247–259
- Wiencke C, Clayton MN, Gómez I, Iken K, Lüder UH, Amsler CD, Karsten U, Hanelt D, Bischof K, Dunton K (2007) Life strategy, ecophysiology and ecology of seaweeds in polar waters. *Rev Environ Sci Biotechnol* 6(1-3):95–126
- Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa FL (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. *Mar Ecol Prog Ser* 197:217–229
- Wiencke C, Roleda MY, Gruber A, Clayton MN, Bischof K (2006) Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments. *J Ecol* 94(2):455–463
- Wängberg SÅ, Selmer JS, Eklund NGA, Gustavson K (1996) UV-B effects on Nordic marine ecosystems – a literature review. *TemaNord* 1996(515):603–613

ATTACHEMENT OF

PUBLICATION I

Fredersdorf J, Karsten U, Bischof K

Physiological responses of the Arctic red alga *Devaleraea ramentacea* to
interactive environmental stress

Polar Biology (under review)

Physiological responses of the Arctic red alga *Devaleraea ramentacea* to interactive environmental stress

Jana Fredersdorf^{1,2,x}, Ulf Karsten³, Kai Bischof¹

¹ Department of Marine Botany, University of Bremen, D-28359 Bremen, Germany;

² Alfred-Wegener-Institute for Polar and Marine Research, D-27570 Bremerhaven, Germany;

³ Institute of Biological Sciences, Applied Ecology, University of Rostock, D-18057 Rostock, Germany

^x corresponding author

fax: +49 421 218 2285, email: Jana.Fredersdorf@awi.de

Key words: Arctic, interactive effects, mycosporine-like amino acids, photosynthesis, red algae

Abstract

The red macroalga *Devaleraea ramentacea* (L.) Guiry represents a typical and abundant species in the Arctic. Previous investigations on these taxa revealed a generally high UV- tolerance and adaptability to gradients of single abiotic factors. Since in the natural environment abiotic conditions are always interconnected, the interactive effects of temperature, radiation and salinity on the physiology of *D. ramentacea* from the Kongsfjord (Spitsbergen) were investigated. Adult macroscopic tetrasporophytes were exposed to three different temperatures between 4 and 17°C which were combined with natural irradiation conditions (photosynthetically active radiation, UV-A radiation, UV-B radiation in the field experiment) and with different diluted salinities (34, 28, 20, in the lab experiment). Measurements of the photosynthetic activity confirmed the high tolerance of *D. ramentacea* against stress caused by either changing a single factor or caused by combined changes of different factors. No interactive effects on photosynthesis, neither of temperature combined with radiation nor with salinity were observed. However, the factor causing most inhibiting influence on the photosynthesis of *D. ramentacea* was temperature,

which could also be an ecologically crucial parameter for this Arctic species. Analysis of the total amount of mycosporine-like amino acids (MAAs) confirmed the function of these UV-absorbing compounds as natural UV-sunscreens. Additionally, a simulating interactive influence of ultraviolet radiation combined with lower temperature on MAA concentration was detected. This could be an indication for a possible low temperature induced MAA accumulation in polar macroalgae. In an ecological context regarding to environmental changes in the Arctic, the adult macroscopic stages of *D. ramentacea* proved to be relatively tolerant and adaptable to increased temperature and UV- radiation and decreased salinity.

Introduction

In Arctic coastal ecosystems marine macroalgae are exposed to distinct seasonal variations in environmental conditions as well as to potential effects of (future) climate changes. The results of assessments conducted by both ACIA (Arctic Climate Impact Assessment, 2005) and the IPCC (Intergovernmental Panel on Climate Change, 2007) indicate that the conditions in the Arctic have been changed in the last decades. Numerous long-term changes in Arctic climate have been observed, of which temperature increases are most conspicuous. Nevertheless the whole ecosystem is affected and changes in the environmental conditions result from the interplay of various physical parameters. Widespread melting of glaciers and sea ice together with a decrease of ocean salinity in the upper 500 m, increases in precipitation and wind patterns, warming of permafrost or increased UV radiation resulting from stratospheric ozone depletion represent additional evidence of expected strong Arctic warming. In general, land areas warm faster than the ocean, but warming has occurred in both systems (ACIA, 2005). The scenarios of the IPCC report (2007) predict that the annual Arctic surface temperatures north of 60°N will be 2-4°C higher by mid-century and 4-7°C higher toward the end of the 21st century compared to the present. An increase of precipitation by about 20% towards the next 100 years is projected. Furthermore, the increased temperature of the Arctic Ocean, including Spitsbergen will lead to earlier ice melt and later freeze-up within the yearly cycle and to a decrease in sea-ice cover, especially in summer (IPCC report, 2007).

Devaleraea ramentacea (L.) Guiry represents one of the few macroalgal species almost endemic to the Arctic, and thus may potentially serve as indicator organism for upcoming environmental changes. This taxa belongs to the family Palmariaceae (Rhodophyta) and its geographical distribution is mainly in the Arctic and circumpolar, but reaches into cold-temperate regions as well. The southern distributional limit in the North Atlantic extends to the Faroe Islands and central Norway (Rueness and Tananger 1984, Lüning 1990).

There are only few studies on *D. ramentacea*, which are focussed to its respective habitat requirements. *Devaleraea ramentacea* is characterised as fast-growing cold-water alga with an optimum temperature range of 6-10°C (Rueness and Tananger 1984, Lüning 1990). Furthermore, *D. ramentacea* is a shallow water species and hence often exposed to high solar radiation, which is reflected in a high UV-tolerance with only little seasonal variations and a high potential of acclimation (Karsten et al. 1999, Bischof et al. 2002). Different adaptive and protective strategies were detected in *D. ramentacea*, such as high antioxidant activities of the enzymes superoxide dismutase (SOD), glutathion reductase (GR) and catalase (CAT) (Aguilera et al. 2002b). An increase in chlorophyll *a* concentrations during a period of decreasing water transmittance was detected (Aguilera et al. 2002a). For UV-protection *D. ramentacea* is capable to synthesize and accumulate UV-absorbing mycosporine-like amino acids (MAAs) (Karsten et al. 1998).

Mycosporine- like amino acids are water-soluble, small molecules absorbing ultraviolet radiation. They act as natural UV- screening compounds and are widespread in nature with the highest diversity of compounds detected in Rhodophyta (reviewed by Bandaranayake 1998, Shick and Dunlap 2002). The occurrence of MAAs in variable concentrations is known from several studies on *D. ramentacea*, also carried out on plants collected from the Kongsfjord (Karsten et al. 1998, 1999, 2003, Hoyer et al. 2001). Seven different MAAs were yet identified in this red alga, but additionally an unknown UV- absorbing compound was found (MAA 357-2, Karsten et al. 1998). Furthermore, the total MAA concentration in Rhodophyta seems to correlate with both, the biogeographic distribution and the water depth (Karsten et al. 1998, 1999, Hoyer et al. 2001). The physiological function of MAAs as natural UV- sunscreens is well investigated, but in recent studies there is evidence for additional functions of mycosporines such as osmolytes, antioxidants, nitrogen reservoir or as protective compatible solutes against thermal stress or desiccation (reviewed by Oren and Gunde-Cimerman 2007). In macroalgae such additional functions of MAAs are not yet documented.

All previous and mainly unifactorial experiments revealed a generally high tolerance and adaptability of *D. ramentacea* to variations in abiotic conditions, especially to changes in radiation stress. However, macroalgae in their natural environment are exposed to multiple abiotic factors which all are interconnected and interdependent. The responses of *D. ramentacea* to simultaneous impacts of environmental stress are badly studied, but are important to better understand algal physiology and ecological implications of environmental changes. The focus of the present study is to identify effects and interactions of temperature, radiation and salinity on photosynthesis and potential acclimation mechanisms of *D. ramentacea* from Spitsbergen.

Materials and methods

Study site and algal material

The study was conducted in the Kongsfjord located at the northwestern coast of Spitsbergen (Norway, 78°55.5'N; 11°56.0'E). The fjord has a salinity of about 34.5psu (practical salinity units) and a summer seawater temperature of about 4°C (Hanelt et al. 2001, Svendsen et al. 2002). During spring and early summer, the fjord is characterised generally as ice-free and shows a high transparency to solar UV radiation. At our study site around Ny Alesund (located at the southern shore of the Kongsfjord), *D. ramentacea* grows in the upper sublittoral, at 1-8 meters depth on bedrock or occasional rocks (e.g. drop-stones) and is accordingly a typical shallow water species (Wiencke et al. 2004). The thallus of *D. ramentacea*, arising from a small basal disc, consists of one or several erect, simple, hollow fronds with a length of about 10cm. Its heteromorphic life cycle includes microscopic female gametophytes, macroscopic male gametophytes and macroscopic tetrasporophytes. Only healthy-looking, dark red-pigmented algal samples were collected by Scuba divers at depths of about 3-5m from sheltered and shaded sites below kelp beds and transported in opaque plastic containers to avoid exposure to high solar irradiance. In June 2005 samples for the field experiment were collected, the laboratory experiment was conducted in July 2006. In the experiments always intact and from epibiota cleaned unialgal tetrasporophytes were used.

Field set-up with combined radiation and temperature treatments

For the field experiment, out on the Old Pier in Ny Alesund, algal material was exposed to natural photosynthetically active radiation (PAR) and ultraviolet radiation (UV) at defined

increasing temperatures for duration of 6 days.

Small plastic baskets containing cleaned algal thalli were placed in three temperature controlled water tanks (mesocosm, 190x80x15cm) supplied with flow-through running seawater causing water circulation and permanent movement. The algae were covered by a water-layer of about 10cm. Temperature in the mesocosms was controlled by cryostats (model 1160S, VWR International GmbH, Germany) and monitored by temperature loggers (testo 175-T1, Testo AG, Lenzkirch, Germany) as well as by digital probe thermometer (TFA Dostmann GmbH Co.KG, Germany). During the experimental run, temperature in the water tanks was adjusted to (1.) 17°C (17.4 ±1.9°C) and (2.) 10°C (9.8 ±1.4°C) and (3.) ambient fjord temperature of 5°C (5.1 ±0.7°C), but with minor variations due to fluctuating environmental conditions e.g. temperatures, rainfall, wind. The natural solar radiation was measured close by on the roof of the NDSC observatory (Network for the Detection of Stratospheric Change) with a LI-190 Quantum Sensor connected to a LI-250 light meter (LI-COR, Lincoln, USA) and a UV-radiometer PMA2100 (Solar light, USA). During the experiment maximum surface irradiance of PAR was 1000-1300 μmol m⁻² s⁻¹ and of UV- radiation 0.7W m⁻² UV-B (±0.17W m⁻², unweighted) and 14.5W m⁻² UV-A (±0.38W m⁻², unweighted). In order to shield the set-up against extremely high solar radiation, the mesocosms were covered by one layer of black net gauze, which led to a reduction in ambient radiation by 40-50%. Consequently, the maximum irradiation of exposed algae was about 600 μmol m⁻² s⁻¹ PAR, 7W m⁻² UV-A and 0.4W m⁻² UV-B. In order to generate different radiation treatments with and without UV- radiation the plastic baskets were additionally shielded with different cut-off foils. Three baskets for each of the three radiation conditions were randomly distributed in each mesocosm. Foils for (1.) PAR treatments (P) were transparent to wavelengths of 400–700 nm (URUV Ultraphan UV farblos, Difrega, Germany). Foils for (2.) PAR+UVA treatments (PA) were transparent to wavelengths of 320–700 nm (Folanorm SF-AS, Folex GmbH, Germany), whereas foils for (3.) PAR+UVA+UVB treatments (PAB) were transparent to wavelengths of 295–700 nm (URT140 Ultraphan UV farblos, Difrega, Germany). The respective transmission spectra of these cut-off foils are shown in Bischof et al. (2002). After six days of exposure, mesocosms were covered by two layers of gauze and cryostats were switched off for a recovery period at dim light and ambient fjord temperature for another two days.

Laboratory set-up with combined temperature and salinity treatments

In the inside laboratory experiment, algae were exposed to different artificially produced salinities at defined temperatures for six days. The basic experimental design was the same as in the field experiment, but with a smaller set-up combined with artificial radiation conditions and different salinities.

After cleaning, alga samples were exposed in plastic beakers (1000 ml, Vitalab GmbH, Germany) containing seawater with three different salinities. These were diluted by mixing fjord water with MilliQ water, controlled by a hand-held Refractometer (ATAGO Co., LTD, Tokyo, Japan) and adjusted to (1.) 34 (fjord salinity), (2.) 28 and (3.) 20. The beakers were placed in temperature controlled water tanks (648x846x160mm, Bürkle GmbH, Germany). Two tanks were established for each of the following three temperature treatments: (1.) 15°C (15.5 ±1°C), (2.) 8°C (8.4° ±0.12°C) and (3.) 4°C (3.5 ±0.2°C). For each temperature three beakers for each of the three salinity conditions were randomly distributed in the two tanks. Above the tanks a set of 15 light-tubes (true light[®] II, 36W, Powertwist, USA) provided permanent irradiation of PAR with a photon fluence density of 80µmol m⁻² s⁻¹ (±10 µmol m⁻² s⁻¹). The algae were covered by about 10cm of water-column, but each beaker was permanently equipped with a bubble stone connected to a self-priming air pump in order to provide water circulation and permanent movement, which minimizes diffusive nutrient limitation. The water in the beakers was exchanged on the fourth experimental day. The defined abiotic factors radiation, temperature and salinity were uniformly distributed across the experimental area and across treatments.

Measurements of photosynthesis

Photosynthetic activity of samples from both experiments was determined by measuring in vivo chlorophyll- fluorescence of photosystem II (PS II) using a PAM 2100 chlorophyll fluorometer (Walz, Effeltrich, Germany) as described by Hanelt et al. (1997). Maximal quantum yield (Fv/Fm) of sporophytes indicating physiological performance (Schreiber et al. 1994 for details) was measured during the experiments. Samples for MAA analyses were simultaneously collected in the field experiment and immediately frozen in liquid nitrogen.

MAA analysis

For extraction and HPLC analysis of total mycosporin-like amino acids (MAAs) contents, the procedure described by Karsten et al. (2003) was applied. In brief, frozen samples were freeze-

dried for 48h and 10-50mg of dry tissue were extracted in 2ml of 25% methanol for 2.5h at 45°C. After centrifugation at 6000x g for 6min, 200µl of supernatants were evaporated to dryness under vacuum and redissolved in 400µl of 100% methanol. These samples were analysed with a Waters HPLC system. The mobile phase consisted of 2.5% methanol (v/v) and 0.1% acetic acid (v/v) in water run isocratically at a flow rate of 0.7ml min⁻¹. MAAs were detected online with a photodiode array detector at 330nm, and absorption spectra (290-400nm) were recorded from HPLC-separated peaks. Identification was done by spectra, retention time and co-chromatography with standard extracts. Concentrations are expressed in mg g⁻¹ dry mass (DM, n=4).

Data processing

Both experiments were bi- factorial with repeated measures and three replicates. The field experiment was arranged as randomized block design and the laboratory experiment as split plot design. Mean values and standard deviations were calculated from all replicates per treatment. Results were analyzed using a two-way analysis of variance with repeated measures on the two factors and their interactions. Statistically significant differences and interactions of means were compared with the Post Hoc test Tukey's (HSD) at $p < 0.05$ (Sokal and Rohlf 1995). All analyses were performed using JMP 6 (SAS Institute, Cary, NC, USA).

Results

Photosynthetic responses. The effects of combined temperature (5, 10, 17°C) and radiation (PAB, PA, P) conditions on maximum quantum yield of photosynthesis (Fv/Fm) in *D. ramentacea* over six days of exposure and two days of recovery are shown in Figure 1. Initial values of Fv/Fm were 0.58 (± 0.076), and a decrease in maximum quantum yield was observed between day 3 and 6 of exposure with the strongest inhibition at 17°C. There was only one significant individual effect of temperature on photosynthesis ($F=8.34$, $p=0.033$). Post-hoc analysis (letters in Fig. 1) revealed that after 6 days of exposure, Fv/Fm of individuals at 10°C was significantly higher than at 17°C ($p=0.024$). Furthermore after two days of recovery, Fv/Fm values generally increased and were consistent with the initial value. However Fv/Fm in algae at 10°C was significantly higher than at 5 and 17°C ($p=0.005$, letters in Fig. 1). There were no

radiation effects ($p=0.1087$) and no interactive effects of temperature and radiation within this first experiment ($p=0.429$).

The maximum quantum yield of photosynthesis at combined temperature (4, 8, 15°C) and salinity conditions (34, 28, 20) decreased only slightly over six days exposure (Fig. 2). All initial values of F_v/F_m were 0.60 (± 0.03). Only one significant effect of temperature ($F=8.81$, $p=0.021$) was found by Post-hoc analysis after two days of exposure (marked in Fig. 2). Thereby, the total F_v/F_m of algae at increased temperature of 15°C was significantly higher compared to 4 and 8°C, but this difference ceased after six days. Moreover, the analysis did not show salinity effects ($p=0.277$) or interactive effects of temperature and salinity ($p=0.107$).

MAA-analysis. The total content of mycosporin-like amino acids (Fig. 3) varied with the radiation conditions and showed interactive effects of radiation with temperature during the field experiment. A total MAA content of 0.65 (± 0.43) mg g⁻¹ dry weight was detected in *D. ramentacea* at the beginning of the experiment. All samples showed a consistent or increasing total content of MAAs, but with large deviations after three and six days of exposure (Fig. 3). Furthermore, MAA-accumulation was clearly recognizable under UV-irradiation, especially at the lowest temperature. The highest MAA amount at 5°C/PA was a 4-fold increase over the initial value. Generally, there was a significant effect of radiation ($F=4.83$, $p=0.017$) and a significant effect by temperature interaction ($F=2.99$, $p=0.038$) on the total MAA content. Post-hoc analysis revealed that, after three days, the total MAA concentration at 5°C and PA with 2.87 (± 0.41) mg g⁻¹ dry weight (as seen in Fig. 3) was significantly higher than at 10 or 17°C and PA ($p=0.0006$) as well as under PAB, P at 5°C ($p=0.0004$) and at the P and PA-treatments at 10°C ($p=0.0212$). After six days of exposure, the total concentration of MAAs at 5°C/PA was with 1.69 (± 0.63) mg g⁻¹ dry weight only significant higher than the total MAAs of the P-treatment at the same temperature ($p=0.018$). However, there were no temperature effects on the MAA synthesis within the experiment ($p=0.40$).

Discussion

Against the background of global change, our study extends the existing knowledge about the damaging impact of UV- radiation on polar seaweeds by analysing combined effects of UV- radiation, temperature and salinity on the physiology of *Devaleraea ramentacea*. The results

demonstrate an increasing influence of temperature as single parameter or in combination with other environmental variables on the photosynthetic efficiency of *D. ramentacea*. In addition, the total amounts of UV- absorbing MAAs are also affected by radiation and temperature.

Temperature effect. In the photosynthetic responses of *D. ramentacea* tetrasporophytes a temperature tolerance ranging from 4 to 17°C (Fig. 1, 2) was detected. For comparison, the average summer seawater temperature in the natural environment of the algae, in the Kongsfjord is around 5°C (Hanelt et al. 2001, Svendsen et al. 2002). The tested and tolerated temperature range is considerably higher than the ambient temperature in situ. Furthermore, Fv/Fm of the studied algae at 10°C was significantly higher than at 5 and 17°C in the field experiment which indicates a temperature optimum for efficient photosynthesis at 10°C. Generally, endemic Arctic species, such as *Laminaria solidungula* or *D. ramentacea* grow up to 10 or 15°C with growth optima between 5 and 10°C (Wiencke et al. 1994). The study of Rueness and Tananger (1984) suggested for *D. ramentacea* a temperature optimum for growth at 6-10°C whereas decreasing growth occurred at 17°C. This upper thermal limit at 18-20°C was confirmed by Wiencke et al. (1994) and by the study of Novaczek et al. (1990) for the European isolates from North Norway. In order to determine the upper temperature limit of *D. ramentacea* from Spitsbergen, a short laboratory experiment was conducted under controlled conditions (data not shown) in which samples were exposed to 20°C, a water temperature unnatural for the Arctic. The algae bleached and disintegrated quickly already after three days, which confirmed that temperatures above 20°C are lethal to *D. ramentacea*. The photosynthetic results shown agree basically with the growth studies cited above. However, it should be noted that temperature optima for growth and photosynthesis could vary to great extent (Davison 1991). A possible reason for this discrepancy of higher thermal limits for photosynthesis could be the ability of algae to acclimate and change the phenotype. The studied isolate of *D. ramentacea* from Spitsbergen can be classified as a polar eurythermal alga (Bischoff-Bäsmann 1997). Arctic and cold temperate macroalgae from the Northern hemisphere are mostly eurythermic and differ from each other by variations of optimum growth temperatures (below or above 10°C) and upper survival temperatures in contrary to the generally stenothermal Antarctic macroalgae (Wiencke and tom Dieck 1989).

Under the impact of combined temperature with different irradiation conditions, Fv/Fm in *D. ramentacea* decreased during the exposure time of six days, but increased again to the initial

value after recovery. Such an efficient recovery under all treatments points to an activated photoprotective mechanism, the dynamic photoinhibition. By rapid and reversible down-regulation of photosynthetic activity, algae protect themselves from excessive PAR radiation and dissipate absorbed energy as physiologically harmless thermal radiation (Osmond 1994; Hanelt et al. 1998). Generally, algae growing in shallow water are more PAR and UV-radiation resistant than algae from deeper waters (Dring 1996, Hanelt et al. 1997, Bischof et al. 1998). Photosynthesis of *D. ramentacea* was also unaffected by UV-radiation in the present study pointing to adaptive mechanisms which is in agreement with earlier studies where this species showed an increasing UV-tolerance by photoacclimation (Aguilera et al. 1999, Karsten et al. 1999, Bischof et al. 2002, Karsten et al. 2003). In contrast, the red alga *Palmaria palmata* that occurs in the same habitat as *D. ramentacea* in the Kongsfjord is not able to photoacclimate.

Devaleraea ramentacea tolerated the examined salinities ranging from fully marine (salinity 34) to hyposaline (28, 20) conditions as reflected in an unaffected photosynthesis. Due to this broad salinity tolerance, this species seems to be well adapted to their Arctic habitat with a strong inflow of freshwater during snow and glaciers melt in spring and summer. Based on the study of Karsten et al. (2003) *D. ramentacea* was characterized as a euryhaline macroalgae showing a photoinhibitory effect only under hypersaline conditions. In strong contrast, *P. palmata* exhibited rather stenohaline features with no survival in hyposaline media. Nevertheless, the photosynthetic efficiency of both species was much more affected by exposure to the combined factors UV radiation and salinity than only to the stressor salinity (Karsten et al. 2003). This influence of several combined stress factors elevated a deleterious effect, also referred to as cross-synergism by Alexieva et al. (2003).

In the present study, there were no interactive effects observed on photosynthesis of *D. ramentacea*, neither of temperature in combination with radiation nor in combination with salinity. In general, there are only few studies on interactions of the variables UV radiation, temperature and salinity on the physiology of polar seaweeds. For instance, the photosynthetic efficiency of the Arctic cold temperate red algae *Palmaria palmata*, *Coccotylus truncatus* and *Phycodrys rubens* as well as of the Antarctic green alga *Ulva bulbosa* was less affected by changes in UV radiation at higher temperatures (van de Poll et al. 2002, Rautenberger & Bischof 2006). Hoffman et al. (2003) also supported the hypothesis that temperature mediates the net biological effect of UV radiation in algae and vice versa.

Acclimation mechanism. Several red macroalgal species including *D. ramentacea* synthesise and accumulate MAAs in the presence of UV radiation, indicating their important role as sunscreen compounds (Bandaranayake 1998, Karsten et al. 1998, Karsten et al. 1999, Aguilera et al. 2002, Hoyer et al. 2002, Shick and Dunlap 2002, Karsten et al. 2003). Also in this study, a generally higher MAA concentration was measured in the UV-treatments. In addition, in both Arctic red algae *D. ramentacea* and *P. palmata* two unknown UV-absorbing compounds (Karsten et al. 1998) were found. In contrast to UV, salinity changes did not influence MAA accumulation in *D. ramentacea* (Karsten et al. 2003).

The important sunscreen function of MAAs is well investigated, but recent studies show that these substances have additional functions, such as antioxidants, nitrogen reservoir or as protective solutes against thermal stress or desiccation (reviewed by Oren and Gunde-Cimerman 2007). In the present study, no individual temperature effect on MAA synthesis was detected, so that a function as thermal protector can be excluded. Nevertheless, the total intracellular MAA accumulation of *D. ramentacea* was affected by interactive effects of radiation and temperature, i.e. particularly under UV-A radiation and the lowest temperature. Hoyer et al. (2002, 2003) reported similar responses for the Antarctic red algae *Iridaea cordata* and *Palmaria decipiens*, i.e. under enhanced PAR and UV-radiation, the MAA concentrations were higher at 5 than at 10°C, which supports the hypothesis of cold-induced MAAs as additional UV-protection.

In summary, the Arctic isolate of *D. ramentacea* from Spitsbergen turns out to be highly tolerant to single and multiple abiotic stress factors. The photosynthetic responses of *D. ramentacea* reflected a wide tolerance of the investigated abiotic factors temperature, UV-radiation and salinity. No interactive effects, neither of temperature in combination with radiation nor in combination with salinity were detected concerning photosynthetic performance. The higher MAA concentrations in the presence of UV-radiation confirmed their potential sunscreen function. Consequently, in view of ozone depletion and global warming with increasing melting of glaciers, tetrasporophytes of *D. ramentacea* exhibit broad tolerances against increased UV-radiation and temperatures as well as to decreased salinity, and hence will most probably be able to acclimate to global change scenarios in Arctic waters. However, more studies on interactive effects and acclimation mechanisms, especially on the yet uninvestigated, probably more sensitive microscopic development stages and gametophytes of *D. ramentacea* are required to make precise predictions about the whole species tolerance pattern.

Acknowledgements. This study was financially supported by the Helmholtz Society of German Research Centres (Project: NG-VH-059) For collecting samples and assistance on site authors are deeply grateful to the AWI Scuba dive team, and in particular to Max Schwanitz. We thank Sebastian Lembcke for HPLC assistance. Furthermore, many thanks go to the International Arctic Environmental Research and Monitoring Facility at Ny Ålesund (Svalbard) for supporting this study.

References

- ACIA (2005). Arctic Council and International Arctic Science Committee. Arctic climate impact assessment—Scientific report. Cambridge University Press, Cambridge, 1042 pp
- Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002a) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Mar Biol* 140(6):1087–1095
- Aguilera J, Dummermuth A, Karsten U, Schriek R, Wiencke C (2002b) Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. *Polar Biol* 25(6):432–441
- Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Mar Ecol Prog Ser* 191:109–119
- Alexieva V, Ivanov S, Sergiev I, Karanov E (2003) Interaction between stresses. *Bulg J Plant Physiol (Special Issue)*:1–17
- Bandaranayake WM (1998) Mycosporines: are they nature's sunscreens? *Nat Prod Rep* 15(2):159–172
- Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Mar Biol* 140(6):1097–1106
- Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol* 20(6):388–395
- Bischoff B, Wiencke C (1993) Temperature requirements for growth and survival of macroalgae

-
- from Disko Island (Greenland). *Helgol. Meeresunters.* 47(2):167–191
- Bischoff-Bäsmann B (1997) Temperature requirements and biogeography of marine macroalgae – Adaptation of marine macroalgae to low temperatures. *Rep Polar Res* 245:134pp
- Breeman AM, Pakker H (1994) Temperature ecotypes in seaweeds: Adaptive significance and biogeographic implications. *Bot. Mar.* 37(3):171–180
- Davison IR (1991) Environmental effects on algal photosynthesis: Temperature. *J Phycol* 27(1):2–8
- Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Mar Biol* 131(2):361–369
- Hanelt D, Wiencke C, Nultsch W (1997) Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *J Photochem Photobiol B: Biol* 38(1):40–47
- Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138(3):649–658
- Hoffman JR, Hansen LJ, Klinger T (2003) Interactions between UV radiation and temperature limit inferences from single-factor experiments. *J Phycol* 39(2):268–272
- Hoyer K (2003) Occurrence, induction and physiological importance of UV-absorbing substances in polar macroalgae. Doctoral thesis. Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, *Rep Polar Mar Res* 440, 155 pp
- Hoyer K, Karsten U, Sawall T, Wiencke C (2001) Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. *Mar Ecol Prog Ser* 211:117–129
- Hoyer K, Karsten U, Wiencke C (2002) MAA synthesis and accumulation in polar macroalgae are controlled by abiotic factors. *J. Phycol.* 38(s1):17
- IPCC (2007). Intergovernmental Panel on Climate Change. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment.* Cambridge University Press, Cambridge, 996 pp
- Karsten U, Bischof K, Hanelt D, Tüg H, Wiencke C (1999) The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). *Physiol Plant* 105:58–66
- Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet

-
- radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. *Polar Biol* 26(4):249–258
- Karsten U, Sawall T, Hanelt D, Bischof K, Figueroa FL, Flores-Moya A, Wiencke C (1998) An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Bot Mar* 41:443–453
- Lüning K (1990) *Seaweeds. Their environment, biogeography, and ecophysiology*. John Wiley & Sons, Inc, New York, 527 pp
- Novaczek I, Lubbers G, Breeman A (1990) Thermal ecotypes of amphi-Atlantic algae. I. Algae of Arctic to cold-temperate distribution (*Chaetomorpha melagonium*, *Devaleraea ramentacea* and *Phycodryis rubens*). *Helgol Mar Res* 44(3):459–474
- Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiol. Lett.* 269(1):1–10
- Osmond CB (1994) What is photoinhibition? Insights from comparisons of shade and sun plants. In: Baker NR, Bowyer JR (eds) *Photoinhibition of Photosynthesis from molecular mechanisms to the field*. BIOS Scientific Publishers, Oxford, pp 1–24
- Rautenberger R, Bischof K (2006) Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol* 29(11):988–996
- Rueness J, Tananger T (1984) Growth in culture of four red algae from Norway with potential for mariculture. *Hydrobiologia* 116-117(1):303–307
- Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E-D, Caldwell MM (eds) *Ecophysiology of photosynthesis*. *Ecol Stud Anal Synth* 100, pp 49–70
- Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu Rev Physiol* 64:223–262
- Sokal R, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*. 887 pp
- Svendsen H, Beszczynska-Moeller A, Hagen JO, Lefauconnier B, Tverberg V, Gerland S, Oerbaeck JB, Bischof K, Papucci C, Zajaczkowski M, Attolini R, Bruland O, Wiencke C, Winther JG, Dallmann W (2002) The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 21(1):133–166
- Van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2002) Temperature dependence of UV

-
- radiation effects in Arctic and temperate isolates of three red macrophytes. *Eur J Phycol* 37(1):59–68
- Wiencke C, tom Dieck I (1989) Temperature requirements for growth and temperature tolerance of macroalgae endemic to the Antarctic region. *Mar Ecol Prog Ser* 54:189–197
- Wiencke C, Bartsch I, Bischoff B, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37(3):247–259
- Wiencke C, Clayton MN, Gómez I, Iken K, Lüder UH, Amsler CD, Karsten U, Hanelt D, Bischof K, Dunton K (2007) Life strategy, ecophysiology and ecology of seaweeds in polar waters. *Rev Environ Sci Biotechnol* 6(1-3):95–126
- Wiencke C, Vögele B, Kovaltchouk NA, Hop H (2004) Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. In: Wiencke C (ed) *The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991-2003*. *Ber Polarforsch Meeresforsch* 492, pp 55–62

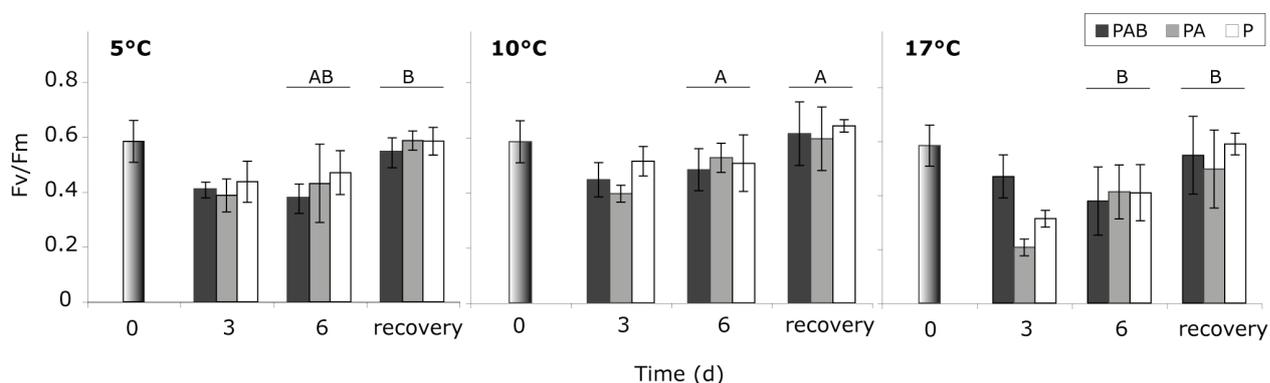


Fig. 1 Maximum quantum yields of photosystem II (Fv/Fm) of macroscopic tetrasporophytes of *Devaleraea ramentacea* at three temperatures (5, 10, 17°C) and radiation conditions (PAB=black bars, PA=grey bars, P=white bars), measured over six days of exposure and after two days of recovery under natural conditions. Standard deviations are represented by vertical bars (n=3). Different letters indicate significant temperature differences ($p < 0.05$) between treatments at the respective measuring date

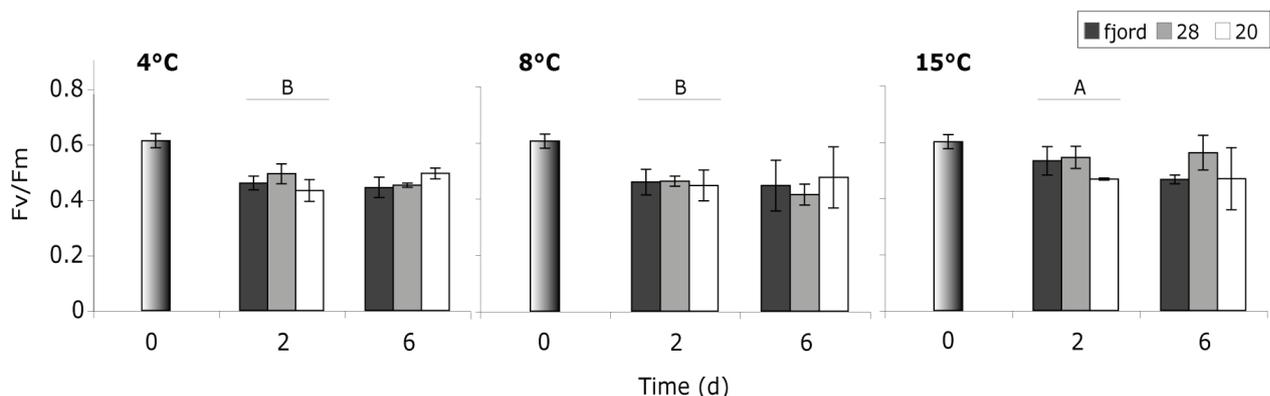


Fig. 2 Maximum quantum yield of photosystem II (Fv/Fm) of macroscopic tetrasporophytes of *Devaleraea ramentacea* at three temperatures (4, 8, 15°C) and salinities (34=black bars, 28=grey bars, 20=white bars), measured over six days of exposure under laboratory conditions. Standard deviations are represented by vertical bars (n=3). Different letters indicate significant differences ($p < 0.05$) between treatments at the respective measuring date

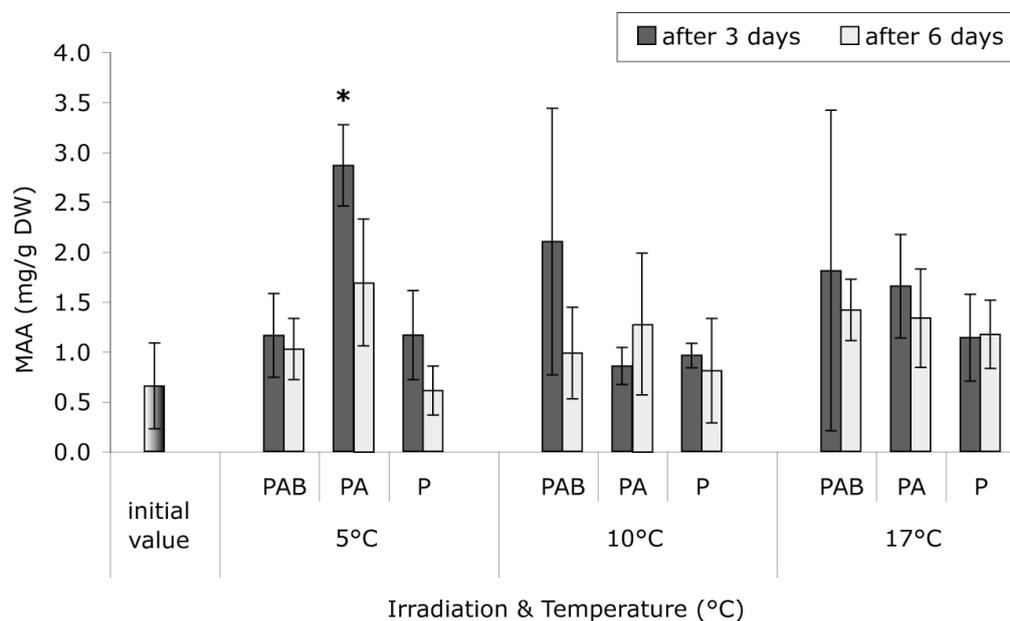


Fig. 3 Total mycosporine-like amino acid contents (MAAs in mg g^{-1} dry weight) in *Devaleraea ramentacea* after exposure (3 days=black bars, 6 days=grey bars) to interactive effects of temperature (5, 10, 17°C) at different radiation conditions (PAB, PA, P) under natural conditions. Standard deviations are represented by vertical bars ($n=4$). Star indicate significant radiation effect and interaction ($p < 0.05$)

ATTACHEMENT OF

PUBLICATION II

Fredersdorf J, Müller R, Becker S, Wiencke C, Bischof K

Interactive effects of radiation, temperature and salinity on different life history
stages of Arctic kelp *Alaria esculenta* (Phaeophyceae)

Oecologia (in press)

DOI: 10.1007/s00442-009-1326-9

Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria esculenta* (Phaeophyceae)

Jana Fredersdorf ^{1,2,*}, Ruth Müller ², Susanne Becker ¹, Christian Wiencke ², Kai Bischof ¹

¹Department of Marine Botany, University of Bremen, Leobener Str. NW2 and Center for Tropical Marine Ecology, Fahrenheitstr. 6, 28359 Bremen, Germany;

²Section Seaweed Biology, Alfred Wegener Institute for Marine und Polar Research (AWI), Am Handelshafen 12, 27570 Bremerhaven, Germany

* corresponding author:

fax: +49 421 218 2285, email: Jana.Fredersdorf@awi.de

Key words: climate change; germination; life cycle; multiple stress; photosynthesis

Abstract

To estimate the potential effects of climate change on polar marine macroalgae, studies on interactive stress effects of multiple climate-related parameters are essential. Interactions of temperature, radiation and salinity on two different life history stages of *Alaria esculenta* (L.) Greville from the Kongsfjord (Spitsbergen) were investigated for the first time within this study. Adult macroscopic sporophytes of *A. esculenta* were exposed to different temperatures between 4°C and 21°C combined with artificial irradiation conditions (photosynthetically active radiation, ultraviolet radiation: UV-A/UV-B, first experiment) and with different salinities (34, 28, 20, second experiment). Effects of photosynthetic activity were determined by measuring variable chlorophyll- fluorescence of photosystem II. Germination success of young microscopic zoospores of *A. esculenta* was studied under multifactorial stress. Zoospore suspensions were exposed to the above-mentioned three different salinities and irradiation conditions at four temperatures between 2°C and 16°C. Overall, *A. esculenta* exhibited a highly stage-specific susceptibility towards the experimental treatments. In both experiments using sporophytes,

photosynthetic activity showed significant temperature effects and only very few significant radiation and salinity effects. Microscopic stages of *A. esculenta* were shown to be more sensitive than the adult macroscopic stages, since germination capacity of zoospores was significantly affected by temperature and salinity changes, and interactions of both. These results suggest that multiple stress factors interact synergistically. Temperature seems to be a predominant environmental parameter for the kelp *A. esculenta*. Overall, *A. esculenta* proved to be relatively tolerant and adaptable to increasing temperature and UV- radiation, as well as to diluted salinities, but only up to a specific limit.

Introduction

“ Kelp forests are phylogenetically diverse, structurally complex and highly productive components of coldwater rocky marine coastlines” (Steneck et al. 2002). They act as food, habitat and nursery for multiple associated organisms. Light, temperature, nutrients, water movement and salinity primarily control the growth and distribution of marine algae (Kirst 1990). Due to their enormous importance within coastal ecosystems, a decrease in seaweed abundance will have dramatic consequences for the sum of associated organisms (Bischof et al. 2006). Meanwhile, climate change has previously caused measurable temporary effects on kelps near thermal limit (Steneck et al. 2002). The Fourth Assessment Report of the United Nations Intergovernmental Panel on Climate Change (IPCC 2007) shows that warming of the climate system is unequivocal and has occurred in both the Northern and Southern Hemispheres and across the oceans. The high scenario of the IPCC report (2007) predicts that the annual Arctic surface temperatures north of 60°N will increase in the range between 2.4 and 6.4°C by 2100. Furthermore, the increased temperature of the Arctic Ocean including Spitsbergen will lead to earlier ice melt and later freeze-up within the yearly cycle, to increases in precipitation, to a decrease in sea-ice cover with a decrease of ocean salinity in the upper 500 m and increased UV radiation resulting from stratospheric ozone depletion (ACIA 2005, IPCC 2007). Changes affect macroalgae, plankton, fish and zooplankton especially due to rising water temperatures and changes in salinity, oxygen levels and water circulation.

Physiochemical changes are happening most rapidly in Polar Regions. Thus, they are considered as a sensitive barometer for processing climate change. Environmental variables are changing simultaneously worldwide, as different abiotic factors are acting in combination and

interdependently. Our interest was to study the interactive effects of multiple abiotic stressors on Arctic marine macroalgae to understand parts of stress physiology and to estimate the ecological implications.

In recent years, the marine coastal ecosystem of the Arctic Kongsfjord was intensively studied and serves currently as a model ecosystem and monitoring site for the effects of climate change in the Arctic (Svendsen et al. 2002, Wiencke 2004a). The underwater flora is composed of at least 50 different macroalgal species (Wiencke et al. 2004b). In the mid sublittoral the brown algae *Laminaria digitata*, *Saccharina latissima* and *Alaria esculenta* are the key species of the dominant kelp forests.

Alaria esculenta (L.) Greville, a large brown seaweed, is a member of the family *Alariaceae* belonging to the order Laminariales (kelp) which is populating sublittoral zones of Arctic and cold temperate coastal ecosystems (Lüning, 1990). The regional distribution of *A. esculenta* is temperature controlled (Munda and Lüning, 1977) and is present in the North Pacific as well as in the North Atlantic, and is absent in the southern North Sea and English Channel due to high summer water temperatures exceeding 16°C (Munda and Lüning, 1977, Lüning, 1990). *Alaria esculenta* has a heteromorphic diplohaplontic life history. The thallus of *A. esculenta* is characterized by an upright stipe and a long blade with a midrib. The morphology of lamina and stipe can vary widely between specimens due to wave exposure (Sundene, 1962).

In the Kongsfjord, *A. esculenta* can be found in depths between 3 and 10 meters (Bischof et al. 1998). Earlier physiological studies on this species proved that sporophytes of *A. esculenta* have a certain potential to acclimate effectively to increasing levels of both, photosynthetically active radiation (PAR) and moderate ultraviolet (UV) radiation fluence (Bischof et al. 1998, Bischof et al. 1999, Roleda et al. 2005). Under ambient solar radiation, the germination and UV tolerance of zoospores was highest in the shallow water species *Saccorhiza dermatodea*, intermediate in the sublittoral *A. esculenta* and lowest in the upper to mid sublittoral *L. digitata* (Wiencke et al. 2006). While UV- damaging effects on seaweed physiology in single- factor experiments are well studied (reviewed by Franklin and Forster 1997, Bischof et al. 2006, Roleda et al. 2007, Wiencke et al. 2007a), the physiological studies on interactive effects of multiple abiotic stressors are scarce (but see Hoffman et al 2003, Müller et al. 2008, Steinhoff et al 2008). Moreover, early developmental stages are known to be most susceptible to environmental stress (Roleda et al. 2007), but there are only very few studies on interactions of several abiotic stressors on microscopic life stages of Laminariales (Hoffman et al 2003, Müller et al. 2008, Steinhoff et al

2008). The purpose of the present study is to explore the interactive effects of abiotic environmental factors on the kelp *A. esculenta*. In particular, we identify the effects and interactions of temperature, radiation and salinity and test potential physiological tolerance limits on two different life history stages of *A. esculenta* from Spitsbergen.

Material & Methods

Study site

The Kongsfjord presents a marine coastal ecosystem located at the northwestern coast of Spitsbergen (78° 55' N 11° 56' E, Svalbard, Norway). During spring and early summer, the fjord is generally free of ice, the water is clear, and thus, its transparency to UV radiation is very high. These factors are combined with relatively low air temperatures at that time (Hanelt et al. 2001). With increasing air temperature in the summer (about 5°C), snow layers and glacier ice melt and cause a high discharge of turbid fresh water and sediments into the fjord. In general, the local water mass has a salinity of about 34.5 psu (practical salinity units) in the spring and drops below 28 psu in the surface water near the glacier in the summer, whereas the seawater temperature increases and varies by about 4°C in the summer (Hanelt et al. 2001, Svendsen et al. 2002).

Algal material

In June 2005 and early August 2006 sporophytes of *A. esculenta* were collected in the Kongsfjord (Spitsbergen, Norway) in the vicinity of Ny Ålesund by Scuba divers and transported to the laboratory in black plastic containers. For bi-factorial experiments with sporophytes of *A. esculenta*, 14 individuals were collected at depths of about 7-9 m (experiment I) and 10 individuals in 9-10 m (experiment II), respectively. After cleaning of sporophytes, several algal discs of 30 mm in diameter were cut right beside the midrib and seven discs from the mixture of all discs were exposed in each plastic beaker (1000 ml, Vitalab GmbH, Germany). Each beaker was permanently equipped with a bubble stone connected to a self-priming pump in order to provide water circulation and permanent movement of the alga discs. Seawater in the beakers was exchanged on the fourth experimental day. The defined abiotic factors radiation, temperature and salinity were uniformly distributed across the experimental area and across treatments in all setups. To determine the vulnerability of zoospores, individuals of *A. esculenta* with fertile sporophylls were sampled by means of Scuba diving in 4-8 m depth in July 2007. Sori from

mature sporophylls were cut, cleaned and dried with tissue papers, as well as stored in a dark moist chamber at 2°C over 1-2 nights. To induce the release of zoospores, sori were subsequently immersed in 0.2 µm filtered seawater (7°C) for a maximum of 30 minutes. After removal of sori zoospore suspensions were filtered through 20 µm gauze (Nytal HD 20, Hydro-Bios, Germany).

Bi- factorial experimental set-ups for sporophytes

In the first laboratory experiment, algal material was exposed to artificial UV-radiation at defined temperatures for the duration of 6 days. The beakers containing algal discs were placed in temperature controlled water tanks (648 x 846 x 160 mm, 39 l, Bürkle GmbH, Germany). The temperature in each tank was controlled by cryostats (model 1160S, VWR International GmbH, Germany) and monitored by temperature loggers (testo 175-T1, Testo AG, Lenzkirch, Germany) and by digital probe thermometer in each beaker. (TFA Dostmann GmbH Co.KG, Germany). Two tanks were established for each of the five temperatures applied. For each temperature three beakers with foils for each of the three radiation conditions were randomly distributed in the two tanks. A set of 15 light-tubes provided permanent uniform irradiation above the tanks: Nine tubes (true light[®] II, 36 W, Powertwist, USA) emitted photosynthetically active radiation (PAR) and six fluorescent tubes (UV A- 340 tubes, 40 W, Q-Panel, USA) UV- radiation. Irradiance was measured with a LI-190 Quantum Sensor connected to a LI-250 light meter (LI-COR, Lincoln, USA) and a UV-radiometer PMA2100 (Solar light, USA). The adjusted temperatures and irradiation conditions are detailed in Table 1. In order to generate three radiation treatments with and without UV- radiation the beakers containing alga discs were shielded with different cut-off foils. Foils were transparent to wavelengths of (1.) 400 – 700 nm (URUV Ultraphan UV farblos, Difrega, Germany) for PAR treatments (P), (2.) 320 – 700 nm (Folanorm SF-AS, Folex GmbH, Germany) for PAR+UVA (PA) treatments and (3.) 295 – 700 nm (URT140 Ultraphan UV farblos, Difrega, Germany) for PAR+UVA+UVB (PAB) treatments. The respective transmission spectra of these cut-off foils are shown in Bischof et al. (2002).

In the second laboratory experiment, algal discs were exposed to different, artificially produced salinities at defined temperatures for six days. The basic experimental set-up was the same as in the first experiment, but the radiation conditions and the ambient salinities of the samples were changed (see Table 1). Above the tanks only PAR- irradiance (15 light tubes) was provided. Algal discs were exposed to three different salinities in separate beakers. These were diluted by

mixing fjord water with MilliQ water and defined by a hand-held Refractometer (ATAGO Co., LTD, Tokyo, Japan).

Photosynthetic measurements of sporophytes

Photosynthetic activity of samples from the two experiments was determined by measuring *in vivo* chlorophyll- fluorescence of photosystem II (PS II) using a PAM 2100 chlorophyll fluorometer (Walz, Effeltrich, Germany) as described by Hanelt et al. (1997). The maximal quantum yields of photosynthesis (F_v/F_m) as an indicator of the physiological status of the blades (Schreiber et al. 1994 for details) was measured initially and after one, two, and five days of exposure. The experiments were arranged as split plot design with repeated measures and three to five replications. Mean values and standard deviations were calculated from the replicates per treatment. Results were analyzed using a two-way analysis of variance with repeated measures on the two factors and their interactions. Statistically significant differences and interactions of means were compared with the Post Hoc test Tukey's (HSD) at $p < 0.05$ (Sokal and Rohlf 1995). All statistical analysis with data from sporophytes was performed using the commercial software JMP 6 (SAS Institute, Cary, NC, USA).

Photosynthetic measurements of zoospores

Mixed zoospore suspensions from five individuals were diluted with distilled water to different salinities (see Table 1, Cond 340i, sensor TetraCon 325, WTW, Germany) to determine the photosynthetic efficiency of zoospores. After darkening the suspensions for three minutes, the maximal quantum yield (F_v/F_m) was calculated in four (experiment I, salinity 20, 26, 33) or five (experiment II, salinity 20, 33) replicates using a Water PAM (Walz, Germany). Data of each experiment were tested for homogeneity with the Levene's test, and means were compared by a one-factorial ANOVA including the Post-Hoc test Tukey's. In addition, F_v/F_m values from zoospore suspensions at salinity 33 from two experiments were statistically tested with Levene's ($p < 0.01$) and t – test ($p < 0.05$). All statistical analysis with data from zoospores was conducted in accordance with Sokal & Rohlf (1995) with the software Statistica Version 7 (StatSoft, Inc., USA).

Germination capacity of zoospores

To study germination success separate zoospore suspensions obtained from five individual sporophylls were adjusted to three different salinities (see Table 1) with distilled water. In parallel, the numbers of zoospores in suspensions were counted with a Neubauer chamber (Brand, Germany) under 200- fold magnification using an Axioplan microscope (Zeiss, Germany). Suspensions were allotted by dispensettes (Brand, Germany) into 35 x 10 mm culture dishes (Corning TM, Corning Inc., USA) containing two cover slips each (after settlement 25 ± 1 zoospores mm^{-2}). Subsequently, suspensions with different salinities were exposed in culture dishes covered with cut-off foils to generate the different radiation treatments (P, PA, PAB) in climate chambers run at four temperatures (see Table 1). After eight hours of UV exposure, six days of UV recovery at dimmed PAR ($6\text{-}10\mu\text{mol m}^{-2} \text{s}^{-1}$) under constant temperatures and salinity, the percentage of germination was ascertained from 300 spores per replicate under 200- or 400-fold magnification using an Axioplan microscope (Zeiss, Germany). Thereby, the germinated spores possessing a germination tube were distinguished from dead and living cells without germination tubes (for details see Müller et al. 2008). Percentage data were arcsine transformed and homogeneity of variances was tested with Cochran's test ($p < 0.01$) prior to testing data with a three- factorial ANOVA ($p < 0.05$) and Post-Hoc Tukey's test (HSD, $p < 0.05$).

Results

Photosynthesis of sporophytes

Our first results demonstrate the interactive effects of two combined stress factors: radiation/temperature and salinity/temperature on sporophytes of *Alaria esculenta* (Fig. 1, 2). In both experiments the maximum quantum yield of photosystem II (Fv/Fm) showed pronounced significant temperature effects, but only little, but still significant radiation or salinity effects. There was no significant interaction between the combined abiotic factors.

The responses of sporophytes of *A. esculenta* to different combinations of temperature and radiation conditions over six days are shown in figure 1. There was a significant effect of time ($F= 12.97$, $p < 0.0001$) with Fv/Fm decreasing over time. Furthermore, the main effect of temperature ($F= 19.33$, $p < 0.001$, letters in Fig. 1) and the time at temperature interaction ($F=$

7.25, $p < 0.001$) were both highly significant. Initial values of Fv/Fm were 0.66 ± 0.04 (day 1, Fig. 1). By the second day of exposure a decrease of Fv/Fm was observed at 4°C, 9°C and 21°C with the strongest inhibition with an Fv/Fm of 0.26 ± 0.02 occurring under the PAB treatment at 4°C on day six (Fig. 1). At higher temperatures, 13°C and 17°C, Fv/Fm values were constant over three days of exposure and decreased only on day six (Fig. 1). With the exception at 21°C, maximum quantum yields after two days of recovery at approx. 5°C and dim light were consistent with values measured at day six, or showed an upward trend (data not shown). However, under the highest temperature of 21°C, Fv/Fm decreased rapidly (Fig. 1), algal discs bleached, disintegrated and died after three days of exposure.

On the second day of exposure (letters in Fig. 1), the Fv/Fm of the algae at 4°C and 21°C were significantly lower than that of the other temperatures ($p < 0.001$). Also after three days of exposure, the Fv/Fm of algal treatments at 4°C were significantly lower than that of the treatments at 13°C and 17°C ($p < 0.001$), and the Fv/Fm of algal treatments at 9°C were significantly lower than that of the treatments at 13°C ($p < 0.001$). However, the lowest Fv/Fm was that of bleached and thin alga discs at 21°C on the third day ($p < 0.001$). The individual effect of radiation on Fv/Fm of *A. esculenta* sporophytes was significant ($F = 4.38$, $p = 0.022$), as well as the interaction of radiation with time ($F(6, 56) = 2.92$, $p = 0.015$). Significant inhibition of Fv/Fm by radiation were observed in algae exposed to the whole light spectrum (PAB) at lower temperatures of 4°C and 9°C (stars in Fig. 1). On the second day of exposure the Fv/Fm of specimens under the PAB treatment at 4°C was significantly lower than under the P treatment at 4°C ($p = 0.017$), and the Fv/Fm of specimens under the PAB treatment at 9°C were lower than under the P and the PA treatments at 9°C ($p = 0.002$), respectively. However, there were no interactive effects of temperature and radiation within the experiment ($p = 0.191$).

The maximum quantum yield of photosynthesis (Fv/Fm) over six days at combined temperature (4 - 15°C) and salinity conditions (34, 28 and 20) decreased over time (Fig. 2). All initial values (day 1, Fig. 2) of Fv/Fm were 0.713 ± 0.013 , then decreased on day two and were constant during the following days. In addition, there was a significant individual effect of temperature ($F(2, 18) = 5.56$, $p = 0.013$) and a significant time by temperature interaction ($F = 3.22$, $p = 0.014$). Post-hoc analysis (letters in Fig. 2) revealed that Fv/Fm of algae at 15°C on day two was significantly higher than at 8°C ($p = 0.020$). Furthermore on day three, Fv/Fm of algae at 15°C was significantly higher than at 8°C and 4°C ($p = 0.002$) (letters in Fig. 2). Nonetheless, the results of

the two-way analysis of variance with repeated measures did not show interactive effects of temperature and salinity ($p = 0.130$), of temperature, time and salinity ($p = 0.151$) nor of time and salinity ($p = 0.064$). Moreover, only one significant individual effect of salinity ($F = 4.51$, $p = 0.026$) was found by Post-hoc analysis on day six, as the total Fv/Fm of algae in diluted salinity of 28 was significantly higher than at the fjord salinity of 34 ($p = 0.018$).

Photosynthesis of zoospores

Fv/Fm values of 0.39 ± 0.06 were measured in zoospores of *Alaria esculenta* exposed to three salinities of 20, 26 and 33 (Fig. 3, grey bars). No significant differences among salinity treatments were detected ($p \geq 0.05$). In a second experiment, photosynthesis of zoospores at ambient salinity of 33 decreased significantly to an Fv/Fm value of 0.31 ± 0.02 compared to the first experiment ($p < 0.001$), due to unidentified environmental influences (Fig. 3, black bars). Moreover, during the second experiment Fv/Fm measured in low salinity (20) treated zoospores was inhibited to 0.15 ± 0.02 (to 50 %), and thus it was significantly different to that of zoospores under ambient salinity conditions of 33 ($p < 0.001$).

Germination of zoospores

Germination rates of zoospores after eight hours of UV exposure and six days of post-culture varied with temperatures and salinity (Fig. 4). Maximally 70-80% of zoospores of *A. esculenta* germinated at 7°C / ambient salinity (34) and at 12°C / moderate salinity (28) under all radiation conditions (Fig. 4). Nevertheless, the germination of zoospores was significantly impaired by temperature ($F = 35.73$, $p \leq 0.001$) and salinity treatments ($F = 50.84$, $p \leq 0.001$) and their interaction ($F = 2.33$, $p = 0.035$). Zoospores exposed to salinities of 34 and 28 at 2-12°C were significantly different from the control (P treatment) at the low salinity of 20 at 16°C ($p \leq 0.03$), where only 30% zoospores germinated. On the other hand, zoospores exposed to P treatment and low salinity (20) at 2-7°C achieved a maximum of 46-48% germination and 61% germination at 12°C, and were equivalent to other salinity treated controls in this temperature range ($p \geq 0.05$). Similarly, the germination of PA and PAB treated zoospores at 2-12°C was 46-75% (Fig. 4) and did not differ between salinity treatments or radiation treatments in the same temperature range ($p \geq 0.05$). Likewise PA and PAB treated zoospores exposed to ambient salinity (34) at 16°C revealed 48-52% germination, and were not different from P, PA and PAB treatments exposed to the three salinity conditions at 2-12°C ($p \geq 0.05$).

However, germination of PA and PAB treated zoospores at diluted salinities of 28 and 20 was strongly inhibited by the high temperature of 16°C. The germination decreased down to 38-43% (28) or 7-11% (20), respectively. In detail, germination of PA/ 16°C treated zoospores at a salinity of 20 (star in Fig. 4) differed significantly from germination of PA/ 16°C treated zoospores at ambient salinity of 34 ($p \leq 0.001$), and from those spores exposed to all other salinity and radiation treatments at 2-12°C ($p \leq 0.019$). Within PAB treated zoospores at 16°C significant differences were likewise obtained between germination under ambient (34) and diluted (20) salinities ($p \leq 0.001$). Moreover, germination of PAB treated zoospores at 16°C and salinity of 20 (star in Fig. 4) differed significantly to that of exposed zoospores at 12°C and all salinities ($p \leq 0.006$). Overall, germination of zoospores was strongly affected by different salinities at a relatively high temperature of 16°C ($F = 3.33$, $p \leq 0.04$). However, only a significant temperature /salinity interaction could be detected ($F = 2.33$, $p = 0.035$).

Discussion

This study demonstrates the importance of research on physiological responses to interactions between two or more environmental stress factors, especially with regard to ecological aspects and against the background of global climate change. It suggests that multiple stress factors interact synergistically or that one factor prevails as a single effect: photosynthetic activity of sporophytes showed significant individual effects, whereas germination capacity of zoospores was additionally affected by interactions. Microscopic zoospores were shown to be more sensitive than adult macroscopic sporophytes as other studies showed before (Dring et al. 1996, Coelho et al. 2000, Veliz et al. 2006, Wiencke et al. 2006).

Temperature effects. Photosynthesis of *A. esculenta* sporophytes reflected the wide range of geographical distribution (Lüning, 1990) and tolerance of temperatures between 4°C and 17°C of this alga (Fig. 1,2). For comparison, 5°C is the average summer temperature of the natural environment of the algae in the investigation area (Hanelt et al. 2001), but the IPCC report (2007) predicts temperature rises of 0.5 to 1.6°C by 2030, rising to 1.1 to 6.4°C by 2100. Under the impact of combined temperature and radiation conditions the Fv/Fm of sporophytes under 4°C and 9°C decreased rapidly compared to the more constant Fv/Fm values at 13°C and 17°C (Fig. 1). Generally, the decrease in Fv/Fm is a response to diverse stress conditions on photosynthesis.

Furthermore, such a decrease also suggests an activated photoprotective mechanism, the dynamic photoinhibition. By rapid and reversible down-regulation of photosynthetic activity, algae protect themselves from excessive PAR radiation (Hanelt et al. 1997). However, different degree of Fv/Fm reduction was observed between the treatments. This indicates that the temperature optimum with efficient photosynthesis of *A. esculenta* sporophytes was in the range between 13°C and 17°C. On the other hand, germination of zoospores of *A. esculenta* exhibits a lower optimal temperature range between 2°C and 12°C (Fig. 4, Müller et al. 2008). There are no data available from the literature to compare thermal optimums of different life stages of Arctic kelp species. There exist only a few temperature studies on Arctic kelps, mainly on growth at the upper or lower survival temperatures (Biebl 1970, Fortes and Lüning 1980, Bartsch 1993, Wiencke et al. 1994, Bischoff-Bäsmann 1997). The zoospores of *A. esculenta* showed a lower upper survival limit (UST) for germination with less than 18°C after 7 days of temperature exposure (Müller et al. 2008), whereas in the present study zoospores survived $\geq 16^\circ\text{C}$ for 6 days (Fig. 4). Similar observations have been made for sporophytes of *A. esculenta* by Munda and Lüning (1977) and Sundene (1962), where temperatures of 16-17°C for a duration of few weeks on Helgoland or Oslo Fjord were lethal to *Alaria* sporophytes. Photosynthesis of sporophytes of *A. esculenta* was unaffected by temperatures of $\leq 17^\circ\text{C}$ for 6 days in the present study. A discrepancy of lower thermal limits for growth and higher limits for photosynthesis were often observed and are discussed in Davison 1991. The ability of algae to change phenotypically may be reason for the discrepancy in this study.

The highest tested temperature of 21°C, which is unrealistic under global change conditions in the Arctic, was tested to reveal the maximal temperature limit. The algal discs bleached and disintegrated quickly, which proved that temperatures over 20°C are lethal to sporophytes of *A. esculenta*. Also the most temperature tolerant male and female gametophytes of *A. esculenta* revealed an UST of 19-21°C after 8 weeks of exposure (tom Dieck 1993). Thus our physiological results agree with Widdowson (1971), who described the northerly distribution pattern of the genus *Alaria* with a southern limit close to the 20°C isotherm of maximum sea temperature.

Temperature and irradiation effects. In addition to significant temperature effects on sporophytes, there were some radiation effects (Fig. 1). Significant inhibitions by radiation conditions were only observed in sporophytes of *A. esculenta* exposed to the whole light spectrum under the lowest temperature. In the set-up, there was a relative low PAR:UV ratio.

UV-B irradiance, however, was similar to or higher than naturally occurring intensities. This suggests that the sensitivity of algae to additional stress factors increases at temperatures lower than the optimum growth temperatures. Hoffman et al. (2003) also supported the hypothesis that temperature mediates the net biological effect of UV radiation and vice versa. Gómez et al. (2001) concluded from their study on *Gelidium pulchellum* that increasing growth temperature might stimulate repair processes. Additionally, the UV- induced inhibition of photosynthesis was much higher in *Ulva clathrata* from Chile than in *Ulva bulbosa* from Antarctica at 0°C, whereas temperatures of 10°C compensated for UV- effects in both species (Rautenberger and Bischof, 2006). Most of the published studies on polar kelp zoospores focus on UV-effects and the impacts on germination pattern, photosynthesis and recovery after exposure (see Swanson and Druehl 2000, Roleda et al. 2006b, Wiencke et al. 2007b, for reviews Roleda et al. 2007, Wiencke et al. 2007a and references therein). There are only very few studies on interactions between temperature and UV- radiation presently available. Hoffman et al. (2003) exposed early life stages of *Alaria marginata* and *Fucus gardneri* to four levels of UV-radiation at three temperatures. For *A. marginata*, 10°C was not a limiting temperature in the absence of UV-radiation, but under high levels of UV-radiation spores were unable to germinate. Furthermore, Steinhoff et al. (2008) and Müller et al. (2008) illustrated that UV-B radiation and high temperatures detrimentally affected the physiology and ultrastructure of zoospores of temperate and Arctic Laminariales. This suggests that UV-radiation and temperature interactions represent a general phenomenon and have important implications for studies on climate change (Hoffman et al. 2003). UV radiation and temperature had no interactive effects on the germination of zoospores in this study (Fig. 4, Müller et al. 2008) and were, in comparison to other important kelp species of the Kongsfjord, highly tolerant to both abiotic factors (Müller et al. 2008). However, it has to be taken into account that a repeated UV-B exposure of 4 hours for three days or higher PAR and UV-B irradiances caused a strong impairment of germination of zoospores of *Alaria* (Hoffman et al. 2003, Wiencke et al 2007b).

Temperature and salinity effects. *Alaria esculenta* sporophytes tolerated examined temperatures between 4°C and 15°C and salinities ranging from fjord salinity 34 to diluted salinities of 28 and 20 in the second experiment, with only one significant individual effect of salinity found on day six (Fig. 2). In contrast, zoospores were affected by the interaction of low salinity combined with high temperature, since the germination of PA and PAB treated zoospores

at a low salinity of 20 and 16°C was significantly different from almost all other treatments (Fig. 4). Nevertheless, a temperature of 16°C is an unrealistic temperature scenario for Arctic seawaters even under the forecasted global warming, and thus, the salinity effects on zoospores are not ecologically relevant. In consequence, both zoospores and sporophytes of *A. esculenta* are relatively tolerant to diluted salinities at environmentally significant conditions. As such, the kelp *A. esculenta* is well adapted to the inflows of melt water into the Kongsfjord during late spring and summer.

Based on the study of Karsten (2007) *Alaria esculenta*, *Saccharina latissima* and *Laminaria solidungula* were characterized as stenohaline macroalgae. *Alaria esculenta* showed a high effective quantum yield between 10 and 50 psu, but bleached and died in 5 psu media. By contrast, *Fucus distichus* with a broad salinity tolerance was characterized as euryhaline and *Laminaria digitata* and *Saccorhiza dermatodea* as stenohalin-euryhaline. Karsten (2007) hypothesized that acclimation responses of temperate or cold temperate organisms are usually slowed down and so the observed responses of algae can be explained by a temperature-limited physiological capacity. Except for that study on *A. esculenta*, interactive effects of salinity and temperature have never been evaluated in polar algae (Karsten 2007). Nevertheless, there are a few, often older studies about effects of salinity and temperature, for example on *Fucus vesiculosus* (Russell 1987). The experiments indicated that *Fucus* is evidently much more susceptible to saline changes at extreme temperatures. Another study by Thomas et al. (1988) showed that *Cladophora rupestris* had a reduced salinity-tolerance range at extreme temperatures, but *Cladophora glomerata* proved better able to tolerate increased salinity at higher temperatures.

Ecological conclusions. In summary, the ecological predominant and most influential environmental factor for the kelp *A. esculenta* is temperature. Temperature is not only responsible for the regulation of metabolism and reproduction but also for the geographical dispersion of kelp species. In both investigated life stages of *A. esculenta*, an upper temperature limit close to 16-20°C was determined, which is considerably higher than in the study area of the Kongsfjord. Temperature dominated as an individual effect and interacted synergistically with radiation and salinity. There are cases observed when plant organisms exposed to a single stress agent were capable of increasing their resistance to subsequent unfavorable impacts (= cross- adaption, reviewed by Alexieva et al. 2003). A trend to a cross-adaptation was detected in sporophytes,

where increasing temperature reduced UV- effects (Fig. 1). Significant UV- inhibitions were only observed in algae under the lowest applied temperatures (Fig. 1). More often, the simultaneous influence of several stress factors elevates their deleterious effect, so it considerably exceeds the simple additive effect of their action alone (= cross-synergism, Alexieva et al. 2003). The damaging and increasing effects of low salinity at relatively high temperature on germination of *A. esculenta* zoospores (Fig. 4) indicate a definite cross-synergism.

In an ecological context, and in particular with regard to environmental changes in the Arctic, *A. esculenta* proved to be tolerant and adaptable to increased temperature, UV- radiation and decreased salinity, which are occurring due to global warming and ozone depletion and faster melting of glaciers. These results are only valid up to a relatively unknown species-specific limit. Many more studies on interactive effects, especially on the most sensitive developmental stages as zoospores and gametes and on other kelp species are required for predictions of correlations, specific limits and effects of global change on seaweed- dominated marine coastal ecosystems.

Acknowledgements. This study was financially supported by the Helmholtz Society of German Research Centres (Project: NG-VH-059) For collecting samples and assistance on site authors are deeply grateful to the Scuba divers, and in particular to Max Schwanitz. The authors thank Dr. Mirta Teichberg for helpful comments. Furthermore, many thanks go to the International Arctic Environmental Research and Monitoring Facility at Ny Ålesund (Svalbard) for supporting this study. All experiments comply with the current laws of Norway and Germany.

References

- ACIA (2005). Arctic Council and International Arctic Science Committee. Arctic climate impact assessment – Scientific report. Cambridge University Press, Cambridge, 1042 pp
- Alexieva V, Ivanov S, Sergiev I, Karanov E (2003) Interaction between stresses. BULG. J. PLANT PHYSIOL. (SPECIAL ISSUE):1–17
- Biebl R (1970) Vergleichende Untersuchungen zur Temperaturresistenz von Meeresalgen entlang der pazifischen Küste Nordamerikas. Protoplasma 69(1):61–83
- Bischof K, Gomez I, Molis M, Hanelt D, Karsten U, Lüder U, Roleda MY, Zacher K, Wiencke C (2006) Ultraviolet radiation shapes seaweed communities. Rev Environ Sci Biotechnol 5(2-

3):141–166

- Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol* 20(6):388–395
- Bischof K, Hanelt D, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biol* 1:435–444
- Bischoff-Bäsmann B (1997) Temperature requirements and biogeography of marine macroalgae – adaptation of marine macroalgae to low temperatures. *Rep Polar Res* 245:134pp
- Coelho SM, Rijstenbil JW, Brown MT (2000) Impacts of anthropogenic stresses on the early development stages of seaweeds. *J. Aquat. Ecosyst. Stress Recovery* 7(4):317–333
- Davison IR (1991) Environmental effects on algal photosynthesis: Temperature. *J Phycol* 27(1):2–8
- Dring MJ, Makarov V, Schoschina E, Lorenz M, Luening K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Mar Biol* 126(1):183–191
- Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol Meeresunters* 34:15–29
- Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur J Phycol* 32(3):207–232
- Gómez I, Figueroa FL, Sousa-Pinto I, Viñegla B, Pérez-Rodríguez E, Maestre C, Coelho S, Felga A, Pereira R (2001) Effects of UV radiation and temperature on photosynthesis as measured by PAM fluorescence in the red alga *Gelidium pulchellum* (Turner) Kützing. *Bot Mar* 44(1):9–16
- Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138(3):649–658
- Hanelt D, Wiencke C, Nultsch W (1997) Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *J Photochem Photobiol B: Biol* 38(1):40–47
- Hoffman JR, Hansen LJ, Klinger T (2003) Interactions between UV radiation and temperature limit inferences from single-factor experiments. *J Phycol* 39(2):268–272
- IPCC (2007). Intergovernmental Panel on Climate Change. *Climate Change 2007: The Physical*

-
- Science Basis. Contribution of Working Group I to the Fourth Assessment. Cambridge University Press, Cambridge, 996 pp
- Jacob A, Kirst GO, Wiencke C, Lehmann H (1991) Physiological responses of the green alga *Prasiola crispa* ssp. antarctica to salinity stress. *J Plant Physiol* 139:57–62
- Karsten U (2007) Research Note: Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55(4):257–262
- Karsten U, Wiencke C, Kirst G (1991) The Effect Of Salinity Changes Upon The Physiology Of Eulittoral Green Macroalgae From Antarctica And Southern Chile .2. Intracellular Inorganic-Ions And Organic-Compounds. *Journal Of Experimental Botany* 42(245):1533–1539
- Kirst G (1990) Salinity Tolerance Of Eukaryotic Marine Algae. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:21–53
- Lüning K (1990) Seaweeds. Their environment, biogeography, and ecophysiology. John Wiley & Sons, Inc, New York, 527 pp
- Müller R, Wiencke C, Bischof K (2008) Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Climate Research*, in press
- Munda IM, Lüning K (1977) Growth performance of *Alaria esculenta* off Helgoland. *Helgol. Wiss. Meeresunters* 29(3):311–314
- Rautenberger R, Bischof K (2006) Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol* 29(11):988–996
- Roleda MY, Clayton MN, Wiencke C (2006a) Screening capacity of UV-absorbing compounds in spores of Arctic Laminariales. *Journal Of Experimental Marine Biology And Ecology* 338(1):123–133
- Roleda MY, Hanelt D, Wiencke C (2005) Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. *Polar Biol* 28:539–549
- Roleda MY, Hanelt D, Wiencke C (2006b) Exposure to ultraviolet radiation delays photosynthetic recovery in Arctic kelp zoospores. *Photosynthesis Research* 88(3):311–322
- Roleda MY, Wiencke C, Hanelt D, Bischof K (2007) Sensitivity of the Early Life Stages of Macroalgae from the Northern Hemisphere to Ultraviolet Radiation. *Photochemistry and Photobiology* 83(4):851–862
- Russell G (1987) Spatial and environmental components of evolutionary change: interactive

-
- effects of salinity and temperature on *Fucus vesiculosus* as an example. *Helgol Mar Res* 41(3):371–376
- Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze E-D, Caldwell MM (eds) *Ecophysiology of photosynthesis*. *Ecol Stud Anal Synth* 100, pp 49–70
- Sokal R, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research*. 887 pp
- Solomon S, Qin D, Mannin M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (2007) *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, available at www.ipcc.ch
- Steinhoff FS, Wiencke C, Müller R, Bischof K (2008) Effects of ultraviolet radiation and temperature on the ultrastructure of zoospores of the brown macroalga *Laminaria hyperborea*. *Plant Biology* 10(3):388–397
- Steneck RS, Graham M. H., Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation* 29(4):436–459
- Sundene O (1962) The implications of transplant and culture experiments on the growth and distribution of *Alaria esculenta*. *Nytt Magasin for Botanikk* 9:155–174
- Svendsen H, Beszczynska-Moeller A, Hagen JO, Lefauconnier B, Tverberg V, Gerland S, Oerbaeck JB, Bischof K, Papucci C, Zajaczkowski M, Attolini R, Bruland O, Wiencke C, Winther JG, Dallmann W (2002) The physical environment of Kongsfjord-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 21(1):133–166
- Swanson AK, Druehl LD (2000) Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient. *Mar Biol* 136(4):657–664
- Thomas DN, Collins JC, Russell G (1988) Interactive effects of temperature and salinity upon net photosynthesis of *Cladophora glomerata* (L.) Kuetz. and *C. rupestris* (L.) Kuetz. *Bot.Mar.* 31(1):73–77
- tom Dieck (Bartsch) I (1993) Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *Mar Ecol Prog Ser* 100:253–264

-
- van den Hoek C, Mann DG, Jahns HM (1995) *Algae. An introduction to phycology*. Cambridge University Press, Cambridge, Cambridge
- Veliz K, Edding M, Tala F, Gomez I (2006) Effects of ultraviolet radiation on different life cycle stages of the south Pacific kelps *Lessonia nigrescens* and *L. trabeculata* (Laminariales, Phaeophytceae). *Mar Biol* 149(5):1015–1024
- Widdowson TB (1971) A taxonomic revision of the genus *Alaria* Greville. *Syesis* 4:11–49
- Wiencke C (2004a) The coastal ecosystem of Kongsfjord, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991-2003. Alfred-Wegener Institut für Meeres- und Polarforschung, Reports on Polar and Marine Research
- Wiencke C, Bartsch I, Bischoff B, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37(3):247–259
- Wiencke C, Clayton MN, Gómez I, Iken K, Lüder UH, Amsler CD, Karsten U, Hanelt D, Bischof K, Dunton K (2007a) Life strategy, ecophysiology and ecology of seaweeds in polar waters. *Rev Environ Sci Biotechnol* 6(1-3):95–126
- Wiencke C, Lüder UH, Roleda MY (2007b) Impact of ultraviolet radiation on physiology and development of zoospores of the brown alga *Alaria esculenta* from Spitsbergen. *Physiologia Plantarum* 130(4):601–612
- Wiencke C, Roleda MY, Gruber A, Clayton MN, Bischof K (2006) Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: evidence through field experiments. *J Ecol* 94(2):455–463
- Wiencke C, Vögele B, Kovaltchouk NA, Hop H (2004b) Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjord, Svalbard. In: Wiencke C (ed) The coastal ecosystem of Kongsfjord, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991-2003. *Ber Polarforsch Meeresforsch* 492, pp 55–62

Table 1 Experimental conditions of irradiance, temperature and salinity with exposure time during the studies on interactive effects on different life cycle stages of *Alaria esculenta*

Life cycle stage	Radiation		Temperature [°C]	Salinity	Exposure time
	PAR [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	UV [W m^{-2} , unweighted]			
Sporophyt (n=14)	46 (± 8)	UV-A: 7 (± 1) UV-B: 0.3 (± 0.07)	4, 9, 13, 17, 21 (± 1)		6d
Sporophyt (n=10)	80 (± 10)		4, 8, 15 (± 1)	34, 28, 20	6 d
Zoospore				33, 26, 20	short-time
Zoospore	20 (± 2)	UV-A: 4.5 (± 0.5) UV-B: 0.4 (± 0.04)			8 h +
	dim light (6-10)		2, 7, 12, 16 (± 1)	34, 28, 20	6d

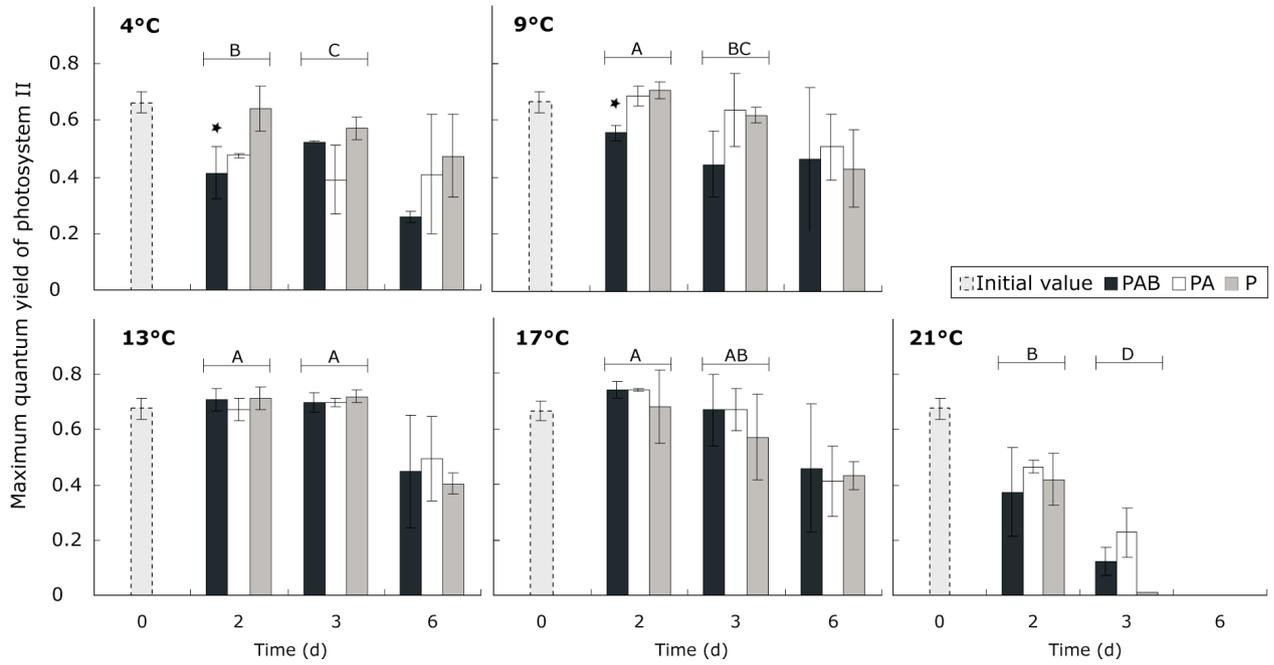


Figure 1 Maximum quantum yield of photosystem II (Fv/Fm) of the vegetative blade lamina of *A. esculenta* at five temperatures (4, 9, 13, 17, 21°C) and three radiation conditions (PAB = black bars, PA = white bars, P = grey bars), measured over six days of exposure. Standard deviations are represented by vertical bars (n=3). Different letters indicate significant temperature differences (p< 0.05) between treatments at the respective measuring date, and stars indicate significant radiation effects (p< 0.05) between treatments

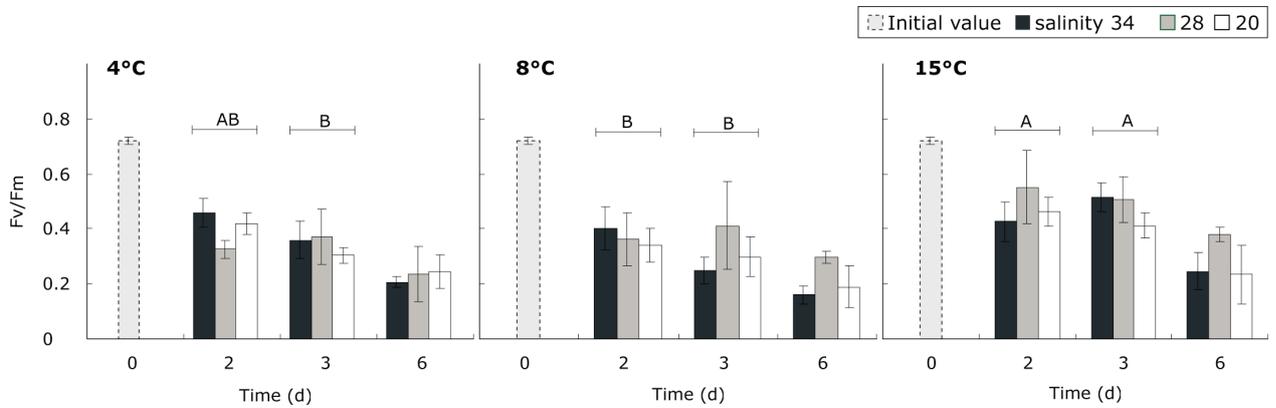


Figure 2 Maximum quantum yield of photosystem II (Fv/Fm) of the vegetative blade lamina of *A. esculenta* at three temperatures (4, 8, 15°C) and three salinities (34 = black bars, 28 = grey bars, 20= white bars), measured over six days of exposure. Standard deviations are represented by vertical bars (n=3). Different letters indicate significant differences (p< 0.05) between treatments at the respective measuring date

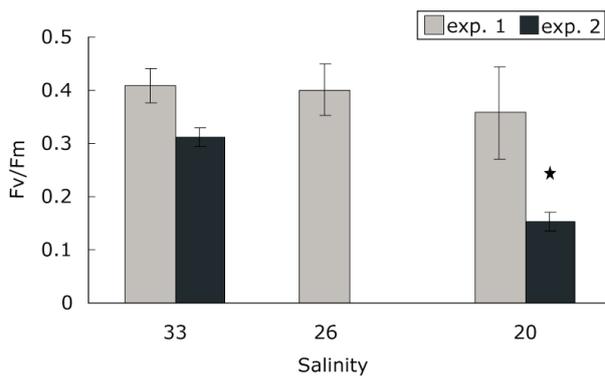


Figure 3 Maximum quantum yields of photosystem II (Fv/Fm) of freshly released zoospore suspensions of *A. esculenta* at different salinities (33, 28, 20) after the first (n=4, grey bars) and second (n=5, black bars) experimental runs. Standard deviations are represented by vertical bars (n=4/5), and stars indicate significant salinity effects (p< 0.05)

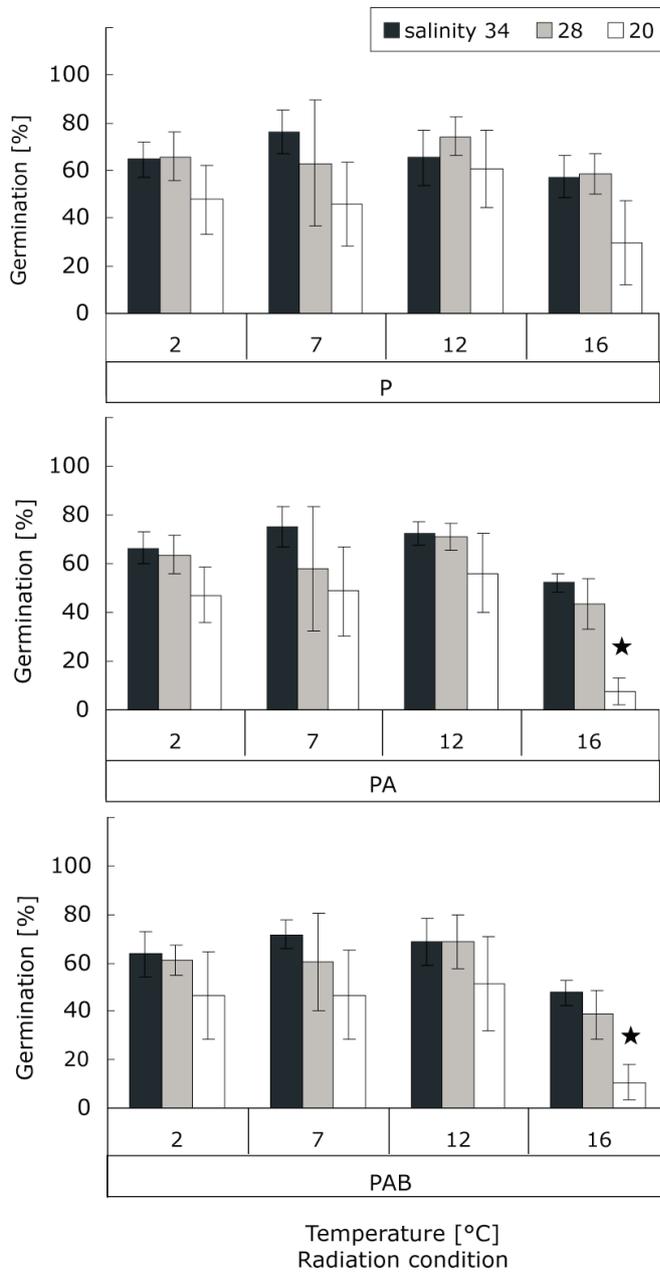


Figure 4 Germination rates of *Alaria esculenta* zoospores (expressed in percentage of control), ascertained after 8 hours of radiation exposure (P, PA, PAB) and 7 days of UV recovery below dim light at constant temperature (2, 7, 12, 16°C) and salinity conditions (34 = black bars, 28 = grey bar, 20 = white bars). Standard deviations are represented by vertical bars (n=3), and stars indicate interactive effects of temperature and salinity ($p < 0.05$)

ATTACHEMENT OF

PUBLICATION III

Fredersdorf J, Bischof K

Impacts of combined abiotic stress factors on adult sporophytes of two Arctic kelp species *Saccharina latissima* and *Laminaria solidungula* (Phaeophyceae)

Polar Research (submitted)

Impacts of combined abiotic stress factors on adult sporophytes of two Arctic kelp species *Saccharina latissima* and *Laminaria solidungula* (Phaeophyceae)

Jana Fredersdorf^{1,2x} & Kai Bischof¹

¹ Department of Marine Botany, University of Bremen, D-28359 Bremen, Germany;

² Alfred-Wegener-Institute for Polar and Marine Research, D-27570 Bremerhaven, Germany

^x corresponding author:

fax: +49 421 218 2285, email: Jana.Fredersdorf@awi.de

Key words: Arctic, interactive effects, Laminariales, photosynthesis, pigment, Spitsbergen

Abstract

In Arctic coastal ecosystems, kelps (Laminariales) are exposed to seasonal variations in environmental factors and additionally to potential stress effects of climate changes. However, the responses of marine macroalgae to the impacts of combined abiotic stress are relatively unknown. In the present study, adult sporophytes of two kelp species - the temperate species *Saccharina latissima* and the Arctic-endemic species *Laminaria solidungula* - were exposed to different artificial radiation conditions at different temperatures. Additionally, *L.solidungula* sporophytes were exposed to different artificially produced salinities at three different temperatures. The photosynthetic performance of both kelp species to interactive stress showed pronounced species-dependent temperature effects, but was unaffected by UV-radiation. Only the maximum quantum yield of *S.latissima* was affected by an interaction of high temperature combined with photosynthetically active radiation. Furthermore, individual salinity effects on photosynthetic efficiency of *L.solidungula* and on the total amount of the accessory pigment fucoxanthin were observed. No interactive effect on photosynthesis of *L.solidungula* was detected, neither of temperature in combination with radiation nor with salinity.

Overall, temperature and salinity dominated as individual effects, but for both kelp species, the influence of temperature seems to be ecologically most important. The temperature tolerance correlates with the geographical distribution pattern of both species. In an ecological

context regarding to environmental changes in the Arctic, the adult macroscopic sporophytes of *L.solidungula* and *S.latissima* proved to be tolerant and adaptable to increased temperature, UV-radiation and decreased salinity. However, the Arctic endemic species *L.solidungula* seems to have a more limited adaptation ability in comparison to the temperate kelp species.

Introduction

Kelps are large seaweeds of the brown algal order Laminariales, which play a vital ecological role in marine coastal environments in polar and temperate regions. Kelp forests represent a dynamic, structurally complex and biologically diverse ecosystem, which is also exposed to distinct seasonal variation in environmental conditions (Lüning 1990, Wiencke et al. 2007).

The most abundant genus within the order Laminariales is *Laminaria*, but recent molecular studies changed the understanding of phylogeny and taxonomy of kelps. In consequence, the current polyphyletic genus was separated into the two genera *Laminaria* Lamouroux and *Saccharina* Stackhouse (Bartsch et al. 2008 and references therein). The new name for *Laminaria saccharina* was proposed to be *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders by Lane et al. (2006). This is a common abundant species in the north-temperate Atlantic and Pacific coastal waters with a broad physiological plasticity. However, the deep-growing Arctic endemic *Laminaria solidungula* J. Agardh with the typical membranous holdfast is restricted in its distribution to Arctic and Northern Atlantic subarctic waters (reviewed by Bartsch et al. 2008). The geographical distributions of both species with their northern and southern limits correlate with their species-specific temperature requirement for growth and reproduction (Fortes and Lüning 1980, Breeman 1988, tom Dieck 1993, Wiencke et al. 1994).

Seasonal variations of abiotic factors, especially the photoperiod, affect algal growth performance of *Laminaria* sporophytes (Fortes and Lüning 1980, Bartsch et al. 2008 and references therein). The growth strategy of *S.latissima* in Arctic waters with rapid growth from winter to spring, during the period of first light after the ice break up with an availability of nutrients is similar to its growth pattern in temperate regions (Dunton 1985, Borum et al. 2002). In contrast, *L.solidungula* grows predominantly in complete darkness during winter and is optimally adapted to low light conditions and long dark periods in the Arctic environment (Chapman and Lindley 1980, Dunton 1985, Dunton and Jodwalis 1988, Korb and Gerard 2000). Moreover, the vertical zonation pattern of kelp species in the Kongsfjord

also results from their responses and tolerance to a number of different factors such as light penetration, wave exposure, competition or grazing. However, the main influence on depth distribution is the radiation intensity, due to the different tolerance against ultraviolet (UV) radiation and high photosynthetically active radiation (PAR), and due to the limiting PAR quantity in the lower sublittoral (Gomez et al. 1997, Hanelt et al. 1997b, Bischof et al. 1998b, Aguilera et al. 1999, Wiencke et al. 2000). Photosynthesis of Laminariales has a certain capability for acclimation to UV-stress and subsequent recovery from inhibition. In contrast to the more resistant shallow-water species *S.latissima*, a very high UV-sensitivity with strong inhibitions of photosynthetic performance, oxygen production and growth was observed in the Arctic deep-water species *L. solidungula* (Bischof et al. 1998a, Aguilera et al. 1999, Bischof et al. 2000, 2002, Karsten et al. 2001, Michler et al. 2002). In addition to this acclimation potential, kelps have also the ability to synthesize or adjust different protective systems, for example protective pigments or UV-absorbing phlorotannins against physiological stress (reviewed by Bischof et al. 2006, Karsten 2008, Bartsch et al. 2008). Carotenoids, the main accessory pigments act as light absorbing compounds in conjunction with chlorophyll *a* in the light harvesting complexes of the photosynthesis apparatus. Additionally they serve as antioxidants or as passive UV-sunscreens (Karsten 2008). One of the most abundant carotenoids in nature is fucoxanthin, especially in brown algae.

While UV-effects on seaweed physiology in single-factor experiments are well investigated (reviewed by Franklin and Forster 1997, Bischof et al. 2006, Roleda et al. 2007, Wiencke et al. 2007, Bartsch et al. 2008), the physiological studies on interactive effects of multiple abiotic stressors are scarce (but see Hoffman et al. 2003, Rautenberger and Bischof 2006, Müller et al. 2008, Steinhoff et al. 2008). Kelp forests in their natural environment are exposed to multiple and often highly variable abiotic factors, which are all interconnected and interdependent. However, our knowledge on interactive effects of temperature combined with UV-radiation or salinity on marine macroalgae is limited, but extremely important in order to understand interrelations of stress physiology and to estimate the possible ecological implications of environmental changes.

In the Arctic Kongsfjord on Spitsbergen, dense kelp forests grow on rocky bedrocks at approximately 1-20m depth and are dominated by the perennial canopy species *Alaria esculenta*, *Laminaria digitata*, *Saccharina latissima* in the upper and mid sublittoral and *Laminaria solidungula* in the lower sublittoral (Wiencke et al. 2004).

In the present study we aimed to identify stress effects, interaction of stress effects and potential physiological tolerance limits on photosynthesis of two kelp species from the

Kongsfjord on Spitsbergen. In detail, we investigated and compared photosynthetic responses of the temperate kelp *S.latissima* and the Arctic-endemic kelp *L.solidungula* to interactive abiotic effects of temperature and UV-radiation. Additionally, we studied the impact of temperature in combination with salinity on photosynthetic performance and pigment quantity of *L.solidungula*.

Material & Methods

Study site and algal material

The present study was conducted in the Kongsfjord located at the northwestern coast of Spitsbergen (Norway, 78°55.5'N; 11°56.0'E) in the vicinity of Ny Alesund. In general, the local water mass of the Kongsfjord has a salinity of about 34.5psu (practical salinity units) during spring and drops below 28psu in the surface water layer close to glaciers in summer, whereas the seawater temperature increases in summer and varies around 4°C. During spring and early summer, the fjord is generally ice-free with clear water, and thus, its transparency to UV-radiation is very high (Hanelt et al. 2001, Svendsen et al. 2002). In summer, melting of surrounding snow layers and glacier ice causes a high discharge of turbid fresh water and sediments into the fjord, thus diminishing water transparency.

Adult sporophytes of *S.latissima* and *L.solidungula* were collected by Scuba divers at different depths within the Kongsfjord in May 2005 and June 2006 (see Table 1), and transported to the laboratory in black plastic containers. After cleaning the sporophyte surface, several algal discs (30mm in diameter) were cut from the central part of the phylloids and a mixture of all discs was produced.

UV-radiation and temperature: Laboratory set up

Algal material of *L.solidungula* and *S.latissima* was exposed to three different artificial UV-radiation (PAB, PA, P) at defined temperatures (see Table 1) for a duration of six days with a subsequent recovery phase of two days. The detailed principal laboratory set-up is described in Fredersdorf et al. (2009). It was assured that the defined abiotic factors radiation, temperature and salinity were homogenous across the whole experimental setup and within all treatments.

Plastic beakers containing the algal discs were placed in temperature controlled water tanks. The temperature in each tank was controlled by cryostats and monitored by temperature loggers and a digital probe thermometer. For each temperature applied two of these tanks were established. Each beaker was equipped with a bubble stone connected to a self-priming

pump in order to provide permanent water circulation and movement of the algal discs. For each of the three different radiation conditions (PAB, PA, P) beakers with different cut-off foils were prepared. Foils were transparent to wavelengths of (1.) 295-700nm for PAR+UVA+UVB (= PAB) treatments, (2.) 320-700nm for PAR+UVA (= PA) treatments and (3.) 400-700nm for PAR (= P) treatments. Three beakers for each of the three radiation conditions (nine all in all) were randomly distributed in each temperature treatment (consisting of two tanks). A set of 15 light-tubes provided permanent and uniform irradiation of photosynthetically active radiation (PAR) and UV-radiation above the tanks. After six days of exposure, cryostats and light-tubes were switched off for a recovery period of two days at dim light ($\leq 10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and ambient fjord temperature.

Temperature and salinity: Laboratory set-up

Algal discs of *L.solidungula* were exposed to three different artificially produced salinities at defined temperatures for six days (details in Table 1). The basic experimental set-up was similar as described above, but with different salinities conditions and only PAR was provided. Algal discs were exposed to three different salinities in separate beakers. These different salinities were produced by mixing fjord water with MilliQ water and defined by a hand-held Refractometer.

Photosynthetic measurements

In both experiments, the photosynthetic activity of samples was determined by measuring *in vivo* chlorophyll-fluorescence of photosystem II (PS II) using a PAM 2100 chlorophyll fluorometer (Walz, Effeltrich, Germany) as described by Hanelt et al. (1997). Maximal quantum yield (F_v/F_m) of sporophytes indicating physiological performance (Schreiber et al. 1994 for details) was measured during the experiments. To estimate the photosynthetic capacity, photosynthesis vs. irradiance curves (PI-curves) were also recorded by the PAM fluorometer in order to reveal initial light saturation points (I_k) of experimental specimens.

Pigment analyses

Samples for pigment analyses of *L.solidungula* were collected at the second laboratory experiment after 6 days of exposure and immediately frozen in liquid nitrogen. For extraction of chlorophyll *a* and fucoxanthin and the following High Performance Liquid Chromatography (HPLC) analysis, a newly established protocol by Rautenberger (2008) was applied. In brief, frozen samples were extracted in 500 μL of 100% N-N-dimethylformamide

(DMF) and incubated in a nitrogen atmosphere at darkness for 22 hours at 4°C. This step was repeated by using 800µL of 100% DMF and afterwards the bleached thalli were washed with 500µL of 100% DMF. To separate pigments extracted from the thalli, the samples were centrifuged (5min, 16,100 x g, 4°C), passed through a filter membrane (0.4 µm pore size in diameter) and finally covered with nitrogen and stored at -20°C until the analysis. HPLC was performed with a Hitachi Elite LaChrom System (VWR International, Darmstadt, Germany), equipped with a temperature controlled auto sampler set to 5°C (L-2200) and a L-2450 photodiode array detector at 440nm. The pigments isolated (40µl) were subjected to the HPLC and separated by using a mobile phase of solvent A (100% acetone, Merck, Germany) and solvent B (10 mM Tris-HCl, pH 7.8; details see Rautenberger 2008). Each substance was identified due to the generated spectrum, the respective retention time, and co-chromatography of standard extracts from DHI Water & Environment (Hørsholm, Denmark). The concentrations are given in mg g⁻¹ fresh weight (FW, n=3-5).

Data processing

Both laboratory experiments were arranged as split plot design with repeated measures and three replications. Mean values and standard deviations were calculated from all replicates per treatment. Results were analyzed by using a two-way analysis of variance with repeated measures and their interactions. Statistically significant differences and interactions of means were compared with the Post-hoc test Tukey's (HSD) at p< 0.05 (Sokal and Rohlf 1995). All statistical analyses were performed using the commercial software JMP 6 (SAS Institute, Cary, NC, USA).

Results

Figure 1 illustrates the effects of combined temperature and radiation conditions on the maximum quantum yield of photosynthesis (Fv/Fm) of *L.solidungula* and *S.latissima* over six days of exposure and two days of recovery. The responses of sporophytes of *L.solidungula* are shown on the left part in figure 1, their initial values of Fv/Fm were 0.76 (± 0.02) with an initial light saturation point (I_k) of 95.5 (±25.4) µmol m⁻² s⁻¹. Only a significant individual effect of temperature (p< 0.001) on photosynthetic performance of *L.solidungula* was observed, but no effects caused by radiation (p= 0.34), or interaction of temperature and radiation (p= 0.069). A clear decrease in the maximum quantum yield was observed in the 20°C treatments at all radiation conditions and the algal discs bleached, disintegrated and died

beginning from the second day of exposure. Post-hoc analysis revealed that after three days of exposure, Fv/Fm of *L.solidungula* individuals at 20°C was significantly lower than at 0, 5 and 10°C ($p < 0.001$). Beside the value at 20°C, all Fv/Fm values increased and were consistent with the initial value after two days of recovery.

The maximum quantum yields of *S.latissima* sporophytes at combined temperature and radiation conditions were relatively constant over the experimental period (see right part in Fig. 1). All initial values of Fv/Fm were 0.66 (± 0.08) with an initial light saturation point (I_k) of 93.5 (± 23.0) $\mu\text{mol m}^{-2} \text{s}^{-1}$. There was a significant individual effect of temperature ($p < 0.0001$), but not of radiation ($p = 0.12$) on the photosynthetic performance of *S.latissima*. On the second and third day of exposure, the Fv/Fm of algal treatments at 20°C were significantly lower than that at 10°C ($p < 0.006$) at all radiation conditions. Furthermore, a significant interactive effect ($p = 0.0014$) was observed after two days of recovery. The Fv/Fm of algal samples exposed at 20°C with only PAR was significantly lower than in all other treatments. Additionally, it is noticeable that the Fv/Fm of both algal species exposed to the whole light spectrum (PAB) at 5°C showed a slight, but not significant downward trend after six days of exposure.

As shown in figure 2, the maximum quantum yield of *L.solidungula* sporophytes at combined temperatures (3, 8, 15°C) and salinities (34, 28, 20) conditions decreased during a period of six days of exposure. The initial values of Fv/Fm were 0.69 (± 0.07) with an initial light saturation point (I_k) of 63.4 (± 14.1) $\mu\text{mol m}^{-2} \text{s}^{-1}$. Post-hoc analysis revealed only one significant effect of salinity ($p = 0.0003$). After two days of exposure, the total Fv/Fm of the sporophytes at 15 and 3°C in fjord salinity of 34 (stars in Fig. 2) was significantly higher than at the diluted salinity of 28 and 20. Additionally, there was a significant main effect of temperature ($p = 0.0034$) after six days of exposure, as the total Fv/Fm of algae at 3 and 8°C were significantly higher than at 15°C (letters in Fig. 2). Nonetheless, the results of the two-way analysis of variance with repeated measures did not show any interactive effects of different temperature and salinity combinations ($p = 0.9$).

The total amount of the pigments chlorophyll *a* and fucoxanthin (see Fig. 3) extracted from *L.solidungula* was analysed by HPLC after 6 days of exposure at combined temperatures (3, 8, 15°C) and salinities (34, 28, 20) conditions. The total content of 1.05 (± 0.6) mg g⁻¹ fresh weight of chlorophyll *a* and 0.89 (± 0.27) mg g⁻¹ fresh weight of fucoxanthin was determined in initial samples. All samples showed a consistent total content of chlorophyll *a* with large

deviations and no significant effects of temperature ($p=0.55$) or salinity ($p=0.23$) and also no interactions of both ($p=0.10$). Furthermore, increased fucoxanthin content was clearly recognizable under ambient fjord salinity (34). There was a significant effect of salinity ($p=0.008$) and a significant tendency of a temperature and salinity interaction ($p=0.055$). Post-hoc analysis revealed that, after six days of exposure, the total fucoxanthin concentration at salinity of 34 was significantly higher than at diluted salinities of 28 and 20 and pigment concentration at $8^{\circ}\text{C}/34$ was significantly higher than at $15^{\circ}\text{C}/28$ and $8^{\circ}\text{C}/20$ (star in Fig. 3). However, the total amount of fucoxanthin was not affected by temperature during this experiment ($p=0.50$). The inset within figure 3 shows the total pigment content plotted against time. Chlorophyll *a* decreased within six days (t_0 - t_6) of exposure, whereas the amount of fucoxanthin tends to increase simultaneously slowly.

Discussion

The photosynthetic responses of the two kelp species *Laminaria solidungula* and *Saccharina latissima* from Spitsbergen to interactive stress showed pronounced species-dependent temperature effects. The temperature response correlates with the geographical distribution pattern of the species, whereas no impairments of photosystem II by UV-radiation was observed. Furthermore, photosynthetic efficiency and the total amount of the accessory pigment fucoxanthin of *L.solidungula* were affected by the impact of salinity.

Temperature was found to continuously influence all photosynthetic measurements on *L.solidungula* and *S.latissima* as a single parameter. A temperature optimum around 10°C (Fig. 1, 2) was detected in both species and only an exposure at 20°C over 6 days resulted in major differences in photosynthetic responses. This high water temperature is unnatural in Arctic waters, but by applying it we aimed for the physiological determination of the upper temperature limit. A clear decrease in the maximum quantum yield of *L.solidungula* was observed, which confirmed that temperatures above 20°C are lethal for this Arctic endemic species. In contrast, photosynthetic efficiency of *S.latissima* was inhibited at 20°C , but this temperature was non-lethal for the temperate macroalga.

The differences in temperature limits concur basically with gametophyte survival studies of different Atlantic and Arctic *Laminaria* isolates (Bolton and Lüning 1982, tom Dieck 1993, Wiencke et al. 1994). In culture, gametophytes of *S.latissima* survived at 18 - 22°C but disintegrated at 23°C , whereas gametophytes of the Arctic species *L.solidungula* survived a

temperature of 18°C but died at 20°C.

The photosynthesis of *S.latissima* sporophytes reflected the wide range of geographical distribution (Lüning 1990) and the tolerance of temperatures between 5°C and 20°C of this temperate macroalga (Fig.1). The study of Bolton and Lüning (1982) confirmed on the basis of growth measurements on *S.latissima* that its optimal temperature is set in the range between 10-15°C, a typical optimum range of cold-temperate North Atlantic species (Wiencke et al. 1994). For the photosynthetic responses of *L.solidungula* sporophytes, a temperature tolerance between 0°C to 15°C (Fig. 1, 2) was detected. Furthermore, Fv/Fm of the algae studied was significantly higher at 3 and 8°C than at 15°C (Fig. 2), which indicates a temperature optimum for efficient photosynthetic activity of *L.solidungula* to be at 0-10°C. In general, endemic Arctic species grow at temperatures up to 10 or 15°C with growth optima between 5 and 10°C (Wiencke et al. 1994). Arctic and cold temperate macroalgae from the Northern hemisphere are mostly eurythermic and differ by variations of optimum growth (below or above 10°C) and elevated upper survival temperatures (Wiencke et al. 1994, Dunton and Dayton 1995).

In the present study, under the influence of combined temperature with different irradiation conditions, the maximum quantum yields of both kelps were unaffected by UV-radiation. However, the ratio of PAR:UV irradiance was unnatural in the set-up, as the proportion of PAR was relatively low in comparison to the UV irradiance than occurring naturally. In contrast to the shallow-water species *S.lattissima*, the deep-water macroalga *L.solidungula* is normally not exposed to UV-radiation in their natural habitat. The results suggested a relatively high photosynthetic ability of these adult sporophytes to acclimate to a short-term stress of UV irradiation. This is in agreement with past studies, in which older kelp sporophytes consisting of multilayered thicker blades with protective mucosal layer acclimated faster to high irradiation conditions than younger life history stages (Dring et al. 1996, Hanelt et al. 1997).

Laminaria solidungula sporophytes tolerated the salinities examined ranging from fully marine (salinity 34) to hyposaline (28, 20) conditions in the second experiment, but with a significant individual effect of salinity appearing after two days of exposure (Fig. 2). The Fv/Fm of algae in fjord salinity of 34 at 15 and 3°C was significantly higher than at the diluted salinities of 28 and 20. Based on one of the few ecophysiological studies on salinity effects or their interactions in polar seaweeds, *Alaria esculenta*, *S.latissima* and *L.solidungula* were characterized as stenohaline macroalgae (Karsten 2007). Due to this limited salinity

tolerance, this Arctic endemic species seemed to have a restricted adaptation potential to their Arctic habitat with a strong inflow of freshwater during snow and glaciers melt in spring and summer. Furthermore, it is noticeable that all maximum quantum yields of *L.solidungula* sporophytes at conditions with combined temperature and salinity decreased over six days of exposure, including the temperature optimum in fjord salinity (Fig. 2). The calculated initial light saturation point (I_k) of the alga discs was somewhat below the artificial PAR intensity as well as lower than the I_k value of *L.solidungula* in the first laboratory experiment. Hence, it could be an indication of additional stress of white light during the experiment or a previous impact of white light in the natural habitat during the summer before collecting. This suggests also an increasing susceptibility of previously damaged algae to additional stress factors.

In the present study, no interactive effects, neither of temperature in combination with radiation nor in combination with salinity, on photosynthesis of *L.solidungula* were observed. However, the maximum quantum yield of *S.latissima* samples was affected by an interaction of high temperature combined with PAR radiation, since F_v/F_m of P-treatments at 20°C was significantly different from almost all other treatments after recovery. A possible reason for this incomplete recovery might be a potential supporting effect of the short UV-radiation wavelength range in the recovery process as hypothesised by Hanelt et al. (2006). A tendency of an inhibitory effect of UV-radiation with low temperature was observed on both macroalgae, the F_v/F_m of PAB treatments at 5°C after the sixth day of exposure was diminishing. The according hypothesis that temperature mitigates the net biological effect of UV-radiation on macroalgae is supported by some studies (Gómez et al. 2001, van de Poll et al. 2002, Hoffman et al. 2003, Rautenberger and Bischof, 2006).

Photosynthetic pigments, especially carotenoids as main accessory pigments are affected by a large variety of stresses (Rmiki et al. 1999). In studies on *Dunaliella salina*, impacts of temperature and salinity were detected not only on the content of chlorophyll, but also on other photosynthetic pigments (Borowitzka et al. 1990, Davison 1991). The total pigment amount of chlorophyll *a* and fucoxanthin in samples of *L.solidungula* at combined temperature and salinity stress was analysed in the present study. However, no individual temperature effect on both pigment contents was detected, but a higher fucoxanthin concentration was measured at ambient fjord salinity, particularly at fjord salinity of 34 at 8°C. Accordingly, the total fucoxanthin concentration was affected by the individual factor salinity and by interactive effects of salinity and temperature. In contrast, salinity changes or interactions did not influence the content of chlorophyll *a*. Fucoxanthin is a major light-

harvesting pigment for photosynthesis, but additional functions are unknown.

In summary, temperature and salinity dominated mainly as individual effects. But for both kelp species, the influence of temperature seems to be ecologically most important. The temperature tolerance correlates with the geographical distribution pattern of the species. In *L.solidungula* as well as *S.latissima*, an upper temperature limit above 15°C or 20°C was determined, which is considerably higher than the ambient temperature in the study area. For comparison, the average summer seawater temperature in the natural environment of the algae, in the Kongsfjord is around 4°C (Hanelt et al. 2001, Svendsen et al. 2002). Simultaneously, the local water mass has a salinity of about 34.5psu in spring and drops below 28psu in the surface water near the glacier in summer (Hanelt et al. 2001, Svendsen et al. 2002). The underwater radiation regime of the Kongsfjord is also subject to strong seasonal variations, sea ice as well as actual weather conditions and the turbidity of the water column. UV-B radiation can penetrate down to 6-10m depths in clear waters conditions in the Kongsfjord, and consequently affect macroalgae inhabiting shallow waters, for example *S.latissima* (Hanelt et al. 2001, Svendsen et al. 2002). In this study, *L.solidungula* sporophytes showed a limited tolerance at diluted salinity conditions. Nevertheless, no impacts of UV-radiation on both kelp species were detected.

However, in the Arctic coastal ecosystem marine macroalgae are also exposed to potential effects of climate changes. Widespread melting of glaciers and sea ice together with a decrease of ocean salinity in the upper 500 m, increases in precipitation and wind patterns, warming of permafrost or increased UV-radiation resulting from stratospheric ozone depletion represent additional evidence of expected strong Arctic warming. The scenarios of the IPCC report (2007) predict that the annual Arctic surface temperatures north of 60°N will be 2-4°C higher by mid-century and 4-7°C higher toward the end of the 21st century compared to the present. Furthermore, the increased temperature of the Arctic Ocean, including Spitsbergen will lead to earlier ice melt and later freeze-up within the yearly cycle and to a decrease in sea-ice cover, especially in summer (ACIA 2005, IPCC 2007).

In an ecological context, and in particular with regard to this possible environmental changes in the Arctic, the adult sporophytes of *L.solidungula* and *S.latissima* proved to be highly tolerant and adaptable to increased temperature, UV-radiation and decreased salinity. However, in comparison to the temperate kelp species, the Arctic endemic species *L.solidungula* seems to have a more limited ability to adapt to its changing Arctic habitat. Nevertheless, further studies on multiple stress effects especially on different developmental

stages of the macroalgae are indispensable to draw physiological and ecological conclusions for the development of kelp communities in the future.

Acknowledgements. This study was performed at the Ny Ålesund International Arctic Environmental Research and Monitoring Facility on Spitsbergen (Svalbard) and logistically and financially supported by the Helmholtz Junior Research Group (VH-NG-059) and the Alfred-Wegener-Institute for Marine and Polar Research. For collecting samples and assistance on site authors are deeply grateful to the AWI Scuba dive team, and in particular to Max Schwanitz. Many thanks go to Alice Schneider for the HPLC analysis.

References:

- Aguilera, J., Karsten, U., Lippert, H., Vögele, B., Philipp, E., Hanelt, D., Wiencke, C., 1999. Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Marine Ecology Progress Series* 191, 109–119.
- Arctic Council and International Arctic Science Committee, 2005. ACIA 2005. Arctic climate impact assessment – Scientific report. Cambridge University Press, Cambridge.
- Bartsch, I., Wiencke, C., Bischof, K., Buchholz, CM., Buck, BH., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, MJ., Schubert, H., Schumann, R., Valentin, K., Weinberger, F., Wiese, J., 2008. The genus *Laminaria* sensu lato: recent insights and developments. *European Journal of Phycology* 43 (1), 1–86.
- Bischof, K., Gomez, I., Molis, M., Hanelt, D., Karsten, U., Lüder, U., Roleda, M.Y., Zacher, K., Wiencke, C., 2006. Ultraviolet radiation shapes seaweed communities. *Reviews in Environmental Science and Biotechnology* 5 (2-3), 141–166.
- Bischof, K., Hanelt, D., Aguilera, J., Karsten, U., Vögele, B., Sawall, T., Wiencke, C., 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Marine Biology* 140 (6), 1097–1106.
- Bischof, K., Hanelt, D., Tüg, H., Karsten, U., Brouwer, P.E.M., Wiencke, C., 1998. Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biology* 20 (6), 388–395.

-
- Bischof, K., Hanelt, D., Wiencke, C., 1998. UV-radiation can affect depth-zonation of Antarctic macroalgae. *Marine Biology* 131 (4), 597–605.
- Bischof, K., Hanelt, D., Wiencke, C., 2000. Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* 211, 555–562.
- Bolton, J.J., Lüning, K., 1982. Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Marine Biology* 66, 89–94.
- Borowitzka, M.A., Borowitzka, L.J., Kessly, D., 1990. Effects of salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*. *Journal of Applied Phycology* 2 (2), 111–119.
- Borum, J., Pedersen, M.F., Krause Jensen, D., Christensen, P.B., Nielsen, K., 2002. Biomass, photosynthesis and growth of *Laminaria saccharina* in a high-arctic fjord, NE Greenland. *Marine Biology* 141 (1), 11–19.
- Breeman, A.M., 1988. Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: experimental and phenological evidence. *Helgoland Marine Research* 42, 199–241.
- Chapman, A.R.O., Lindley, J.E., 1980. Seasonal growth of *Laminaria solidungula* in the Canadian High Arctic in relation to irradiance and dissolved nutrient concentrations. *Marine Biology* 57 (1), 1–5.
- Davison, I.R., 1991. Environmental effects on algal photosynthesis: Temperature. *Journal of Phycology* 27 (1), 2–8.
- Dring, M.J., Makarov, V., Schoschina, E., Lorenz, M., Luening, K., 1996. Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Marine Biology* 126 (1), 183–191.
- Dunton, K.H., 1985. Growth of dark-exposed *Laminaria saccharina* (L.) Lamour. and *Laminaria solidungula* J. Ag. (Laminariales: Phaeophyta) in the Alaskan Beaufort Sea. *Journal of Experimental Marine Biology and Ecology* 94 (1-3), 181–189.
- Dunton, K.H., Dayton, P.K., 1995. The biology of high latitude kelp. In: Skjoldal, H.R., Hopkins, C., Erikstad, K.E., Leinaas, H.P. (Eds.), *Ecology of Fjords and Coastal Waters: Proceedings of the mare Nor Symposium on the Ecology of Fjords and Coastal Aters*, Tromsø, Norway, 5-9 December, 1994. Elsevier Science B.V., Amsterdam, pp. 499–508.
- Dunton, K.H., Jodwalis, C.M., 1988. Photosynthetic performance of *Laminaria solidungula* measured *in situ* in the Alaskan High Arctic. *Marine Biology* 98 (2), 277–285.

-
- Fortes, M.D., Lüning, K., 1980. Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgoland Marine Research* 34, 15–29.
- Franklin, L.A., Forster, R.M., 1997. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *European Journal of Phycology* 32 (3), 207–232.
- Fredersdorf, J., Müller, R., Becker, S., Wiencke, C., Bischof, K., in press. Interactive effects of radiation, temperature and salinity on different life history stages of Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia*
- Gomez, I., Wiencke, C., 1997. Seasonal growth and photosynthetic performance of the Antarctic macroalga *Desmarestia menziesii* (Phaeophyceae) cultivated under fluctuating Antarctic daylengths. *Botanica Acta* 110, 25–31.
- Gómez, I., Figueroa, F.L., Sousa-Pinto, I., Viñebla, B., Pérez-Rodríguez, E., Maestre, C., Coelho, S., Felga, A., Pereira, R., 2001. Effects of UV radiation and temperature on photosynthesis as measured by PAM fluorescence in the red alga *Gelidium pulchellum* (Turner) Kützinger. *Botanica Marina* 44 (1), 9–16.
- Hanelt, D., Wiencke, C., Nultsch, W., 1997. Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *Journal of Photochemistry and Photobiology B: Biology* 38 (1), 40–47.
- Hanelt, D., Hawes, I., Rae, R., 2006. Reduction of UV-B radiation causes an enhancement of photoinhibition in high light stressed aquatic plants from New Zealand lakes. *Journal of Photochemistry and Photobiology B: Biology* 84 (2), 89–102.
- Hanelt, D., Melchersmann, B., Wiencke, C., Nultsch, W., 1997. Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Marine Ecology Progress Series* 149 (1-3), 255–266.
- Hanelt, D., Tüg, H., Bischof, K., Groß, C., Lippert, H., Sawall, T., Wiencke, C., 2001. Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Marine Biology* 138 (3), 649–658.
- Hanelt, D., Wiencke, C., Karsten, U., Nultsch, W., 1997. Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). *Journal of Phycology* 33 (3), 387–395.
- Hoffman, J.R., Hansen, L.J., Klinger, T., 2003. Interactions between UV radiation and temperature limit inferences from single-factor experiments. *Journal of Phycology* 39 (2),

268–272.

- Intergovernmental Panel on Climate Change, 2007. IPCC 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment. Cambridge University Press, Cambridge, 996pp.
- Karsten, U., 2007. Research Note: Salinity tolerance of Arctic kelps from Spitsbergen. *Phycological Research* 55 (4), 257–262.
- Karsten, U., 2008. Defense Strategies of Algae and Cyanobacteria Against Solar Ultraviolet Radiation. In: Amsler, C.D. (ed.), *Algal Chemical Ecology*. pp. 273–296.
- Karsten, U., Bischof, K., Wiencke, C., 2001. Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters. *Oecologia* 127 (1), 11–20.
- Korb, R.E., Gerard, V.A., 2000. Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments. *Marine Ecology Progress Series* 198, 83–92.
- Lane, C.E., Mayes, C., Druehl, L.D., Saunders, G.W., 2006. A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *Journal of Phycology* 42 (2), 493–512.
- Lüning, K., 1990. Seaweeds. Their environment, biogeography, and ecophysiology. John Wiley & Sons, Inc, New York, 527pp.
- Michler, T., Aguilera, J., Hanelt, D., Bischof, K., Wiencke, C., 2002. Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. *Marine Biology* 140 (6), 1117–1127.
- Mueller R, Wiencke C, Bischof K, 2008. Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Climate Research* 37 (2-3), 203–213.
- Rautenberger, R., 2008. Physiological reactions of marine macrophytes along abiotic stress gradients. Doctoral thesis. University of Bremen, Bremen, Germany.
- Rautenberger, R., Bischof, K., 2006. Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biology* 29 (11), 988–996.
- Rmiki, N.E., Schoefs, B., Lemoine, Y., 1999. Carotenoids and stress in higher plants and algae. In: M. Pessarakli (ed.), *Handbook of Plant and Crop Stress*. CRC Press Inc., pp. 465–482.
- Roleda, M.Y., Wiencke, C., Hanelt, D., Bischof, K., 2007. Sensitivity of the Early Life Stages of Macroalgae from the Northern Hemisphere to Ultraviolet Radiation. *Photochemistry*

-
- and Photobiology 83 (4), 851–862.
- Schreiber, U., Bilger, W., Neubauer, C., 1994. Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.-D., Caldwell, M.M. (eds.), Ecophysiology of photosynthesis. Ecological studies: analysis and synthesis 100, pp. 49–70.
- Sokal, R., Rohlf, F.J., 1995. Biometry: the principles and practice of statistics in biological research. 887pp.
- Steinhoff, F.S., Wiencke, C., Müller, R., Bischof, K., 2008. Effects of ultraviolet radiation and temperature on the ultrastructure of zoospores of the brown macroalga *Laminaria hyperborea*. Plant Biology 10 (3), 388–397.
- Svendsen, H., Beszczynska-Moeller, A., Hagen, J.O., Lefauconnier, B., Tverberg, V., Gerland, S., Oerbaeck, J.B., Bischof, K., Papucci, C., Zajaczkowski, M., Attolini, R., Bruland, O., Wiencke, C., Winther, J.G., Dallmann, W., 2002. The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. Polar Research 21 (1), 133–166.
- tom Dieck(Bartsch), I., 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. Marine Ecology Progress Series 100, 253–264.
- Van de Poll, W.H., Eggert, A., Buma, A.G.J., Breeman, A.M., 2002. Temperature dependence of UV radiation effects in Arctic and temperate isolates of three red macrophytes. European Journal of Phycology 37 (1), 59–68.
- Wiencke, C., Bartsch, I., Bischoff, B., Peters, A.F., Breeman, A.M., 1994. Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. Botanica Marina 37 (3), 247–259.
- Wiencke, C., Clayton, M.N., Gómez, I., Iken, K., Lüder, U.H., Amsler, C.D., Karsten, U., Hanelt, D., Bischof, K., Dunton, K., 2007. Life strategy, ecophysiology and ecology of seaweeds in polar waters. Reviews in Environmental Science and Biotechnology 6 (1-3), 95–126.
- Wiencke, C., Gómez, I., Pakker, H., Flores-Moya, A., Altamirano, M., Hanelt, D., Bischof, K., Figueroa, F.L., 2000. Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Marine Ecology Progress Series 197, 217–229.

Wiencke, C., Vögele, B., Kovaltchouk, N.A., Hop, H., 2004. Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. In: Wiencke, C. (ed.), The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991-2003. Reports on Polar and Marine Research 492, pp. 55–62.

Table 1 Experimental conditions of irradiance, temperature and salinity with exposure time during the studies on interactive effects on sporophytes of *Laminaria solidungula* and *Saccharina latissima*

Species	Collecting date; water depth (m)	Radiation		Temperature [°C]	Salinity	Exposure time [days]
		PAR [$\mu\text{mol m}^{-2}\text{s}^{-1}$]	UV [W m^{-2} , unweighted]			
<i>Saccharina latissima</i> (n=15)	May 2005; 3-4m	33.4 (± 6.7)	6.7 (± 1.1) UV-A; 0.25 (± 0.06) UV-B	5°C (5.3 \pm 0.3); 10°C (10.6 \pm 0.3); 20°C (21.5 \pm 0.8)		6 + 2 recovery
<i>Laminaria solidungula</i> (n=15)	May 2005; 14-18m	29.3 (± 5.4)	5.4 (± 0.9) UV-A; 0.15 (± 0.03) UV-B	0°C (0.47 \pm 0.35); 5°C (4.6 \pm 0.4); 10°C (10.9 \pm 0.4); 20°C (20.6 \pm 1.2)		6 + 2 recovery
<i>Laminaria solidungula</i> (n=11)	July 2006; 14-18m	80		3°C (3.4 \pm 0.2); 8°C (8.1 \pm 0.4); 15°C (14.6 \pm 0.6)	34; 28; 20	6

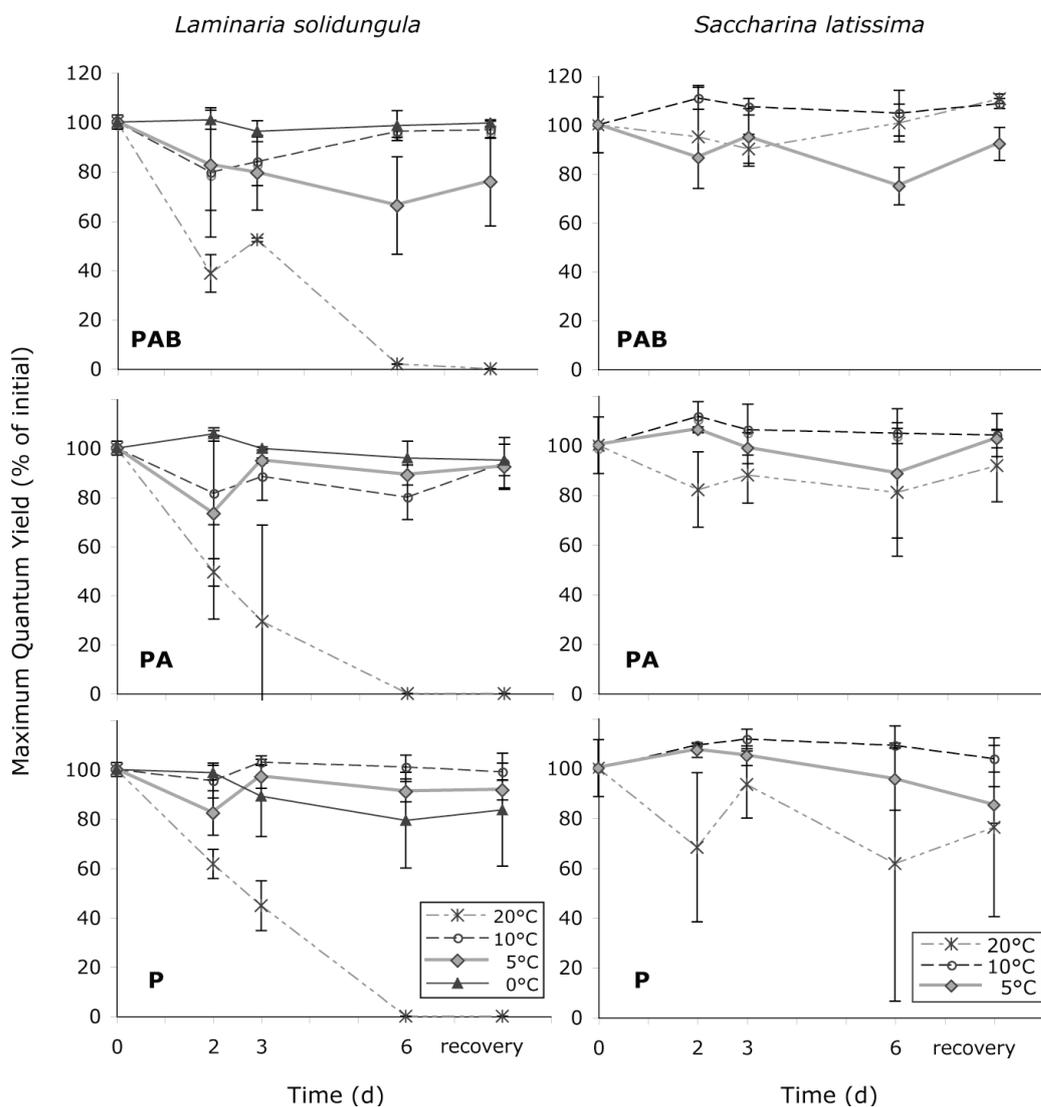


Fig. 1 Changes in maximum quantum yields of photosystem II of *Laminaria solidungula* (left) and *Saccharina latissima* (right) sporophytes at different temperatures (0, 5, 10, 20°C) and radiation conditions (PAB= PAR+UV-A+UV-B; PA= PAR+UV-A; P= PAR), measured over six days of exposure and after two days of recovery under laboratory conditions. Standard deviations are represented by vertical bars (n=3).

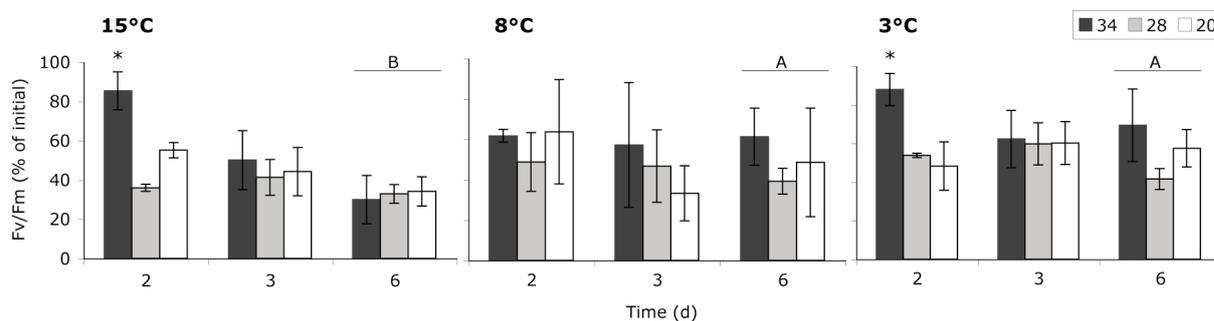


Fig. 2 Changes in maximum quantum yields of photosystem II (Fv/Fm) of *Laminaria solidungula* sporophytes at different temperatures (3, 8, 15°C) and salinities (34= black bars, 28= grey bars, 20= white bars), measured over six days of exposure under laboratory conditions. Standard deviations are represented by vertical bars (n=3). Stars indicate significant salinity effects; different letters indicate significant temperature differences ($p < 0.05$) between treatments at the respective measuring date.

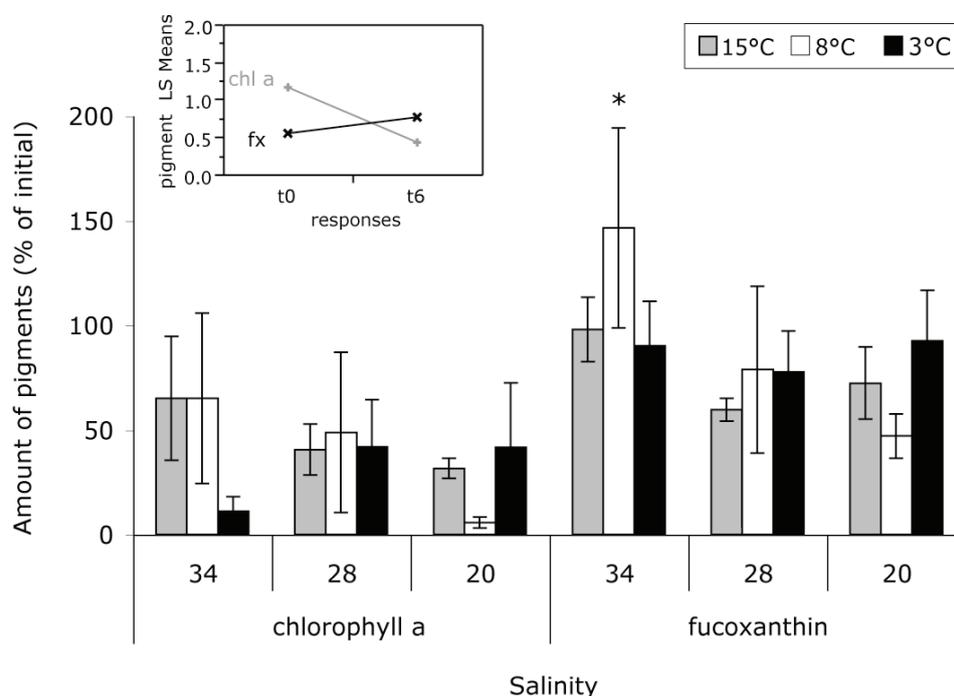


Fig. 3 Changes in total pigment concentration (% of initial) of chlorophyll *a* and fucoxanthin in *L. solidungula* after six days of exposure to different temperatures (3°C= black bars, 8°C= white bars, 15°C= grey bars) at different salinities (34, 28, 20) under laboratory conditions. Standard deviations are represented by vertical bars (n=3-5). Star indicates significant salinity effects and interaction ($p < 0.05$). A plot of total pigment content against the time is given in the small diagram.

ATTACHEMENT OF

PUBLICATION IV

Fredersdorf J, Bischof K

Irradiance of photosynthetically active radiation determines UV-susceptibility of
photosynthesis in *Ulva lactuca* L. (Chlorophyta)

Phycological Research (2007) 55(4): 295–301

RESEARCH NOTE

Irradiance of photosynthetically active radiation determines ultraviolet-susceptibility of photosynthesis in *Ulva lactuca* L. (Chlorophyta)

Jana Fredersdorf^{1,2*} and Kai Bischof²

¹Institute for Polar Ecology, University of Kiel, Wischhofstr. 1-3, Building 12, 24148, Kiel, Germany and ²Department of Marine Botany, University of Bremen Leobener Str. NW2 and Center for Tropical Marine Ecology, Fahrenheitstr. 6, 28359, Bremen, Germany

SUMMARY

The respective ratio of photosynthetically active to ultraviolet radiation is of crucial importance to results obtained in ultraviolet (UV)-research on photoautotrophic organisms. Specimens of the green macroalga *Ulva lactuca* L. were exposed to a constant irradiance of UV-radiation at increasing irradiances of photosynthetically active radiation (PAR). The effects of experimental irradiance and spectral composition on photoinhibition of photosynthesis and its recovery were monitored by chlorophyll fluorescence measurements and the activity of the xanthophyll cycle was assessed by high performance liquid chromatography-(HPLC) based pigment analysis. Results indicate a UV-induced delay in recovery from PAR-induced photoinhibition and a deceleration of violaxanthin conversion within the xanthophyll cycle due to the presence of UV-radiation. Also the concentration of the protective pigment lutein increased considerably and could be indicative of the existence of an additional light-protective mechanism, as, for example, the lutein-epoxid cycle in *Ulva*. In total, results clearly show that the extent of UV-induced inhibition of photosynthesis to be found in UV-exposure experiments is highly dependent on the irradiance of background photosynthetically active radiation: with increasing irradiance of PAR the UV-effects were diminished. Exemplified by the green algae *Ulva lactuca* this study demonstrates the crucial importance of the ratios of PAR:UV applied in UV-research, particularly when conducting laboratory experiments in an ecological context.

Key words: photosynthesis, ultraviolet radiation, *Ulva lactuca*, xanthophyll cycle.

INTRODUCTION

Continuous records of ongoing stratospheric ozone depletion and the concomitant increase in solar

ultraviolet-(UV) radiation reaching the earth's surface have led to substantial research efforts in order to explore the related consequences on the biosphere. Within coastal ecosystems, marine macroalgae have been identified as a group of organisms of vital importance to ecosystem function but potentially vulnerable under increased UV-levels (see Franklin & Forster 1997 and Bischof *et al.* 2006b for review). In many studies conducted so far, representatives of the green algal genus of *Ulva* have been used as some kind of model organisms in order to assess UV effects in marine macroalgae (Franklin *et al.* 1992; Franklin 1994; Han & Kain 1996; Han 1996, 1998, 2003; Altamirano *et al.* 2000a,b; Beer *et al.* 2000; Bischof *et al.* 2002a,b,c, 2003, 2006a). Among the multitude of effects caused by UV-exposure on *Ulva*, changes in pigment composition and internal carbon and nitrogen content (Altamirano *et al.* 2000a,b), an inhibition of the xanthophyll cycle, which increases reactive oxygen species (ROS) production and oxidative damage (Bischof *et al.* 2003), different photoadaptive strategies during sporulation (Han *et al.* 2003), UVB (280–320 nm) absorbing compounds (Han & Han 2005) or differential habitat specific interactive effects of UVB and temperature (Rautenberger & Bischof 2006) were found.

Representatives of this genus have been extensively used in laboratory as well as in field experiments. However results from field and laboratory experiments are often inconsistent, or even contradictory, even when studies are conducted on the same species (Fiscus & Booker 1995). The reasons for that might partly be due to the extremely different experimental conditions applied in the respective studies. Radiation conditions,

*To whom correspondence should be addressed.

Email: jfredersdorf@ipoe.uni-kiel.de

Communicating editor: H. Miyashita.

Received 23 October 2006; accepted 29 March 2007.

doi: 10.1111/j.1440-1835.2007.00474.x

both for spectral quality as well as irradiance, are of crucial importance for the generation of UV effects on photosynthesis (Fiscus & Booker 1995). Artificial radiation conditions in laboratory studies are hardly able to mimic natural solar radiation. Furthermore, the different ratios of PAR:UV radiation may result in artefacts and incorrect conclusions from the viewpoint of ecology. Previous field studies on *Ulva* and *Chaetomorpha* indicated the presence of marked interactive effects of PAR and UV radiation acting synergistically in the inhibition of photosynthesis (Bischof *et al.* 2002b, 2003, 2006a), which might be neglected in studies applying unnatural radiation conditions. In the latter studies a marked UVB induced inhibition of the xanthophyll cycle was found, which might imply an impaired protection system against high PAR radiation. As a consequence, the related increases in ROS production may cause oxidative damage to components of the photosynthetic apparatus.

The study presented aims to illustrate the importance of the respective ratio of PAR:UV applied in UV-research in order to avoid overestimation of UV effects in an ecological context. Despite the wide awareness of this problem in UV research, this is the first study addressing the importance of PAR irradiance in assessment of UV susceptibility of green algal photosynthesis. Therefore, specimens of *Ulva lactuca* were exposed to a constant irradiance of UV radiation and at varied irradiances of background PAR.

MATERIALS AND METHODS

Algal material

Ulva lactuca originally isolated from Disko Bay, Greenland, were grown from stock cultures kept at the Alfred Wegener Institute for Polar and Marine Research (Bremerhaven, Germany; collection number 1128). Algae were cultivated in Provasoli enriched seawater at 0°C and 10 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ in a light/dark cycle of 16/8 h.

Experimental conditions

The experiment was set up in two temperature controlled climate chambers (Uniphyt, Heraeus-Vötsch, Balingen, Germany) at the Institute for Polar Ecology, Kiel (Germany). For acclimation to experimental conditions, algae were cultivated in one of the chambers at 5°C ($\pm 0.5^\circ\text{C}$) and 20 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ in a 16 h light/8 h dark-cycle for three days prior to the start of the experiment. In the other chamber, the experimental radiation conditions were set-up at identical temperature conditions. UV-radiation was provided by two UVA (320–400 nm)-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA), and PAR was generated by Osram Powerstar

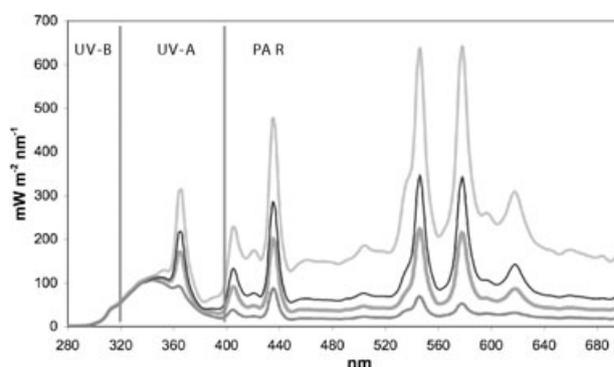


Fig. 1. Spectra of experimental radiation conditions corresponding to 10 W m^{-2} UVA (320–400 nm), 1.0 W m^{-2} UVB (280–320 nm) and 30, 100, 200 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR), respectively.

HQI-R bulbs (Osram, Munich, Germany). The emitted irradiance in the UV-range was adjusted to 1.0 W m^{-2} UVB ($\pm 0.02 \text{ W m}^{-2}$, unweighted) and 10 W m^{-2} UVA ($\pm 1.6 \text{ W m}^{-2}$, depending on the number of PAR-bulbs switched on/off, unweighted). The experiment was designed to expose algal material to different irradiance levels of PAR under constant irradiances of UV-radiation. For each experiment the background irradiance of PAR was changed on the different days of exposure and set at 30, 100, 200 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Irradiance was recorded by a Licor 2π sensor connected to a LI-1400 data logger (LI-COR, Lincoln, USA). In addition, spectral irradiance measurements from 280 to 700 nm were conducted by a RAMSES ACC UV/Vis spectroradiometer (Trios, Oldenburg, Germany). The spectral distribution of irradiance applied during the experimental treatments is shown in Figure 1. A set of four different types of glass filters (Schott, Mainz, Germany) was used to generate different spectral radiation conditions: the UG five filter transmits below 400 and above 680 nm, and thus almost excludes PAR (=UVA + UVB treatment, AB), the WG 280 filter exhibits a cut-off below 280 nm and thus includes all wavelength ranges (=PAR + UVA + UVB treatment, PAB), the WG 320 with a cut-off below 320 nm excludes UVB from the treatment (=PAR + UVA treatment, PA) and finally the filter GG 400 (cut-off below 400 nm) excludes all UV-radiation and thus represents the PAR treatment (P). The respective transmission spectra of these filters are shown in Bischof *et al.* (2002b). After acclimation, algae were directly transferred to the respective radiation conditions for 6 h of exposure. Subsequently, the algae were transferred back to the first climate chamber for 2 h of recovery in 20 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$.

Measurements of photosynthesis

Photosynthetic performance was determined by pulse-amplitude-modulation (PAM)-fluorescence mea-

measurements of maximal quantum yield of photosynthesis (Fv/Fm) using a PAM 2100 chlorophyll fluorometer (Walz, Effeltrich, Germany) as described previously by Hanelt *et al.* (1997). During the period of 8 h in each experimental treatment, Fv/Fm was measured initially and after 1, 3 and 6 h of exposure, and after 2 h of recovery. Simultaneously, samples for pigment analyses were collected and immediately frozen in liquid nitrogen. All the measurements were carried out three times. Prior to the experiments, photosynthesis versus irradiance curves (PI-curves) were recorded by the PAM fluorometer (Schreiber *et al.* 1994) in order to reveal light saturation levels (Ik-values) of experimental specimens. Based on the initial Ik-value of about $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ samples exposed to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR were selected for further analysis of photosynthetic pigments.

Pigment extraction

For extraction and high performance liquid chromatography (HPLC) analysis of photosynthetic pigments (chlorophyll *a* and *b*, lutein, violaxanthin and zeaxanthin), the protocol described by Bischof *et al.* (2002a) was applied. In short, frozen samples were transferred to 2 mL of 100% N-N-dimethylformamide (DMF) and stored in darkness for approximately 18 h. Afterwards, extracts were centrifuged at 5000 g for 5 min at 4°C, the supernatant was subsequently mixed with diethyl-ether and distilled water. Under a stream of gaseous nitrogen pigments were precipitated and re-dissolved in 800 μL of acetonitrile/methanol/tetrahydrofuran (75:15:10). Sample volumes of 95 μL were injected into a photodiode array (PDA) equipped HPLC system (Elite LaChrom, VWR-Merck, Darmstadt, Germany). The protocol and binary gradient system was described in detail by Bischof *et al.* (2002a). For pigment identification, chromatograms and retention times of samples were compared to respective pigment standards obtained from DHI Water & Environment (Horsholm, Denmark).

Data treatment

All measurements were carried out in triplicate. Mean values and standard deviations were calculated. Statistically significant differences were tested using ANOVA and posthoc comparisons of means (Tukey Posthoc-test) at $P < 0.05$ (Sokal & Rohlf 1995). Analyses were carried out using Jump 6.0 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Our results show the effects of different irradiances of PAR on photosynthesis of *Ulva lactuca* under constant

levels of UV-radiation. These findings clearly show the importance of background PAR for the significance of UV-effects.

The effects of experimental exposure and subsequent recovery on maximal quantum yield of photosynthesis (Fv/Fm) under the different radiations conditions are shown in Figure 2. All treatments tested resulted in a substantial reduction in Fv/Fm, however, the extent and the velocity of inhibition and recovery were dependent on irradiance and spectral quality (Fig. 2). Strongest inhibition was observed in algae exposed to the combination of high PAR and UV-radiation (PAB and PA treatment). For example, exposure to the whole light spectrum (PAB) with the highest intensity of PAR ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$), the maximum quantum yield decreased rapidly within the first hour of exposure down to 24% of the initial value, and by the end of 6 h only 7.3% of the initial Fv/Fm value was measured. Under these conditions recovery was not completed after 2 h in dim white light ($20 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$), as during that period values just increased to 34% of the initial values. Under conditions of high PAR, recovery in specimens exposed to a combination of PAR and UV was slowed down compared to the samples exposed under UV exclusion. In all PAR treatments, which included UV-radiation (PAB, PA, Fig. 2) the differences in the degree of inhibition and recovery were minor, whereas most pronounced differences in the rate of inhibition were observed in the PAR treatment without UV (P, Fig. 2). However, the rate of recovery does not reflect these large differences under the different irradiances of PAR. In samples exposed under the UG-5 filter (AB), the inhibition observed was solely based on UV-induced impairment, as PAR was excluded from the treatment. By this we could separate the exclusive effect of UV-radiation from potential interactions with background PAR and therefore, no differences between PAR treatments were observed in these samples. In all specimens in the AB treatment, maximum quantum yields decreased slowly but continuously and to a similar extent, irrespective of PAR levels. Photosynthetic measurements in samples exposed to PAR alone showed a marked dependence of the extent of photo-inhibition on impinging irradiance (Fig. 2). Under all treatments, efficient recovery is indicative for ongoing dynamic photoinhibition. Dynamic photoinhibition, now also termed 'photoprotection', is considered a protective mechanism in higher plants and algae exposed to high irradiances of PAR (Osmond 1994; Hanelt *et al.* 1997, 2003; Häder *et al.* 1998; Franklin *et al.* 2003). By rapid and reversible down-regulation of photosynthetic activity, plants protect themselves from excessive radiation and prevent long-lasting, irreversible damage to photosynthetic or cellular components. Subsequently, recovery proceeds rapidly after stressful conditions have ceased. The mechanism behind this is

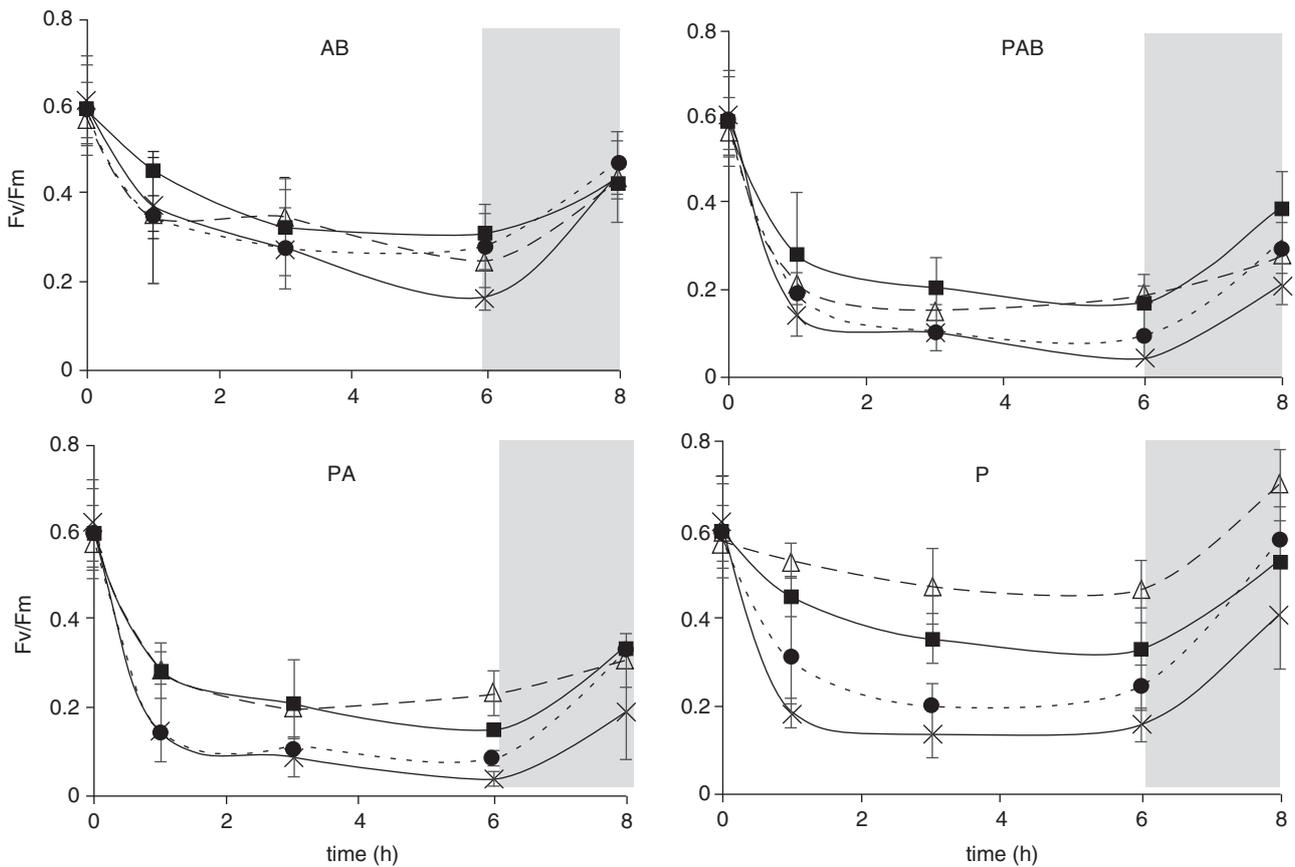


Fig. 2. Maximal quantum yield of photosynthesis (F_v/F_m) in *Ulva lactuca* during exposure to different radiation conditions and subsequent recovery in dim white light (gray). Photosynthetically active radiation (PAR) intensities: Δ , $30 \mu\text{mol m}^{-2} \text{s}^{-1}$; \blacksquare , $100 \mu\text{mol m}^{-2} \text{s}^{-1}$; \bullet , $200 \mu\text{mol m}^{-2} \text{s}^{-1}$; \times , $500 \mu\text{mol m}^{-2} \text{s}^{-1}$; UV-radiation was adjusted to 10 W m^{-2} UVA (320–400 nm), 1.0 W m^{-2} UVB (280–320 nm).

likely to involve a rapid conversion of violaxanthin to zeaxanthin within the xanthophyll cycle, leading to the dissipation of excessively absorbed energy as heat (Demmig-Adams 1990; Demmig-Adams & Adams 1992; Vershinin & Kamnev 1996). This dynamic component during inhibition, the rapid down-regulation of photosynthetic activity also appears in the PAR + UV treatments (PAB + PA), but recovery does not proceed at the same rate as in samples exposed under UV exclusion. This UV-induced delay in recovery from photoinhibition was previously described for the marine brown alga *Alaria esculenta* (Bischof *et al.* 1999) as well as for the red alga *Palmaria palmata* (Hanelt *et al.* 1997). Apparently UV-radiation either interferes with repair or regulatory mechanisms, which might include amongst others an increase in D1 degradation.

Comparison of the PAR and the PAR + UV treatments show the differential contribution of UV radiation to the total extent of photoinhibition (Fig. 2), with a stronger inhibitory contribution of UV under the lowest and a minor share under the highest irradiances of PAR. In order to show the share of UV-radiation in total inhibition of maximal quantum yield of PSII, the F_v/F_m

value of the PAR-treatment was subtracted from the respective values under the PAR-UV-treatment ($F_v/F_{m_{\text{PAB}}} - F_v/F_{m_{\text{P}}}$, Bischof *et al.* 1998). However, this calculation represents a simplification as potential synergies or antagonisms between PAR and UV-radiation become neglected. Figure 3 demonstrates the contribution of UV-radiation to the overall inhibition of photosynthesis. With increasing PAR-intensity the share of UV-induced inhibition of F_v/F_m is reduced. These findings correspond with data from other experiments on higher plants (Teramura 1986; Fiscus & Booker 1995) in which significant interactions between UVB and photosynthetically active radiation was observed with respect to photoinhibition of photosynthesis. There, the extent of UVB-effects in soybean depended on PAR-irradiances and, furthermore, adverse UV-effects could be ameliorated under PAR-levels approaching normal natural conditions.

In previous field studies conducted on *Ulva rotundata* and *Chaetomorpha linum*, marked interactive effects of PAR and UV-radiation on photosynthesis were observed in field experiments (Bischof *et al.* 2002b, 2006a). It was found that the UV-induced deceleration

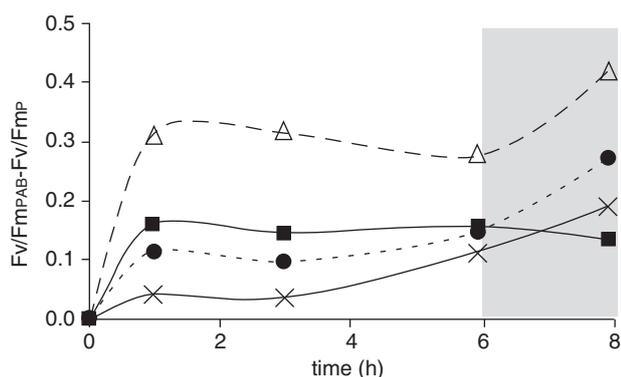


Fig. 3. Relative share of ultraviolet-(UV) induced inhibition to the total inhibition of photosynthesis in *Ulva lactuca* exposed to different photosynthetically active radiation (PAR) intensities, recovery phase marked gray. PAR intensities: Δ , $30 \mu\text{mol m}^{-2} \text{s}^{-1}$; \blacksquare , $100 \mu\text{mol m}^{-2} \text{s}^{-1}$; \bullet , $200 \mu\text{mol m}^{-2} \text{s}^{-1}$; \times , $500 \mu\text{mol m}^{-2} \text{s}^{-1}$; UV-radiation was adjusted to 10 W m^{-2} UVA (320–400 nm), 1.0 W m^{-2} UVB (280–320 nm).

of the xanthophyll cycle may be one important mechanism for how UV radiation may increase susceptibility of the photosynthetic machinery to high irradiances of PAR. This delay in violaxanthin conversion may be explanatory for an impaired ability of high light-exposed algae for dynamic photoinhibition (Demmig-Adams & Adams 1992; Hanelt *et al.* 1997). This finding is clearly supported by the present study: In Figure 4 concentrations of violaxanthin (vx) and lutein (lu) are expressed per mol chlorophyll a (chl a). The contents of chlorophyll a were all along constant. Within the first hour of exposure the content of the xanthophyll cycle pigment violaxanthin (Fig. 4a) increased under all treatments, which included UV-radiation, whereas under the PAR-treatment violaxanthin decreased significantly (two-way ANOVA, d.f.: 2, F: 6.8, P : 0.0405). After 6 h of exposure, violaxanthin content was also diminished in the UV-exposed samples. Significant differences in concentration were absent between the different UV-treatments. Consequently 1 h of exposure to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR resulted in an efficient conversion of violaxanthin within the xanthophyll cycle. In contrast, supplemental UVA and UVA + B radiation as well as UV radiation without PAR resulted in an accumulation of violaxanthin. However, these differences were minimized in the further course of exposure, indicating the absence of persisting damaging effects on the xanthophyll cycle but rather a delay of xanthophyll conversion.

In addition, changes in the amount of the xanthophyll lutein (Fig. 4b) may contribute to photoacclimation and photoprotection (Niyogi *et al.* 1997; Müller *et al.* 2001). The concentration of lutein increased under all radiation-treatments during the course of the

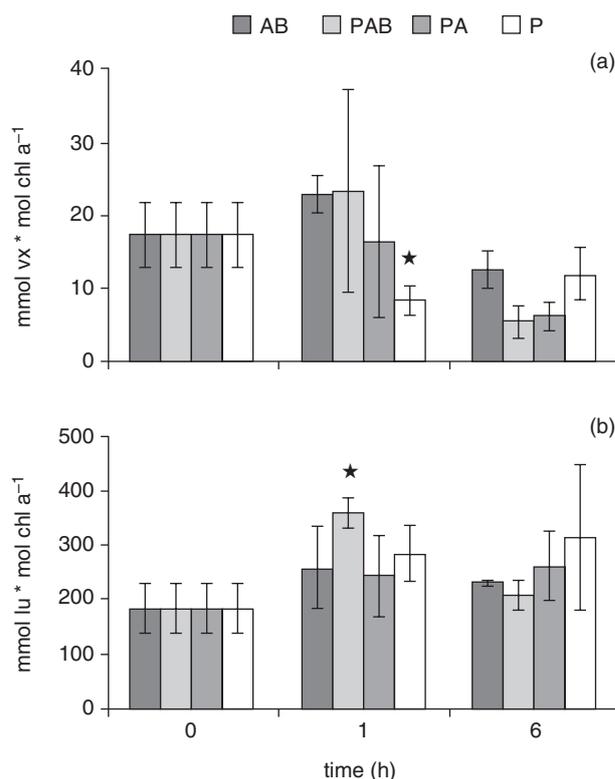


Fig. 4. Changes in the content of violaxanthin (vx, Fig. 4a) and lutein (lu, Fig. 4b) after 1 and 6 h of exposure to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) irradiation, ultraviolet-(UV) radiation was adjusted to 10 W m^{-2} UVA (320–400 nm), 1.0 W m^{-2} UVB (280–320 nm). Values are normalized to the content of mol Chl a; \star indicates statistically significant differences.

experiment. Especially in the PAR + UV-treatment (PAB) the content increased quickly and significantly (two-way ANOVA, d.f.: 2, F: 27.47, P : 0.0026) after 1 h. The considerable increase in lutein could be indicative of the existence of an additional light-protective mechanism, as for example, the lutein-epoxid cycle, as originally discovered in green tomato fruit by Rabinowitch *et al.* (1975). This cycle, described as a new xanthophyll cycle involves the de-epoxidation of lutein-5, 6-epoxid (Lx) to lutein by the xanthophyll cycle enzyme violaxanthin de-epoxidase (Bungard *et al.* 1999) in the light and subsequent epoxidation in the dark. This was also demonstrated in the parasitic plants *Cuscuta reflexa* (Bungard *et al.* 1999; Snyder *et al.* 2005) and in mistletoes (Matsubara *et al.* 2003) and as well in non-parasitic plants like in several tree species of *Quercus* (García-Plazaola *et al.* 2002, 2003) and in the microalgae *Dunaliella tertiolecta* (Antia & Cheng 1983). Presently, our data just allow hypothesizing on the formation of Lx based on comparisons of HPLC spectra with the ones published by Bungard *et al.* (1999) and the increasing concentration of lutein. To

our knowledge there is no commercially available pigment standard for Lx yet. Therefore, more studies on the existence of the lutein-luteinopoxid cycle in marine macroalgae should be conducted.

In conclusion, results from our experiment on *Ulva lactuca* show the particular importance of the ratios PAR:UV, especially for comparisons between laboratory and field experiments and the estimations of UV-effects. It is likely that the inconsistency of PAR:UV ratios applied in laboratory studies result in significant variations of UV effects observed in the different studies conducted so far (see reviews by Fiscus & Booker 1995; Wängberg *et al.* 1996; Franklin & Forster 1997; Allen *et al.* 1998). Furthermore, the particularly low PAR:UV ratios often applied in laboratory studies result in a substantial overestimation of UV effects. Fiscus and Booker (1995) stated that high UVB exposures at very low PAR-levels are necessary to produce many of the UV-effects described so far.

In nature high irradiances of UV-radiation do only occur in combination with high levels of PAR (Hanelt *et al.* 2003) and the natural solar spectrum exhibits ratios of energy distribution over the different wavelength ranges of UVB : UVA : PAR = 0.6:10:100 at the surface of the earth (Franklin & Forster 1997). In this study, the PAR:UVR ratios applied were still low compared to ambient solar radiation. Contrary to other studies, we attempted to apply as much PAR ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) as possible obtaining a ratio of approximately 1:10:100, closer to the ecologically relevant ratio. Franklin *et al.* (2003) stated that future UV-studies will need more ecologically-relevant contexts with realistic, appropriate spectral composition and with other ecologically relevant factors, for example, realistic nutrient concentrations, temperature or competitors.

As a result of our study, the apparent effects of UV radiation clearly depend on the irradiance of background PAR. Future laboratory studies on UV effects will need to increase their ecological significance by improving the radiation conditions applied in exposure experiments.

ACKNOWLEDGMENTS

This study was financially supported by the Helmholtz Society of German Research Centres (Project: NG-VH-051) and Deutsche Forschungsgemeinschaft (DFG Bi 772/2-1,2). The authors are most grateful to Alice Schneider for pigment analysis.

REFERENCES

- Allen, D., Nogues, S. and Baker, N. R. 1998. Review article. Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *J. Exp. Bot.* **49**: 1775–88.
- Altamirano, M., Flores-Moya, A., Conde, F. and Figueroa, F. L. 2000a. Growth seasonality, photosynthetic pigments, and carbon and nitrogen content in relation to environmental factors: a field study of *Ulva olivascens* (Ulvales, Chlorophyta). *Phycologia* **39**: 50–8.
- Altamirano, M., Flores-Moya, A. and Figueroa, F. L. 2000b. Long-Term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated *in situ*. *Bot. Marina* **43**: 119–26.
- Antia, N. J. and Cheng, J. Y. 1983. Evidence for anomalous xanthophyll composition in a clone *Dunaliella tertiolecta* (Chlorophyceae). *Phycologia* **22**: 235–42.
- Beer, S., Larsson, C., Poryan, O. and Axelsson, L. 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. *Eur. J. Phycol.* **35**: 69–74.
- Bischof, K., Hanelt, D., Tüg, H., Karsten, U., Brouwer, P. E. M. and Wiencke, C. 1998. Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol.* **20**: 388–95.
- Bischof, K., Hanelt, D. and Wiencke, C. 1999. Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biol.* **1**: 435–44.
- Bischof, K., Kräbs, G., Wiencke, C. and Hanelt, D. 2002a. Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* **215**: 502–9.
- Bischof, K., Peralta, G., Kräbs, G., van de Poll, W. H., Pérez-Lloréns, J. L. and Breeman, A. M. 2002b. Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. *J. Exp. Bot.* **53**: 2411–21.
- Bischof, K., Hanelt, D. and Wiencke, C. 2002c. UV radiation and Arctic marine macroalgae. In Hessen, D. O. (Ed.) UV radiation and Arctic ecosystems. *Ecol. Stud.* **153**: 227–44.
- Bischof, K., Janknegt, P. J., Buma, A. G. J., Rijstenbil, J. W., Peralta, G. and Breeman, A. M. 2003. Oxidative stress and enzymatic scavenging of superoxide radicals induced by solar UV-B radiation in *Ulva* canopies from southern Spain. *Scientia Marina* **67**: 353–9.
- Bischof, K., Rautenberger, R., Brey, L. and Pérez-Lloréns, J. L. 2006a. Physiological acclimation to gradients of solar irradiance within mats of the filamentous green macroalga *Chaetomorpha linum* from southern Spain. *Mar. Ecol. Prog. Ser.* **306**: 165–75.
- Bischof, K., Gomez, I., Molis, M. *et al.* 2006b. Ultraviolet radiation shapes seaweed communities. *Rev. Environ. Sci. Biotechnol.* **5**: 141–66.
- Bungard, R. A., Ruban, A. V., Hibberd, J. M., Press, M. C., Horton, P. and Scholes, J. D. 1999. Unusual carotenoid

- composition and a new type of xanthophyll cycle in plants. *Proc. Natl Acad. Sci. USA/Plant Biol.* **96**: 1135–9.
- Demmig-Adams, B. 1990. Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. *Biochem. Biophys. Acta Bioenerg.* **1020**: 1–24.
- Demmig-Adams, B. and Adams, W. W. III. 1992. Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43**: 599–626.
- Fiscus, E. L. and Booker, F. L. 1995. Is increased UV-B a threat to crop photosynthesis and productivity? *Photosynth. Res.* **43**: 81–92.
- Franklin, L. A. 1994. The effect of temperature acclimation on the photoinhibitory responses of *Ulva rotundata* Blid. *Planta* **192**: 324–31.
- Franklin, L. A. and Forster, R. M. 1997. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur. J. Phycol.* **32**: 207–32.
- Franklin, L. A., Levavasseur, G., Osmond, C. B., Henley, W. J. and Ramus, J. 1992. Two components of onset and recovery during photoinhibition of *Ulva rotundata*. *Planta* **186**: 399–408.
- Franklin, L. A., Osmond, C. B. and Larkum, A. W. D. 2003. Photoinhibition, UV-B and Algal Photosynthesis. In Larkum, A. W. D., Douglas, S. E. and Raven, J. A. (Eds) *Photosynthesis in Algae*. Kluwer Academic Publishers, Dordrecht, pp. 351–84.
- García-Plazaola, J. I., Hernández, A., Errasti, E. and Becerril, J. M. 2002. Occurrence and operation of the lutein epoxide cycle in *Quercus* species. *Funct. Plant Biol.* **29**: 1075–80.
- García-Plazaola, J. I., Hernández, A., Olano, J. M. and Becerril, M. B. 2003. The operation of the lutein epoxide cycle correlates with energy dissipation. *Funct. Plant Biol.* **30**: 319–24.
- Häder, D. P., Kumar, H. D., Smith, R. C. and Worrest, R. C. 1998. Effect on aquatic ecosystems. *J. Photochem. Photobiol. B: Biol.* **46**: 53–68.
- Han, T. 1996. Effect of ultraviolet-B radiation on *Ulva pertusa* Kjellman (Chlorophyta) I. growth and pigment content. *Algae (Korean J. Phycol.)* **11**: 155–9.
- Han, Y.-S. and Han, T. 2005. UV-B induction of UV-B protection in *Ulva pertusa* (Chlorophyta). *J. Phycol.* **41**: 523–30.
- Han, T. and Kain, J. M. 1996. Far-UV action on growth, pigmentation and photosynthesis of *Ulva lactuca* (Chlorophyta). *Algae (Korean J. Phycol.)* **11**: 203–6.
- Han, T., Chung, H. and Kang, S.-H. 1998. UV photobiology of marine macroalgae. *Korean J. Polar Res.* **9**: 37–46.
- Han, T., Han, Y.-S., Kim, K.-Y. *et al.* 2003. Influences of light and UV-B on growth and sporulation of the green alga *Ulva pertusa* Kjellman. *J. Exp. Mar. Biol. Ecol.* **290**: 115–31.
- Hanelt, D., Wiencke, C. and Nultsch, W. 1997. Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *J. Photochem. Photobiol. B: Biol.* **38**: 40–7.
- Hanelt, D., Wiencke, C. and Bischof, K. 2003. Photosynthesis in marine Macroalgae. In Larkum, A. W., Douglas, S. E. and Raven, J. A. (Eds) *Photosynthesis in Algae. Advances in Photosynthesis and Respiration 18*. Kluwer Academic Publishers, Dordrecht. pp. 413–35.
- Matsubara, S., Morosinotto, T., Bassi, R. *et al.* 2003. Occurrence of the lutein-epoxide cycle in mistletoes of the Loranthaceae and Viscaceae. *Planta* **217**: 868–79.
- Müller, P., Li X.-P. and Niyogi, K. K. 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiol.* **125**: 1558–66.
- Niyogi, K. K., Bjoerkman, O. and Grossman, A. R. 1997. The roles of specific xanthophylls in photoprotection. *Proc. Natl Acad. Sci. USA* **94**: 14162–7.
- Osmond, C. B. 1994. What is photoinhibition? Insights from comparisons of shade and sun plants. In Baker, N. R. and Bowyer, J. R. (Eds) *Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field*. BIOS Scientific Publishers, Oxford, pp. 1–24.
- Rabinowitch, H. D., Budowski, P. and Kedar, N. 1975. Carotenoids and epoxide cycles in mature-green tomatoes. *Planta* **122**: 91–7.
- Rautenberger, R. and Bischof, K. 2006. Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol.* **29**: 988–996. (Online First), 10.1007/s00300-006-0141-6.
- Schreiber, U., Bilger, W. and Neubauer, C. 1994. Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis. *Ecol. Stud.* **100**: 49–70.
- Snyder, A. M., Clark, B. M. and Bungard, R. A. 2005. Light-dependent conversion of carotenoids in the parasitic angiosperm *Cuscuta reflexa* L. *Plant, Cell Environ.* **28**: 1326–33.
- Sokal, R. and Rohlf, F. J. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. Library of Congress Cataloging. W.H. Freeman, New York, 887 pp.
- Teramura, A. H. 1986. Interaction between UV-B radiation and other stresses in plants. In Worrest, R. C. and Caldwell, M. M. (Eds) *Stratospheric ozone reduction, solar ultraviolet radiation and plant life*. Springer-Verlag, Berlin. pp. 327–343.
- Vershinin, A. O. and Kamnev, A. N. 1996. Xanthophyll cycle in marine macroalgae. *Bot. Marina* **39**: 421–5.
- Wängberg, S. Å., Selmer, J. S., Eklund, N. G. A. and Gustavson, K. 1996. UV-B effects on Nordic marine ecosystems – a literature review. *Temandord* **515**: 60.

ATTACHEMENT OF

PUBLICATION V

Karsten U, Wulff A, Roleda M, Müller R, Steinhoff FS, **Fredersdorf J**,

Wiencke C

Physiological responses of polar benthic micro- and macroalgae to ultraviolet
radiation

Botanica Marina (Special Issue) (under review)

Review:**Physiological responses of polar benthic micro- and macroalgae to ultraviolet radiation**

Ulf Karsten^{1,*}, Angela Wulff², Michael Y. Roleda³, Ruth Müller⁴, Franciska S. Steinhoff⁵, Jana Fredersdorf^{3,5} and Christian Wiencke⁴

¹University of Rostock, Institute of Biological Sciences, Applied Ecology, Albert-Einstein-Strasse 3, D-18057 Rostock, Germany, Email: ulf.karsten@uni-rostock.de

²University of Gothenburg, Marine Ecology, P.O. Box 461, SE-405 30 Göteborg, Sweden

³University of Kiel, Institute for Polar Ecology, Wischhofstrasse 1-3, D-24148 Kiel, Germany

⁴Alfred-Wegener-Institute for Polar and Marine Research, Department Seaweed Biology, Am Handelshafen 12, D-27570 Bremerhaven, Germany

⁵University of Bremen, Institute of Marine Botany, Leobener Str., D-28359 Bremen, Germany

* Corresponding author

Abstract

Stratospheric ozone depletion and the concomitant increase in UVB radiation at the earth's surface represent major threats to polar marine ecosystems. While in coastal rocky shore environments, macroalgae constitute a community of particular significance to ecosystem function, benthic diatoms dominate microphytobenthic communities which typically grow on top of shallow-water sediments as highly productive and stabilising phototrophic biofilms. This review summarises the present knowledge on how UV radiation affects the physiology of polar benthic micro- and macroalgae with an emphasis on cell biological and structural changes, molecular targets and repair mechanisms, induction of reactive oxygen species and antioxidative strategies, photosynthesis and growth, photoprotective mechanisms, interactive effects between UVR and other abiotic factors, and finally ecological consequences. Although the data presented already indicate that specific characteristics and adaptations in polar benthic micro- and macroalgae exist that explain their ecological success and limits under environmentally extreme conditions, much more research is needed to understand the underlying mechanisms. Particularly more ecosystem approaches and studies on interactive effects as well as modern genomic, proteomic and metabolomic approaches may help to address the open questions and to draw a more holistic picture.

Keywords

Avoidance, DNA repair, growth, interactive effects, mycosporine-like amino acids, phlorotannins, photosynthesis, ultrastructure, UV-sunscreens, life cycle

Introduction

The stratospheric accumulation of ozone is primarily responsible for absorbing parts of the solar ultraviolet radiation (UVR) before it can reach the marine biosphere. Emission of anthropogenic halogenated volatile substances in the last century have – apart from natural sources of these compounds (Laturnus 2001, Laturnus et al. 2002, Gribble 2003) - resulted in a stratospheric enrichment of these compounds, which may persist for many decades. Because of the high chemical reactivity of halogens they efficiently destroy ozone in the protective layer. This is particularly well reflected in the strong ozone decline over Antarctica each spring, which can account for more than 75% depletion, a phenomenon known to the public as ‘ozone hole’ (Wessel et al. 1998, for details Whitehead et al. 2000). But also the Arctic is currently affected by ozone depletion and consequently increasing UVR (McKenzie et al. 2003, Zacher et al., this issue).

UVR is differentiated according to the CIE definition (Commission Internationale de l’Eclairage 1935) into three wavebands – UVC: 190–280 nm, UVB: 280–315 nm and UVA: 315–400 nm. UVC is strongly mutagenic and lethal to most organisms, however, due to its complete absorption through the atmospheric ozone layer it does not reach the biosphere. In contrast, UVA is not attenuated by ozone, and hence its fluence will be unaffected by any ozone layer reduction reaching polar organisms. It is the UVB range that increases as a consequence of stratospheric ozone destruction. Although this waveband represents less than 1% of the total solar flux reaching the earth's surface, it is biologically extremely harmful (Franklin and Forster 1997). Calculations indicate that a 10% decline in column ozone would result in an approximate 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).

Physical and chemical environment in benthic habitats of polar regions

Sea ice is probably the most important factor affecting polar benthic algae. Benthic organisms can be completely removed by ice-scouring (Teixido et al. 2007), and, furthermore, during ice break-up in early summer benthic algae are suddenly exposed to very high intensities of both photosynthetically active radiation (PAR, 400-700 nm) and UVR (Bischof et al. 2002a, Aguilera et al. 2002a). Although irradiance intensities logarithmically decrease with increasing water depth, still 1% penetration depth of UVB was recorded down to 19 m in Potter Cove, King George Island (Richter et al. 2008). For benthic microalgae, the sediment has been proposed to be a refuge to escape harmful radiation by migration (Wulff et al. 2008a) but UVR has been shown to penetrate ca. 0.6 mm (UVB) and 1 mm (UVA) into a

sandy sediment (Wulff et al. 1999). However, not only high radiation conditions are stressful, but polar benthic algae also have to survive the dark winter months which account about 4 months. Heterotrophic growth has been suggested as survival strategy for diatoms and other algae (Mortain-Bertrand et al. 1988, Tuchman 1996). In addition, for various macroalgae a life strategy as annual anticipator has been reported, i.e. these plants grow in winter on the expense of storage products that were formed during the previous summer (Wiencke et al. this issue). Temperature changes can be substantial in rock pools and in the upper eulittoral but are comparably small in the subtidal, ranging from ca 2°C to -1.8°C. Macronutrients (nitrogen, phosphate and silicate) are seldom limiting for benthic algal growth in polar areas (Drew and Hastings 1992), but lack of micronutrients such as iron may reduce primary productivity. In addition, in dense microalgal mats also macronutrients could be a limiting factor for growth due to the compact nature of these communities. From different biological processes such as photosynthesis, respiration, fermentation and sulfate-sulfur reduction a set of very steep and diurnally changing chemical gradients are created in the top layer of sediments such as for oxygen concentration, soluble sulfide and hydrogen ions (Garcia-Pichel et al. 1994 and references therein) that may affect nutrient availability and uptake.

Structure and function of benthic micro- and macroalgal communities

The shallow water zone of polar regions is dominated by two types of algal communities. While macroalgal species of the Chlorophyta, Rhodophyta and Phaeophyceae primarily settle on hard bottom substrate, such as rocky shorelines, their microscopic pendants preferentially cover extensive sediment areas. At their growth site seaweeds often form submersible, complex structured underwater forests characterized by high primary productivity, thereby providing food, shelter and habitat to many marine invertebrates and fish (Bischof et al. 2006). The macroalgal communities typically show distinct vertical zonation patterns exhibiting a characteristic sequence of species with increasing depth which is related to the vertically changing environmental parameters (Bischof et al. 2006). Being at the basis of marine food webs, macroalgae are directly consumed by a diversified suite of micro- and macrograzers (Duffy and Hay 2000), while algal exudates might fuel the microbial loop if they are used by free-living and alga-associated bacteria. In addition, seaweeds serve many species as habitat, to which sessile forms attach directly, and may host motile animals by provision of shelter from predators.

Microphytobenthic biofilms in shallow waters are typically dominated by Bacillariophyceae. This phototrophic community is generally known from temperate marine regions as highly

productive providing a major food source for benthic suspension- or deposit-feeders (Cahoon 1999), as control barrier for oxygen fluxes at the sediment/water interface (Risgaard-Petersen et al. 1994), and as stabilizer of sediment surfaces against erosion by the excretion of extracellular polymeric substances (De Brouwer et al. 2005). Consequently, the microphytobenthos represents a key component in the functioning of trophic webs in coastal zones which are characterized by sediments (Cahoon 1999). However, in contrast to temperate to tropical regions, structure and function of microphytobenthic communities are poorly studied in polar waters (Glud et al., this issue).

UVR effects on benthic micro- and macroalgae

Solar radiation is essential for life in polar regions. In the process of photosynthesis, the microphytobenthic and macrophytobenthic communities absorb visible light energy with their respective photosynthetic antennae to chemically fix inorganic carbon into energy-rich organic compounds. However, an increase in UVR can inhibit many biological processes. The major cellular targets of UVB are different biomolecules, which directly absorb this radiation, or which are indirectly affected by various UV-induced photochemical reactions. The biological and, ultimately, ecological consequences are numerous.

Cell biological and ultrastructural changes

The cell biological and ultrastructural changes due to UVR-exposure depend on the general physiological constitution, the life-history and developmental stage of the individual under study. The most susceptible stages in the life history of macroalgae are their spores, gametes and zygotes. This has been demonstrated in species of the genus *Fucus*, and in various Laminariales and Gigartinales from Spitsbergen, King George Island (Antarctica) and the North Sea (Schoenwaelder et al. 2003, Müller et al. 2008b, Roleda et al. 2007b, 2008a, Zacher et al. 2008). As discussed below, multiple cellular processes are negatively affected by UVR, in particular photosynthesis (Roleda et al. 2006d), nuclear division (Huovinen et al. 2000) and motility (Makarov and Voskoboynikov 2001). On the other hand, there are repair mechanisms operating, which mitigate damage. This is shown by the effective recovery of photosynthesis and the repair of DNA damage (Roleda et al. 2004, 2006a, 2008a, Zacher et al. 2008). Damage may also be prevented by UV-absorbing phlorotannins in brown algae (Swanson and Druehl 2002, Roleda et al. 2005, 2006c) and mycosporine-like amino acids in red algae (Roleda et al. 2008a, Zacher et al. 2008). The balance between the damaging effects of UVR and the repair and protective mechanisms can be measured by the integrative

parameter 'germination'. If spores or zygotes germinate after UVR exposure, the repair and protective mechanisms are strong enough to cope with the damaging effects of UVR.

So, the effects on the ultrastructure of an algal cell can be quite diverse ranging from no to very strong damage, the latter usually reflecting cell death. But one should always keep in mind that an electron micrograph shows a snap shot only of the moment the organism was fixed, and hence gives no information about physiological processes such as UVR acclimation. Ongoing repair or degradation processes, for instance, can be easily overseen or misinterpreted. Therefore, when studying UVR-induced ultrastructural effects additional physiological information should always be taken into account.

UVR affects all cellular components, especially the chloroplasts, the mitochondria, the nucleus and also the cytoplasm. In Table 1 the present knowledge about UVR-effects on the fine structure of marine algal cells of different systematic position from polar to cold temperate regions is summarized. When comparing the results it must be taken into consideration that the data were obtained on different life-history and developmental stages and that different exposure times and radiation conditions were used in the various studies.

Numerous investigations were done within the Rhodophyta. Poppe et al. (2002, 2003) investigated laboratory grown *Palmaria decipiens*, *Palmaria palmata*, *Phycodrys austrogeorgica* and *Bangia atropurpurea*, whereas Holzinger et al. (2004) studied field-grown *Odonthalia dentata* and *Palmaria palmata*. The most striking effect of UVR in the studied species was the disturbance of the fine structure of the chloroplasts. The intrathylakoidal space was generally enlarged and the thylakoid membranes became wrinkled. In UVR-sensitive species or after strong UVR-exposure the thylakoids became tubular, e. g. in *B. atropurpurea* (Poppe et al. 2003) or disintegrated into "inside-out" vesicles, obviously a general phenomenon in red algal chloroplasts. In these vesicles the phycobilisomes, which are normally attached on the outside of the thylakoids, were exposed to the inside. In such cases the photosynthetic apparatus was strongly damaged and photosynthesis was impaired if not fully inhibited. In some cases these fine structural changes were, however, partly reversible reflecting acclimation of photosynthesis to UVR as shown in *P. decipiens* (Poppe et al. 2002). Another effect of UVR was the formation of protrusions of the chloroplast envelope as shown in *P. palmata* and *O. dentata* (Holzinger et al. 2004) or a disintegration of both envelope membranes as shown in *Phycodrys austrogeorgica* (Poppe et al. 2003). In the latter species the protein crystals generally occurring in the cytoplasm became corroded after UVR-treatment indicating either damage or remobilization of the stored protein for repair processes (Poppe et al. 2003). Within the mitochondria the cristae

appeared swollen under UVR and were transformed into sacculi (Holzinger et al. 2004, Poppe et al. 2002).

In contrast, the foliose thalli of the green alga *Prasiola crispa* (Holzinger et al. 2006) showed no effect after mild UVR exposure. After stronger exposure only slight alterations appeared within the chloroplasts, such as dilatations of thylakoids and a reduced number of plastoglobuli. Under these conditions photosynthetic efficiency decreased. The mitochondria showed slight damages and cytoplasmic globules increased in size and became more abundant. So in contrast to most of the red algae discussed above *P. crispa* can cope with UV stress relatively well.

In brown algae two different life history stages were examined electron microscopically, zoospores and filamentous gametophytes. After UVB exposure the zoospores of *Laminaria hyperborea* showed an enhanced formation of plastoglobuli in the chloroplast, the nucleoplasma became mottled and the structure of the mitochondria changed from the tubulus- to the sacculus-type (Steinhoff et al. 2008). Phlorotannin containing physodes were present, but did not show any change after UVR exposure, so that their contribution to UV-protection is doubtful. The fine structure of gametophytes of *Saccharina latissima* and *Laminaria digitata* did not change very much under UVR. The only change was the increase in size of plastoglobuli in the chloroplast (Müller et al. 2008c), generally regarded as indication for the up-regulation of plastid lipid metabolism in response to oxidative stress.

As no data on these UVR effects on benthic diatoms are available we summarize here results obtained on the three pelagic diatom species *Cyclotella* sp., *Nitzschia closterium* (*Cylindrotheca closterium*) and *Thalassiosira nordenskjoldii* (Buma et al. 1996). Exposure of these microalgae to UVB resulted in an increased cell size. Moreover, although the chloroplast number rose the organelle size decreased and became disoriented. The nucleus appeared enlarged and the contrast between nucleolus, chromatin and nucleoplasm faded with increasing UVB exposure. These ultrastructural changes mirror the fact that cell division was inhibited, whereas growth still continued as the general cell metabolism was not or only little affected by UVR. Similar results were obtained on the coccolithophore *Emiliana huxleyi* (Buma et al. 2000).

Changes in chloroplast ultrastructure after UV stress seem to be a general phenomenon also in fresh water algae, e.g. *Micrasterias denticulata* (Lütz et al. 1997) and in endosymbiotic dinoflagellates (Hannack et al. 1997). Other features include the formation of large ER-cisternae in *Micrasterias denticulata* (Meindl and Lütz 1996) and of lipid globules containing secondary carotenoids in snow algae e.g. *Chlamydomonas nivalis* (Remias et al. 2005). For

further information of these algal groups the reader is directed to the review of Holzinger and Lütz (2006).

Molecular targets and repair mechanisms

DNA represents one of the most UV-sensitive biomolecules, and UV-induced damage occurs directly through absorption of UVB quanta by the aromatic residues. The absorbed energy can be dissipated by different mechanisms involving single bases (e.g. single-strand breaks) or interactions between adjacent bases (e.g. dimerization) and between non-adjacent bases (i.e. inter- or intrastrand crosslinks) (Karentz et al. 1991). The structural consequences are conformational alterations such as the often observed formation of cyclobutane dimers and pyrimidine (6–4)–pyrimidone (6–4)–photoproducts (CPDs) (Lois and Buchanan 1994). Such UV-induced DNA damage can significantly affect transcription, causing erroneous replication and promoting mutations, which finally increase mortality of algal cells.

Life stage-dependent susceptibility to UV-B-induced DNA damage was observed in several polar macroalgae. Zoospores of the sublittoral kelps from Spitsbergen (*Alaria esculenta*, Wiencke et al. 2007; *Laminaria digitata*, Lüder et al. 2008; *Saccorhiza dermatodea*, Roleda et al. 2006a) are more susceptible to UV-B-induced DNA damage compared to their respective young sporophytes (Roleda et al. 2005, 2006b). Recently, filamentous *Urospora penicilliformis* gametophytes from Antarctica were also reported to sustain significantly lesser DNA damage compared to propagules exposed to the same UV-B dose (Roleda et al. 2008c). In multicellular life stages, the relatively thick cell wall may be able to selectively filter short UV-wavelengths from reaching the UV-sensitive targets (i.e. nucleus) compared to the “naked” propagules. On the other hand, an inverse relationship between thallus thickness and remaining DNA damage was also reported in young sporophytes of different kelp species (Roleda et al. 2005, 2006b, 2007a). Aside from UV screening by cell walls, intracellular mechanisms such as synthesis of UV-absorbing compounds (see below) are also important for UV protection against DNA damage. Moreover, release of a cloud of spores (e.g. kelp zoospores with phlorotannin-containing physodes) can self-shade each other, thereby forming a kind of “biofilter” against harmful UVR (Roleda et al. 2006c).

Sensitivity of different propagules to UVR-induced DNA damage is related to size of the propagules, ploidy levels and depth distribution of the adult plants (Roleda et al. 2007a, 2008a, Zacher et al. 2007a). Propagules exposed to comparable UV-B dose showed significantly lower DNA damage as observed in eulittoral *Urospora penicilliformis* (Roleda et al. 2008c) compared to the gametes of the sublittoral *Ascoseira mirabilis* (Roleda et al.

2007b). Among kelps, the reported prevalence of larger, more UV-tolerant meiospores originating from species or populations from sites exposed to high UV radiation suggest that kelp meiospores are pre-adapted to the UV conditions of the parent plant (Swanson and Druehl 2000, Wiencke et al. 2006). Comparison between spores of different ploidy levels in Antarctic *Gigartina skottsbergii* showed less DNA damage in diploid carpospores compared to haploid tetraspores (Roleda et al. 2008a). Haploid cells are reported to be efficient replicators, while diploid cells are resistant to damage (Long and Michod 1995). The higher tolerance of carpospores to UVR confirms the genetic buffering hypothesis, which proposes that diploids benefit from better cellular regulation and are, therefore, more vigorous and tolerant to radiation stress (Raper and Flexer 1970). During the diploid state DNA damage can be repaired, since there are two copies of this biomolecule in each cell and one copy can be presumed to be undamaged.

Repair of UV-induced DNA photoproducts can take place by various mechanisms that include photoreactivation (light-mediated repair) or nucleotide and base excision repair (Britt 1996). The light-induced DNA repair is based on the activity of the key enzyme photolyase, which binds to complementary DNA strands and efficiently breaks pyrimidine dimers. However, this enzyme only functions as a DNA repair mechanism when blue light is available for activation. Significant removal of CPDs indicating repair of DNA damage was observed in Arctic seaweeds investigated after recovery in low white light. DNA damage repair could either be mediated by light-dependent photolyases or light-independent nucleotide excision repair (Pakker et al. 2000, van de Poll et al. 2002a).

In the planktonic Antarctic diatom *Chaetoceros dichaeta* no CPD accumulation was detected in high light-acclimated cells (Buma et al. 2006). For polar benthic microalgae, only one study has addressed UV effects on DNA damage (Wulff et al. 2008a). In this study, only minimal CPD accumulation was observed under unnaturally high intensities of UVB, and the effect was completely reversed when allowed to recover under photoreactivating radiation (Wulff et al 2008a).

Besides UV-induced DNA photoproducts, damage to protein molecules represents the second major direct effect of UVB in algae. Typical target proteins are those associated with the plasmalemma or involved in photosynthesis such as the D1 protein of photosystem II and the enzyme Rubisco in the Calvin cycle (Bischof et al. 2000). The native three dimensional structure of proteins is a prerequisite for any specific function, and hence any structural damage can be reflected in loss of cell vitality because these molecules have multiple roles as enzymes, hormones and structural components. However, since proteins typically occur in

numerous copies inside the algal cell, any UV-induced damage is less severe compared with DNA-damage. On the other hand, degradation and replacement of damaged protein molecules require energy, which might otherwise support more essential processes such as DNA repair. The replacement of any UV-damaged protein by de novo biosynthesis contributes to counteract UV damage. The molecular mechanisms are well studied in cyanobacteria, and might be similar in polar micro- and macroalgae. Exposure to moderate doses of UVB resulted in an increased turnover rate of the D1 and D2 reaction centre subunits of photosystem II, thus rapidly replacing damaged proteins by newly synthesised polypeptides (Campbell et al. 1998; Máté et al. 1998). A key step in this repair process is the UVB induced differential transcription of reaction centre protein encoding genes.

Other biomolecules such as photosynthetic pigments can also be destroyed under UVR with the phycobilins (main pigments of Rhodophyta) being the most sensitive, and carotenoids generally being less affected than chlorophylls. In contrast, lipids, which are major compounds of all biological membranes, cannot absorb UVR, but can be easily peroxidized through UV-induced reactive oxygen species (see below) (Bischof et al. 2006).

Induction of reactive oxygen species (ROS) and antioxidative strategies

Polar micro- and macroalgae perform oxygenic photosynthesis using water as an electron donor thereby releasing molecular oxygen, which can be accumulated and chemically easily converted to potentially damaging reactive oxygen species (ROS). Photooxidative stress, including UVB, stimulates various cellular processes leading to the production of superoxide radicals (O_2^-), as well as singlet-oxygen (1O_2) and hydroxyl radicals (OH^\cdot). The sources and production sites of ROS are mainly related to photosynthetic activities such as the pseudocyclic photophosphorylation and the Mehler reaction, which stimulate the accumulation of hydrogen peroxide (Asada 1994; Elstner 1990).

Besides these internal processes, formation of ROS may also take place in the environment by UV-induced photoactivation of dissolved organic matter, photochemical degradation, and liberation of excited electrons that initiate the reduction of molecular oxygen resulting in superoxide anion radicals (Cooper and Zika 1983). A second reduction step of the superoxide radical followed by protonation yields hydrogen peroxide, which is a powerful oxidant because of its relatively long half-life that allows long distance diffusion (Asada 1994). There are indications that UVB in Antarctic summer can stimulate the input of hydrogen peroxide from photochemical reactions and atmospheric wet deposition into shallow waters increasing its concentration from typical 20-300 nM up to 5.000 nM (Abele et al. 1999).

ROS can cause extensive damage to DNA, proteins, and other biological molecules and structures (Bischof et al. 2006). Polar micro- and macroalgae have evolved a complex defence system against ROS including non-enzymatic antioxidants like ascorbate (vitamine C), tocopherol (vitamine E), carotenoids and reduced glutathione. However, these low-molecular weight antioxidants are chemically consumed and hence not considered the most efficient detoxifying agents. In contrast, antioxidant enzymes can efficiently counteract all UV-induced ROS, and include superoxide dismutase (SOD), catalase, glutathione peroxidase and the enzymes involved in ascorbate-glutathione cycle like ascorbate peroxidase (APX), mono-dehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Schriek 2000, Aguilera et al. 2002b, Dummermuth et al. 2003). While Antarctic diatoms exhibited high enzymatic antioxidant activities even at low temperatures due to low activation energies (Schriek 2000), Arctic macroalgae are capable to up-regulate some of their antioxidative enzymes under UVR, thereby allowing fast acclimation to changes in environmental radiation conditions (Aguilera et al. 2002b). In addition, some polar seaweeds may even synthesize and accumulate specific antioxidants such as bromophenolic compounds (Dummermuth et al. 2003).

Physiological processes and acclimation

The effects of UVB exposure on biological systems are manifold, and any damage or disturbance of biomolecules will strongly affect physiological processes such as photosynthesis, growth and reproduction.

Photosynthesis

Photosynthesis is probably the most intensively studied process under UVR stress in polar algae. Due to its biochemical complexity, numerous sites can be affected by this waveband. These include, for example, inhibition of energy transfer within the PS II reaction centre, the water splitting complex, or the light-harvesting complex. In addition, key enzymes of the Calvin-Cycle such as Rubisco are typical molecular targets. The response of polar micro- and macroalgae towards UVB exposure is determined by the interplay of genetically fixed adaptation and physiological acclimation (Bischof et al 2006). Moreover, sensitivity of photosynthetic and accessory pigments to UVR is species specific and dose dependent. Pigment damage can result either (1) when protein-based pigments absorb UV energy directly and undergo photochemical degradation; (2) by photosensitizer action; or (3) oxygen radical production in addition to singlet oxygen (Vincent and Neale 2000). Accessory pigments such

as carotenoids are involved in several aspects of photosynthesis such as light absorption and energy transfer to the reaction center complex but also in protection of the photosynthetic apparatus from damage by strong illumination.

Many micro- and macroalgae, particularly from the intertidal zone, can cope with enhanced radiation conditions in summer including UVB because of their ability for dynamic photoinhibition, which represents a photoprotective mechanism by which excessive energy absorbed is dissipated harmless as heat (Krause and Weis 1991). Dynamic photoinhibition is characterized by a pronounced decrease in photosynthetic activity during high radiation stress especially at noon, followed by a fast and full recovery during subsequent exposure to low radiation conditions, for example, at late afternoon as documented for Arctic seaweeds in the field (Hanelt et al. 1997). Macroalgae from the intertidal and supralittoral zone such as the Chlorophyta *Urospora penicilliformis* and *Prasiola crispa*, and the Phaeophyceae *Fucus distichus* are only mildly affected by UVR indicating a broad photophysiological tolerance (Hanelt et al. 1997, Holzinger et al. 2006, Roleda et al. 2008c). Nevertheless, in most polar seaweed species studied so far UVB contributes significantly to some degree of photoinhibition of photosynthesis; while recovery in photosynthetic efficiency, regardless of life history phase, is always lower in deep water species compared to species from shallow waters. However, the underlying photoprotective mechanisms involved, whether physical or biochemical, remain to be elucidated. At least in higher plants dynamic photoinhibition may also be regulated by an increase in the zeaxanthin content of the PSII antenna (Adams and Demmig-Adams 1992) and/or by increasing the amount of inactive PSII centres which dissipate a surplus of absorbed energy as heat to protect the photosynthetically active centres (Öquist and Chow 1992). Whether these mechanisms are also acting in polar micro- and macroalgae is still an open question. In contrast to intertidal seaweeds, all deep water and understory algae from the sublittoral recover only partly and slowly or even bleach, indicating chronic photoinhibition (Karsten et al. 2001). While dynamic photoinhibition is a reversible physiological mechanism, chronic photoinhibition is reflected by photodamage of components of the photosynthetic apparatus such as the D1 protein (Bischof et al. 2006) and requires de novo synthesis of proteins.

The sensitivity of photosynthesis in reproductive cells of Antarctic macroalgae to PAR and UVR is related to the observed vertical zonation pattern of the adult plants (Roleda et al. 2008b, Zacher et al. 2007a). This response was also reported in the early life stages of Arctic macroalgae (Roleda et al. 2006d). It seems that low light adaptation of photosynthesis is a typical feature of reproductive cells of macroalgae (Roleda et al. 2006a, 2006d, Wiencke et al.

2007, Zacher et al. 2007a), which can be explained by differences in chlorophyll antenna size and number of chloroplasts present in reproductive cells compared to multicellular macroscopic stages. *In situ* solar radiation can, therefore, exert a significant effect on survival of spores in the field (Wiencke et al. 2006). Consequently, survival of propagules will be dependent on their immediate settlement on substrate at depths in between rock crevices, under boulders or under algal canopies where the prevailing low-light microenvironment is suitable for their germination.

The few studies of UVR effects on photosynthesis in benthic diatoms from polar waters indicate only temporal, small photoinhibition facilitated by dynamic recovery and efficient DNA damage repair (Wulff et al. 2008a,b). In benthic microalgae, photosynthetic performance measured as maximum quantum yield of PSII (F_v/F_m) seems to be affected by UVA and UVB but observed effects are mostly transient and disappear after a couple of days treatment (Wulff et al 2008a,b,c). In a repeated time series measurement of photosynthetic response to UVR, Waring et al. (2006) reported a reduction in UVB-induced photoinhibition with increasing UVB exposure period. These authors speculated about a possible recovery process or an acclimation to UVB taking place within the exposure. The diatom species in this study were not of polar origin but at least 2 of the 4 species tested have been found in Antarctic sediments (Wulff et al 2008b, Al-Handal and Wulff 2008). For the planktonic polar diatom, *Thalassiosira antarctica*, a 2h recovery period was required to regain photosynthetic capacity comparable to the initial value (van de Poll et al. 2006). The ability to cope with UVR after a period of darkness was tested in an Antarctic benthic diatom community where the diatoms were kept in total darkness for 15 and 64 days, respectively, and then exposed for relatively high intensities of UVR (Wulff et al 2008c). The diatom community was dominated by the large species *Gyrosigma fasciola* and *Pleurosigma obscurum* and these cells were able to resume photosynthetic activity after 64 days in darkness and coped with relatively high intensities of UVR compared with their natural habitat. However, although the cells surviving the darkness could deal with the applied UVR, each individual cell in the population did not survive the long dark period.

The prevailing environmental factors in different habitats along the vertical gradient of the shore are important in conditioning the photophysiological optimum and conferring fitness for the organism's survival. Acclimation of photosynthesis to UVR has been documented in sporophytes of Arctic Laminariales collected at different depths (Bischof et al. 1998, 1999). A common response observed in brown algal species during acclimation to UVR 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, or by the formation of UV-screening compounds.

The common consequences of UVB on photosynthetic function are decreased or even fully inhibited CO₂-fixation, and hence a decline in primary and biomass production (Franklin and Forster 1997, Bischof et al. 2006).

Growth

Since the photosynthetic process in many species of polar micro- and macroalgae can acclimate to variable radiation stress, long-term effects of increasing UVR should measure growth and reproduction rather than photosynthesis alone. Growth and reproduction typically characterise the ecological success of individual algal species under the given environmental conditions. In contrast to all other cellular processes, such as photosynthesis, respiration, anabolic pathways etc., growth well integrates the impact of all positive and negative abiotic factors and, thus, represents the most important physiological key parameter to describe the performance of algae in their polar ecosystem.

A simple growth model: $G = P - R - L$, proposed by Carr et al. (1997), follows a growth differentiation balance, where growth increment over time (G) is a function of the rate of biomass production through gross photosynthesis (P) and loss due to respiration (R) and tissue loss or decay (L). Under radiation stress (high PAR + UVR), photoinhibition of photosynthesis already decreases potential carbon acquisition (P) into plant dry matter (G) (Long et al. 1994). Dark respiration (R) represents the energy used to synthesize new biomass (growth respiration) and to maintain metabolic activity (maintenance respiration). Exposure to UVR causes molecular and cellular damage which could further increase loss due to respiration (R) by diverting more photosynthates for repair and/or protection (e.g. production of UV-sunscreen compounds), all of which results in decreasing growth.

Similar to the photosynthetic performance also growth sensitivity of polar seaweeds showed a strong correlation to the vertical distribution (Aguilera et al. 1999, Michler et al. 2002, Roleda et al. 2007a). Moreover, growth rates of juvenile life stages of macroalgae were more susceptible to UVR exposure compared to adult plants, which is well documented for young kelp sporophytes (Roleda et al. 2005, 2006b). Since many growth studies on seaweeds were performed for only short periods on intact young plants or on discs excised from adult macroalgal thalli UV-induced morphological deformation and tissue damage can be easily overlooked (Aguilera et al. 1999). However, pronounced tissue necrosis and loss of parts of the thalli was reported in the UV-sensitive Arctic *Laminaria solidungula* after 1 week of daily

exposure to 18 h UVR (Michler et al. 2002), indicating that such changes have a strong effect on growth performance.

In contrast to polar seaweeds and planktonic microalgae, studies of UVR effects on growth of benthic diatoms from Antarctic habitats are rare (Wulff et al. 2008 a,b) and for the Arctic region even virtually lacking. On the Antarctic Peninsula, Zacher et al (2007c) studied UVA and UVB effects in a 15 weeks field experiment on an intertidal hard bottom platform. The growth of the microalgal assemblage dominated by the diatom genera *Cocconeis* and *Navicula* was unaffected both by UVA and UVB radiation. In a similar subtidal experiment, Campana et al (2008) reached the same conclusion, the subtidal community being dominated by the diatom species *Fragilaria striatula*, *Navicula cf perminuta* and *Navicula cf hanseni*. Also in laboratory studies, growth of Antarctic benthic diatoms seems to be unaffected by UVR. There are basically two strategies for these microalgae to respond to enhanced UVR: adaptation or avoidance. The first process includes repair and protection mechanisms, and as recently shown for Antarctic benthic diatoms they exhibited only minimal DNA damage under UVB treatment and probably a high and efficient repair capacity (Wulff et al. 2008a). The second process includes migration and vertical movement (see below). In addition, since growth was always unaffected, these authors concluded that UVR does not seem to be a threat to benthic marine Antarctic diatoms.

Photoprotective mechanisms

Avoidance

In contrast to sessile seaweeds benthic diatoms can physically move away from harmful UVB which represents one of the most effective avoidance strategies (Wulff et al. 2008a). However, it always requires an ability to detect this waveband. Furthermore, all algae have a dilemma with regard to solar radiation. While sufficient PAR is an essential prerequisite for photosynthesis, too much UVR can be harmful. Migration and vertical movement in sediments in combination with self-shading definitely appears to be an efficient way for motile diatoms to receive the optimum light requirements and to avoid long-term exposure to high UVR thereby saving energy for photoprotective acclimation.

In contrast to motile benthic diatoms, most macroalgae have to cope with the prevailing radiation conditions. If these plants grow deep in the water column they are never exposed to UVR (Karsten et al. 2001). In contrast, in the intertidal zone (eulittoral) or in sheltered coastal lagoons, macroalgae have to cope with high radiation. Here, green algae of the annual genera *Ulva*, *Acrosiphonia* or *Urospora* often form mat- or turf-like communities, which exhibit

steep gradients of UVB, but also of physiological responses. While top layers are exposed to high surface irradiance and hence often bleach, bottom layers permanently experience very low radiation conditions or even remain in darkness (Bischof et al. 2002b, 2006). Another strategy for small macroalgae to avoid high radiation in more shallow water is to grow under the protecting canopy of larger macroalgal taxa such as kelps. Indeed, recruitment of juvenile kelps under the canopy of adult plants is considered to be an adaptive behaviour, which effectively protects these early life stages from radiation stress, therefore minimizing ecological cost for protection and hence enabling allocation of more photosynthate for growth (Herms and Mattson 1992).

Another mechanism that may be involved in UVR protection and acclimation is the establishment of a physical barrier which shields the photosynthetic apparatus against damaging radiation (Karentz 1994). For example, increasing thallus thickness was observed to minimize UV-B-induced DNA damage as outer cell layers can shade inner cells and present a longer pathlength for UVR absorption (Franklin and Forster 1997, Roleda et al. 2007a). Thallus translucence or opacity can also influence reflection, attenuation, scattering, absorption or transmittance of UV radiation (Caldwell et al. 1983). Among kelps, optically dark pigmented juvenile sporophytes show strong absorbance in the UV waveband (Roleda et al. 2005, 2006b), characteristic of the UV-absorbing phlorotannins accumulated within the outer cortical layer of the thalli of Laminariales (Lüder and Clayton 2004, Shibata et al. 2004). In *Homosira banksii* (Fucales), a brown layer consisting of oxidized phenolic compounds released from the peripheral cells forms a protective lamina for the photosynthetic tissue beneath (Schoenwaelder 2002). UVR can, therefore, be attenuated by cellular UV-absorbing compounds and cell walls of the epidermal tissue effectively reducing UV fluence before reaching important physiological targets. UV-absorbing compounds located in the epidermis are reported to protect Arctic vascular plants from UV-B radiation (Nybakken et al. 2004). Preliminary studies on different Arctic turf algae showed similar UV-protective mechanism on the cell walls of *Urospora penicilliformis* (Bilger W. personal communication, Bilger and Roleda, unpublished data).

Photoprotective substances

One of the most important physiochemical acclimation mechanisms against UVR represents the biosynthesis and accumulation of UV-sunscreen 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 many polar micro- and 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 Rhodophyta (Karentz et al. 1991; Hoyer et al. 2001), Phaeophyceae and most Chlorophyta typically lack these compounds, except the green alga genus *Prasiola* which contains high concentrations of a unique MAA with an absorption maximum at 324 nm (Hoyer et al. 2001, Karsten et al. 2005). Most Phaeophyceae synthesize photoprotective phlorotannins under UV exposure (Pavia et al. 1997), these compounds will be reviewed in detail below. While planktonic centric diatoms from Antarctica also accumulate MAAs under UVR (Buma et al. 2006), their benthic pennate pendants are surprisingly poor in these compounds or even lack them at all (Wulff et al. 2008a).

Field and laboratory experiments in the Arctic with the intertidal red alga *Devaleraea ramentacea* showed a continuous decrease in photosynthetic performance under UVR with increasing collecting depths between 1 and 5 m. The total MAA concentration was also correlated with the original sampling depth, i.e. shallow water isolates contained much higher amounts than algae from deeper waters, also indicating a strong correlation between the MAA contents and the degree of sensitivity of photosynthetic activity in *D. ramentacea* (Karsten et al. 1999). The sunscreen function of MAAs has been further inferred in other polar red algae from a decrease in concentration with increasing depth (Hoyer et al. 2001, 2003). Supra- and eulittoral Rhodophyta typically experience the strongest insolation, and consequently synthesise and accumulate highest MAA values, which are positively correlated with the natural UV doses (Karsten et al. 1998). In contrast, many red algal taxa growing in deep waters are biochemically not capable of producing MAAs (Hoyer et al. 2001, 2002; Karsten et al. 2001), which explains their strong sensitivity to high ambient solar radiation. These species do not experience UV exposure in nature, and, hence, there is no physiological need to synthesise and accumulate metabolically expensive nitrogen-containing MAAs. This in turn saves energy to better support other essential pathways such as the biosynthesis of light-harvesting phycobilisomes to guarantee sufficient PAR absorption under the prevailing low-light conditions.

In the Antarctic red alga *P. decipiens*, juveniles collected in the upper to mid sublittoral during winter contained low MAA concentrations while mature plants collected in late spring and summer exhibited significantly higher values, indicating strong seasonal effects, which are related to the changing daylengths and radiation conditions (Post and Larkum 1993). Based on the MAA concentrations and the induction patterns after exposure to different defined radiation conditions red algae can be physiologically classified in three 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 of the lower sublittoral, *Type II* and *III* species grow from the supra- and eulittoral to the upper and mid sublittoral zone. Experiments under defined radiation conditions proved that the induction, biosynthesis and accumulation of MAAs are very flexible and species-specific processes. While some polar Rhodophyta synthesize MAAs particularly under UVB, others prefer UVA or higher PAR only (Hoyer et al. 2003). Although experimental evidence for a particular trigger mechanism is still missing, it is reasonable to assume that a signal transduction pathway must be involved in the overall process leading to high MAA concentrations. Based on the different types of MAA induction patterns in polar red algae the presence of various photoreceptors, most probably between the blue light and UVB range, must be considered (Kräbs et al. 2002).

The entire algal thallus does not respond uniformly to ambient UVR. Young apical or marginal zones, i.e. growing cells, synthesise and preferentially accumulate MAAs leading to cross sectional and longitudinal concentration gradients (Hoyer et al. 2001). Populations of *D. ramentacea* collected from very shallow water typically exhibit green apices and red basal parts. The more exposed green tips contain 5-fold higher MAA contents than the red bases (Karsten et al. 1999). Older tissue regions usually exhibit thicker cell walls and a leathery texture, and are therefore optically opaque. The higher MAA concentrations in the most exposed thallus regions are essential to guarantee protection of the more delicate meristematic cells.

From an ecological standpoint, the ephemeral, tufty *Prasiola* is intriguing due to its capability to grow in polar regions subaerially on soil and rocks underneath or near seagull or penguin colonies (Holzinger et al. 2006), i.e. habitats rich in nitrogen containing faeces. Considering a relation between the MAA concentration and nitrogen availability in different species of the red alga *Porphyra* (Korbee et al. 2005), it seems that this nutrient might be a critical factor for the broad photophysiological tolerance of *Prasiola* under subaerial conditions (Holzinger et al. 2006). Seasonal studies on *Prasiola* in Antarctica indicate some variation in MAA

concentrations, but always with high minimum steady-state amounts (Jackson and Seppelt 1997). Consequently, it appears that the 324-nm MAA in *Prasiola* is expressed constitutively. As well as functioning as natural UV-protective compounds, some MAAs such as mycosporine-glycine also have moderate antioxidant activity (Dunlap and Yamamoto 1995). In addition, the presumed biochemical precursor of MAAs, 4-deoxygadusol exhibits strong antioxidant activity (Dunlap et al. 1998). The photo-physicochemical properties of MAAs guarantee both a high UV-protective effectiveness in combination with antioxidant capabilities.

Phlorotannins are exclusively produced by brown algae and include hundreds of phenolic compounds (126–650 kDa) composed of the monomer phloroglucinol (Amsler and Fairhead 2005). The primary biological function of phlorotannins is the strengthening of cell walls and one of several suggested secondary functions is the protection of algae against harmful UV-B radiation (Ragan and Glombitza 1986, Swanson and Druehl 2002). This role is based on the absorbance of phlorotannins in the UV region with peaks at approximately 195 nm and 270 nm, whereas the absorbance shoulders extend from < 200 nm to 400 nm (Ragan and Glombitza 1986, Pavia et al. 1997).

Experimental evidence documented that intra- and/or extracellular phlorotannins within very dense kelp spore suspensions used as biofilters shielded underlying kelp spores from UVB thereby allowing germination (Roleda et al. 2006c, Swanson and Druehl 2002). Such UVB protection of kelp spores by exudated phlorotannins may also occur in dense kelp beds (Swanson and Druehl 2002), although the sunscreen capability of phlorotannins diminishes with decreasing concentrations (Roleda et al. 2006c). Thus, different UVR responses of spore germination of the Arctic *A. esculenta* and *L. digitata* at different dates within 2 weeks (Wiencke et al. 2006) are probably attributed to changing phlorotannin contents in the water column.

Whether UVR exposure can induce the formation and accumulation of phlorotannins in Phaeophyceae is still an open question because of contradictory results in the literature. Light-microscopic analysis of zoospores of the Arctic kelps *A. esculenta*, *S. dermatodea* and *L. digitata* indicated that UVB treatment led to an enlargement of phlorotannin-containing physodes, an aggregation of several small physodes to bigger ones as well as to an exudation of physodes into the surrounding seawater (Wiencke et al. 2004, 2007; Roleda et al. 2006c). These observations were, however, not confirmed by an ultrastructural analysis of UVB exposed zoospores of the UVR sensitive, cold-temperate *L. hyperborea* (Steinhoff et al. 2008).

Moreover, zoospore suspensions of *A. esculenta*, *L. digitata* and *S. latissima*, juveniles and embryos of *Fucus gardneri*, and sporophytes of *Desmarestia anceps* and *D. menziesii* did not show any concentration increase after UVB treatment (Henry and Alstynne 2004, Fairhead et al. 2006, Müller et al. 2008a). An accumulation of phlorotannin contents after treatment with UVB was also not detected in the seawater surrounding blades of *Macrocystis integrifolia* and zoospores of *A. esculenta*, *L. digitata* and *S. latissima* (Swanson and Druehl 2002, Müller et al. 2008a). Otherwise, an induction of phlorotannins by UVR was noticed in the blades of the giant kelp *M. integrifolia* (Swanson and Druehl 2002) and in sporophytic tissues of *Ascophyllum nodosum*, *S. latissima* and *Nereocystis luetkeana* (Pavia et al. 1997, Pavia and Brock 2000, Swanson and Fox 2007).

Generally, the often observed discrepancies between studies on UVR-sunscreen function of phlorotannins may be attributed to low replicate numbers, different exposure times and irradiation intensities, analytical methods and/or selected species/life stages studied, although ontogenetic, intra- and interspecific phlorotannin levels are known to be highly variable (Amsler and Fairhead 2005). Particularly the standard measurement of hundreds of compounds pooled as phlorotannins could lead to false negative or positive results, whereas the analysis of single phlorotannin compounds after UVB treatment might help to better understand the UV protective role of phlorotannins. Therefore, future studies should pay more attention to the simultaneous analysis of UVR- responses of different algal species in the field, of their intra- and extracellular phlorotannin levels and compositions, and of their absorption properties to substantiate the role of phlorotannins as UV protectants in brown algae from polar environments.

Interactive effects of UVR with other abiotic and biotic factors

Under natural conditions, plants are subject to the influence of a multitude of abiotic and biotic factors (Alexieva et al. 2003). Stress factors do not usually operate individually or independently, so that spatial or temporal variations and interactions between and co-variation of stresses are the norm in the natural environment (Vinebrooke et al. 2004; Jones et al. 1993). Stressors act synergistically when their combined effect on biological components is larger than the responses to each single stressor and antagonistically when the impact is smaller (Folt et al. 1999). It is also possible that plant organisms exposed to a single stress agent are capable of increasing their resistance to subsequent unfavourable impacts (cross-adaptation, Alexieva et al. 2003).

In the polar regions, several environmental factors already changed and are still changing in parallel, such as the ozone depletion and the concomitant rise in UVB, as well as the increase in greenhouse gases (e.g. CO₂) and the resulting global warming and acidification of the oceans. Consequently, any rise in UVB is not exclusively affecting marine benthic micro- and macroalgae since changes in the other environmental factors have to be considered as well (Zacher et al. this issue). Already in 1994, Bothwell and colleagues found that benthic diatoms benefit from UVR since the grazers were more susceptible to the applied radiation (Bothwell et al. 1994). Also in the field grazers had a larger impact than UVR although no negative effect of UVR on the grazers was apparent (Zacher et al. 2007c).

Hoffman et al. (2003) supported the hypothesis that temperature mitigates the net biological effect of UVR on macroalgae and *vice versa*. Correspondingly, the photosynthetic performance of sporophytes of the Antarctic green alga *Ulva bulbosa*, the Subantarctic *U. clathrata*, the Arctic kelp *A. esculenta*, and the Arctic red algae *P. palmata*, *Coccotylus truncatus* and *Phycodrys rubens* was less impaired by UVB at higher temperatures (van de Poll et al. 2002b; Rautenberger and Bischof 2006; Fredersdorf et al. 2008). In the latter three red algae, the recovery of photosynthesis after UVB stress was likewise improved at higher temperatures, although the influence of UVB was predominant regarding their relative growth rate and CPD accumulation as marker of DNA damage (van de Poll et al. 2002b). In contrast, the germination of zoospores and photosynthetic performance of sporophytes of the UVB tolerant *A. esculenta* were mainly determined by temperature if exposed to both, UVR and temperature (Fredersdorf et al. 2008; Müller et al. 2008b). Otherwise, the germination of highly UVB vulnerable zoospores of the Arctic kelps *S. latissima* and *L. digitata* were more UVB affected at unfavourable lower and higher temperatures rather than at the ambient temperature 7°C (Müller et al. 2008b). The germination of zoospores of the Helgolandic ecotype of *L. digitata* was, however, less harmed by UVB at temperatures higher than 2°C (Müller et al. 2008b). By contrast, the sensitivity of germlings of three *Fucus* species from Helgoland to UVB radiation was enhanced with increasing temperature (Altamiro et al. 2003). Thus, the temperature dependence of UVR effects on seaweeds varies highly within and among algal species and genera.

There are only few ecophysiological studies on interactive effects of UVR and/or temperature with salinity in polar seaweeds although significant amounts of freshwater regularly flow into polar waters due to rivers and melting of glaciers and snow (Karsten et al. 2003; Fredersdorf et al. 2008). These studies reveal likewise life stage-, intra- and interspecies-specific responses to interactive stress. In the study of Karsten et al. (2003) interactive effects of UVR

and salinity on two shallow water Arctic red macroalgae, *D. ramentacea* and *P. palmata*, were investigated. While *D. ramentacea* exhibited euryhaline features and acclimated well to the UVR applied, *P. palmata* can be characterised as stenohaline plant because of its high mortality already under mild hyposaline conditions. In addition, the latter species showed a limited ability to acclimate to changing PAR/UV radiation pointing to a relatively low physiological plasticity.

Within the life history of the Arctic kelp *A. esculenta*, sporophytes revealed a significant main temperature and only small salinity effect but no interacting effects of both stressors (Fredersdorf et al. 2008). In contrast, the germination of zoospores of *A. esculenta* was strongly affected by an interactive and synergistic effect of low salinity at the limiting temperature of 16°C, but no interaction of salinity or temperature with UVR could be identified (Fredersdorf et al. 2008).

At the community level, species-specific UVB responses of algae and distinct food preferences of grazers result in complex changes (Zacher et al. 2007b, Zacher and Campana 2008). For instance, the preference of snails to feed on UVB sensitive green algal recruits favoured the upcoming of leathery red algal recruits in the Antarctic intertidal phytobenthos (Zacher et al. 2007b). Although the UVR effects on the diversity and species composition of macroalgal assemblages were less pronounced, the biomass and recruit density of the community was in long-term more substantially affected by grazing (Zacher et al. 2007b; Zacher and Campana 2008). The effects of UVB and temperature on the recruitment and succession of the green alga *Ulva intestinalis* from Nova Scotia (Canada) were likewise mitigated by consumers, while nutrients supplementary enhanced the grazing pressure (Lotze et al. 2002). High levels of nutrients also abated detrimental UVB effects on a diatom-dominated, soft bottom community at the Swedish west coast (Wulff et al. 2000). In Antarctic waters which are characterised by generally high nutrient levels (Korb and Gerard 2000), a diatom-dominated, hard bottom community was almost unaffected by UVB, although grazing structured the community almost entirely (Zacher et al. 2007c). These few studies on interactive effects on micro- and macrophytobenthos of polar latitudes indicate that grazing pressure may have a generally stronger impact on the community than UVB, whereas nutrients may influence the impact of grazing on algae. Comparable observations were made in the Arctic microbial food web, where negative UVB effects on the growth of nano- and picocautotrophs as well as heterotrophic nanoflagellates were much less substantial than the mortality imposed by grazing (Wickham and Carstens 1998).

Nevertheless, there are numerous fundamental and still unanswered questions about the

tolerance of benthic micro- and macroalgae to combined stress, especially at the respective ratio of PAR:UVR. One unsolved problem is related to the comparability of experiments undertaken in the field and in the laboratory because natural solar radiation (waveband ratios, intensities) is very difficult to artificially mimic. Consequently, PAR:UVR ratios in both approaches can be quite different and hence in the laboratory often so unnatural, that the resulting data have to be taken with caution. Clearly, much more ecological relevant studies on interactive effects, especially on the most sensitive developmental stages and on the algal communities are required for predictions of the effects of global change on polar marine ecosystems.

Ecological consequences

To conclude what ecological effects could be the result from increased UVR is a difficult task. Most likely, effects on the community or even ecosystem level have already taken place before the official discovery of the ozone hole over Antarctica in the 70's. While the few existing studies on benthic microalgae point to a capacity to adapt to ambient and increased UVR, more studies are needed to identify the underlying mechanisms and, furthermore, to include several trophic levels under natural conditions. For macroalgae, colonization stages and spores seem to be more sensitive to UVR compared to adult macroalgae. Consequently, in polar environments influenced by ice and scouring (frequent colonization events), UVR could be an important controlling factor. In addition, a changed zonation pattern has been suggested (e.g. Bischof et al. 2006) with possible cascade effects on the community level, including epiphytes and grazers. Although the number of field studies has increased over the last years, still very few, if any, take the ecosystem approach. For future studies, this is clearly one of the greatest challenges to elucidate UV effects on polar benthic primary producers.

Conclusions

Although considerable progress has been achieved in recent years on UVR effects, our present knowledge about the physiological capabilities of benthic micro- and macroalgae from polar regions is still fragmentary. Due to the extreme remoteness of the polar regions and the infrequency of scientific studies many open questions have still to be addressed. Particularly, more experimental evidence is needed from both field and controlled laboratory studies to precisely document susceptibility, tolerances, acclimation and adaptation with an emphasis on the underlying mechanisms which can only be elucidated by modern genomic, proteomic and metabolomic approaches. In addition and as already mentioned, more

ecosystem approaches and studies on interactive effects are needed to draw a more holistic picture.

Acknowledgements

The authors like to thank the German Research Foundation (DFG), the Bremen International Graduate School for Marine Sciences (GLOMAR) and the Helmholtz Association for financial support through several projects over the last decade as well as the Ny-Ålesund International Research and Monitoring Facility for their support and the AWI diving team for providing all types of field samples.

References

- Abele, D, G.A. Ferreira and I. Schloss. 1999. H₂O₂ accumulation from photochemical production and atmospheric wet deposition in Antarctic coastal and off-shore waters of Potter Cove, King George Island. *Antarc. Sci.* 11: 131–139.
- Adams III, W.W., and B. Demmig-Adams. 1992. Operation of xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. *Planta* 186: 390-398.
- Aguilera, J., U. Karsten, H. Lippert, B. Vögele, E. Philipp, D. Hanelt and C. Wiencke. 1999. Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Mar. Ecol. Prog. Ser.* 191: 109–119.
- Aguilera, J., K. Bischof, U. Karsten, D. Hanelt and C. Wiencke. 2002a. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Mar. Biol.* 140: 1087-1095.
- Aguilera, J., A. Dummermuth, U. Karsten, R. Schriek and C. Wiencke. 2002b. Enzymatic defenses against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. *Polar Biol.* 25: 432–441.
- Alexieva, V., S. Ivanov, I. Sergiev and E. Karanov. 2003. Interaction between stresses. *Bulg. J. Plant Physiol.* Special Issue: Proceedings of the European Workshop ESSA: 1–17.
- Al-Handal, A. and A. Wulff. 2008. Marine benthic diatoms from Potter Cove, King George Island, Antarctica. *Bot. Mar.* 51: 51-68.
- Altamirano, M., A. Flores-Moya and F.L. Figueroa. 2003. Effects of UV radiation and temperature on growth of germlings of three species of *Fucus* (Phaeophyceae). *Aquat. Bot.* 75: 9–20.
- Amsler, C.D. and V.A. Fairhead. 2005. Defensive and sensory chemical ecology of brown algae. *Adv. Bot. Res.* 43: 1-91.
- Asada, K. 1994. Production and action of active oxygen species in photosynthetic tissues. In: (C.H. Foyer and P.M. Mullineaux, eds) *Causes of photooxidative stress and amelioration of defence systems in plants*. CRC Press, Boca Raton. pp. 77–104.

- Bandaranayake, W.M. 1998. Mycosporines: are they nature's sunscreens? *Nat. Prod. Rep.* 15: 159-172.
- Bischof, K., D. Hanelt, H. Tüg, U. Karsten, P.E.M. Brouwer and C. Wiencke. 1998. Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol.* 20: 388-395.
- Bischof, K., D. Hanelt and C. Wiencke. 1999. Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biol.* 1: 435-444.
- Bischof, K., D. Hanelt and C. Wiencke. 2000. UV-effects on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* 211: 555-562.
- Bischof, K., D. Hanelt, J. Aguilera, U. Karsten, B. Vögele, T. Sawall and C. Wiencke. 2002a. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Mar. Biol.* 140: 1097-1106.
- Bischof, K., G. Kräbs, C. Wiencke and D. Hanelt. 2002b. Solar ultraviolet radiation affects the activity of ribulose-1,5-biphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* 215: 502-509.
- Bischof, K., I. Gomez, M. Molis, D. Hanelt, U. Karsten, U. Lüder, M.Y. Roleda, K. Zacher and C. Wiencke. 2006. Ultraviolet radiation shapes seaweed communities. *Rev. Environ. Sci. Biotechn.* 5: 141-166.
- Bothwell, M.L., D.M.J. Sherbot and C.M. Pollock. 1994. Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. *Science* 265: 97-100.
- Britt, A.B. 1996. DNA damage and repair in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 75-100.
- Buma, A.G.J., H.J. Zemmeling, K. Sjollema and W.W.C. Gieskes. 1996. UVB radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms. *Mar. Ecol. Prog. Ser.* 142: 47-54.
- Buma, A.G.J., T. van Oijen, W. van de Poll, M.J.W. Veldhuis and W.W.C. Gieskes. 2000. The sensitivity of *Emiliana huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. *J. Phycol.* 36: 296-303.
- Buma, A.G.J., S.W. wright, R. van den Enden, W.H. van den Poll and A.T. Davidson. 2006. PAR acclimation and UVBR-induced DNA damage in Antarctic marine microalgae. *Mar. Ecol. Prog. Ser.* 315: 33-42.
- Cahoon, L.B. 1999. The role of benthic microalgae in neritic ecosystems. *Oceanogr. Mar. Biol. Annu. Rev.* 37: 47-86.
- Caldwell, M.M., R. Robberecht and S.D. Flint. 1983. Internal filters: Prospects for UV-acclimation in higher plants. *Physiol. Plant.* 58: 445-450.

- Campana, G., M.L. Quartino, A. Yousif and A. Wulff. 2008. Impacts of UV radiation and grazing on the structure of a subtidal benthic diatom assemblage in Antarctica. *Ber. Polarforsch. Meeresforsch.* 571: 302-310.
- Campbell, D., M.J. Eriksson, G. Öquist, P. Gustafsson and A.K. Clarke. 1998. The cyanobacterium *Synechococcus* resists UV-B by exchanging photosystem II reaction-center D1 proteins. *Proc. Nat. Acad. Sci.* 95: 364–369.
- Carr, G.M., H.C. Duthie and W.D. Taylor. 1997. Models of aquatic plant productivity: A review of the factors that influence growth. *Aquat. Bot.* 59: 195–215.
- Conde, F.R., M.S. Churio and C.M. Previtali. 2000. The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. *J. Photochem. Photobiol.* 56B: 139-144.
- Cooper, J.C. and R.G. Zika. 1983. Photochemical formation of hydrogen peroxide in surface and ground waters exposed to sunlight. *Science* 220: 711–712
- DeBrouwer, J.F.C., K. Wolfstein, G.K. Ruddy, T.E.R. Jones and L.J. Stal. 2005. Biogenic stabilization of intertidal sediments: the importance of extracellular polymeric substances produced by benthic diatoms. *Microb. Ecol.* 49: 501-512.
- Drew, E.A. and R.M. Hastings. 1992. A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. *Phycologia* 31: 262-277.
- Duffy J.E. and M.E. Hay. 2000. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecol. Monogr.* 70: 237-263.
- Dummermuth, A.L., U.Karsten, K.M. Fisch, G.M. König and C. Wiencke. 2003. Responses of marine macroalgae to hydrogen-peroxide stress. *J. Exp. Mar. Biol. Ecol.* 289: 103-121.
- Dunlap, W.C. and Y. Yamamoto. 1995. Small-molecule antioxidants in marine organisms: Antioxidant activity of mycosporine-glycine. *Comp. Biochem. Physiol., B* 112: 105–114.
- Dunlap, W.C., B.E. Chalker, W.M. Bandaranayake and J.J. Wu Won. 1998. Nature's sunscreen from the Great Barrier Reef, Australia. *Int. J. Cosmetic Sci.* 20: 41–51.
- Elstner, E.F. 1990. *Der Sauerstoff*. BI Wissenschaftlicher Verlag, Mannheim.
- Fairhead, V.A., D.A. Amsler and J.B. McClintock. 2006. Lack of defence or phlorotannin induction by UV radiation or mesograzers in *Desmarestia anceps* and *D. menziesii* (Phaeophyceae). *J. Phycol.* 42: 1174-1183.
- Folt, C.L., C.Y. Chen, M.V. Moore and J. Burnaford. 1999. Synergism and antagonism among multiple stressors. *Limnol. Oceanogr.* 44: 864–877.
- Franklin, L.A. and R.M. Forster. 1997. The changing irradiance environment: Consequences for marine macrophyte physiology, productivity and ecology. *Eur. J. Phycol.* 32: 207–232.
- Frederick, J.E., H.E. Snell and E.K. Haywood. 1989. Solar ultraviolet radiation at the earth's surface. *Photochem. Photobiol.* 50: 443–450.
- Fredersdorf, J., R. Müller, S. Becker, C. Wiencke and K. Bischof. 2008. Interactive effects of

- radiation, temperature and salinity on different life history stages of Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia* (submitted).
- García-Pichel, F., M. Mechling and R.W. Castenholz. 1994. Diel migrations of microorganisms within a benthic, hypersaline mat community. *Appl. Environ. Microb.* 60: 1500-1511.
- Gribble, G.W. 2003. The diversity of naturally produced organohalogenes. *Chemosphere* 52: 289-297.
- Hanelt, D., C. Wiencke and W. Nultsch. 1997. Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. *J. Photochem. Photobiol. B, Biol.* 38: 40-47.
- Hannack, K., P. Kestler, O. Sicken and W. Westheide. 1998. The influence of UV radiation on number and ultrastructure of the endosymbiotic dinoflagellates in the sea anemone *Cereus pedunculatus* (Anthozoa: Actiniaria). *Helgol. Mar. Res.* 51: 487-502.
- Henry, B.E. and K.L. van Alstyne. 2004. Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *J. Phycol.* 40: 527-533.
- Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* 67: 283-335.
- Hoffman, J.R., L.J. Hansen and T. Klinger. 2003. Interactions between UV radiation and temperature limit inferences from single-factor experiments. *J. Phycol.* 39: 268-272.
- Holzinger, A., C. Lütz, U. Karsten and C. Wiencke. 2004. The effect of ultraviolet radiation on ultrastructure and photosynthesis in the red macroalgae *Palmaria palmata* and *Odonthalia dentata* from Arctic waters. *Plant Biol.* 6: 568-577.
- Holzinger, A., U. Karsten, C. Lütz and C. Wiencke. 2006. Ultrastructure and photosynthesis in the supralittoral green macroalga *Prasiola crispa* from Spitsbergen (Norway) under UV exposure. *Phycologia* 45: 168-177.
- Holzinger, A. and C. Lütz. 2006. Algae and UV radiation: Effects on ultrastructure and related metabolic functions. *Micron* 37: 190-207.
- Hoyer, K., U. Karsten, T. Sawall and C. Wiencke. 2001. Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. *Mar. Ecol. Prog. Ser.* 211: 117-129.
- Hoyer, K., U. Karsten and C. Wiencke. 2002. Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions. *Mar. Biol.* 141: 619-627.
- Hoyer, K., U. Karsten and C. Wiencke. 2003. Inventory of UV-absorbing mycosporine-like amino acids in polar macroalgae and factors controlling their concentration. In: (A.H.L. Huiskes, W.W.C. Gieskes, J. Rozema, R.M.L. Schorno, S.M. Van der Vies SM and W.J. Wolff, eds) Proceedings of the VIII SCAR Meeting. Backhuys Publ. Leiden, The Netherlands. p 56-62.
- Huovinen, P.S., A.O.J. Oikari, M.R. Soimasuo and G.N. Cherr. 2000. Impact of UV radiation on the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes. *Photochem. Photobiol.* 72: 308-213.

- Jackson, A.E. and R.D. Seppelt. 1997. Physiological adaptations to freezing and UV radiation exposure in *Prasiola crispa*, an Antarctic terrestrial alga. In: (B. Battaglia, J. Valencia and D.W.H. Walton, eds) *Antarctic communities: species, structure, and survival*. Cambridge University Press, Cambridge. pp. 226–233.
- Jones, H.G., T.J. Flowers and M.B. Jones. 1993. *Plants under Stress, Biochemistry, Physiology and Ecology and their Application to Plant Improvement*. Cambridge University Press, Cambridge, U.K.
- Karentz, D. 1994. Ultraviolet tolerance mechanisms in Antarctic marine organisms. *Antarct. Res. Ser.* 62: 93-110.
- Karentz, D., J.E. Cleaver and D.L. Mitchell. 1991. Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *J. Phycol.* 27: 326–341.
- Karsten, U., T. Sawall, D. Hanelt, K. Bischof, F.L. Figueroa, A. Flores-Moya and C. Wiencke. 1998. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Bot. Mar.* 41: 443-453.
- Karsten, U., K. Bischof, D. Hanelt, H. Tüg and C. Wiencke. 1999. The effect of UV-radiation on photosynthesis and UV-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). *Physiol. Plant.* 105: 58-66.
- Karsten, U., K. Bischof and C. Wiencke. 2001. Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters followed by exposure to natural solar radiation. *Oecologia* 127: 11-20.
- Karsten, U., A. Dummermuth, K. Hoyer and C. Wiencke. 2003. Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. *Polar Biol.* 26: 249–258.
- Karsten, U., T. Friedl, R. Schumann, K. Hoyer and S. Lembecke. 2005. Mycosporine-like amino acids (MAAs) and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). *J. Phycol.* 41: 557-566.
- Korb, R.E. and V.A. Gerard. 2000. Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments. *Mar. Ecol. Prog. Ser.* 198: 83–92.
- Korbee, N., P. Huovinen, F.L. Figueroa, J. Aguilera and U. Karsten. 2005. Availability of ammonium influences photosynthesis and the accumulation of MAAs in two *Porphyra* species (Bangiales, Rhodophyta). *Mar. Biol.* 146: 645-654.
- Kräbs, G., K. Bischof, D. Hanelt, U. Karsten and C. Wiencke. 2002. Wavelength-dependent induction of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus*. *J. Exp. Mar. Biol. Ecol.* 268: 69-82.
- Krause, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis, the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42: 313–349.
- Laturnus, F. 2001. Marine macroalgae in polar regions as sources for volatile organohalogenes. *Environ. Sci. Pollut. Res.* 8: 103-108.

- Laternus, F., K.F. Haselmann, T. Borch and C. Grøn. 2002. Terrestrial natural sources of trichloromethane (chloroform, CHCl_3) – An overview. *Biogeochem.* 60: 121-139.
- Lois, R. and B.B.N. Buchanan. 1994. Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation: II. Mechanisms of UV-resistance in *Arabidopsis*. *Planta* 194: 504–509.
- Long, A. and R. E. Michod. 1995. Origin of sex for error repair I. Sex, diploidy, and haploidy. *Theor. Popul. Biol.* 47: 18–55.
- Long, S.P., S. Humphries and P.G. Falkowski. 1994. Photoinhibition of photosynthesis in nature. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 45: 633–662.
- Lotze, H.K. and B. Worm. 2002. Complex interactions of climatic and ecological controls on macroalgal recruitment. *Limnol. Oceanogr.* 47: 1734–1741.
- Lüder, U.H. and M.N. Clayton. 2004. Induction of phlorotannins in the brown algae *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory—The first microscopic study. *Planta* 218: 928–937.
- Lüder, U.H., M.Y. Roleda and C. Wiencke. 2008. Impact of ultraviolet radiation on photosynthesis, DNA, cell structure and germination, and the role of phlorotannins as UV-protecting substances in zoospores of *Laminaria digitata* (Laminariales, Phaeophyta) *Polar Biol.* (submitted).
- Lütz, C., H.K. Seidlitz and U. Meindl. 1997. Physiological and structural changes in the chloroplast of the green alga *Micrasterias denticulata* induced by UV-B simulation. *Plant Ecol.* 128: 54-64.
- Makarov, M.V. and G. M. Voskoboinikov. 2001. The influence of ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. *Bot. Mar.* 44: 89-94.
- Máté, Z., L. Sass, M. Szekeres, I. Vass and F. Nagy. 1998. UV-B induced differential transcription of psbA genes encoding the D1 protein of photosystem II in the cyanobacterium *Synechocystis* 6803. *J. Biol. Chem.* 273: 17439–17444.
- McKenzie, R.L., L.O. Björn, A. Bais, and M. Ilyas. 2003. Changes in biologically-active ultraviolet radiation reaching the Earth's surface. *Photochem. Photobiol. Sci.* 2: 5-15.
- Meindl, U. and C. Lütz. 1996. Effects of UV irradiation on cell development and ultrastructure of the green alga *Micrasterias*. *J. Photochem. Photobiol.* 36: 285-292.
- Michler, T., J. Aguilera, D. Hanelt, K. Bischof and C. Wiencke. 2002. Long-term effects of ultraviolet radiation on growth and photosynthetic performance of polar and cold-temperate macroalgae. *Mar. Biol.* 140: 1117–1127.
- Mortain-Bertrand, A., C. Descolas-Gros and H. Jupin. 1988. Pathway of dark inorganic carbon fixation in two species of diatoms: influence of light regime and regulator factors on diel variations. *J. Plank. Res.* 10: 199-217.
- Müller, R., C. Wiencke, K. Bischof and B. Krock. 2008a. Zoospores of three Arctic Laminariales under different UV radiation and temperature conditions: Exceptional

- spectral absorbance properties and lack of phlorotannin induction. *Photochem. Photobiol.* (in press).
- Müller, R., C. Wiencke and K. Bischof. 2008b. Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Clim. Res.* (in press).
- Müller, R., C. Desel, F.S. Steinhoff, C. Wiencke and K. Bischof. 2008c. UVB- and temperature-induced reactive oxygen species in gametophytes of three Arctic kelp species (Laminariales, Phaeophyceae). *Plant Biol.* (submitted).
- Nybakken, L., W. Bilger, U. Johanson, L.O. Björn, M. Zielke and B. Solheim. 2004. Epidermal UV-screening in vascular plants from Svalbard (Norwegian Arctic) *Polar Biol.* 27: 383-390.
- Öquist, G. and W.S. Chow. 1992. On the relationship between the quantum yield of Photosystem II electron transport, as determined by chlorophyll fluorescence, and the quantum yield of CO₂-dependent O₂ evolution. *Photosynth. Res.* 33: 51-62
- Pakker, H., R.S.T. Martins, P. Boelen, A.G.J. Buma, O. Nikaido and A.M. Breeman. 2000. Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). *J. Phycol.* 36: 334–341.
- Pavia, H., G. Cervin, A. Lindgren and P. Aberg. 1997. Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Prog. Ser.* 157: 139-146.
- Pavia, H. and E. Brock. 2000. Extrinsic factors influencing phlorotannin production in the brown alga *Ascophyllum nodosum*. *Mar. Ecol. Prog. Ser.* 193: 285–294.
- Poppe, F., D. Hanelt and C. Wiencke. 2002. Changes in ultrastructure, photosynthetic activity and pigments in the Antarctic Red alga *Palmaria decipiens* during acclimation to UV radiation. *Bot. Mar.* 45: 253-261.
- Poppe, F., R.A.M. Schmidt, D. Hanelt and C. Wiencke. 2003. Effects of UV radiation on the ultrastructure of several red algae. *Phycol. Res.* 51: 11-19.
- Post, A. and A.W. Larkum. 1993. UV-absorbing pigments, photosynthesis and UV exposure in Antarctica: comparison of terrestrial and marine algae. *Aquat. Bot.* 45: 231-243.
- Ragan, M.A. and K.W. Glombitza. 1986. Phlorotannins, brown algal polyphenols. *Prog. Phycol. Res.* 4: 129-241.
- Raper J.R. and A.S. Flexer. 1970. The road to diploidy with emphasis on a detour. *Symp. Soc. Gen. Microb.* 20: 401–432.
- Rautenberger, R. and K. Bischof. 2006. Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol.* 29: 988–996.
- Remias, D., U. Lütz-Meindl and C. Lütz. 2005. Photosynthesis, pigments and ultrastructure of the alpine snow alga *Chlamydomonas nivalis*. *Eur. J. Phycol.* 40: 259 -268.

- Richter, A., S. Wuttke and K. Zacher. 2008. Two years of *in situ* UV measurements at the Dallmann Laboratory/Jubany Station. *Ber. Polarforsch. Meeresforsch.* 571:12–19.
- Risgaard-Petersen, N., S. Rysgaard, L.P. Nielsen and N.P. Revsbech. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol. Oceanogr.* 39: 573-579.
- Roleda, M.Y., W.H. van de Poll, D. Hanelt and C. Wiencke. 2004. PAR and UVBR effects on photosynthesis, viability, growth and DNA in different life stages of two coexisting Gigartinales: implications for recruitment and zonation pattern. *Mar. Ecol. Prog. Ser.* 281: 37-50.
- Roleda, M.Y., D. Hanelt and C. Wiencke. 2005. Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. *Polar Biol.* 28: 539–549.
- Roleda, M.Y., C. Wiencke and U.H. Lüder. 2006a. Impact of ultraviolet radiation on cell structure, UV-absorbing compounds, photosynthesis, DNA damage and germination in zoospores of Arctic *Saccorhiza dermatodea*. *J. Exp. Bot.* 57: 3847–4856.
- Roleda, M.Y., C. Wiencke and D. Hanelt. 2006b. Thallus morphology and optical characteristics affect growth and DNA damage by UV radiation in juvenile Arctic *Laminaria* sporophytes. *Planta* 223: 407–417.
- Roleda, M.Y., M.N. Clayton and C. Wiencke. 2006c. Screening capacity of UV-absorbing compounds in spores of Arctic Laminariales. *J. Exp. Mar. Biol. Ecol.* 338: 123–133.
- Roleda, M.Y., D. Hanelt and C. Wiencke. 2006d. Exposure to ultraviolet radiation delays photosynthetic recovery in Arctic kelp zoospores. *Photosynth. Res.* 88: 311–322.
- Roleda, M.Y., C. Wiencke, D. Hanelt and K. Bischof. 2007a. Sensitivity of the early life stages of macroalgae from the Northern Hemisphere to ultraviolet radiation. *Photochem. Photobiol.* 83: 851-862.
- Roleda, M.Y., K. Zacher, A. Wulff, D. Hanelt and C. Wiencke. 2007b. Photosynthetic performance, DNA damage and repair in gametes of the endemic Antarctic brown alga *Ascoseira mirabilis* exposed to ultraviolet radiation. *Austral. Ecol.* 32: 917-926.
- Roleda, M.Y., K. Zacher, A. Wulff, D. Hanelt, and C. Wiencke. 2008a. Susceptibility of spores of different ploidy levels from Antarctic *Gigartina skottsbergii* (Gigartina, Rhodophyta) to ultraviolet radiation. *Phycologia* 47: 361-370.
- Roleda, M.Y., K. Zacher, G.L. Campana, A. Wulff, D. Hanelt, M.L. Quartino and C. Wiencke. 2008b. Photosynthetic performance and impact of ultraviolet radiation on the reproductive cells of Antarctic macroalgae. *Ber. Polarforsch. Meeresforsch.* 571: 254-262.
- Roleda M.Y., G. Campana, C. Wiencke, D. Hanelt, M.L. Quartino, A. Wulff. 2008c. Sensitivity of Antarctic *Urospora penicilliformis* (Ulotrichales, Chlorophyta) to ultraviolet radiation is life stage dependent. *J. Phycol.* (in press).

- Schoenwaelder, M.E.A. 2002. Physode distribution and the effect of 'thallus sunburn' in *Homosira banksii* (Fucales, Phaeophyceae). *Bot. Mar.* 45: 262-266.
- Schoenwaelder, M.E.A., C. Wiencke, M.N. Clayton and K.W. Glombitza. 2003. The effect of elevated UV radiation on *Fucus* spp. (Fucales, Phaeophyta) zygote and embryo development. *Plant Biol.* 5: 366-377.
- Schriek, R. 2000. Effects of light and temperature on the enzymatic antioxidative defense systems in the Antarctic ice diatom *Entomoneis kufferathii* Manguin. *Rep. Polar Res.* 349: 1-130.
- Shibata, T., S. Kawaguchi, Y. Hama, M. Inagaki, K. Yamaguchi and T. Nakamura. 2004. Local and chemical distribution of phlorotannins in brown algae. *J. Appl. Phycol.* 16: 291-296.
- Steinhoff, F.S., C. Wiencke, R. Müller and K. Bischof. 2008. Effects of ultraviolet radiation and temperature on the ultrastructure of zoospores of the brown macroalga *Laminaria hyperborea*. *Plant Biol.* 10: 388-397.
- Swanson, A.K. and L.D. Druehl. 2000. Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient. *Mar. Biol.* 136: 657-664.
- Swanson, A.K. and L.D. Druehl. 2002. Induction, exudation and the photoprotective role of kelp phlorotannins. *Aqua. Bot.* 73: 241-253.
- Swanson, A.K. and C.H. Fox. 2007. Altered kelp (Laminariales) phlorotannins and growth under elevated carbon dioxide and ultraviolet-B treatments can influence associated intertidal food webs. *Glob. Change Biol.* 13(8): 1696 – 1709.
- Teixido, N., J. Garrabou, J. Gutt and W.E. Arntz. 2007. Iceberg disturbance and successional spatial patterns: the case of the shelf Antarctic benthic communities. *Ecosystems* 10: 142-157.
- Tuchman, N.C. 1996. The role of heterotrophy in algae. In: (R.J. Stevenson, M.L. Bothwell and R.L. Lowe, eds). *Algal ecology: freshwater benthic ecosystems*. Academic Press, New York. pp. 299-319.
- van de Poll, W.H., D. Hanelt, K. Hoyer, A.G.J. Buma and A.M. Breeman. 2002a. Ultraviolet-B-induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. *Photochem. Photobiol.* 76: 493-501.
- van de Poll, W.H., A. Eggert, A.G.J. Buma and A.M. Breeman. 2002b. Temperature dependence of UV radiation effects in Arctic and temperate isolates of three red macrophytes. *Eur. J. Phycol.* 37: 59-68.
- Vincent, W.F. and P.J. Neale. 2000. Mechanisms of UV damage to aquatic organisms. In: (S.S. de Mora, S. Demers and M. Vernet, eds). *The effects of UV radiation in the marine environment*. Cambridge University Press, Cambridge. pp. 149-176.
- Vinebrooke, R.D., K.L. Cottingham, J. Norberg, M. Scheffer, S.I. Dodson, S.C. Maberly and U. Sommer. 2004. Impacts of multiple stressors on biodiversity and ecosystem

- functioning: the role of species co-tolerance. *Oikos* 104: 451-457.
- Waring J., G.J.C. Underwood and N.R. Baker. 2006. Impact of elevated UV-B radiation on photosynthetic electron transport, primary productivity and carbon allocation in estuarine epipellic diatoms. *Plant Cell Environ.* 29: 521-534.
- Wessel, S., S. Aoki, P. Winkler, R. Weller, A. Herber, H. Gernandt and O. Schrems. 1998. Tropospheric ozone depletion in polar regions. A comparison of observations in the Arctic and Antarctic. *Tellus B* 50: 34–50.
- Wiencke, C, K. Dunton and I. Gómez. 2009. Phenology and seasonal physiological performance of polar seaweeds. *Bot. Mar.* (this issue)
- Wiencke, C., M.N. Clayton and M.E.A. Schoenwaelder. 2004. Sensitivity and acclimation to UV radiation of zoospores from five species of Laminariales from the Arctic. *Mar. Biol.* 145: 31-39.
- Wiencke, C., M.Y. Roleda, A. Gruber, M.N. Clayton and K. Bischof. 2006. Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: Evidence through field experiments. *J. Ecol.* 94: 455–463.
- Wiencke, C., U.H. Lüder and M.Y. Roleda. 2007. Impact of ultraviolet radiation on physiology and development of zoospores of the brown alga *Alaria esculenta* from Spitzbergen. *Physiol. Plant.* 130: 601-612.
- Whitehead, R.F., S.J. de Mora and S. Demers. 2000. Enhanced UV radiation – a new problem for the marine environment. In: (S. de Mora, S. Demers and M. Vernet, eds). *The effects of UV radiation in the marine environment*. Cambridge University Press, Cambridge. pp. 1–34.
- Wickham, S. and M. Carstens. 1998. Effects of ultraviolet-B radiation on two Arctic microbial food webs. *Aquat. Microb. Ecol.* 16:163–171.
- Wulff A., C. Nilsson, K. Sundbäck, S.A. Wängberg and S. Odmark. 1999. UV radiation effects on microbenthos - a four month field experiment. *Aquat. Microb. Ecol.* 19: 269-278.
- Wulff, A., S.A. Wängberg, K. Sundbäck, C. Nilsson and G.J.C. Underwood. 2000. Effects of UVB radiation on a marine microphytobenthic community growing on a sand-substratum under different nutrient conditions. *Limnol. Oceanogr.* 45: 1144–1152.
- Wulff, A., M.Y. Roleda, K. Zacher and C. Wiencke. 2008a. UV radiation effects on pigments, photosynthetic efficiency and DNA of an Antarctic marine benthic diatom community. *Aquat. Biol.* 3: 167-177.
- Wulff, A., K. Zacher, D. Hanelt, A. Al-Handal and C. Wiencke. 2008b. UV radiation - a threat to Antarctic benthic marine diatoms? *Antarc. Sci.* 20: 13-20.
- Wulff A., M.Y. Roleda, K. Zacher and C. Wiencke. 2008c. Exposure to sudden light burst after prolonged darkness - A case study on benthic diatoms in Antarctica. *Diat. Res.* 23: (in press).

- Zacher, K., M.Y. Roleda, D. Hanelt and C. Wiencke. 2007a. UV effects on photosynthesis and DNA in propagules of three Antarctic seaweeds (*Adenocystis utricularis*, *Monostroma hariatii* and *Porphyra endiviifolium*). *Planta* 225: 1505-1516.
- Zacher, K., A. Wulff, M. Molis, D. Hanelt and C. Wiencke. 2007b. Ultraviolet radiation and consumer effects on a field-grown intertidal macroalgal assemblage in Antarctica. *Global Change Biol.* 13(6):1201–1215.
- Zacher, K., D. Hanelt, C. Wiencke and A. Wulff. 2007c. Grazing and UV radiation effects on an Antarctic intertidal microalgal assemblage: a long-term field study. *Polar Biol.* 30:1203–1212.
- Zacher, K. and G. Campana. 2008. UV radiation and consumer effects of an intertidal and subtidal macroalgal assemblage: a comparative study. *Ber. Polarforsch. Meeresforsch.* 571: 287-294.
- Zacher, K., M.Y. Roleda, A. Wulff, D. Hanelt and C. Wiencke. 2008. Responses of Antarctic *Iridaea cordata* (Rhodophyta) tetraspores exposed to ultraviolet radiation. *Phycol. Res.* (in press).

Table 1. UVR-effects on the ultrastructure of marine macroalgae of different systematic position from polar to cold temperate regions.

Phylum/ Class	Species	Development stage	UV exposure	Chloroplast	Nucleus	Mitochondria	others	Reference	
Rhodophyta	<i>Palmaria decipiens</i>	male gametophytes	2-23 h 0.57 W m ⁻² UVB 6.84 W m ⁻² UVA 3.35 W m ⁻² PAR	After 2 h: Chloroplast thylakoids dilated, vesicle like formations at the chloroplast margins After 6-8 h thylakoids disrupted and connected to vesicles After 12-23 h changes disappeared, thylakoids irregular in shape	-	After 4 h tubuli swollen sacculus appearance, after 23 h changes disappeared	-	Poppe et al. 2002, 2003	
		vegetative thalli	6/24 h 0.20 W m ⁻² UVB 4.7 W m ⁻² UVA 25-30 μmol photons m ⁻² s ⁻¹	Thylakoid lumen enlarged, thylakoid membranes wrinkled, outer chloroplast envelope with protrusions into the cytoplasm	-	Cristae visible, swollen	-	Holzinger et al. 2004	
		vegetative thalli	2-48 h 0.6-0.8 W m ⁻² UVB 6.24-8.41 W m ⁻² UVA 3.12-4.66 W m ⁻² PAR	Vesiculation of thylakoids after 2 h, after 6 h increased vesiculation, after 24 h, growth of vesicles and expansion of intrathylakoidal space	-	After 2 h swollen tubuli, inner mitochondrial membrane sacculus type	-	Poppe et al. 2003	
	<i>Phycodrys austrorogatica</i>	gametophytes	2-12 h 0.6-0.8 W m ⁻² UVB 6.24-8.41 W m ⁻² UVA 3.12-4.66 W m ⁻² PAR	After 2-7 h: dilated thylakoids, formation of inside-out thylakoidal vesicles; after 12 h detached phycobilisomes, thylakoid and chloroplast envelope disintegrated	-	-	-	Crystalline inclusions in cytoplasm	Poppe et al. 2003
		gametophytes	2-96 h 0.6-0.8 W m ⁻² UVB 6.24-8.41 W m ⁻² UVA 3.12-4.66 W m ⁻² PAR	Thylakoids change from parallel/central organisation to vesicle-like appearance adjacent to the pyrenoid, tubular arranged thylakoids	-	-	-	-	Poppe et al. 2003
	<i>Odonthalia dentata</i>	vegetative thalli	6/24 h 0.20 W m ⁻² UVB 4.7 W m ⁻² UVA 25-30 μmol photons m ⁻² s ⁻¹	Irregularities in thylakoid membranes, granular structures and interrupted appearance of thylakoid membranes, reduction and branching of thylakoid membranes; small protrusions at outer chloroplast membrane	-	-	-	-	Holzinger et al., 2004
		zoospores	8 h 0.31±0.05 W m ⁻² UVB 5.7±0.7 W m ⁻² UVA 18.9±1.2 μmol photons m ⁻² s ⁻¹	Formation of plastoglobuli, disrupted and fragmented thylakoids at 17 °C, vesicle like structures	Mottled nucleio-plasma, nucleopores varied in size	tubulus/ sacculus type, swollen cristae	-	-	Steinhoff et al. 2008
		gametophytes	8 h 0.35 W m ⁻² UVB 5.5 W m ⁻² UVA 20 μmol photons m ⁻² s ⁻¹	Formation of plastoglobuli	-	-	-	-	Müller et al. 2008c
	<i>Laminaria digitata</i>	gametophytes	8 h 0.35 W m ⁻² UVB 5.5 W m ⁻² UVA 20 μmol photons m ⁻² s ⁻¹	Formation of plastoglobuli	-	-	-	-	Müller et al. 2008c
	Chlorophyta	<i>Prasiola crispa</i>	vegetative thalli	6/24 h 0.20 W m ⁻² UVB 4.7 W m ⁻² UVA 25-30 μmol photons m ⁻² s ⁻¹	No effects after 6 h, after 24 h: slight alterations; reduced number of plastoglobuli, dilatations of thylakoids	-	After 24 h: slight damage	Globules increased 3- to 4-fold, more abundant	Holzinger et al. 2006

Jana Fredersdorf
Emanuelstrasse 15
28213 Bremen

Erklärung gemäß § 6 (5) PromO:

(vom 14. März 2007)

Ich erkläre hiermit, dass ich

1. die vorliegende Doktorarbeit mit dem Titel *Interactive abiotic stress effects on Arctic marine macroalgae - Physiological responses of adult sporophytes* ohne unerlaubte fremde Hilfe angefertigt habe,
 2. keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe,
- und
3. die den benutzen Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremen, 13.03.2009

Jana Fredersdorf

Acknowledgements

Bedanken möchte ich mich zunächst bei meinem Doktorvater Prof. Dr. Kai Bischof für die Bereitstellung dieses spannenden Themas, für die ansteckende Begeisterung für Makroalgen, für jegliche Unterstützung und intensive Betreuung meiner Doktorarbeit. Auch meinem zweiten Betreuer und Gutachter Prof. Dr. Christian Wiencke möchte ich danken für die fachliche und auch finanzielle Unterstützung.

Ein Dankschön geht an alle meine Co-Autoren für die gute Zusammenarbeit!

Weiterhin möchte ich den Mitarbeitern der Koldewey-Station und Kings Bay AS in Ny Ålesund auf Spitzbergen für die logistische Unterstützung während meiner Expeditionen danken! Unerlässlich für diese Arbeit waren natürlich die Makroalgen in, für mich unerreichbaren Tiefen. Daher danke ich natürlich besonders den verschiedenen AWI-Tauchteams auf Spitzbergen, unter der Leitung von Max Schwanitz für die gute Versorgung mit Versuchsobjekten!

Ich danke allen Kollegen im Institut für Polarökologie in Kiel für die schöne IPÖ-Zeit dort in der Arbeitsgruppe mit unvergesslichen Kaffeerunden! Ein besonderer Dank geht vor allem: an Frank-Peter für die technische und handwerkliche Unterstützung bei allen Versuchsaufbauten, an Michael für die viele Computerhilfe & Ratschläge, an Matthias für die beste Literaturdatenbank und an Alice für die Geduld & Hilfe alle meine Proben zu bearbeiten und ihre Unterstützung bei Laborversuchen in Ny Alesund.

Auch möchte ich allen Kollegen in meinen Arbeitsgruppen am AWI Bremerhaven und in der Meeresbotanik der Universität Bremen danken für die schöne Zeit, die tolle Arbeitsatmosphäre sowie die fachliche Unterstützung und Hilfe bei allen Fragen.

Ein besonders liebes Dankeschön geht an alle meine Freunde für die vielen hilfreichen Tipps und Ideen, fürs Korrekturlesen, für die Motivation und vor allem die super Ablenkungen! Kurz, aber in Liebe: Danke Max für Alles! Natürlich geht auch ein riesengroßer Dank an meine Familie, besonders an meine Eltern, die mich immer unterstützten und mir zu jeder Zeit mit Rat und Tat zur Seite standen.