

Adaptation to ultraviolet radiation in marine green macroalgae: Screening and tolerance

Dissertation

Zur Erlangung des Doktorgrades an der Mathematisch-Naturwissenschaftlichen Fakultät der
Christian-Albrechts-Universität zu Kiel

vorgelegt von

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Kiel, September 2014

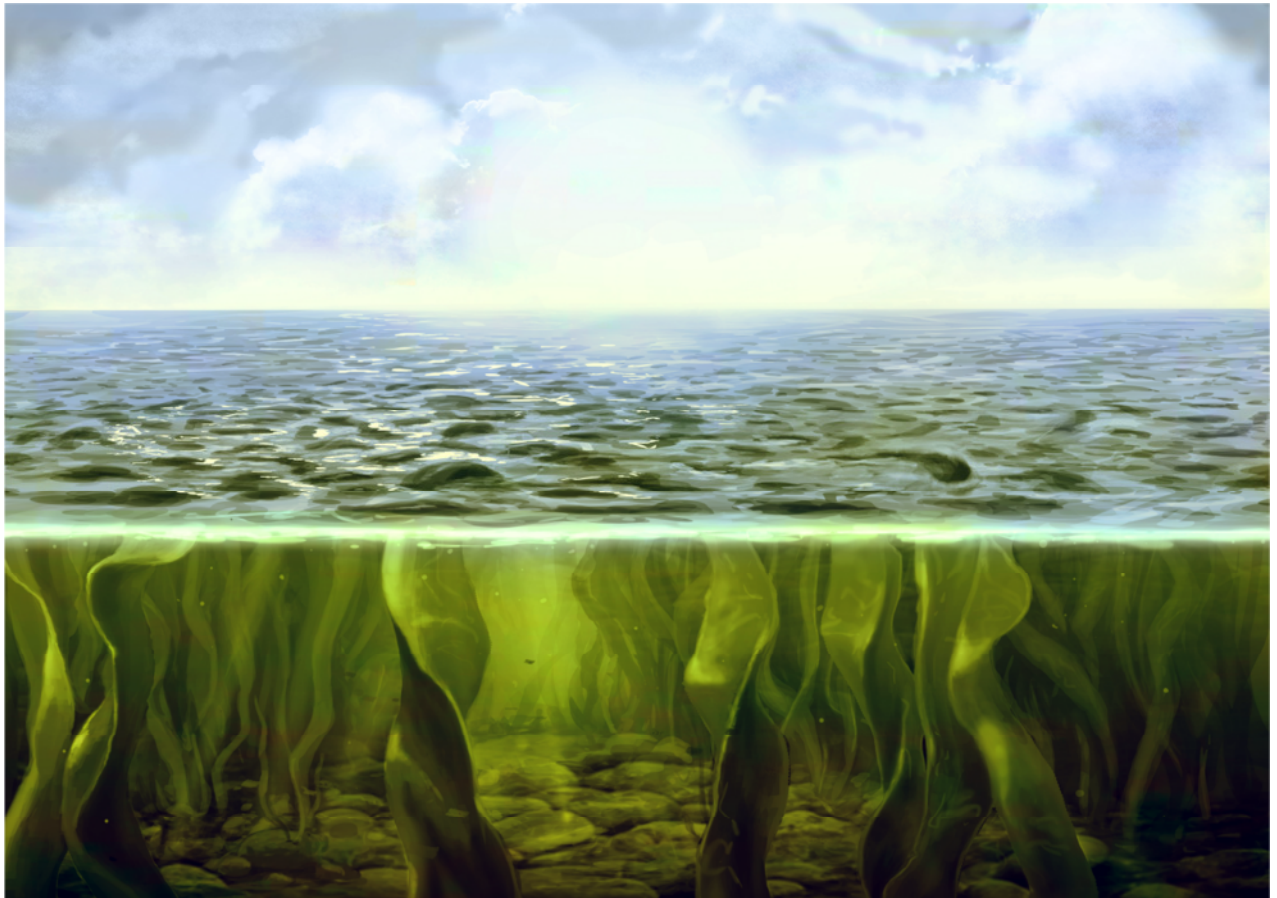
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Tag der mündlichen Prüfung: 05.12.2014

Zum Druck genehmigt: 05.12.2014

gez. _____, Dekan



Painting by Lasse Kanitz

Contents

Chapter 1 – Introduction	1
Green macroalgae and the intertidal habitat	1
Effects of sunlight on photosynthesis	6
UVB-induced DNA damages.....	9
UV screening	11
Adaptation and acclimation mechanisms of UVB resistance in macroalgae	13
This thesis.....	15
Chapter 2 – Screening of ultraviolet-A and B radiation in marine green macroalgae (Chlorophyta).....	17
Chapter 3 – UVB-induced DNA and photosystem II damage in two intertidal green macroalgae: distinct survival strategies in UV-screening and non-screening Chlorophyta	30
Chapter 4 – Relative sensitivity of DNA and photosystem II in <i>Ulva intestinalis</i> Linnaeus (Chlorophyta) to natural solar irradiation	40
Chapter 5 – UVB resistance mechanisms in four green macroalgae with different ecological niches	61
Chapter 6 – Discussion	84
UV screening in green macroalgae	84
Protective function of UV screening in Cladophorales	86
Cellular UVB tolerance mechanisms in non-screening macroalgae.....	87
DNA damages in <i>Ulva intestinalis</i> in the field	88
Relative sensitivity of DNA and PS II <i>in situ</i>	89
UVB resistance strategies of green macroalgae.....	89
Conclusion.....	95
Chapter 7 – Summary / Zusammenfassung.....	97
References.....	100

Declaration of own contributions to the manuscripts

Chapter 2 – Screening of ultraviolet radiation

Chapter 3 – UVB-induced DNA and photosystem II damage

Chapter 4 – Relative sensitivity of DNA and photosystem II

Chapter 5 – UVB resistance mechanisms in four green algae

Chapter	2	3	4	5
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Chapter 1 – Introduction

Green macroalgae and the intertidal habitat

Green macroalgae (phylum Chlorophyta, classes Trebouxiophyceae and Ulvophyceae) are one of five major groups of benthic primary producers in the marine environment together with the red macroalgae (phylum Rhodophyta, classes Bangiophyceae and Florideophyceae), the brown macroalgae (phylum Ochrophyta, class Phaeophyceae), the seagrasses (phylum Tracheophyta) and the very diverse benthic microalgae (Duarte and Cebrián 1996, Lewis and McCourt 2004). Green algae encompass more than 2200 species with most of them from the class Ulvophyceae in marine as well as freshwater habitats (www.algaebase.org¹). Within the Ulvophyceae very diverse morphologies and cell structures are present ranging from unicellular, to filamentous, sheet-like and corticated forms with either a single nucleus or being multinucleate (Cocquyt et al. 2010). Also among the Ulvophyceae quite different life cycles and ecological strategies are realized with heteromorph or isomorph alternations of generations and opportunistic (e.g. *Ulva*) as well as late successional species (e.g. *Codium*) (Littler and Littler 1980). Nevertheless, the group of marine green macroalgae is often generalizing referred to as “bloom-forming” (Worm and Lotze 2006), “green-tide” (Nelson et al. 2003a), “biofouling” (Dobretsov et al. 2005) or even “nuisance” (Valiela et al. 1997, Stevenson 2014) algae without a distinct differentiation. The genera *Ulva* and *Cladophora* are the most widespread ones with the highest potential of mass developments (Dodds and Gudder 1992, Valiela et al. 1997, Nelson et al. 2008, Wang et al. 2012). In their distribution green macroalgae like all marine phototrophs are restricted to the euphotic zone, meaning the zone where enough light is received for a positive net photosynthesis and growth on a yearly basis.

In water sunlight is exponential decreasing with increasing depth due to absorption and scattering called attenuation. Absorption by water, independent if salty or not, is strongest in the red and infrared spectral region but can be dramatically changed by pigments of planktonic organisms and colored detrital and dissolved organic matter (CDOM) as e.g. *gelbstoff*. *Gelbstoff* mainly originates from terrestrial input of humic acids but other polyphenolic substances can also be released from marine organisms like brown macroalgae (Ragan and Jensen 1979, Blough et al. 1993). While photosynthetic pigments from phytoplankton are absorbing predominantly in the blue spectral region

¹ <http://www.algaebase.org/browse/taxonomy/?id=97241>, 01.08.2014

the absorption by CDOM increases strongly with decreasing wavelength, so that ultraviolet-B radiation is attenuated much more than visible radiation (Blough et al. 1993). CDOM and phytoplankton each contribute roughly 50 % to the blue light absorption (Siegel et al. 2002) and coastal waters, which are rich in *gelbstoff*, thus appear green in comparison to oceanic waters as e.g. shown for Helgoland by Lüning and Dring (1979) and the Baltic Sea by Piazena and Häder (1994). For the western Baltic with high riverine input very high diffusive attenuation coefficients above 10 m^{-1} have been reported for ultraviolet-B radiation (Molis et al. 2003). On the other hand small particles ($\sim 2 \mu\text{m}$ and larger) cause classical Mie scattering of underwater sunlight basically independent of wavelength increasing the path length and thereby the probability of absorption (Kirk 1981). These particles can be plankton or particulate organic matter. Taken together the spatial extension of the euphotic zone strongly depends on water transparency and the depth profile of the shore. Moreover, on most coasts the sea level is not constant due to tides and periodic water level changes contribute to the dimensions of the euphotic zone creating the intertidal zone. Accordingly, the euphotic zone and especially the intertidal is not a homogenous habitat but rather a gradient of all sorts of abiotic and biotic factors. Hence, in the literature the intertidal is divided into several horizontal strips mainly differing in the degree of disturbance (e.g. desiccation, herbivory) and light availability resulting in a zonation pattern of organisms (overview in Lüning 1985). This division can either be based on tidal water level changes or the presence of key species:

First coming from land, the supralittoral can be defined as the zone between the beginning of the spray zone and the level of the mean high tide; or by the uppermost occurrence of marine organisms and the upper limit of barnacles (after Stephenson and Stephenson 1949). This means the supralittoral is flooded only from time to time requiring an exceptional tolerance to emergence. Next, the eulittoral extends from the mean high water to the mean low water mark resulting in very regular alternations of submergence and emergence for the populating organisms. Typical species from this zone are barnacles and mussels, fucoid brown algae and green algae like *Ulva*, *Urospora* or *Cladophora* as exemplified in Portugal by Boaventura et al. (2002), or for Helgoland by Reichert et al. (2008). The beginning of the sublittoral is given by the appearance of brown kelps, especially from the order Laminariales. Within the sublittoral the upper part of it can further be defined as the zone between the level of the mean low tide and the extreme low tide providing a much more stable environment with respect abiotic factors but high mechanical stress due to the strong currents of the surf. The mechanically strengthened midribs of typical sublittoral species like *Alaria esculenta* mirror this. Finally, the mid sublittoral is never emerged and only on the basis of light attenuation separated from the lower sublittoral with the 5 % depth of surface irradiance as the border (from Lüning 1985). The phototrophic species inhabiting this zone have a higher ratio of photosynthetically active to respiring tissue to reduce their minimum light requirement (Markager and Sand-Jensen 1992, Gómez et al. 1997). This zonation pattern can be found with some minor

changes at most shores worldwide, even when no tides are occurring (Stephenson and Stephenson 1949).

In an example for the upper part of the littoral from the western Baltic **Figure 1.1** sketches the dominant green macroalgae (*Ulva intestinalis*, *Cladophora sericea*, *Acrosiphonia spec.* and *Bryopsis hypnoides*) at their respective positions. Additionally the abundant brown macroalga, *Fucus spiralis*, is shown as it was regularly observed at this site. The presented location is at the northern canal bank in Kiel-Holtenau close to the lock (N54° 22', E10° 8', L1 in **Figure 1.2**).

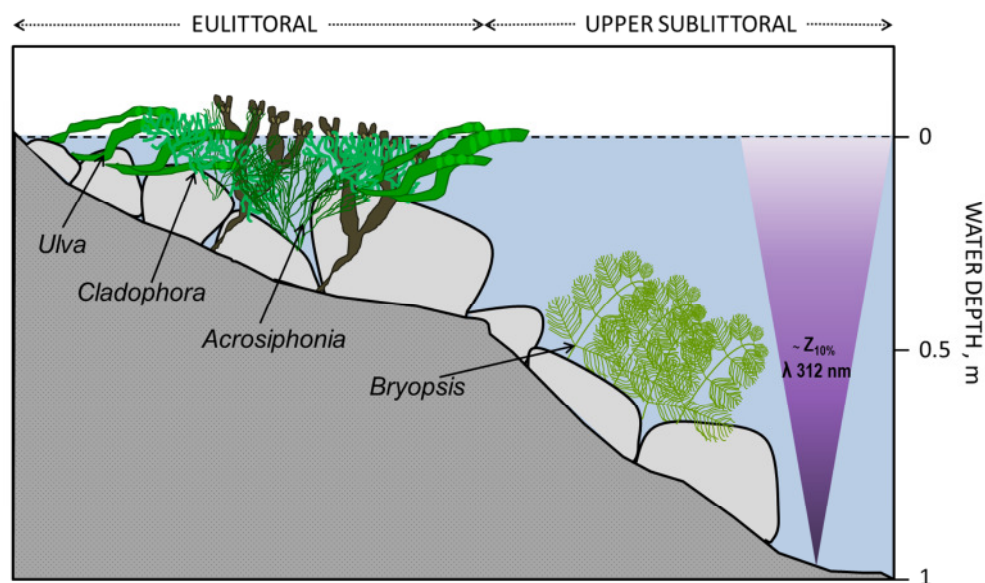


Figure 1.1. Detail of the upper littoral zonation in the Kiel Canal in Holtenau (L1 in **Figure 1.2**) with the positions of the investigated green macroalgae (*Ulva intestinalis*, *Cladophora sericea*, *Acrosiphonia spec.* and *Bryopsis hypnoides*) and the abundant brown alga (*Fucus spiralis*). At this location no water level changes occur as it is inside of the Kiel Canal. The approximate depth for 10 % of the surface irradiance at λ 312 nm is given as determined from spectrophotometric absorption measurements for water samples from the Kiel Fjord. The picture resembles the situation as it was observed in early summer.

At this site the water level is only influenced by waves generated by wind or passing ships. These waves are also stirring up the sediment making the water along the water front relatively turbid although the water transparency at this end of the canal is higher than in the west (Gocke et al. 2010). The depth at which 10 % of surface irradiance of ultraviolet-B radiation ($Z_{10\%}$, $\lambda_{312 \text{ nm}}$) is left is indicated at 0.5 m water depth as it was determined from water samples taken at the lock in Kiel-Holtenau on the side of the Kiel Fjord (L2 in **Figure 1.2**). The canal bank was regularly visited and the vegetation inspected from 2008 to 2014. Macrothalli of the four labelled species were sampled here to be employed in UVB stress experiments (see **chapter 5**).

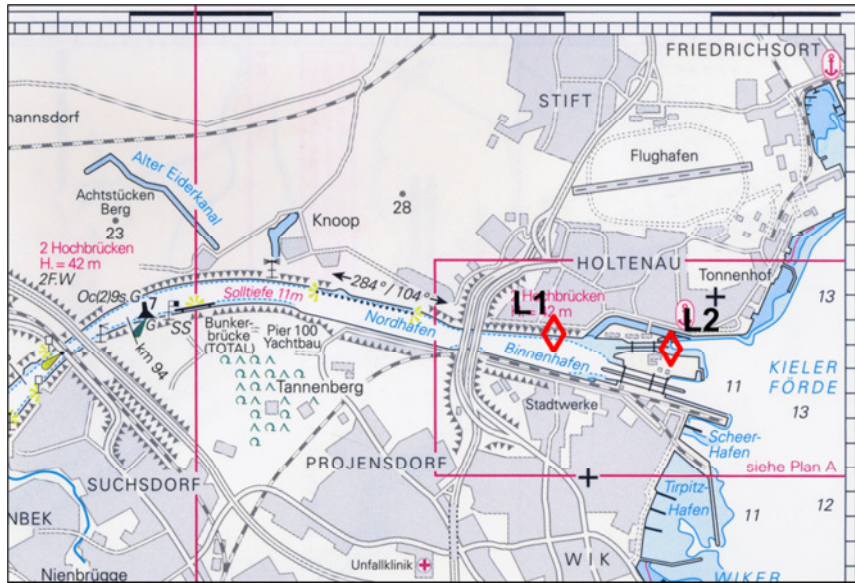


Figure 1.2. Map detail of Kiel Canal at the mouth in Kiel-Holtenau (Nautische Veröffentlichung, Verlagsgesellschaft mbH, Arnis, Germany, 2009, 1 : 55000). L1 and L2 denote the sampling site for different species of green macroalgae used throughout the study (of which a depicted depth profile is shown in Figure 1) and the site of a long-term experiment located at the lock island in Kiel-Holtenau in summer 2013 (see chapter 4), respectively.

For macroalgae as photoautotrophs one of the most important abiotic factors is the light availability within the presented zonation. For them light is as vital as the air we breathe as it drives photosynthesis. Furthermore, sunlight has functions as a signaling and regulating factor (Rico and Guiry 1996, Franklin et al. 2005, Jansen and Bornman 2012) but also exhibits a damaging potential (e.g. reviews by Powles 1984, Aro et al. 1993). All of these roles are determined by the strength of irradiance, the exposure duration and the spectral composition in combination with the absorption properties of the biomolecules (Peak and Peak 1983).

The damaging potential of sunlight can be ascribed to either the visible range (400 – 700 nm, photosynthetically active radiation, PAR) or the short-wavelength range of ultraviolet-A (UVA, 315 – 400 nm) and ultraviolet-B (UVB, 280 – 315 nm) radiation. Longer wavelengths above 750 nm are not absorbed efficiently by plants but quickly attenuated by water and thus do not play an important role for aquatic phototrophs. Also UVB radiation is attenuated strongly (Jerlov 1950), especially in the presence of high concentrations of *gelbstoff* and the penetration of 10% of surface irradiance ($Z_{10\%}$) of UVB radiation varies between less than 1 m and 15 m, as shown for the Baltic or the open water of the eastern Mediterranean or the Gulf of Mexico (overview in Franklin and Forster 1997, cf. Figure 1.1 this thesis). In extremely clear waters, as e.g. the Antarctic Ocean the biological impact of UVB radiation can be significant down to about 10 m (Karentz and Lutze 1990).

As pointed out sunlight quality and quantity change with water depth creating a vertical gradient which is known to be one key factor for determining the zonation of macroalgae (reviews from Markager and Sand-Jensen 1992, Bischof et al. 2006a). In this community structure very often green algae are found in the upper eulittoral or even supralittoral (Metaxas and Scheibling 1993, Hanelt et al. 1997a, Gómez and Huovinen 2011). A common feature of the Ulvophyceae is the composition of photosynthetic pigments with chlorophyll a and b and the accessory carotenoids and xanthophylls, similar to higher plants (Lichtenthaler et al. 1982, Bianchi et al. 1997). This pigment equipment enables the species of green algae to absorb within a wide range of visible light but, as in higher plants, the lowest absorption is in the green spectral region of sunlight which, on the other hand, is dominating in the sublittoral (Lüning and Dring 1985). Historically, this led to the suggestion that green algae are typical eulittoral algae in comparison to red and brown algae which possess accessory pigments with higher absorption of green light (Engelmann 1884 as resumed e.g. in Lüning 1985, and Lobban and Harrison 1994). But arguments against this hypothesis were that green algae can also use the blue light from deeper waters for photosynthesis if the water is relatively clear; the green gap is filled by additional carotenoids like siphonaxanthin (Ramus 1983, Han et al. 2007); and in thick thalli with higher pigment contents the photosynthetic action spectrum is largely wavelength independent, as e.g. in *Codium* species (Lüning and Dring 1985). This means the vertical position of green algae may result not from their inability to grow in greater depths but more probably from their ability to withstand the high irradiances at the exposed positions (Bischof et al. 1998, Hanelt 1998, Gómez and Huovinen 2011).

Effects of sunlight on photosynthesis

Diurnal changes of photosynthetic efficiency synchronous to the diel variation in irradiance have been described in green, brown and red macroalgae by many researchers (e.g. Henley et al. 1991a (*Ulva rotundata*), Hanelt et al. 1997b (several polar brown and red algae), Gómez et al. 2004 (18 cold-temperate green, brown and red algae)). A common response of all studied algae was a substantial reduction of optimal quantum yield of photosystem II (PS II) during the hours of maximal irradiances followed by a recovery in the afternoon. These changes can be monitored by chlorophyll fluorescence measurements as variations in the ground fluorescence (F_0) and maximal fluorescence (F_M) (Demmig and Björkman 1987). From these and similar observations in higher plants and proven by experimental exposure to high irradiances the conclusion was drawn that light above the saturating irradiance is inhibiting photosynthesis (Powles 1984, Neale 1987). A reversible part of photoinhibition is described as 'dynamic' photoinhibition and known to be an important photoprotective mechanism in algae and higher plants (Osmond and Grace 1995, Hanelt 1998).

Several physiological mechanisms exist in algae, cyanobacteria and plants that contribute to downregulation of photosynthetic energy utilization (Jahns and Holzwarth 2012). These reduce the photosynthetic efficiency of PS II resulting in non-photochemical quenching (NPQ) of variable chlorophyll fluorescence ($F_V = F_M - F_0$) of PS II (Horton and Hague 1988, Osmond et al. 1993). This way a major portion of absorbed energy can be safely converted to heat. It is known that the carotenoid zeaxanthin is mediating this mechanism although the detailed molecular mechanism is still unresolved (Ruban et al. 2007, Niyogi and Truong 2013). Zeaxanthin is formed in high-light through de-epoxidation of the carotenoid violaxanthin by the violaxanthin-deepoxidase (VDE). Because the VDE is activated under acidic conditions the formation of zeaxanthin is triggered by the pH gradient that builds up over the thylakoid membrane due to photosynthetic electron transport (review Pfündel and Bilger 1994). Additionally a fraction of the light harvesting complex (LHC) of PS II can be functionally detached from the inner antennae of PS II to reduce the excitation pressure on the reaction center in a process designated as state transition (Horton and Hague 1988). The overall capacity of NPQ was shown in *Ulva rotundata* to be acclimated to the prevailing light conditions (Osmond et al. 1993) predominantly by an increase of xanthophyll cycle pigments (Franklin et al. 1992, Bischof et al. 2002a). If the capacity of these photoprotective reactions against high-light is exhausted another type of photoinhibition by strong irradiances appears, which is considered destructive (Demmig et al. 1987).

In particular, PS II is also subject to photodamage, termed 'chronic' photoinhibition, or photoinactivation, which is also lowering the optimal quantum yield but with a much slower recovery rate (Demmig and Björkman 1987). Under high light conditions, especially if the photon flux density (PFD) is higher than growth PFD the capacity of dark reactions (consumption of ATP and reducing

equivalents in form of NADPH +H⁺ by carbon fixation) restricts the photosynthetic activity. That means the equilibrium of energy supply and utilization is unbalanced. The surplus of absorbed light energy is defined as excessive light (Krause 1988). As a consequence of the over-reduction of the electron transport chain excessive light may induce acceptor side photoinhibition of PS II (Aro et al. 1993). The fully reduced primary electron acceptor (Q_A⁻) can recombine with the oxidized reaction center chlorophyll a (Chl_{P680}⁺) molecule (Vass et al. 1992). This reaction results in excited triplet chlorophyll (³Chl*) which might induce spin-reversal in molecular oxygen forming singlet oxygen (¹O₂) (Hideg et al. 1998). This molecule is highly reactive and may oxidize proteins and lipids (Nishiyama et al. 2011). Another possibility for ROS generation in the chloroplasts is at the photosystem I (PS I) centers where under electron acceptor limitation (e.g. high-light) electrons might be transferred to oxygen yielding superoxide (O₂⁻) in the so-called 'Mehler reaction' (Mehler 1951). This will quickly disproportionate into hydrogen peroxide (H₂O₂) and O₂ catalyzed by the superoxide dismutase (SOD) and H₂O₂ can further be detoxified in the water-water cycle (Miyake and Asada 1994). Overall acceptor-side photoinhibition is most likely at very high irradiances which are for algae especially relevant during low tide and cloudless skies (Henley et al. 1992).

Besides these effects of high irradiances as they are often occurring in direct sunlight there is another mechanism by which photodamage can be induced at PS II. It was observed that photoinhibition is (under addition of chloroplast protein synthesis inhibitors) also occurring at very low light and increasing linearly with light intensity (Tyystjärvi and Aro 1996). The oxygen evolving complex at the luminal side of PS II is strongly absorbing UVB radiation with a continued absorption into the blue spectral region (Ohnishi et al. 2005). The absorbed energy has the potential to release the manganese ions from the complex leading to a loss of water splitting (Hideg et al. 1993). This reaction is linearly depending on light intensity as it is in the first step a pure photophysical process (Tyystjärvi and Aro 1996, Hakala et al. 2005). Without intact water splitting the supply of electrons to PS II is stopped and after further absorption of PAR and subsequent charge separation the oxidized P680⁺ radical will accumulate (Ohnishi et al. 2005). This radical is highly reactive and might oxidize its neighboring molecules (Hideg and Vass 1996).

All these radical producing (ROS and others) photoreactions are directly located at or in the close vicinity of the PSII reaction center with the heterodimer of D1/D2 core proteins (reviewed in Asada 2006). The life time of the D1 protein is rather short, as it is continuously degraded and replaced by new synthesized molecules also in low light (Mattoo et al. 1984, Greenberg et al. 1989). Under high-light conditions this turnover is increased (Tyystjärvi et al. 1992, Komenda and Barber 1995) and the protein components of the repair process itself may be a crucial site for ROS-induced photodamage. In cyanobacteria it was shown that ROS do not directly increase photoinhibition (Allakhverdiev and Murata 2004) or stimulate the D1 degradation. Rather the *de novo* synthesis, especially the elongation during translation of the D1 protein is shown to be sensitive to oxidative

stress (Nishiyama et al. 2001, 2011). If the rate of D1 degradation exceeds the rate of renewal PS II reaction center damage and photoinhibition is occurring (Allakhverdiev and Murata 2004). Other components of the thylakoid membrane that can be attacked by the radicals are the pigments or the lipids of the membrane itself (Hideg et al. 1993). These damages are referred to as photooxidative damages (Triantaphylides et al. 2008). As a protective mechanism against this photooxidation it was shown that green macroalgae from the upper eulittoral, like *Acrosiphonia penicilliformis* or *Chaetomorpha linum*, have remarkably high antioxidative capacities in comparison to deeper growing red algae (Aguilera et al. 2002).

Not only light reactions are sensitive to sunlight or especially UVB radiation but also different components of the dark reactions in photosynthesis are impaired. As an essential enzyme the ribulose 1,5 bisphosphate carboxylase (Rubisco) activity and concentration is reduced by approximately 50 % in *Ulva lactuca* under full solar irradiation as compared to samples under PAR only (Bischof et al. 2002a). A similar impairment of Rubisco was demonstrated in higher plants (Strid et al. 1990). Another example of an UVB-inhibited enzyme is the VDE which is central for the photoprotective activity of the xanthophyll cycle (Pfündel et al. 1992, Bischof et al. 2002a). This circumstance reveals that UVB has the potential to amplify the radiative stress of high PAR by impairing the protective mechanisms against high irradiances. On the other hand on special occasions and only in true high-light adapted aquatic phototrophs an ameliorating effect of UVB on photosynthesis under natural radiation conditions was observed, indicating an important but unknown regulation impact of UVB radiation on the light defensive mechanisms in these species (Hanelt et al. 2006, Hanelt and Roleda 2009).

However, the various processes of photoinhibition in the light or dark reactions, either by UVB radiation or PAR, and the respective protective responses influence the photochemical efficiency of PS II and can be quantified as the optimal quantum yield by chlorophyll fluorescence (F_V/F_M). Changes in the optimal quantum yield of PS II are therefore a valuable tool for assessing the UVB sensitivity of an alga and the rates of recovery may be interpreted as an essential component of the species-specific cellular tolerance capacity.

UVB-induced DNA damages

Chromatin of the nuclear, but also of organellar DNA has an absorption maximum at 260 nm and still efficiently absorbs UVB radiation. As with decreasing wavelength the energy content per photon increases a high amount of energy is absorbed by DNA molecules which can modify the structure of the double helix. The most abundant UVB-induced photoproduct is a cyclobutane-pyrimidine dimer (CPD) (Brash and Haseltine 1982, Besaratinia et al. 2011). It can be formed by a (2+2) cycloaddition between the C5-C6 double bonds of pyrimidines where two pyrimidine bases (either thymine or cytosine) are neighboring (Setlow and Carrier 1966, review Cadet et al. 2012). Alternatively to the ring formation, a covalent electron bond can connect the two pyrimidine bases resulting in a pyrimidine (6-4) pyrimidone photoproduct (6,4-PP) (Mitchell and Nairn 1989). In both cases the hydrogen bond to the complementary base (adenine or guanine) of the second DNA strand is lost and the DNA strand carrying the dimer is bended in a larger curve. The dimer formation makes the site impassable for polymerases and inhibits transcription and replication (Setlow et al. 1963). This means already one DNA dimer has the potential to suppress the expression of the respective gene located on this DNA segment. With an average gene length of 1 kb to 2.5 kb as e.g. in cyanobacteria or rice (Yu et al. 2005, Vass et al. 2013) a dimer frequency of 1 – 2.5 CPD/1 kb (equal to 1000 – 2500 CPDs/1 Mb) would be statistically enough to block transcription of all genes.

The biological effects of DNA dimers are manifold being cytotoxic or even genotoxic and lethal. Of especial importance is that CPDs and 6,4-PP are cancerogenic and mutagenic (Pfeifer and Besaratinia 2012). Reduced cell survival and growth was found in dependence of CPD frequency in Antarctic diatoms or an herbaceous plant from Chile, respectively (Karentz et al. 1991, Giordano et al. 2004). For the cyanobacterium *Synechocystis* a negative relationship of CPD concentration and PS II recovery was observed which was attributed to an inhibited expression of the *psbA* gene, coding for the D1 protein (Vass et al. 2013). Brown and red macroalgae and their reproductive propagules also show an impairment of growth that was connected to increased CPD frequencies (Roleda et al. 2007). In addition to UVB-induced dimerization other forms of damage might occur in DNA due to environmental stress. Prominent example among a vast variety of others is the formation of 8-oxo-7,8, dihydroguanine (8-oxoGua) which results from oxidation reactions of guanine with hydroxylradicals or superoxide (Cadet et al. 2003). Finally, single or double strand breaks represent another type of UV-induced DNA damage which leads to a reduction in DNA stability.

Bacteria, plants, and animals possess several repair pathways for radiation-induced DNA dimers (reviews by Sancar 2003, and Britt 2004). Among those occurring in plants one mechanism is outstandingly efficient, the photoreactivation (Pang and Hays 1991, Taylor et al. 1996, Dany et al.

2001). Photoreactivation represents an error-free and fast cellular UVB tolerance mechanism and is also known in bacteria, fungi, insects and vertebrates except for placental mammals. The direct reversal of dimers into their monomeric forms is carried out by a specific light-activated DNA binding enzyme, the photolyase. All photolyases contain one catalytic flavin adenine dinucleotide (FAD) cofactor in the two-electron reduced form FADH⁻, and one chromophore which can be a 5,10-methylentetrahydrofolate (MTHF) or a 8-hydroxy-7,8-didemethyl-5-deazariboflavin (8-HDF) (for a review see Weber 2005). Evolutionary, photolyases are closely related to blue light receptors, the cryptochromes which are derived from them (Cashmore et al. 1999). The identity of the chromophore determines the action spectrum of the photolyase always expressing a dominant peak in the UVA or blue light range (Takeuchi et al. 1998, Sancar 2003). This means in sunlight the damaging UVB radiation is constantly accompanied by the reversing radiation. For CPDs and 6,4-PP distinct enzymes exist and the removal is reported to be faster for CPDs than for 6,4-PP (Taylor et al. 1996). As any enzymatic process the photoreactivation is temperature-dependent (Pang and Hays 1991, Takeuchi et al. 1996) and the enzyme itself can be upregulated by exposure to UVB radiation (Kang et al. 1998, Giordano et al. 2003) or inactivated e.g. by ROS (Takeuchi et al. 2007).

The alternative repair pathways for UVB-induced DNA lesions are referred to as 'dark repair' and can be divided into nucleotide or base excision repair (NER and BER, respectively) (for a review see Britt 2004). These processes rely on a complex machinery of DNA binding enzymes and cofactors. The action of this excision repair is therefore easily influenced by the physiological state of the DNA as e.g. degree of condensation (Bohr et al. 1985). In bacteria and humans a correlation of NER and transcription was found entering the literature as transcription coupled repair (TCR) (review by Hanawalt and Spivak 2008).

The most often used method for CPD detection in plants in the past 20 years is an immunoassay (Cadet et al. 2005) employing specific primary antibodies and subsequent detection by enzyme conjugated secondary antibodies with chemiluminescent properties in a southern dot blot. The method is suited to detect CPDs in a small amount of DNA and down to 1 lesion/10Mb (Wani et al. 1987). However the quantitative comparability often is not possible and reported dimer frequencies vary relatively strong. On the other hand, the used irradiation protocols are diverse. This limitation of the existing data becomes clear comparing four studies which included green macroalgae: Although CPDs were quantified with the same primary antibody (H3 clone, Roza et al. 1988) the concentrations ranged from 0.5 CPDs/Mb after 8 h exposure to 0.4 W m⁻² UVB (gametophytes of *Urospora penicilliformis*, Roleda et al. 2009), to 1.5 CPDs/Mb in full sunlight of southern Spain (macrothalli of *Ulva rotundata*, Bischof et al. 2002b), over 25 CPDs/Mb under mild UVB supplementation in the Antarctic (*Prasiola crispa ssp. antarctica*, Lud et al. 2001) and 75CPDs/Mb after 45 min to 2.3 W m⁻² experimental UVB (macrothalli of *Monostroma arcticum*, van de Poll et al. 2002). A comprehensive examination of the sensitivity of DNA in green algae is missing.

UV screening

In phototrophic organisms the effects of strong irradiances of sunlight and especially UVB radiation have led to the incurrence of protective mechanism against this stress. Aside from cellular tolerance mechanisms UVB absorption by protective compounds represents an alternative resistance strategy. Physical UV screening is widespread in higher (Day et al. 1992) as well as lower plants (lichens (Solhaug and Gaussla 1996) and mosses (Newsham 2003), microalgae (Jeffrey et al. 1999) and red (Karsten et al. 1998) and brown macroalgae (Swanson and Druehl 2002)). On the other hand, after a broad survey of green macroalgae by Karsten et al. (1998) it was generally accepted that in most green macroalgae no or only trace amounts of screening substances are present (e.g. Bischof et al. 2006a).

In vascular plants primarily flavonoids are accumulated in the vacuoles of the epidermal cells (Robberecht and Caldwell 1978). This tissue localization reduces the penetration of UV radiation into the leaves and the photosynthetically active mesophyll is shielded. Thereby the induction of UV-induced damages is efficiently prevented as demonstrated in flavonoid deficient mutants of *Arabidopsis thaliana* (Landry et al. 1995) or soybean cultivars with diminished screening capability (Xu et al. 2008). Likewise in Antarctic mosses the UV tolerance increased with a high concentration of cell-wall bound absorbing compounds (Clarke and Robinson 2008) reducing the concentration of DNA lesions after a UV challenge (Turnbull et al. 2009). Also Rhodophytes from the upper eulittoral are able to raise the concentration of UV-shielding mycosporine-like amino acids (MAAs) (Hoyer et al. 2001) which are preventing UVB-induced photoinhibition (Bischof et al. 2000a). Overall, the chemical nature of the substances is various in phototrophs (Cockell and Knowland 1999). For example, an ancient pigment only found in cyanobacteria is scytonemin which is a symmetrical dimer with eight aromatic rings (Proteau et al. 1993). The absorption maximum is in the UVA *in vivo* and it is accumulated in the outer sheets of cyanobacterial mats (Garcia-Pichel and Castenholz 1991). In brown macroalgae polyphloroglucinols called phlorotannins which are contained in cytoplasmatically located physodes or insoluble bound to the cell walls (Swanson and Druehl 2002) are supposed to be involved in UV protection (Abdala-Díaz et al. 2006). As these UV-absorbing substances are also excreted in substantial amounts they may provide protection also for other organisms in the macroalgal bed.

For green algae only a few UV-shielding species had been encountered. One of these rare examples is the green macroalga *Dasycladus vermicularis* (Menzel et al. 1983, Perez-Rodriguez et al. 2003). The Dasycladales are a group of siphonous algae forming a sister group to the Bryopsidales and Cladophorales (Cocquyt et al. 2010). In *Dasycladus vermicularis* the UVA-absorbing trihydroxycoumarins (THCs) are localized in or near to the cell wall and an outer membrane (Perez-Rodriguez et al. 2003) which makes them suitable UV-screens. Aside from

shielding the inner tissues the phenolic substances are also excreted into the seawater sharing their protective function with other organisms inhabiting this vicinity. It was shown that an increased concentration of THCs in the water of tide pools can reduce UV-induced photoinhibition in other algae (Perez-Rodriguez et al. 2003). Another exceptional UV screening green alga is the genus *Prasiola* from the class Trebouxiophyceae which can synthesize MAAs, like many Rhodophytes (Sivalingam et al. 1974). The Trebouxiophyceae are more distantly related to the other green macroalgae (Cocquyt et al. 2010) and the species from the genus *Prasiola* encompass only a few marine representatives, being mainly distributed in freshwater and terrestrial habitats or as photosymbionts of lichens (Karsten et al. 2005). The MAA of *Prasiola crispera* was identified as a unique 324 nm-absorbing molecule inducible by UVB radiation (Gröniger and Häder 2002). Last, in *Ulva pertusa* the UVB-specific induction of an unidentified UVB-screening substance was reported to protect PS II activity from UVB-induced damage (Han and Han 2005). Similar as observed for flavonoids, a correlation between the UVB absorption of methanolic cell extracts and antioxidative radical scavenging capacity was found in this study. Unfortunately, this lead was not pursued in other green algae, also not from the genus *Ulva* in the following years and the publication remained as a single observation.

The absence of UV screening in the majority of marine green macroalgae was in strong contrast to the expectation as this group has at least the same need for UVB resistance than the other macroalgae. Maybe after the failure of Karsten et al. (1998) to find screening species in a wide variety of green macroalgae by employing an extraction based procedure the ambition on this question was satisfied. The method used was well suited for compounds dissolved in the cytoplasm or vacuoles but could have overlooked cell wall bound substances or chemically very resistant compounds of high molecular weight such as sporopollenins or sporopollenin-like substances (Atkinson et al. 1972, Derenne et al. 1992). Such compounds are very difficult to determine but are known from cell walls of spores or green microalgae (Atkinson et al. 1972, Xiong et al. 1997). The common characteristic of sporopollenin or sporopollenin-like compounds is an increasing absorption with decreasing wavelength (Gorton and Vogelmann 2003). Such substances might be contained in green macroalgae without being noticeable in established extraction procedures. Overall, it seems worth to scrutinize the field of UV screening in green macroalgae again as there might be some undiscovered findings.

Adaptation and acclimation mechanisms of UVB resistance in macroalgae

As sessile organisms plants and macroalgae are continuously exposed to the environmental conditions of their growth site. This means they need to sustain these stressful conditions by resistance mechanisms to establish a persistent population in the respective habitat. With regard to UVB radiation basically three strategies are employed: avoidance by protective UV-absorbing substances, tolerance by physiological processes that keep the balance of UV-induced damages and repair mechanisms, or escape from the radiation by growth in the shade or outside of the summer season (Jansen et al. 1998). Certainly, all strategies can be combined to various degrees and the sum of these mechanisms will result in the species-specific UVB resistance (Cockell and Knowland 1999).

For macroalgae, it is accepted that the species-specific resistance to experimental short-term UVB exposure can be related to depth distribution on a community level, i.e. the escape strategy plays an important role (review Bischof et al. 2006a). Besides living in greater depths another escape mechanism might be mat or canopy formation which is a common feature of several species from the orders Ulvales but also Cladophorales (Vergara et al. 1998, Bischof et al. 2006b). In these multilayered packages the penetration of light is substantially diminished and also depleted in short wavelength radiation (Bischof et al. 2002b). Thalli from the inner parts of these mats exhibit strongly decreased signs of radiation stress in comparison to top layers. Otherwise, in shallower growing species cellular UVB tolerance and avoidance mechanisms are increasingly vital, as e.g. shown in a set of 21 species of seaweeds from the Antarctic Peninsula (Huovinen and Gómez 2013). Therein the eulittoral algae showed less photoinhibition and a faster recovery after UVB exposure. This investigation focused on the species-specific potential of several physiological defense mechanisms (e.g. the recovery kinetics of PS II and the antioxidative capacity) but also included possible UV shielding by phlorotannins in brown algae. Further referring to the UV protection by MAAs in red algae (Hoyer et al. 2001), it was comprehensively speculated that a synergism of the latter two resistance mechanisms is determining the UV susceptibility of red and brown macroalgae (Huovinen and Gómez 2013).

In the same study, like in numerous others, green algae have been reported to exhibit very high cellular UVB tolerance employing a mosaic of physiological processes (Huovinen and Gómez 2013). Vigorous dynamic photoinhibition (Hanelt 1998), reported in *Chaetomorpha linum* also in response to UVB radiation (Bischof et al. 2006b), a UV-robust D1 turnover as e.g. described in *Ulva bulbosa* and *U. clathrata* (Rautenberger and Bischof 2006), accompanied by high antioxidative capacities (Aguilera et al. 2002) are acting as cellular defenses in green algae. Many of these

results have been produced with laboratory grown algae indicating that the observed specific reactions represent adaptive differences.

On the other hand differences of the degree of UVB resistance can either be genetic or phenotypic due to acclimation. Acclimation can be described as the response of an organism to a sublethal level of stress. It is well accepted that macroalgae like higher plants are able to photoacclimate to the prevailing irradiance of their growth site within a short period (Henley et al. 1991a, Vergara et al. 1998, Han et al. 2007). This acclimation involves the pigment content and composition of the photosynthetic apparatus (Ramus et al. 1976). Acclimation to high or low photon fluences is not limited to PAR but rather can also occur due to changes in UVB radiation (Grobe and Murphy 1998, Altamirano et al. 2000, Figueroa et al. 2014). For UVB radiation a dominant negative effect on Rubisco activity and concentration was observed in *Monostroma arcticum*, *Ulva lactuca* and *Ulva rotundata* (Bischof et al. 2000b, 2002a, 2002b). Synchronous with the inhibition of Rubisco an increase in a structural enzyme-binding protein, chaperonin 60 (CPN 60) which promotes the quaternary stability of Rubisco, was detected in *U. rotundata*, pointing to a protective acclimation of cellular tolerance (Bischof et al. 2002b). With respect to UVB-induced DNA damages the capacity of photoreactivation was repeatedly found to acclimate in higher plants. In *Gunnera magellanica* grown under UV-exclusion for several weeks the photoreactivation rate was only 50 % of the plants from ambient UVB control plot (Giordano et al. 2003). The same decreased cellular UVB tolerance of the DNA was reported from growth chamber alfalfa seedlings in comparison to field grown individuals (Takayanagi et al. 1994). These results point to an induction of the photolyase by UVB radiation but evidence for this is lacking for macroalgae.

To what extent these different strategies are present in green algae and how different species of green algae are geared up against UVB radiation remains an open question. The observation that the relative abundance of green algae was increased under UVB enhancement conditions in a successional analysis by Molis et al. (2003) proves their resistance to this abiotic stress factor. But only a few attempts were made to explain this high resistance up to now. In exclusion studies utilizing optical filters for UVB very specific UVB responses were observed but mostly only one or two genera were included (Grobe and Murphy 1998, Choo et al. 2004, 2005, Bischof et al. 2002b, 2006b). Also the mechanism by which the green algae *Cladophora glomerata* and *Chaetomorpha linum* (both from the order Cladophorales) gained their high UVB resistance was not further investigated (Choo et al. 2005, Bischof et al. 2006b).

This thesis

To investigate the adaptation to ultraviolet radiation in marine green macroalgae and their defense mechanisms several experimental approaches have been pursued to answer the following hypotheses (H1-H6):

H1: Species with UV screening exist among green macroalgae.

The ability to synthesize UVB and/or UVA screening substances is very widespread in the marine and terrestrial phototrophs (Cockell and Knowland 1999) and it seems difficult to imagine that green macroalgae should be one of the very exceptions without screening, especially in view of their prevailing shallow-water distribution. Previous attempts failed to detect UV screening compounds in this group employing extraction procedures (Karsten et al. 1998) possibly because the absorbing substances might be bound to the cell walls. Therefore a non-invasive *in vivo* method based on chlorophyll fluorescence was applied which was adopted from measurements of epidermal UV transmittance for leaves of higher plants (Bilger et al. 1997). The investigations covered a wide taxonomic range of green macroalgae with species from six orders and included 71 isolates. The results are presented in chapter 2 as published in the Journal of Phycology (Pescheck et al. 2010).

H2: UVB screening detected by the fluorescence approach protects cellular targets like the DNA and photosystem II against UVB-induced damages.

A reduced fluorescence yield from UVB excitation of chlorophyll implies attenuation of UVB radiation by UV absorbing compounds. The protective function of these compounds for cellular targets has to be proven because optical or ultrastructural properties of the cells might influence the screening effect (Garcia-Pichel 1994). By comparing the dose responses of UVB-induced DNA damages and reductions of PS II activity in a screening and a non-screening alga the protective effectiveness of UVB screening was evaluated under controlled laboratory conditions. The exposure to artificial UVB radiation with different fluence rates was chosen as a mechanistical approach to quantify the cellular protection by screening in *Rhizoclonium riparium* (order Cladophorales). This species was identified in the transmittance analysis as the alga with the highest screening and was compared to *Ulva clathrata* (order Ulvales) isolated from the same original habitat but without screening (chapter 3).

H3. The non-screening alga *Ulva clathrata* employs cellular UVB tolerance mechanisms to compensate for the missing screening.

An outbalanced damage induction by cellular tolerance relies on repair or replacement rates of UVB-sensitive structures (Lesser et al. 1994). In recovery experiments the repair kinetics for UVB-induced DNA damages and PS II inhibition of *U. clathrata* were determined in comparison to the screening alga *R. riparium*. The cosmopolitan and very abundant genus *Ulva* was chosen as a key

genus to investigate alternative mechanisms of UVB resistance. The results for hypothesis 2 and 3 are given in **chapter 3** which is a contribution to the Journal of Photochemistry and Photobiology: B-Biology (Pescheck et al. 2014).

H4: In *U. intestinalis* DNA damages will accumulate depending on the UVB dose under natural solar irradiation.

Experimental UVB exposure is a valuable tool for the analysis of UVB-sensitive targets but such results have limited validity under natural irradiance conditions as shown in soybean (Caldwell et al. 1994). The absence of protective UVB screening provoked the question how much UVB-induced DNA damages are accumulating in *U. intestinalis* in sunlight in its natural habitat. To test for the question if there is an accumulation depending on the UVB dose short-term observations over daily cycles and a long-term study over four weeks were conducted. A diurnal variation of lesion frequency and possibly a seasonal variation was expected both depending on sunlight irradiance.

H5: The relative UVB sensitivity of the DNA in comparison to photosystem II is increased in *U. intestinalis* in the field.

As derived from the former finding of a very efficient PS II recovery in *U. intestinalis* and a rather slow DNA repair (cf. to H3 and H6) the importance of both targets under solar irradiation was comparatively analyzed in the field. To this end in parallel the DNA damage and PS II efficiency were determined and the possible interactions were discussed from the same individual thallus. The results covering H4 and H5 are contained in **chapter 4** as a manuscript prepared for submission to Marine Ecology-Progress Series.

H6: The species-specific UVB resistance of green macroalgae is related to their ecological niche.

From the UV screening analysis and the experiments with *U. clathrata* and *R. riparium* (cf. to H1, H2 and H3) it was clear that not all green macroalgae have the same degree and strategy of UVB resistance. It was assumed that this capability can be related to the relative encounter of UVB radiation in the habitat and thus to the requirement of UVB protection. In four species from a natural UVB gradient of their ecological niches (*Acrosiphonia spec.*, *Bryopsis hypnoides*, *Cladophora sericea* and *Ulva intestinalis*) different mechanisms of UVB resistance were compared. As the experiments were conducted with field collected algae from the Kiel Canal (cf. to **Figure 1.1**) acclimation to natural sunlight during development of the thalli probably was influencing the UVB resistance which could not be separated from adaptive differences. Resistance was quantified as UVB dose response and the potential of screening and cellular tolerance were analyzed. The results are described in **chapter 5** as a manuscript prepared for submission to the Journal of Experimental and Environmental Botany.

Chapter 2 – Screening of ultraviolet-A and B radiation in marine green macroalgae (Chlorophyta)

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(Manuscript as published in the *Journal of Phycology*, 46, 444–455 (2010),

DOI: 10.1111/j.1529-8817.2010.00825.x)

Abstract

Ultraviolet screening capacity of macrothalli from marine chlorophytes was analyzed using an *in vivo* technique based on chlorophyll fluorescence. The method, originally introduced to assess epidermal UV-transmittance in leaves from higher plants, is extended to macroalgae. Validation of the method was obtained by measuring unprotected samples, i.e. isolated chloroplasts from six algal species. It is shown, that in a total of 71 investigated green macroalgae, including cultured and field collected material from six systematic orders, only 40 % or 60 % displayed significant screening of UV-A or UV-B radiation, respectively. Generally, the extent of screening was low in most of these species. Data analysis resulted in a clear phylogenetic pattern with minor influence of climatic origin of a given species. For some species comparison between field collected and culture grown samples was possible. Only in 11 of 25 species of field collected algae had appreciably higher screening than those grown in the absence of UV-B radiation. For the first time very efficient UV-A and UV-B screening is demonstrated for the order of the Cladophorales. Their UV-B screening potential varied between 40 % and 85 % of incoming UV-B radiation. However, the nature and localization of the detected UV-absorbing compounds are still unknown. Long-term UV-exposure experiments pointed to a negative correlation of UV-B screening capacity and UV-induced inhibition of photosynthetic efficiency. Thus, species with pronounced screening were more UV-resistant than species with lower screening.

Key index words: chlorophyll fluorescence, Cladophorales, green macroalgae, UV-absorbing compounds, UV-B radiation, UV-resistance

SCREENING OF ULTRAVIOLET-A AND ULTRAVIOLET-B RADIATION IN MARINE GREEN MACROALGAE (CHLOROPHYTA)¹

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Ultraviolet-screening capacity of macrothalli from marine chlorophytes was analyzed using an *in vivo* technique based on chl fluorescence. The method, originally introduced to assess epidermal UV transmittance in leaves from higher plants, is extended to macroalgae. Validation of the method was obtained by measuring unprotected samples (i.e., isolated chloroplasts from six algal species). It is shown that in a total of 71 investigated green macroalgae, including cultured and field-collected material from six systematic orders, only 40% or 60% displayed significant screening of ultraviolet-A (UVA) or ultraviolet-B (UVB) radiation, respectively. Generally, the extent of screening was low in most of these species. Data analysis resulted in a clear phylogenetic pattern with minor influence of climatic origin of a given species. For some species, comparison between field-collected and culture-grown samples was possible. Only in 11 of 25 species field collected algae had appreciably higher screening than those grown in the absence of UVB radiation. For the first time, very efficient UVA and UVB screening is demonstrated for the order of the Cladophorales. Their UVB-screening potential varied between 40% and 85% of incoming UVB radiation. However, the nature and localization of the detected UV-absorbing compounds are still unknown. Long-term UV-exposure experiments pointed to a negative correlation of UVB-screening capacity and UV-induced inhibition of photosynthetic efficiency. Thus, species with pronounced screening were more UV resistant than species with lower screening.

Key index words: chlorophyll fluorescence; Cladophorales; green macroalgae; UV-absorbing compounds; UVB radiation; UV resistance

Abbreviations: F_{BG} , the fluorescence induced by blue-green excitation; F_{UVA} or F_{UVB} , the chl fluorescence induced by UVA or UVB radiation; THCs, trihydroxycoumarins; T_{UVA} , apparent transmittance for UVA radiation; T_{UVB} , apparent transmittance for UVB radiation; UVB_{BE} , biologically effective UVB radiation

Ultraviolet radiation is a common but potentially harmful environmental factor for intertidal phototrophic organisms from polar to tropical regions. Although incident UV radiation declines with decreasing solar elevation toward higher latitudes, the increased UVB irradiance (290–315 nm, UVBR) related to seasonally reduced ozone concentration over the Antarctic and Arctic counteracts this global trend (McKenzie et al. 2003). In comparison to red and brown macroalgae, green macroalgae are regularly exposed to high solar radiation, including high doses of UVB and UVA (315–400 nm), due to their prevailing distribution in shallow-water habitats. Many investigations have shown that species-specific depth distribution and resistance to ultraviolet (Larkum and Wood 1993, Bischof et al. 1998b) and high PAR (Hanelt et al. 1997) are strongly correlated in macroalgae and aquatic vascular plants (Rae et al. 2001). However, research has focused on brown (Bischof et al. 1998a) and red algae (Maegawa et al. 1993, van de Poll et al. 2003) as well as on reproductive, unicellular propagules (Wiencke et al. 2000, Roleda et al. 2007). UV susceptibility was determined to be most pronounced in deep-water species and reduced in shallow-water species. Resistance to UV radiation in algae can be caused either by cellular tolerance mechanisms or avoidance of UV-induced damages (Davison and Pearson 1996). For green algae, photosynthesis and related enzyme reactions were reported to be very UV resistant (Bischof et al. 1998b, 2000), and levels of antioxidant enzymes and related antioxidant metabolites were notably high

¹Received 23 October 2008. Accepted 18 November 2009.

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(Aguilera et al. 2002). Furthermore, low amounts of cyclobutane-pyrimidine dimers (CPDs) after exposure to natural solar irradiance were described for *Ulva rotunda* (Bischof et al. 2002). Sporulation and growth have been shown to be remarkably resistant to UVBR in *Ulva expansa* (Han et al. 2003).

While there have been a considerable number of investigations with regard to UV-tolerance mechanisms, only a few studies have addressed potential UV screening in marine green macroalgae (Perez-Rodriguez et al. 1998, Gröniger and Häder 2002). Thallus extracts from several green algae exhibited absorbance at ~320–335 nm attributed to mycosporine-like amino acids (MAAs) (Sivalingam et al. 1974). An absorption peak at 294 nm was reported for *Ulva pertusa*, but no identification of UV-absorbing substances was carried out (Han and Han 2005). For *Prasiola crispa* (Post and Larkum 1993) and its close relatives (Karsten et al. 2005), the presence of MAAs is documented, but this seems to be an exclusive phenomenon as other green algae have no or only trace amounts of MAAs (Karsten et al. 1998). This contrasts with the widespread capability of marine and freshwater organisms to synthesize or accumulate MAAs (Shick and Dunlap 2002). The opportunistic life strategy, and the mat-forming growth forms that provide self-shading (Vergara et al. 1998), may compensate for the absence of UV-absorbing pigments in green algae. On the other hand, only a small number of species have been studied so far, covering only a narrow taxonomic range.

Green freshwater microalgae, for example, *Scenedesmus communis* (Blokker et al. 1999) and *Chlamydomonas nivalis* (Gorton and Vogelmann 2003), contain very resistant macromolecules suspected to function as a UV screen (Xiong et al. 1995). These are possibly sporopollenins or sporopollenin-like substances (algenans) that absorb from the ultraviolet to the blue region of the spectrum and are bound to the cell walls of these algae (VanWinkle-Swift and Rickoll 1997). Difficult extraction for cell-wall-bound substances impedes wide investigations of potentially existing UV-absorbing pigments in green macroalgae.

Therefore, in the present study, an *in vivo* method using chl fluorescence not requiring an extraction or any knowledge about the chemical properties of the UV-screening pigments is applied on green macroalgae for the first time. In this approach, apparent UVA and UVB transmittances are calculated from the ratio of blue-green and UVR excited chl fluorescence. This method was originally introduced to determine UV screening in leaves of higher plants (Bilger et al. 1997, 2001). With this fast nondestructive method, macrothalli from 71 green algae from six different orders from polar to tropical regions, including freshly collected field material, were surveyed for their UV-screening capacities. Photosynthetic efficiency after experi-

mental UV exposure was used to address UV resistance in four isolates and related to the UV screening of absorbing pigments.

MATERIALS AND METHODS

Algal material and culture conditions. A total of 46 isolates from 29 species originally isolated at polar, temperate, or tropical sites and from different water depths were investigated (Table S1 in the supplementary material). According to Hayden et al. (2003), *Enteromorpha* species are assigned to the genus *Ulva*. Algae were grown from stock cultures kept at the Alfred-Wegener-Institute for Polar and Marine Research (Bremerhaven, Germany) in 1 L beakers with sterile filtered seawater (0.2 µm, Sartobran 300, Sartorius, Göttingen, Germany) enriched with Provasoli nutrient solution (PES) under continuous aeration. Culture temperatures were 0°C for polar isolates, 9°C for temperate isolates, and 21°C for tropical isolates. PAR with $50 \pm 10 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ was provided by fluorescent tubes in a 16:8 light:dark (L:D) cycle (polar isolates: OSRAM L36/10, Daylight, Munich, Germany; temperate isolates: OSRAM L58W/840, Lumilux, cool white; tropical isolates: OSRAM, L58W/830, Lumilux, warm white).

Field collections. Fresh material of field-grown thalli was collected in the Kongsfjord and at a bird cliff close to Ny Ålesund (Spitsbergen, Norway) in August 2006 and 2008, at a beach in Kiel Bight (Germany) in July 2006 and May 2008, at low tide from the shore of Helgoland (Germany) in June 2007, at Cromarty (Scotland) in September 2007, and at Roscoff (France) in October 2007 (Table S2 in the supplementary material). In the lab, all material was washed thoroughly with seawater and checked under a binocular eyepiece for epiphytes and other macroalgal contaminations. Analyses of freshly collected thalli were carried out within 36 h after sampling.

UV screening in macrothalli of green algae. The UV screening of algal macrothalli was determined with a Xenon-PAM fluorometer (Walz, Effeltrich, Germany). The measurements followed the method described by Bilger et al. (1997) for the determination of epidermal UV transmittance of leaves from higher plants. Since no epidermis exists in algae, "transmittance" refers in their case to transmittance of the cell wall or the cytoplasm and is in the following called "apparent transmittance" without defining the location of the screening compounds. Screening can be defined as $1 - \text{transmittance}$. This *in vivo* method uses the ratio of chl fluorescence induced by ultraviolet and visible radiation as a relative measure of UV transmittance. Whereas a blue-green reference beam can penetrate into the chloroplasts freely, a UVA or UVB measuring beam will be attenuated if the algal thallus contains UV-absorbing substances in the cell wall or cytoplasm. Therefore, the chl fluorescence induced by UVA or UVB radiation, F_{UVA} or F_{UVB} , is lowered with respect to the fluorescence induced by blue-green excitation, F_{BG} . The setup of optical filters, light source, sample holder, and fluorescence detector followed the descriptions of Burchard et al. (2000). The following filter combinations produced three different excitation light beams: UVB ($\lambda_{\text{max}} = 314 \text{ nm}$, 18 nm halfbandwidth [hbw]): DUG11, GG19, UG11 (2 mm, Schott, Mainz, Germany); UVA ($\lambda_{\text{max}} = 366 \text{ nm}$, 32 nm hbw): DUG11, BG39 (5 mm, Schott), neutral filter foil (LEE Filter, Hampshire, England); and broadband blue-green ($\lambda = 420\text{--}550 \text{ nm}$): BG39, neutral filter foil, UV-blocking filter (Balzers, Liechtenstein). Transmittance spectra of these filters are shown in Bilger et al. (1997). The filters were fixed in a custom-made rotatable mount, which was attached to the Xenon flash lamp and allowed for consecutive exposure of the sample to the three excitation beams. The excitation beams impinged on the sample with an angle of 45°,

and the emitted chl fluorescence was detected at an angle of 45° through RG9, R65, and RG645 filters (2 mm, Schott).

The thalli were kept in the dark 15 min prior to the measurements, and adherent water was removed from the algal surface before mounting on the sample holder. The signal produced by a green nonfluorescing foil was subtracted from all measurements. All signals were normalized with respect to those obtained upon excitation of a blue plastic foil with emission properties similar to those of chl (fluorescence standard, Walz). From the fluorescence signals F_{UVA} , F_{UVB} , and F_{BG} , the ratios F_{UVA}/F_{BG} and F_{UVB}/F_{BG} were calculated as a relative measure for the transmittance of UVA and UVB radiation of the thalli, respectively.

In total, culture and field material of 71 species and isolates from polar to tropical habitats from six taxonomic orders were investigated. The apparent transmittance of the thalli, T_{UVA} and T_{UVB} , was obtained by relating the calculated fluorescence ratios F_{UVA}/F_{BG} and F_{UVB}/F_{BG} to a 100% reference value of unprotected samples (Markstädter et al. 2001). This reference was measured as the maximal chl fluorescence ratios F_{UVA}/F_{BG} and F_{UVB}/F_{BG} in isolated chloroplasts from six different species of marine green macroalgae.

Chloroplast isolation. Chloroplast isolation was performed following the protocol by Redebjerg et al. (2006) with some modifications. All steps were conducted under dim light and on ice. Included species were *Acrosiphonia sonderi*, *Acrosiphonia coalita*, *Blidingia minima*, *Rhizoclonium riparium*, *Ulva clathrata*, and *Ulva intestinalis*. Material from *A. sonderi*, *A. coalita*, *R. riparium*, and *U. clathrata* was taken from a culture at 9°C. *U. intestinalis* was freshly collected at the Kiel Canal, *B. minima* was collected at Helgoland, and chloroplasts were isolated after 1 month of cultivation at 9°C. Small pieces of thalli with a total fresh weight of 4–15 g were rinsed three times with sterile filtered seawater, cut into pieces of ~1 mm² with a razor blade, and ground with mortar and pestle under addition of 15–30 mL isolation buffer (1.2 M sorbitol except 0.8 M for Baltic *U. intestinalis*, 50 mM Hepes buffer, pH = 8.0). The homogenate was filtered through a nylon mesh with a pore size of 10 µm, and the filtrate centrifuged 6 min with 500g at 4°C (Megafuge 1.0 R; Heraeus, Osterode, Germany) to remove cell debris. The supernatant was centrifuged 5 min with 4,500g at 4°C to collect the chloroplasts. Chloroplasts were resuspended in isolation buffer and were checked for cell-wall remains under a light microscope (model 46 70 85; Carl Zeiss, Oberkochen, Germany).

Fluorescence excitation ratios of chloroplasts. The chloroplast suspensions with different concentrations were layered on filter paper (Sartorius) and kept dark and cool until dried. F_{UVA}/F_{BG} and F_{UVB}/F_{BG} were measured as described for whole thalli.

Chl extraction. Immediately after preparation, the chl concentration of each chloroplast suspension was determined in 100% methanol with the equations from Porra et al. (1989). A total of 10 µL of chloroplast suspension was extracted in two steps with a total volume of 990 µL solvent. The absorbance at 652.0, 665.2, and 750 nm of unified extracts was measured in an UVIKON spectrophotometer (Kontron Inst., Watford, England). From each chloroplast suspension, a dilution series with final chl concentrations from 0.05 to 5 µg chl · µL⁻¹ was prepared using isolation buffer as thinner. Chl content of algal thalli used in Xe-PAM measurements was determined in four species to compare these concentrations with the chl concentrations of the chloroplast suspensions. From *C. tortuosa*, *U. clathrata*, *U. intestinalis*, and *Ulothrix implexa*, a fresh weight between 25 and 80 mg was placed on the 1.5 cm² sample holder of the Xe-PAM fluorometer, and UV transmittances were registered. Afterward, samples were frozen in liquid nitrogen, homogenized using a cell mill (MM2; Retsch, Haan, Germany), and extracted in two steps adding a total volume of

1.5 mL 100% methanol with a spatula tip of 4 MgCO₃ · Mg(OH)₂ · 5H₂O (Merck, Darmstadt, Germany).

UV-exposure experiment. Culture-grown material from four tropical isolates was exposed at 21°C to artificial UV radiation, produced by two Q-Panel UVA-340 fluorescent tubes (Cleveland, OH, USA), for 3 h in the middle of the light period over 8 d. Included isolates were *Cladophoropsis membranacea* from Hainan, *Cladophoropsis herpestica* from Syria, *Boergesenia forbesii* from Hainan, and *Cladophora vagabunda* from Curaçao. Day-light-fluorescent tubes (Philips, HI-VISION, New York, NY, USA) generated PAR (45–48 µmol photons · m⁻² · s⁻¹) in a 16:8 L:D cycle. Irradiance spectra were recorded with a Bentham DM 150 spectroradiometer (Bentham Instruments LTD, Berkshire, UK). From 286 nm to 313 nm, the respective biologically effective UVB doses (UVB_{BE}) were calculated using the weighting function of the generalized action spectrum for plant damage from Caldwell (1971) normalized at 300 nm after Green et al. (1974). UV irradiances were 7.3 W · m⁻² UVA, 0.48 W · m⁻² UVB, and 0.63 kJ · m⁻² · d⁻¹ UVB_{BE}. Low PAR background values resulted in unnatural high UV/PAR ratios but were used to avoid high-light stress in low-light cultured algae. Algal samples were allowed to acclimate to experimental conditions 1 d prior to the start of the UV treatment. Exposure was in 50 mL petri dishes covered with WG 280 glass filters (5 mm, Schott), or for control samples with GG 400 filters (5 mm, Schott). The respective transmittance spectra of these filters are shown in Bischof et al. (2002). Culture medium was replaced every third day. UV transmittance of algal thalli was measured before the first UV exposure and after 8 d. Photochemical efficiency of PSII was determined with a PAM-101 chl fluorometer (Walz). To access the degree of photoinhibition, optimal photochemical quantum yield (F_v/F_m) was measured on samples predarkened for 30 min. For determination of F_0 , measuring light intensity ranged from 0.3 to 1.2 µmol photons · m⁻² · s⁻¹, and a saturation pulse of 0.8 s with an intensity >7,000 µmol photons · m⁻² · s⁻¹ was used to obtain F_m . Photosynthetic efficiency was recorded at day 0 and day 8 after the last UV treatment.

Statistical analysis. In the survey of cultured and field-grown algae, apparent UV transmittances were tested for a significant difference to 1.0 applying a *t*-test (Microsoft Excel; Microsoft Corp. Redmond, WA, USA). Further, screening data from cultured isolates were analyzed with a main factor analysis of variance with phylogenetic position, climatic origin, and habitat as factors, and transmittances as response variables (Statistica; Statsoft, Hamburg, Germany). Values for UVA transmittances were Box-Cox transformed with lambda = 3.034 to derive normal distribution. If a significant effect was observed, Tukey's test was used as a post hoc comparison to determine the differences of the means. For analysis of UV screening in field-collected material, of maximal fluorescence ratios in chloroplasts and the results of the UV-exposure experiment, a one-way analysis of variance (ANOVA) was performed, again with Tukey's test as a post hoc comparison (Sigmastat; Systat, Erkrath, Germany). If not otherwise stated, a level of probability of $P \leq 0.05$ was applied.

RESULTS

Maximal fluorescence ratios in algal chloroplasts. Isolated chloroplasts from six species were measured to obtain reference values for the fluorescence ratios F_{UVA}/F_{BG} and F_{UVB}/F_{BG} in the absence of screening. These ratios of the chloroplast suspensions from *A. coalita*, *A. sonderi*, *B. minima*, *R. riparium*, *U. clathrata*, and *U. intestinalis* varied in a narrow range from 2.135 to 2.496 for UVA, and from 1.390 to 1.697 for

TABLE 1. Mean F_{UVA}/F_{BG} and F_{UVB}/F_{BG} (\pm SD) in isolated chloroplasts from six species; different letters denote significantly different values ($P < 0.05$).

Species	F_{UVA}/F_{BG}	F_{UVB}/F_{BG}
<i>Acrosiphonia coalita</i> (n = 17)	2.439 \pm 0.139 ^a	1.444 \pm 0.100 ^{bc}
<i>A. sonderi</i> (n = 14)	2.382 \pm 0.087 ^a	1.548 \pm 0.084 ^b
<i>Blidingia minima</i> (n = 13)	2.396 \pm 0.235 ^a	1.390 \pm 0.145 ^c
<i>Rhizoclonium riparium</i> (n = 4)	2.135 \pm 0.148 ^b	1.405 \pm 0.117 ^{bc}
<i>Ulva clathrata</i> (n = 13)	2.496 \pm 0.096 ^a	1.657 \pm 0.093 ^a
<i>U. intestinalis</i> (n = 22)	2.460 \pm 0.103 ^a	1.697 \pm 0.046 ^a
Mean	2.422 \pm 0.154	1.553 \pm 0.152

UVB radiation (Table 1). The highest ratios were found in chloroplasts from *U. clathrata* for both UVA and UVB radiation. The lowest F_{UVA}/F_{BG} occurred in *R. riparium*, and F_{UVB}/F_{BG} in *B. minima*. The algal chloroplast suspensions showed decreasing fluorescence ratios with increasing chl concentrations in the measuring beam (Fig. 1). But as this influence was minor, the mean values for F_{UVA}/F_{BG} and F_{UVB}/F_{BG} over the whole range of chl concentrations were taken as reference values. They amounted to 2.422 ± 0.154 and 1.553 ± 0.152 , respectively. Declining fluorescence ratios with increasing chl content in the measuring beam have been described already by Bilger et al. (1997) for UVA radiation in isolated spinach chloroplasts. However, these authors also reported increasing F_{UVB}/F_{BG} with increasing chl concentration, which was not reproduced in this study. For all species in the survey, UV screening was calculated by dividing measured fluorescence ratios by these mean reference values, or, if this was available, by the respective value from the chloroplasts of the given species.

UV screening of cultured green algae. In the survey of algal macrothalli from the culture of 46 species and isolates of marine green algae, a wide range of apparent UVA and UVB transmittances was observed (Table 3). Different fresh weight quantities and corresponding chl contents in the range from 20 to 300 $\mu\text{g chl} \cdot \text{cm}^{-2}$ in the Xenon-PAM measurements had no significant influence on the fluorescence ratios F_{UVA}/F_{BG} and F_{UVB}/F_{BG} as tested in four different species (data not shown). Therefore, the fresh weight and chl content were not controlled in the remaining investigated isolates. In 17 isolates, apparent UVA transmittance was significantly <1.0 , and in 23 isolates, this was the case for apparent UVB transmittance. This finding means 64% and 50% of the algae had no significant screening of UVA and UVB radiation, respectively. There was a significant effect of phylogenetic position on UVA and UVB screening ($P < 0.001$, analysis of variance for main effects, see Table 2 and Fig. 2). UVA screening was also influenced by climatic origin ($P < 0.01$), which is indistinguishable from growth temperature in this study. Unexpectedly, polar and tropical isolates, growing at 0°C and 21°C, had identical levels of UVA screening, while

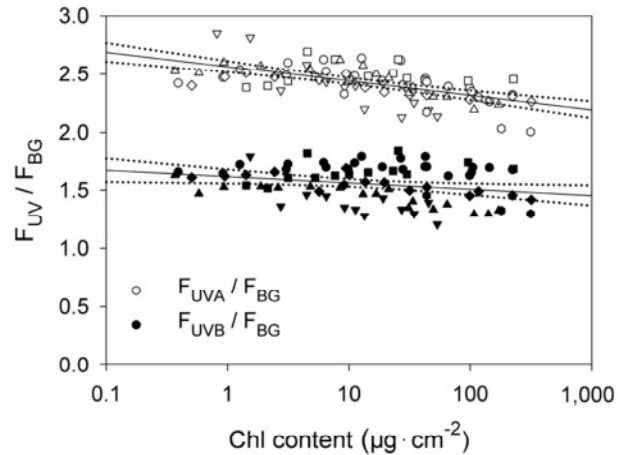


FIG. 1. F_{UVA}/F_{BG} (open symbols) and F_{UVB}/F_{BG} (filled symbols) in chloroplasts from *Acrosiphonia coalita* (triangles up), *Acrosiphonia sonderi* (diamonds), *Blidingia minima* (triangles down), *Rhizoclonium riparium* (hexagonals), *Ulva intestinalis* (circles), and *Ulva clathrata* (squares) against chl concentration on a log scale. Solid lines: $F_{UVA}/F_{BG} = 2.57 - (0.124 \cdot x)$, $r^2 = 0.369$; $F_{UVB}/F_{BG} = 1.62 - (0.054 \cdot x)$, $r^2 = 0.0696$; and dotted lines, 95% confidence intervals. F_{UVA} or F_{UVB} , the chl fluorescence induced by UVA or UVB radiation; F_{BG} , the fluorescence induced by blue-green excitation.

TABLE 2. *P* values from main factor analysis of variance for survey data from culture isolates with order, climatic origin, and habitat as factors, and UVA and UVB transmittance as response variable.

	<i>P</i> value	
	T_{UVA}	T_{UVB}
Order	<0.001	<0.001
Climatic origin	<0.01	0.349
Habitat	0.703	0.943

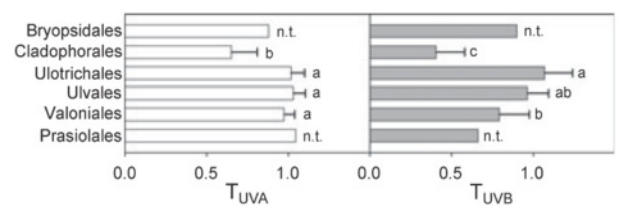


FIG. 2. Mean apparent UVA transmittance (white bars) and UVB transmittance (gray bars) (\pm SD) from culture samples from Bryopsidales (n = 1), Cladophorales (n = 6), Ulotrichales (n = 17), Ulvales (n = 9), Valoniales (n = 11), and Prasiolales (n = 2). Letters denote significantly different values from Tukey's test ($P < 0.05$); n. t., not tested. T_{UVA} or T_{UVB} , apparent transmittance for UVA or UVB radiation.

temperate isolates were less protected against UVA radiation. Habitat with regard to the vertical distribution of the species had no significant influence on UV screening ($P = 0.88$ and 0.96 for UVA and UVB, respectively, Table 2). For the majority of examined species from the orders Ulotrichales and

TABLE 3. Mean apparent UVA and UVB transmittances (T_{UVA} and T_{UVB}) (\pm SD) in 46 cultured isolates.

Species, cultured material	Origin	T_{UVA}	SD	T_{UVB}	SD
Class Bryopsidophyceae					
Order Bryopsidales					
<i>Lambia antarctica</i>	Potter Cove	0.879**	0.033	0.899*	0.058
Class Ulvophyceae					
Order Cladophorales					
<i>Chaetomorpha tortuosa</i>	Disco Island	0.676***	0.065	0.519***	0.094
<i>Chaetomorpha tortuosa</i> b	Disco Island	0.473***	0.023	0.242***	0.036
<i>Cladophora flexuosa</i>	Teneriffe	0.655***	0.010	0.384***	0.023
<i>Cladophora submarina</i>	Bonaire	0.803***	0.010	0.588***	0.008
<i>Cladophora vagabunda</i>	Curacao	0.839***	0.039	0.541***	0.049
<i>Rhizoclonium riparium var. validum</i>	Disco Island	0.460***	0.022	0.151***	0.019
Order Ulotrichales					
<i>Acrosiphonia arcta</i>	Faroe Islands	0.933*	0.048	0.998	0.065
<i>Acrosiphonia arcta</i>	Helgoland	0.901*	0.074	0.963	0.126
<i>Acrosiphonia arcta</i>	Helgoland	1.009	0.032	1.047	0.033
<i>Acrosiphonia arcta</i>	Helgoland	1.009	0.023	1.085	0.036
<i>Acrosiphonia arcta</i>	Roscoff	1.079	0.018	1.227	0.031
<i>Acrosiphonia coalita</i>	Friday Harbor	1.017	0.016	1.186	0.033
<i>Acrosiphonia sonderi</i>	Helgoland	1.063	0.029	1.087	0.066
<i>Acrosiphonia sonderi</i>	Kongsfjord	0.823***	0.032	0.829**	0.049
<i>Acrosiphonia sonderi</i>	Japan	1.065	0.031	1.100	0.042
<i>Acrosiphonia</i> sp.	Puerto Williams	0.912**	0.025	0.984	0.051
<i>Monostroma arcticum</i>	Kongsfjord	0.969**	0.015	1.010	0.019
<i>Ulothrix flacca</i>	Fuerte Bulnes	1.096	0.013	1.289	0.036
<i>Ulothrix implexa</i>	King-George Island	1.134	0.079	1.458	0.112
<i>Urospora penicilliformis</i>	Friday Harbor	1.065	0.009	0.887***	0.013
<i>Urospora wormskioldii</i>	Helgoland	1.016	0.019	0.865***	0.015
<i>Urospora wormskioldii</i>	Halifax	1.088	0.017	0.893***	0.029
<i>Spongomorpha aeruginosa</i>	Grotta Island	1.093	0.029	1.265	0.062
Order Ulvales					
<i>Ulva bulbosa</i>	King-George Island	1.057	0.035	0.826***	0.037
<i>Ulva clathrata</i>	Puerto Williams	0.926***	0.012	0.675***	0.026
<i>Ulva clathrata</i>	Disco Island	1.045	0.018	0.999	0.017
<i>Ulva compressa</i>	Kongsfjord	0.899	0.111	0.991	0.112
<i>Ulva compressa</i>	Hainan	1.139	0.007	0.980	0.020
<i>Ulva fasciata</i>	Hainan	1.064	0.024	1.060	0.051
<i>Ulva intestinalis</i>	Disco Island	1.078	0.035	1.069	0.073
<i>Ulva lactuca</i>	Disco Island	1.036	0.008	0.995	0.032
<i>Ulva rigida</i>	Puerto Williams	1.030	0.056	1.070	0.091
Order Valoniales					
<i>Boergesenia forbesii</i>	Hainan	1.037	0.088	0.943	0.056
<i>Boergesenia forbesii</i>	Hainan	0.961	0.058	0.706***	0.063
<i>Boergesenia forbesii</i>	Hainan	0.955	0.062	0.843**	0.059
<i>Boodlea composita</i>	Hainan	0.945**	0.016	0.673***	0.021
<i>Cladophoropsis herpestica</i>	Hainan	1.074	0.049	0.998	0.070
<i>Cladophoropsis membranacea</i>	Bonaire	0.883***	0.016	0.531***	0.024
<i>Cladophoropsis membranacea</i>	Latakia	0.984	0.022	0.798***	0.043
<i>Cladophoropsis membranacea</i>	Cap Verde Islands	0.930***	0.018	0.658***	0.063
<i>Cladophoropsis membranacea</i>	Red Sea	0.971	0.041	0.643***	0.086
<i>Dictyophaeria cavernosa</i>	Hainan	0.883**	0.031	0.752**	0.076
<i>Struvea</i> sp.	Hainan	1.076	0.075	1.162	0.133
Class Trebouxiophyceae					
Order Prasiolales					
<i>Prasiola crispa</i>	Alps	1.004	0.066	0.610***	0.062
<i>Prasiola crispa</i> ssp. <i>antarctica</i>	King-George Island	1.085	0.011	0.712***	0.041

$n \geq 5$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Ulvales, UVA and UVB screening was absent, or, if present significantly, only very low. From these, the polar isolate of *U. clathrata* from Puerto Williams, Chile, cultured at 0°C showed the lowest apparent UVB transmittance with 0.675, which is equivalent to a screening of 32.5%. In the examined Prasiolales, UVB screening attained ~34%, while UVA radiation was not screened. Apparent transmittances for

UVA radiation were reduced to 0.460, and for UVB radiation to 0.151, corresponding to a screening of 54% and 85% in species from the order Cladophorales. With regard to apparent UVA and UVB transmittance, the Cladophorales stood out from most groups ($P < 0.001$, Fig. 2) and were only indistinguishable from the Bryopsidales and Prasiolales. This latter result is caused by the fact that too low a

number of isolates was investigated in the Bryopsidales and Prasiolales, thereby rendering a statistical differentiation impossible. The Valoniales had significant UVB screening with values down to an apparent transmittance of 0.531 in *C. membranacea* from Bonaire, an island of the Netherlands Antilles. UVA screening was negligible.

UV screening of field-collected algae. The survey of field-collected macrothalli of 25 species and isolates from five different sites showed similar screening as in the cultures for all orders, except for the Ulotrichales and Prasiolales (Table 4, Fig. 3). Collections originated exclusively from supra- or eulittoral positions. Therefore, and because no tropical species were sampled in the field, field data were analyzed solely with regard to phylogeny. *Acrosiphonia arcta* and *Urospora penicilliformis* from the Ulotrichales displayed very high UVB screening in the field, contrasting with their high apparent transmittances found under culture conditions. In other species from the Ulotrichales, like *Monostroma arcticum*, high apparent transmittances were observed in the field, resulting in negligible differences between cultured and field-collected material. In the Prasiolales, both samples from the field displayed UVB screening,

more than twice as high as under culture conditions. But the apparent UVA transmittance was not reduced considerably in comparison to cultured specimens in this group. With regard to the orders Ulvales and Cladophorales, there was no statistical difference for the extent of apparent UVA or UVB transmittance between field and culture material. In the Ulvales, screening varied between 0% and 30% for UVBR (Table 4). Apparently, members of the Ulvales are principally able to synthesize UV-screening

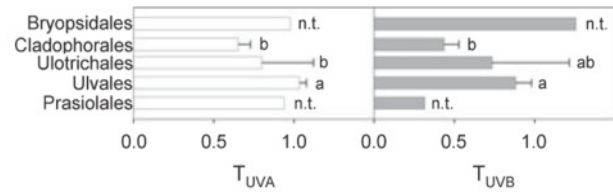


FIG. 3. Mean apparent UVA transmittance (white bars) and UVB transmittance (gray bars) (\pm SD) from field material from Bryopsidales ($n = 1$), Cladophorales ($n = 6$), Ulvales ($n = 11$), Ulotrichales ($n = 5$), and Prasiolales ($n = 2$). Letters denote significantly different values from analysis of variance (ANOVA) followed by Tukey's test ($P < 0.001$); n.t., not tested. T_{UVA} or T_{UVB} , apparent transmittance for UVA or UVB radiation.

TABLE 4. Mean apparent UVA and UVB transmittances (T_{UVA} and T_{UVB}) (\pm SD) in 25 field-collected samples.

Species, field-collected material	Origin	T_{UVA}	SD	T_{UVB}	SD
Class Bryopsidaceae					
Order Bryopsidales					
<i>Codium fragile</i>	Roscoff	0.975*	0.034	1.256	0.066
Class Ulvophyceae					
Order Cladophorales					
<i>Chaetomorpha aerea</i>	Helgoland	0.706***	0.027	0.520***	0.066
<i>Chaetomorpha aerea</i>	Roscoff	0.666***	0.021	0.426***	0.088
<i>Chaetomorpha melagonium</i>	Roscoff	0.684***	0.044	0.430***	0.064
<i>Cladophora rupestris</i>	Helgoland	0.702***	0.020	0.483***	0.055
<i>Cladophora rupestris</i>	Roscoff	0.655***	0.029	0.494***	0.059
<i>Cladophora serica</i>	Kiel	0.500***	0.023	0.263***	0.018
Order Ulotrichales					
<i>Acrosiphonia arcta</i>	Helgoland	0.266***	0.018	0.088***	0.012
<i>Acrosiphonia arcta</i>	Roscoff	0.543***	0.050	0.306***	0.070
<i>Monostroma arcticum</i>	Spitsbergen	1.045	0.069	1.149	0.039
<i>Ulvaria obscura</i>	Spitsbergen	1.038	0.034	1.128	0.039
<i>Urospora penicilliformis</i>	Spitsbergen	0.918***	0.044	0.55***	0.044
Order Ulvales					
<i>Blidingia marginata</i>	Roscoff	1.069	0.032	0.766***	0.029
<i>Blidingia minima</i>	Helgoland	1.002	0.030	0.895**	0.046
<i>Ulva clathrata</i>	Helgoland	0.994	0.014	0.918**	0.028
<i>Ulva intestinalis</i>	Helgoland	1.080	0.012	0.816***	0.024
<i>Ulva intestinalis</i>	Kiel	1.089	0.045	0.697***	0.061
<i>Ulva intestinalis</i>	Cromarty	0.991	0.016	0.869***	0.032
<i>Ulva lactuca</i>	Helgoland	1.031	0.047	0.830***	0.075
<i>Ulva linza</i>	Helgoland	1.042	0.018	0.916***	0.028
<i>Ulva prolifera</i>	Helgoland	0.943***	0.010	0.941**	0.019
<i>Ulva ramulosa</i>	Roscoff	1.055	0.023	1.000	0.017
<i>Ulva rigida</i>	Roscoff	1.059	0.036	1.035	0.036
Class Trebouxiophyceae					
Order Prasiolales					
<i>Prasiola crispa</i>	Spitsbergen	0.987	0.027	0.380***	0.024
<i>Prasiola stipitata</i>	Helgoland	0.890***	0.009	0.249***	0.013

$n \geq 5$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

substances but don't exceed a certain low content even under natural UV levels (Fig. 3). The Cladophorales seem to express a constitutive high level of UVA- and UVB-screening substances as all investigated cultured isolates and field samples shared a very efficient protection capacity.

Relationship of UV resistance and UVB transmittance. During an 8 d period, samples of four isolates were exposed to experimental UVR. Apparent UV transmittances were determined before and after the treatment as well as F_v/F_m . UV screening showed some acclimation with a reduction in apparent UVB transmittance of 0.22 only in *B. forbesii* (data not shown). Apparent transmittances and

damage to PSII after the treatment period are shown in Figure 4. The two species with a significantly better UVB photoprotection were also significantly less damaged. Corresponding observations were also obtained with temperate and polar isolates (data not shown). However, 50% UVB screening was still not sufficient to completely avoid damage under the given experimental conditions (Fig. 4). There seemed to exist no correlation between damage and UVA transmittance as the latter was high in all species and displayed a different pattern (Fig. 4a).

DISCUSSION

Establishment of the method in green macroalgae. In order to assess potential UV-screening mechanisms in green algae, a chl fluorescence method was used, which had been originally introduced for higher plants. So far, this method has been applied only once for phytoplankton (Mohovic et al. 2006), but not in macroalgae. In higher plant leaves, screening compounds are predominantly located in the chl-free epidermis (Hutzler et al. 1998), thereby assuring that the fluorescence excitation beam has to pass the potential screening compounds before it can induce fluorescence from chl. In algae, no epidermis exists, and the only location for screening substances to efficiently hinder chl excitation is the cell wall. On the other hand, it has often been assumed that MAAs located within the algal cells could serve as UV-screening compounds (Garcia-Pichel et al. 1993, Garcia-Pichel 1994, Neale et al. 1998). In rye leaves, no indication for a substantial screening by flavonoids located in the mesophyll was detected (Burchard et al. 2000). However, it has recently been demonstrated that anthocyanins located in the palisade parenchyma of leaves (i.e., side by side with chloroplasts) can lower the fluorescence yield of a green excitation beam (L. Nichelmann and W. Bilger, unpublished data). This may be due to the strong light scattering within tissues and makes the screening function of intracellular compounds feasible. For *Prasiola*, MAAs have been documented (Post and Larkum 1993), and the reduction in apparent transmittance observed in this alga in our study may be explained by their presence.

In order to interpret the fluorescence data quantitatively (i.e., calculate screening or apparent transmittance), it is necessary to use the fluorescence excitation ratio of a reference object of which the absence of screening is known. Such an object is isolated chloroplasts. We have here established reference values using isolated chloroplasts from the algae. These values lie well in the range of measurements of leaves without epidermis and isolated chloroplasts from higher plants (Nybakken et al. 2004b, data not shown). This good agreement supports the suitability of the method for green macroalgae. The

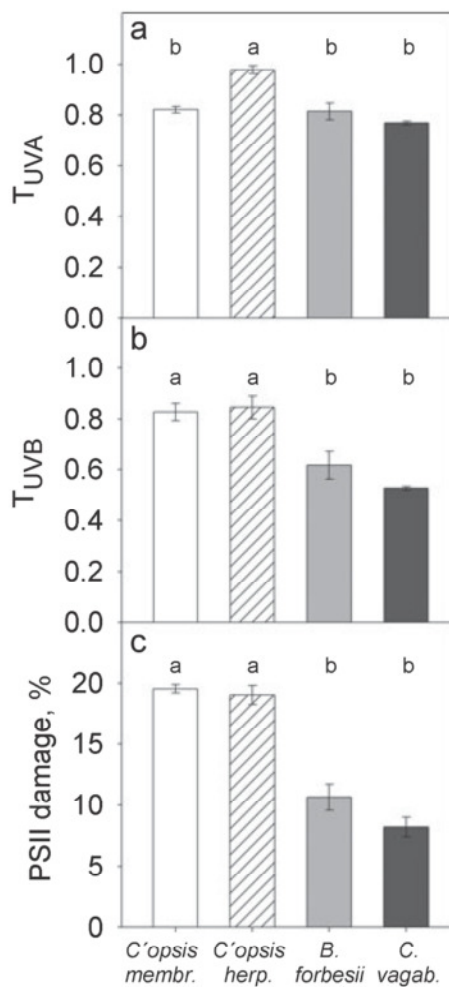


FIG. 4. Apparent UVA (a) and UVB (b) transmittance and PSII damage (c) of four tropical isolates after 8 d culture with UVB irradiation. PSII damage was calculated with respect to a control treated with only PAR ($45 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) in the absence of UVA and UVB. UV irradiances were $7.3 \text{ W} \cdot \text{m}^{-2}$ UVA and $0.48 \text{ W} \cdot \text{m}^{-2}$ UVB. Data are means \pm SE; $n = 4$. Letters denote significantly different values from analysis of variance (ANOVA) followed by Tukey's test ($P < 0.01$). T_{UVA} or T_{UVB} , apparent transmittance for UVA or UVB radiation. *C'opsis membr.*, *Cladophoropsis membranacea*; *C'opsis herp.*, *Cladophoropsis herpeticica*; *B. forbesii*, *Boergesenia forbesii*; *C. vagab.*, *Cladophora vagabunda*.

variance between chloroplasts from six species was low, and it may be assumed that they can be used as representative for 100% transmittance for all green macroalgae (Table 2). Some of the variation in the F_{UV}/F_{BG} ratios may be caused by differences in pigment composition between the species (see below). Surprisingly, it was observed that fluorescence ratios in the thalli were often higher than in isolated chloroplasts of the same species. This was probably not due to differences in chl concentration between the chloroplast preparation and the algal sample. The chl concentration of the investigated chloroplast samples extended well over that of the intact algae used. Furthermore, for whole thalli there was no significant effect of chl concentration at all (data not shown). Rather, the effect indicates the presence of optical differences between intact thalli and chloroplast suspensions applied to white filter paper. Such differences might arise from differential light scattering of the intact thalli and the isolated chloroplasts. As long as the structures scattering the light are distinctly larger than the wavelength of the light, Mie scattering occurs, which may have a small wavelength dependence (Gates 1980). Algal cells, filter paper, and even chloroplasts will probably only cause Mie scattering. Increased Mie scattering in the reference measurements on filter paper, as compared to intact algae, might have reduced UV-induced, more than blue-induced, fluorescence in relation to the differential scattering at these wavelengths. As this contribution was presumably rather small, the procedure for the reference measurements would still yield acceptable results. However, very small particles in the cells induce Rayleigh scattering, which increases strongly with decreasing wavelength. As soon as there was a difference in the contribution of Rayleigh scattering, between intact thalli and chloroplasts, the latter would not serve any longer as a good reference for 100% transmittance.

On the other hand, Mie scattering may add to UVB protection by scattering this radiation away from the thallus surface before it enters the cells. This possibility would not be detected by the fluorescence method, as this scattering affects the UV and blue spectral region equally. Accordingly, the method might underestimate UV protection in finely structured thalli with high surface-to-volume ratios. Also, protection by scattering by very finely structured particles causing Rayleigh scattering would partially be missed. However, as the ratios between fluorescence excited by a BG and a red beam were constant among algal samples with widely different apparent transmittance (data not shown), we assume that Rayleigh scattering was not contributing to the observed differences in F_{UV}/F_{BG} ratios.

The observed low F_{UV}/F_{BG} ratios with chloroplasts would lead to an underestimation of UV protection in whole thalli. For *A. coalita*, this discrepancy was

most pronounced, and a deviation of 7%–8% between calculated and actual UVB screening is possible for this species. Such effects may also explain the very high transmittance values observed in some of the Ulotrichales. In total, an accuracy of screening determined by the fluorescence method of ~10% may be assumed.

Absorption of blue-green measuring light without energy transfer to chl *a* would lower the blue-green-induced chl fluorescence and lead to a drastic underestimation of UV screening. Therefore, pigments like carotenoids, which absorb much more in the visible than in the UV region and are not or only loosely associated with the light-harvesting complex of PSII, would cause problems with the method (Bilger et al. 1997, Pfündel et al. 2007). Siphonocladal and siphonal algae from the Chlorophyceae and also some species from the Ulvales contain an additional carotenoid, siphonaxanthin (and/or its esterified form siphonein), which absorbs in the green region of the spectrum (~540 nm) (Lüning and Dring 1985, Smith and Alberte 1994). This carotenoid has a very efficient energy transfer to chl *a* in the reaction centers (Anderson 1985), and a reduced chl fluorescence emission due to blue-green absorption by this carotenoid is not probable. For example, *Codium fragile* has a flat action spectrum of photosynthesis with no minima in the green region (500–550 nm) due to the presence of siphonaxanthin (Lüning and Dring 1985). However, high transmittance values >1.0 point to a possible interference of carotenoids with the blue-green excitation beam.

UV screening in cultured and field-grown algae. In a first approach, UV transmittance was measured from algae grown under controlled culture conditions. As only low light and no UVR were applied in culture, a baseline of apparent UV transmittance for all included species was obtained. This means all observed differences are genetically fixed and provide insight into constitutive screening mechanisms excluding phenotypic variability. With this, a clear phylogenetic pattern was revealed, but no adaptive differences between polar and tropical isolates were detected. For the first time, it is shown that algae from the Cladophorales display a considerable UVB screening. In other studies, the UV sensitivity of polar and cold-temperate macroalgae (Michler et al. 2002) and natural phytoplankton communities (Mohovic et al. 2006) was also not closely related to their latitudinal distribution. In phytoplankton, UV protection through screening depended definitively more on water quality (Mohovic et al. 2006). In phaeophytes, higher contents of phloroglucinol, which possesses UV-absorbing capacities, were observed in temperate as compared to tropical algae (van Alstyne and Paul 1990). Also, an accumulation of UV-absorbing trihydroxycoumarins (THCs) in the green alga *Dasycladus vermicularis* during the winter months points to an inverse relationship of screening with temperature (Gomez et al. 1998).

As a second approach, field-grown material of 25 species with a large overlap with investigated cultured species was examined. Higher UV screening observed in field-collected samples is pointing to an acclimation potential in some species of the Ulotrichales, Prasiolales, and partially also in the Ulvales. Within the Ulotrichales, in *Acrosiphonia* and *Urospora*, species of the Ulotrichaceae, UVA and UVB screening was increased in field samples. In contrast, for *Monostroma* and *Ulvaria*, members of the Gomontiaceae, high apparent transmittances were observed in the field. Therefore, a specific acclimation potential differentiated at a closer degree of kinship than order could be an explanation for this. While in the Ulotrichales UVA and UVB screening was increased in field samples, in the Prasiolales and Ulvales, only UVB screening was responding, while UVA screening remained unaffected. This indicates the presence of UVB-absorbing pigments without significant UVA absorption under field conditions. *Prasiola crista* and close relatives contain MAAs with maximum absorption at ~ 325 nm (Sivalingam et al. 1974, Karsten et al. 2005). This compound should be detected by the UVB-measuring beam with a wavelength of ~ 314 nm. Other species, like *A. arcta*, *U. penicilliformis*, and *U. intestinalis*, lack MAAs (Karsten et al. 1998). While this agrees with the absence of acclimation in *U. intestinalis*, one has to conclude that *Acrosiphonia* and *Urospora* species use other screening compounds of hitherto unknown chemical nature. Due to these differences in culture and field samples for the Prasiolales and Ulotrichales, an accumulation of UV-screening substances apparently dependent on growth conditions is probable. In several species, an attempt was made to induce UV screening in culture by UVBR. This was not successful. Possibly, the applied UVB irradiance was not sufficient. Natural levels of UVBR vary considerably between tropical and polar regions and with water depth and quality, but the applied irradiances were always somewhat lower, although being already strong enough to induce damage. Therefore, it may be speculated that acclimation of screening is controlled by other factors, such as PAR or temperature.

No difference in UV protection was observed for the Cladophorales from culture and field material. A constitutive high expression of screening pigments is therefore probable. Alternatively, if pigment synthesis was controlled by light, its induction already at $50 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ white light must be postulated. An additional function of UV-absorbing substances, such as protection against herbivores or epiphytes or an antimicrobial function, will lead to a distribution pattern not directly correlated to possible UV stress at the natural habitat (Menzel et al. 1983, van Alstyne and Paul 1990). Other abiotic factors, like salinity and temperature, have been shown to induce the excretion and accu-

mulation of UV-absorbing coumarins in *Dasycladus vermicularis* (Perez-Rodriguez et al. 2001).

Generally, UV screening in green algae was low or even absent in most of the examined species even when they were exposed to natural UVB irradiance. Whereas in some species, UVB screening may not be necessary since they live in depths where UVBR is negligible, for the supra- and eulittoral species, this finding is rather surprising. Even in those species, where screening was detected, such as *C. tortuosa* or *U. penicilliformis*, its extent was far lower than that encountered in higher plants exposed to similar UVB irradiances. Accordingly, these species have to employ additional resistance mechanisms.

UV resistance. UV resistance was assessed by the degree of photoinhibition caused by experimental UV exposure in four tropical isolates. As the degree of photoinhibition correlated much better with UVB screening than UVA screening, damage seemed to be predominantly caused by UVB. In a comparison of *Ulva procerca* and *Cladophora glomerata* from the Baltic, photoinhibition in *Ulva* was determined to be about four times stronger after experimental UV exposure (Choo et al. 2005). In our survey, *Ulva* species proved to have low UV screening, whereas *Cladophora* species showed constitutively high screening. Species distributed in the eulittoral, without screening and further cellular tolerance mechanisms must apply other strategies than UV screening to establish successful populations in the intertidal. It has been shown in *Ulva rotunda* that due to the canopy formation thalli below the uppermost layer are protected from UV stress due to selective filtering of UV radiation by bleached covering thalli (Bischof et al. 2002). In *Chaetomorpha linum*, filaments are also closely interwoven, but in this species, no UV-induced bleaching and disintegration of the outermost parts occurs (Bischof et al. 2006). Since we found high screening in other species of the genus *Chaetomorpha*, one may speculate that *C. linum* also is employing this resistance mechanism. It can be concluded that 50% screening, as observed in several species of the genus *Chaetomorpha* in our study, is adequate to significantly reduce PSII damage under the given conditions (see also Fig. 4). On the other hand, in *D. vermicularis*, 90% UVA screening by THCs was necessary to significantly lower damage to DNA and photosynthesis (Perez-Rodriguez et al. 2003). This could mean that UVB screening functions more effectively in UV protection than UVA screening.

A comparison of species with different UV screening under natural irradiance levels could elucidate in an ecologically meaningful context the benefit of the detected UV-screening capacity.

UV-absorbing compounds. In the Cladophorales, significant screening was observed in several species (Table 3). The absorbance maximum of the responsible unknown screening substance is probably close

to the UVB region of the spectrum, because observed apparent transmittances for UVBR were always lower than for UVAR. This finding would be consistent with the absorption properties of MAAs exhibiting maxima between 310 nm and 360 nm (Caretto et al. 1990). However, circumstantial observations on UVB and UVA screening in some algae from our study contradict this hypothesis. *Chaetomorpha aerea* sampled from southern Spain had a low content of a single MAA, mycosporine-glycine, with a maximal absorption at 310 nm (Karsten et al. 1998). In comparison, the content of 324 nm MAA in *Prasiola crispa* ssp. *antarctica* was almost 100x higher than in *C. aerea* (Hoyer et al. 2001). In our observations, the apparent transmittance of *C. aerea* from Helgoland or Roscoff varied at ~0.65 and 0.48 for UVAR and UVBR, respectively, while *P. crispa* showed a UVB-transmittance of 0.38. But with respect to UVA transmittance, *P. crispa* had much higher values of 0.9. These findings together suggest that the observed lower transmittance in *C. aerea* is probably not due to the presence of mycosporine-glycine. Furthermore, other species from the Cladophorales, like *C. tortuosa*, *C. melagonium*, and *Cladophora rupestris*, sampled from the field do not contain MAAs but showed strong screening in our hands (Karsten et al. 1998). Therefore, we assume that the measured UV screening in the Cladophorales may not be attributed to mycosporine-glycine but to another additional substance. Its identity and localization is not clear. In the cell walls of green microalgae as well as their zygospores, the occurrence of sporopollenins was reported (Xiong et al. 1995, VanWinkle-Swift and Rickoll 1997). This almost undegradable macromolecule is probably responsible for the observed UV resistance of these organisms (Xiong et al. 1997, Daniel et al. 2007). Potentially, sporopollenins may be present in green macroalgae as well. Whatever the screening compound may be, its screening ability due to absorption may be enhanced by light scattering caused by the macromolecular structure of the compound itself and the cell walls. It has been shown that in light-scattering suspensions the absorbance of chromophores is strongly enhanced (Butler 1962).

CONCLUSION

We have shown that from 71 surveyed green macroalgae, 43 and 28 did not screen UVAR and UVBR, respectively. This supports previous findings (e.g., Karsten et al. 1998) that UV screening can be regarded as not as important in green algae as, for example, in red algae or higher plants. In the orders of the Cladophorales and Valoniales, we consistently detected significant screening for the first time, which was, however, less pronounced than in higher plants (Nybakken et al. 2004a, Bilger et al. 2007, Barnes et al. 2008). UV resistance of these species

confirmed the efficiency of this screening. This result also supports the reliability of the newly applied method to detect screening efficiency. With the new knowledge about UV screening in green macrophytes, comparative UV-exposure experiments can be evaluated more precisely.

We thank Prof. Dr. Christian Wiencke for provision of stock culture material from the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven. Thanks to Dr. Maike Roleda for sampling field specimens at Spitsbergen, and to Henrike Beesk and Sandra Treydte for help with measurements at Spitsbergen. For support with the main factor analysis of variance, we thank Dr. Florian Weinberger, and for help with the species identification in Helgoland and Roscoff, we thank Andreas Wagner. Dr. Mirta Teichberg is thanked for correction of the language.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Investigated isolates from species in culture with geographic origin, climate/culture temperature, and habitat.

Table S2. Investigated species collected in the field with geographic origin, collection site, and date.

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Chapter 3 – UVB-induced DNA and photosystem II damage in two intertidal green macroalgae: distinct survival strategies in UV-screening and non-screening Chlorophyta

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(Manuscript as published in the Journal of Photochemistry and Photobiology B: Biology 132, 85–93 (2014), DOI: 10.1016/j.jphotobiol.2014.02.006)

Abstract

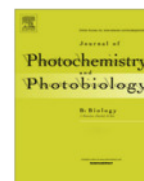
Ultraviolet-B (UVB, 280 – 315 nm) induced accumulation of cyclobutane pyrimidine dimers (CPDs) and deactivation of photosystem II (PS II) was quantified in two intertidal green macroalgae, *Ulva clathrata* and *Rhizoclonium riparium*. The species were chosen due to their shared habitats but contrasting UVB screening potentials. In the non-screening *U. clathrata* CPDs accumulated and PS II activity declined as a linear function of applied UVB irradiance. In *R. riparium* UVB-induced damage was significantly lower than in *U. clathrata*, demonstrating an efficient UVB protection of DNA and PS II by screening. Based on the UVB irradiance reaching the chloroplasts, both species showed an identical intrinsic sensitivity of PS II towards UVB, but DNA lesions accumulated slower in *U. clathrata*. While repair of CPDs was similar in both species, *U. clathrata* was capable of restoring its PS II function decidedly faster than *R. riparium*. In *R. riparium* efficient screening may represent an adaptation to its high light habitat, whereas in *U. clathrata* high repair rates of PS II appear to be important to survive natural UVB exposure. The role of shading of the nucleus by the large chloroplasts in *U. clathrata* is discussed.

Keywords: *Rhizoclonium riparium*; *Ulva clathrata*; cyclobutane pyrimidine dimers; green alga; photosynthesis; repair; ultraviolet radiation; screening



Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

UVB-induced DNA and photosystem II damage in two intertidal green macroalgae: Distinct survival strategies in UV-screening and non-screening Chlorophyta



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ARTICLE INFO

Article history:

Received 30 October 2013

Received in revised form 29 January 2014

Accepted 4 February 2014

Available online 15 February 2014

Keywords:

Rhizoclonium riparium

Ulva clathrata

Cyclobutane pyrimidine dimers

Green alga

Photosynthesis

Repair

Ultraviolet radiation

Screening

ABSTRACT

Ultraviolet-B-induced (UVB, 280–315 nm) accumulation of cyclobutane pyrimidine dimers (CPDs) and deactivation of photosystem II (PS II) was quantified in two intertidal green macroalgae, *Ulva clathrata* and *Rhizoclonium riparium*. The species were chosen due to their shared habitats but contrasting UVB screening potentials. In the non-screening *U. clathrata* CPDs accumulated and PS II activity declined as a linear function of applied UVB irradiance. In *R. riparium* UVB-induced damage was significantly lower than in *U. clathrata*, demonstrating an efficient UVB protection of DNA and PS II by screening. Based on the UVB irradiance reaching the chloroplasts, both species showed an identical intrinsic sensitivity of PS II towards UVB, but DNA lesions accumulated slower in *U. clathrata*. While repair of CPDs was similar in both species, *U. clathrata* was capable of restoring its PS II function decidedly faster than *R. riparium*. In *R. riparium* efficient screening may represent an adaptation to its high light habitat, whereas in *U. clathrata* high repair rates of PS II appear to be important to survive natural UVB exposure. The role of shading of the nucleus by the large chloroplasts in *U. clathrata* is discussed.

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1. Introduction

Ultraviolet-B (UVB, 280–315 nm) radiation comprises only a small part of the solar spectrum but has multiple negative effects on the biosphere. Especially the potential to damage DNA can be critical for the growth and survival of an organism [57,10,26,33]. Different types of DNA lesions are induced by absorbing high energy quanta of the UVB range of the solar spectrum [19,15]. The most frequent ones are cyclobutane-pyrimidine dimers (CPDs) as measured in irradiated isolated DNA as well as in intact cells [22,3]. Other possible DNA lesions are the 6,4 photoproducts (6,4-PPs) which comprise about 25% of the total dimers [49,48]. Both types of lesions impede the progression of DNA and RNA polymerases and therefore limit DNA replication and transcription [69]. As a cytotoxic consequence, UVB-induced DNA damage leads to drastic restrictions in metabolism. Due to imprecise repair or damage bypass by polymerases, DNA damage accumulation can

also exhibit mutagenicity that is clearly related to carcinogenesis [59]. Thus, the quantification of DNA damage is an important parameter for determining the UVB resistance of an organism.

In plants, another sensitive target of UVB radiation is the photosystem II (PS II) complex, especially the manganese cluster of the water splitting apparatus [55,75,77]. After absorption of UVB the manganese ions are released from the cluster and electron flow towards PS II reaction center is inhibited [27]. Furthermore, aromatic components of PS II like the donor to P680, tyrosine, Y₂, or plastoquinones Q_A and Q_B on the acceptor side, may absorb UVB and be damaged, also resulting in impaired electron transport [76]. As a consequence P680⁺ may accumulate and an oxidative chain reaction is activated degrading the associated proteins of PS II, e. g. the D1 reaction center protein [55]. Measuring the photosynthetic efficiency of PS II can therefore give valuable insights into the UV resistance or susceptibility of a plant species.

Resistance against UVB radiation can be achieved either by cellular tolerance mechanisms or by screening of UVB radiation. Cellular tolerance towards UVB radiation depends on the balance between the rates of damage induction and repair processes [43].

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With respect to CPDs a very important UV tolerance mechanism is the so called photoreactivation. During this process the enzyme photolyase is activated by UVA radiation and blue light and splits the covalent bond of a pyrimidine dimer (reviewed in [67,11]). In *Ulva pertusa* this mechanism is suggested to be very efficient in restoring spore germination [28]. In the dark, CPDs can be removed from the DNA strand by nucleotide excision repair (NER) or base excision repair (BER) [68]. However, photoreactivation is much faster than dark repair [56]. The rate of NER and BER depends on factors as type of lesion, methylation, transcription, condensation degree and DNA–histone interaction [9,29,17,40]. For photosynthesis, it was shown that the D1 protein has a higher turnover in UVB tolerant microalgae than in sensitive species [78].

The other type of resistance mechanism, UV screening by photoprotective pigments, is widespread among aquatic and terrestrial phototrophs [21,66,71]. UVB absorbing compounds have been shown to increase the UV resistance of *Arabidopsis thaliana* [44], rice [32], the dinoflagellate *Gymnodinium sanguineum* [53] or the Antarctic moss *Ceratodon purpureus* [20]. On the other hand, most green macroalgae are lacking UV protective compounds with only a few exceptions [38,34]. This is supported by an *in vivo* UV-screening study for a large number of marine green macroalgal species [58]. However, in this study it was also found that species of the order Cladophorales displayed considerable screening capacity for UVB and UVA radiation. So far, it has not been shown that this screening provides photoprotection.

Therefore, susceptibility of two different targets of UVB radiation, DNA and PS II, was investigated in a green macroalga of the order Cladophorales, *Rhizoclonium riparium*. For comparison the non-screening species *Ulva clathrata* (Ulvales) was used. Both species inhabit the upper eulittoral zone from temperate coasts [50]. There, emergence occurs regularly during low tide and the thalli are exposed to direct sunlight, including UVB radiation. For that reason, they are at high risk to accumulate DNA damages or reductions in photosynthetic efficiency [42] and have to employ UVB defense mechanisms. Exposed eulittoral species are reported to be very stress-resistant, especially towards high light stress [30,54] and UVB radiation [4,6,63]. It has been observed that at high photosynthetically active radiation (PAR) the relative contribution of UVB radiation to damage is decreased (reviewed in [23]). Therefore, we deliberately chose an artificial light regime lacking significant proportions of PAR. This approach enables assessing UVB induced damages to PS II separated from PAR driven confounding photoinhibitory effects.

We hypothesized that the UV-screening species *R. riparium* displays a significantly higher resistance than the non-screening species *U. clathrata*. The species-specific response is expected to be correlated to the observed difference in screening. Since both algae share the same habitat, we further hypothesized that *U. clathrata* employs alternative physiological resistance mechanisms to survive UV radiation (UVR) stress, e.g. efficient repair mechanisms.

Furthermore, we also want to highlight a methodological aspect of DNA damage measurements. UVB-induced CPDs have been detected routinely with a well-established dot blot immunoassay in various organisms ([14] (bacterio- and phytoplankton), [61] (macrophytes), [73] (maize)). This chemiluminescence detection assay relies on a calibrated CPD standard that allows obtaining absolute values and comparability between studies. Otherwise only relative data obtained within single blots are comparable (e.g., [74,17]). This limits the investigations severely with respect to sample size and reproducibility. As there was no calibrated CPD standard available, we made the effort to go back to the original protocol of Sinha et al. [72] and created a new standard. As this problem may be encountered also by others we describe in detail how this standard was prepared and how it was used to quantify CPDs.

2. Material and methods

2.1. Algal material

Unialgal cultures of clones from *U. clathrata* (Roth) C. Agardh (order Ulvales) and *R. riparium* (Roth) Harvey (order Cladophorales) were grown in sterile PES medium in a climate chamber at 9 °C and 20–25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (L58 W/830 Daylight, Osram, München, Germany) and a 16/8 h light/dark regime in aerated 1 L glass beakers. Stock cultures of both isolates were kindly provided by Prof. Wiencke from the Alfred-Wegener Institute in Bremerhaven (Isolate numbers: 1086 for *U. clathrata* and 1118 for *R. riparium*; both from Disco Island, Greenland). Hereafter, these species will be referred to as *Ulva* and *Rhizoclonium*.

2.2. Dose response and recovery experiments in *Ulva* and *Rhizoclonium*

Thalli of the two species were simultaneously exposed to artificial UVB radiation produced by fluorescent tubes (TL40/12RS, Philips, Amsterdam, Netherlands) in a growth cabinet (GroBanks, CLF plant climatics, Emersacker, Germany) at 9 °C. In the dose response experiments (experiments D1–4) five different fluence rates ranging from 0 (=control samples) to 10 W m^{-2} UVB_{BE} (weighted after [25]) were applied for 1 h. The UVB fluorescence tubes also emit a constant proportion of visible light which depending on UVB irradiance varied between 2 and 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ as measured with a quantum sensor (LI-190, LI-COR, Lincoln, Nebraska, USA). We also weighted the applied UVB irradiances with the weighting function for DNA damage in alfalfa seedlings of Quate et al. [62] as it was formulated by Musil [52]. This gave virtually the same results and we only show the results from the weighting with the Ghatti spectrum. As the employed lamps emit a spectrum enriched in short wavelength UVB compared to daylight, 1 W m^{-2} weighted UVB from the fluorescence tubes equals 3.15 W m^{-2} unweighted solar UVB. For each species and each UVB irradiance level two open Petri dishes were covered with WG 295 glass filters (Schott, Mainz, Germany) and two Petri dishes with control samples were kept below a UV-blocking Plexiglas filter (GS 321, Röhm, Darmstadt, Germany). The thalli were carefully spread out homogeneously in the Petri dishes to minimize self-shading effects. For DNA damage analysis four subsamples taken from each Petri dish were frozen in liquid nitrogen immediately after the exposure and subsequently stored at –85 °C until DNA extraction. Thereafter, the optimal quantum yield of PS II (F_v/F_m) was determined for each treatment using an Imaging PAM chlorophyll fluorometer (Walz GmbH, Effeltrich, Germany). After 20 min predarkening F_0 was assessed with a measuring light between 0.3 and 1.7 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and F_m with a saturation pulse of >2200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

In a second set of experiments the repair of DNA damage and the recovery of PS II efficiency after 1 h of moderate UVB treatment were investigated under low white light (F15W/35 white, General Electrics, Budapest, Hungary, 10–15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and at 9 °C. UVB exposure was conducted as described above. This time 12 Petri dishes per species were irradiated uniformly and afterwards placed in a second temperature controlled light cabinet for recovery (Type 3000, Rumed, Laatzen, Germany). At different time points two randomly chosen Petri dishes per species were removed from the cabinet and from each dish again four subsamples were frozen in liquid nitrogen. Samples for DNA analysis were taken before the start of the UV exposure (control samples) and at five different time points during recovery up to 48 h after UVB (experiments R1–4/5). For the determination of repair rates of PS II the same set-up as for removal of DNA damages was used but

this time the plastidal protein biosynthesis inhibitor lincomycin (Sigma Aldrich, Taufkirchen, Germany) was added at a final concentration of 2 mM to half of the dishes (experiments L1–4). The damage was calculated as percent to unexposed control samples. The protein synthesis dependent repair of PS II was calculated as difference of F_v/F_m between control and lincomycin treated samples. A model with three parameters was fitted to the repair kinetics in accordance to an equation from Campbell and Tyystjärvi [16].

For each species both types of experiments were repeated at least 4 times independently (D1–4, R1–4/5 and L1–4; Table 1). This replication over time was necessary as WG 295 glass filters were too small to expose enough Petri dishes homogeneously. In experiment D2 the dot blot assay failed, presumably due to impurities of the extracted DNA. Therefore, only the results of the photosynthesis measurements are shown for this experiment. In experiment R1 the removal of CPDs Mb^{-1} was only followed for 24 h.

Additionally, the apparent UVB transmittance (T UVB) of cell walls and/or cytoplasm was determined in *Ulva* and *Rhizoclonium* with a Xenon PAM chlorophyll fluorometer (Walz GmbH) as described in Pescheck et al. [58] one day before each experiment was conducted. Briefly, the ratio of chlorophyll fluorescence induced by a weak UVB measuring beam and a weak blue green measuring beam was calculated and related to the signals recorded from unprotected samples, i.e. chloroplasts isolated from the respective species and transferred to filter paper (Whatman, Dassel, Germany).

2.3. Extraction of DNA and CPD detection

For each treatment DNA of eight replicates was extracted. In 2 mL reaction tubes the frozen algal tissue was ground with 5 glass beads (2 and 4 mm in diameter) using a cell mill (SPEX 2000 Geno/Grinder, type C3, München, Germany). Pulverized samples were extracted with 0.75 mL extraction buffer (2% cetyl-trimethylammonium bromide [CTAB], 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid [EDTA], 100 mM Tris-HCl pH 8, 0.2% β -mercaptoethanol, all Roth, Karlsruhe, Germany) for 45 min at 26 °C. Afterwards samples were washed with 0.75 mL chloroform-isoamyl alcohol (24:1, vol/vol, Roth) followed by centrifugation (16,000g) at 15 °C for 10 min twice. DNA was precipitated with 0.45 mL isopropanol (Roth) for 30 min at –20 °C and pelleted by centrifugation (16,000g) at 5 °C for 30 min. The pellet was washed with 0.2 mL 70% ethanol (Merck, Darmstadt, Germany), vacuum dried in a SpeedVac apparatus (Savant Instruments,

Farmingdale, USA) and suspended in 0.2 mL TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). Before storage at –85 °C samples were treated with 0.25 mg ml^{-1} RNase (Sigma Aldrich) for 30 min at 37 °C. The DNA concentration was determined fluorometrically using the Picogreen assay (Molecular Probes, Eugene, USA) and a TECAN GENius microplate reader (Tecan, Salzburg, Austria).

The immunoassay followed the protocol from van de Poll et al. [60]. 50 ng heat denatured sample DNA was transferred onto a nitrocellulose membrane (pore size 0.1 μm , Whatman) with a Minifold I SRC96 dot blot apparatus (Schleicher & Schuell, Keene, Netherlands) and was immobilized at 80 °C for 2 h. Unspecific binding sites were blocked by a 30 min treatment with 5% skimmed milk powder (Roth) in PBST (PBS + 0.1% Tween 20 (vol/vol), Roth). After washing the membrane three times with PBST it was incubated with the primary antibody H3 (H3 clone 4F6, Affitech, Oslo, Norway) over night at 4 °C. The following day the membrane was washed again three times with PBST and incubated for 2 h with horseradish peroxidase conjugated rabbit anti-mouse serum (1:1600, Dako, Glostrup, Denmark) at room temperature. Before addition of ECL (GE Healthcare, Freiburg, Germany) the membrane was washed again three times with PBST. Then it was sealed in a transparent plastic foil and hyperfilms (GE Healthcare) were exposed to it for different periods of time. After development the films were scanned (hp Scanjet 4890) and the greyscale values were quantified using the software ImageJ (National Institute of Health, Bethesda, USA). On each blot the plasmid based CPD standard (TE diluted) was included in duplicate.

2.4. Generation of the cyclobutane pyrimidine dimer standard

To be able to calculate CPD frequencies in the DNA from UV-irradiated macro algae we created a new calibrated plasmid based CPD standard following the protocol of Sinha et al. [72]. The rationale was to calculate the maximal number of CPDs Mb^{-1} that can be detected by using a DNA sample with known sequence where all sites of neighboring pyrimidine bases have undergone dimerization. For this purpose the pBSK II SK(+) plasmid (Stratagene, La Jolla, USA), containing a total of 2961 base pairs, was exposed to strong UVB radiation emitted by a transilluminator device (ECX-20.M, Peqlab, Erlangen, Germany). In detail, 1.2 mL plasmid DNA solution in TE with a concentration of $1.75 \mu\text{g mL}^{-1}$ were filled in a 1.5 mL quartz cuvette covered with a plastic lid (Hellma, Mühlheim, Germany) which was placed 6 cm distant from the light source. This setup resulted in 62 W m^{-2} weighted UVB (after [25])

Table 1

Apparent UVB transmittance (mean \pm 1 SD, $n = 8$) in *Ulva* and *Rhizoclonium* measured one day before the respective experiments (D1–4: dose response experiments, R1–5: CPD removal experiments, L1–4: PS II repair experiments).

Experiment	Apparent UVB transmittance	
	<i>Ulva</i>	<i>Rhizoclonium</i>
D1	1.031 \pm 0.034	0.350 \pm 0.159
D2	0.880 \pm 0.041	0.221 \pm 0.027
D3	0.900 \pm 0.016	0.327 \pm 0.028
D4	0.856 \pm 0.023	0.103 \pm 0.018
R1	0.910 \pm 0.046	0.163 \pm 0.036
R2	0.846 \pm 0.051	0.169 \pm 0.027
R3	0.901 \pm 0.046	0.233 \pm 0.037
R4	0.911 \pm 0.011	0.234 \pm 0.029
R5	–	0.116 \pm 0.014
L1	0.933 \pm 0.039	0.212 \pm 0.034
L2	0.906 \pm 0.016	0.226 \pm 0.063
L3	0.838 \pm 0.016	0.189 \pm 0.014
L4	0.929 \pm 0.035	0.428 \pm 0.039

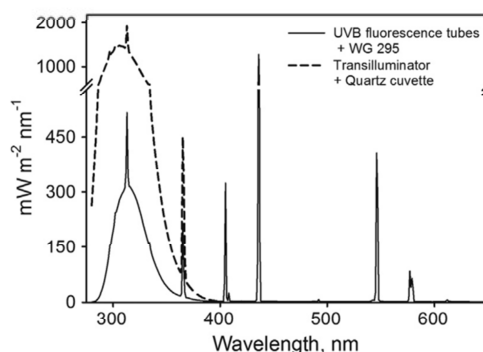


Fig. 1. Spectra of UVB treatments (solid line). UVB fluorescent tubes were used to expose the macroalgae which were covered with WG 295 glass filters. A transilluminator was used to dimerize the pbsk plasmid DNA, the spectrum (dashed line) was corrected for the transmittance of the quartz cuvette. The integrated visible photon irradiance of the UVB fluorescent tubes amounted to $14.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

inside the cuvette. The spectrum representing the energy distribution emitted by the transilluminator was measured with a Bentham DM 150 double monochromator spectroradiometer (Bentham Instruments, LTD, Berkshire, UK) (Fig. 1). UVB exposure was performed under controlled temperature conditions at 23 °C. Every 5 min during exposure and before every sampling the DNA was thoroughly mixed. To follow saturation of pyrimidine dimer formation in the plasmid DNA, samples of 80 μL were drawn from the cuvette and frozen at $-85\text{ }^\circ\text{C}$ at various time points from 0 to 90 min of exposure for immunoblot analysis. DNA concentration was determined fluorometrically (PicoGreen assay) in the same samples. The complete dimerization was performed seven times independently.

The maximum number of detectable dimers in the pBSK II SK(+) plasmid was calculated separately for both complementary strands under certain assumptions: First, there is a difference between the maximal inducible number of CPDs per plasmid and the maximal number of CPDs that can be detected by the primary H3 antibody (H3 clone 4F6, Affitech). This antibody specifically binds to T[^]T and 5'T[^]C on the antisense strand and T[^]T and 3'C[^]T on the sense strand, respectively, contained in single stranded DNA molecules. Between two possible binding sites at least a two nucleotides distance is required for binding of two separate antibodies [65,39]. Otherwise one of the dimers will be spatially blocked by the other one. Second, different putative photoproducts compete with each other (e. g., formation of cytosine dimers may compete with the formation of thymine–cytosine dimers, when the sequence is, e.g., 5'T-C-C). To account for this we did a probability calculation using the relative abundances of pyrimidine dimers found by Setlow and Carrier [70] in UVB ($\lambda = 280\text{ nm}$) exposed purified *E. coli* DNA of 68% T>T, 13% T>C, 13% C>T and 6% C>C. Moreover, at neighboring thymine or cytosine bases CPDs compete with 6–4 photoproducts, resulting in relative abundances of 75–25%, respectively [49,10]. With these assumptions the maximum amount of H3-detectable CPDs per pBSK II SK(+) plasmid is 332 which equals 56,009 CPDs Mb⁻¹.

In order to find the right range of CPDs Mb⁻¹ for the unknown amount of DNA lesions in sample DNA from macroalgae a dilution series of the maximally dimerised plasmid was prepared. From the 30 min UVB-irradiated plasmid solution ten dilution steps between 1:60 and 1:4800 with unexposed plasmid DNA at the same concentration were produced to obtain a standard from 0 to 4031 CPDs Mb⁻¹. Additionally, an equal serial dilution with calf thymus DNA (Sigma Aldrich) was analyzed in parallel. Because both of these dilution methods are very DNA consuming and possibly more prone to errors or degradation, a third dilution with TE-buffer only was added as it was also used in the original publication of Sinha et al. [72]. In five independent experiments the three different dilution methods were checked against each other to examine if there is unequal detection efficiency by the H3 antibody. From the serial dilutions of the standard 100 μL were included in all following blots as calibration in duplicates.

2.5. Statistical analyses

All statistics were performed using Sigmaplot 11 (Systat Software GmbH, Ekrath, Germany) or GraphPad Prism 5 (GraphPad Software, San Diego, USA). The repair kinetics of PS II were fitted in a model with three parameters according to Campbell and Tyystjärvi [16] to describe the initial level of PS II inactivation (y_0), the amplitude (a) and the rate (b) of recovery for each experiment. With the mean of these parameters the solid lines in Fig. 7 and 8 were drawn. Calculation of the log normal function of the saturation curve of dimerization of the plasmids and the repeated measures ANOVA of the different exposure times of the plasmid in Fig. S1 were followed by Tukey post hoc comparisons.

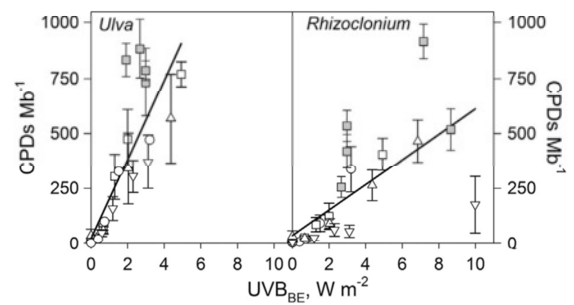


Fig. 2. Dose–response of CPD induction in *Ulva* and *Rhizoclonium* to UVB radiation. Algae were exposed to five different UVB irradiance levels for 1 h each (0 W m^{-2} as control to 4.93 W m^{-2} for *Ulva* and to 9.99 W m^{-2} for *Rhizoclonium*). Open symbols indicate different dose response experiments (D1–4), filled squares represent repair experiments after 0 h recovery, values are means ($n = 8$) ± 1 SD. Regression lines: *Ulva*: $y = 22.4 + 169.2x$, $r^2 = 0.69$, *Rhizoclonium*: $y = 25.8 + 57.7x$, $r^2 = 0.51$.

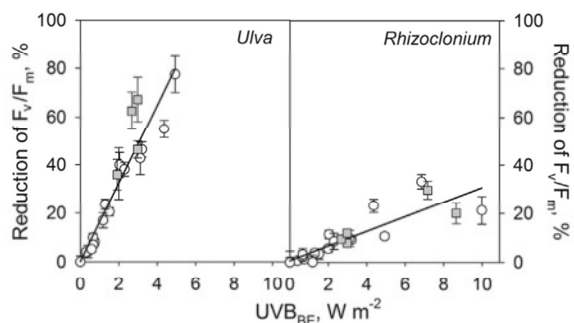


Fig. 3. Dose–response of photosynthetic efficiency to UVB radiation. Reduction of F_v/F_m was calculated in relation to control samples kept under the UV-absorbing plexiglass filters, which had mean F_v/F_m values of 0.69 and 0.67 in *Ulva* and *Rhizoclonium* respectively. Open circles represent dose response experiments, filled squares represent repair experiments after 0 h recovery, values are means ($n = 8$) ± 1 SD. Regression lines: *Ulva*: $y = 0.17 + 16.1x$, $r^2 = 0.88$, *Rhizoclonium*: $y = 0.69 + 3.0x$, $r^2 = 0.72$.

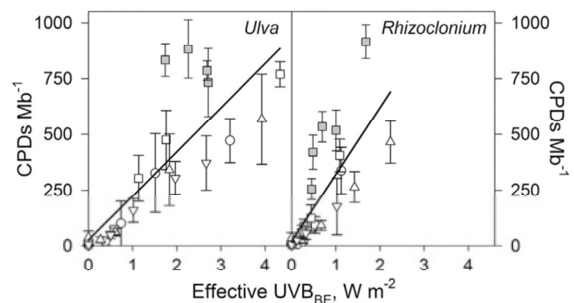


Fig. 4. Dose–response of CPD induction in *Ulva* and *Rhizoclonium* to effective UVB radiation calculated as applied UVB \times UVB transmittance (see Table 1). Data are derived from the same experiments as in Fig. 2. Symbols as in Fig. 2. Regression lines: *Ulva*: $y = 24.8 + 192.2x$, $r^2 = 0.65$, *Rhizoclonium*: $y = 12.6 + 299.6x$, $r^2 = 0.59$.

3. Results

3.1. Calibration of a CPD standard

The standard has to fulfill several requirements to ensure the determination of a correct concentration of CPDs Mb⁻¹. One primary qualification is the verification of complete dimerization during the UVB exposure. Thus, we followed the antibody-

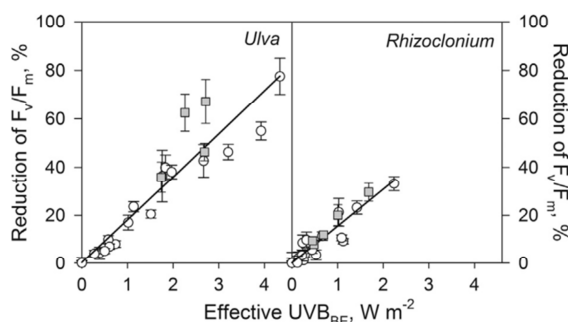


Fig. 5. Dose–response of reduction in photosynthetic efficiency to effective UVB radiation calculated as applied UVB \times UVB transmittance (see Table 1). Data are derived from the same experiments as in Fig. 3. Regression lines: *Ulva*: $y = 0.29 + 17.8x$, $r^2 = 0.86$, *Rhizoclonium*: $y = 0.07 + 15.4x$, $r^2 = 0.82$.

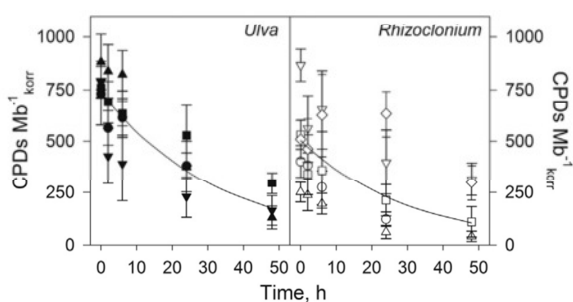


Fig. 6. Remaining DNA damage in CPDs Mb⁻¹ corrected for control concentration (before UVB) in *Ulva* and *Rhizoclonium* after 1 h exposure to UVB during 48 h recovery under low light conditions. Circles, R1; triangle up, R2; square, R3; triangle down, R4; diamond, R5. Values are means ($n = 8$) \pm 1 SD. Each experiment was fitted to the exponential decay function $f(x) = a \exp(-bx)$ and the solid line is drawn from the mean of these independent fits.

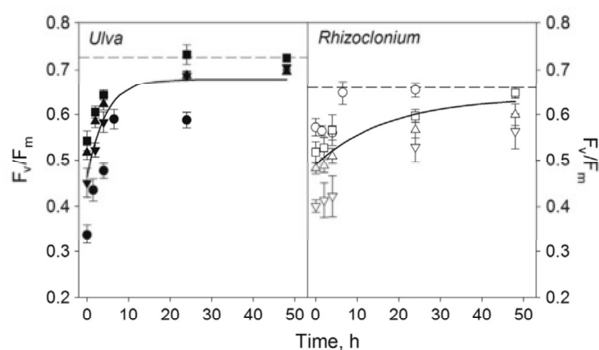


Fig. 7. F_v/F_m during recovery under low light conditions in *Ulva* (filled symbols) and *Rhizoclonium* (open symbols) after 1 h exposure to UVB radiation. The dashed line indicates the mean level of F_v/F_m before the UVB treatment. Circles, L1; squares, L2; triangle up, L3; triangle down, L4. Values are means ($n = 8$) \pm 1 SD. Each experiment was fitted to the model $f(x) = y_0 + a(1 - \exp(-bx))$ and the line drawn is the mean of these four independent fits. For further details, see text.

detectable DNA damage over an extended exposure time. It increased during the first 19 min (Fig. S1a) and started to fall off after 30 min. There was no significant difference in the level of detectable dimers between 15 and 30 min of exposure (RM ANOVA, $p = 0.999$). Simultaneously we controlled the remaining concentration of double-stranded DNA using the Picogreen fluorescence assay. It decreased rapidly from the initial value of $1.75 \text{ ng } \mu\text{L}^{-1}$ to about half of this value during the first 30 min

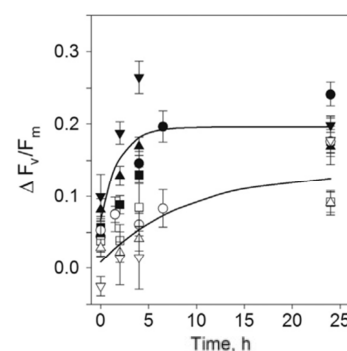


Fig. 8. Recovery of F_v/F_m after UVB treatment at varying irradiances as difference between control and lincomycin treated samples. Filled symbols represent data for *Ulva*, open symbols refer to data from *Rhizoclonium*. The curve fitting was applied as described in the legend of Fig. 7. For *Ulva*: $f(x) = 0.067 + 0.129(1 - \exp(-0.544x))$, for *Rhizoclonium*: $f(x) = 0.008 + 0.125(1 - \exp(-0.112x))$. Identical experiments have identical symbols in Figs. 7 and 8.

(Fig. S1b). For the consecutive calculations the actual DNA concentration of the 30 min irradiated plasmid solution ($0.85 \text{ ng } \mu\text{L}^{-1}$) was used. The applied UVB radiation was not only very efficient in the dimerization of pyrimidine bases but also in the induction of single-strand breaks and denaturation of the plasmids. The length of the plasmid was not altered as after all exposure times two defined bands corresponding to the supercoiled and open circular form with the right size were visible on an agarose gel (not shown). In our experiments the 30 min irradiated plasmid was set as 100% dimerised plasmid DNA. The balance between hitting all possible dimers and the decay of double-stranded plasmid DNA was still acceptable at that time point.

Another very important aspect is the biochemical processing during detection of the dimers. It should resemble the situation existing in the sample DNA as much as possible, e.g. with respect to the composition of the sample. It was found that diluting the exposed plasmid DNA with either un-irradiated DNA (plasmid or calf thymus) or TE-buffer affected the signal intensity differentially. In Fig. S2 the signals produced by the two different DNA dilutions are compared to the TE-buffer dilution. Dilution with unexposed plasmid DNA or calf thymus DNA resulted in almost identical signals whereas dilution with TE-buffer increased the signals per amount of exposed plasmid (Fig. S2a). The ratio between both dilution methods was quite stable over a broad range of greyscale values produced by successive dilution steps (Fig. S2b). From this relation a linear correlation was derived which was used as correction factor to calculate the respective CPD frequencies in the used TE-diluted calibration.

3.2. Apparent UVB transmittance

In *Ulva* apparent UVB transmittances close to 1.0 were determined throughout all experiments (Table 1). This means virtually no UVB screening was detectable and the cell walls and cytoplasm of the thalli were transparent for UVB radiation. In contrast, for *Rhizoclonium* low UVB transmittances varying between 0.428 (experiment L4) and 0.103 (D4) were determined. This implies that only 10–43% of the incident UVB radiation passed through the walls and cytoplasm before reaching the chloroplasts.

3.3. UVB exposure experiments

The dose dependency of CPD induction was investigated in *Ulva* and *Rhizoclonium* (Fig. 2). Including the four data points from the repair experiments in *Ulva* the slope of the induction was almost

three times as steep as in *Rhizoclonium*. This means in *Ulva* the DNA lesions accumulated at lower UVB irradiances than in *Rhizoclonium*, in line with the higher screening capacity in *Rhizoclonium*. For the reduction of F_v/F_m both species also showed very different sensitivities of their photosystems, the slopes differing more than five times (Fig. 3). While the reduction of F_v/F_m reached very high values of around 80% in *Ulva* after exposure to 5 W m^{-2} UVB for 1 h, the reduction of F_v/F_m did not exceed 35% in *Rhizoclonium* although irradiances up to 10 W m^{-2} were applied (Fig. 3).

With the help of the transmittance data (Table 1) we calculated the irradiance of UVB that reached the plastids, which we term effective UVB. After this correction for optical screening one can relate the induction of damage to UVB irradiance on an intracellular level. In *Ulva* only a weak screening was observed and accordingly the degree of photoinhibition and DNA damages was directly related to the incident radiation. On the other hand, for *Rhizoclonium* the slope of the regressions in Figs. 4 and 5 increased considerably as compared to Figs. 2 and 3, respectively. Interestingly, we found in both species a very similar dependency of PS II function on effective UVB whereas the accumulation of CPDs Mb^{-1} was 1.5 times higher in *Rhizoclonium* than in *Ulva* at corresponding effective UVB irradiances (Fig. 4, $p > 0.001$). In addition, for *Rhizoclonium*, the scatter between damage and UVB irradiance was reduced when effective UVB was employed (compare r^2 values of Figs. 2 and 3 with those of Figs. 4 and 5, respectively). This may indicate that variable screening was an important cause for the scatter in Figs. 2 and 3.

3.4. Repair of CPDs

For both species repair of DNA damage was determined as decline in CPDs Mb^{-1} following exposure to UVB for 1 h. This decline was followed over 48 h (24 h for experiment R1) at low light and at 9°C which corresponds to growth conditions. Destructive sampling means that the data vary more than if the same algae were followed over the whole recovery period. The repair was relatively inefficient and not complete in both species even after 48 h (Fig. 6). Additionally, we tested the effect of different UVB doses on the repair capacity as it may depend on the initial amount of damage induced in the two species. For each experiment the two parametric exponential model, $f(x) = a \exp(-bx)$, was fitted to the data and the results for coefficient b are listed in Table 2 as repair rates h^{-1} of both species. The mean of this fit is drawn in Fig. 6 as solid line. There was no significant difference in the repair rates of *Ulva* and *Rhizoclonium* (student t -test, $p = 0.82$).

3.5. Recovery of F_v/F_m

The recovery of F_v/F_m was also followed over 48 h after 1 h UVB exposure in 4 independent experiments for each species (Fig. 7). The UVB irradiance applied to *Rhizoclonium* was higher than that

used for *Ulva* in order to obtain similar reduction of F_v/F_m for both species. Recovery kinetics were fitted to an exponential model ($f(x) = y_0 + a(1 - \exp(-bx))$), where y_0 corresponds to initial damage, a to the amplitude of recovery and b describes the rate of recovery). The model shown in Fig. 7 (solid line) represents the mean values of the three parameters of the fits to the single experiments. In *Rhizoclonium* recovery was clearly slower (Fig. 7). Parameter b was significantly smaller than that of *Ulva* and amounted to only about one third (t -test: $p < 0.001$). In parallel to each experiment shown in Fig. 7 samples were pretreated for 14 h and UVB-irradiated in the presence of 2 mM lincomycin to inhibit repair by protein synthesis. Lincomycin inhibited recovery almost completely for the first 8 h. Later, especially after 24 h some recovery was also observed in these treated samples (data not shown). In Fig. 8, the differences in F_v/F_m between control samples (shown in Fig. 7) and lincomycin treated samples are shown, representing only the lincomycin sensitive part of repair. Due to the later recovery of the lincomycin treated algae only the first 24 h are shown in Fig. 8. Also here the exponential model was fitted to the data. In one of the experiments with *Rhizoclonium* (open circles) the data could not be converged to the model. Therefore, for this species only the means of three experiments were used to draw the line. Again it is apparent that the repair rate of *Rhizoclonium* is much slower than that of *Ulva* ($p = 0.158$). The positive start values for $\Delta F_v/F_m$ of *Ulva*, indicate that lincomycin enhanced the maximal damage. This reveals that in the absence of lincomycin some repair occurred already during exposure. *Ulva* apparently had positive $\Delta F_v/F_m$ values even when the model was extrapolated over the predarkening time of 20 min, before the first F_v/F_m was determined.

4. Discussion

4.1. Influence of screening on the accumulation of CPDs and damage at PS II

The data presented here show that in *Rhizoclonium*, belonging to the Cladophorales, the previously detected screening [58] is fully functional and indeed serves to reduce UVB-induced damage at two different important targets. In *Rhizoclonium* showing screening up to 90% (Table 1) CPD accumulation was almost three times lower than in the non-screening *Ulva* (Fig. 2). For PS II efficiency the difference was even larger, namely five times (Fig. 3). The size of PS II damage in *Ulva* and *Rhizoclonium* coincided when damage was related to effective UVB irradiance calculated using UVB transmittance data (Fig. 5). On one hand this strongly supports the validity of the fluorescence approach to quantify UVB screening. On the other hand it suggests that PS II has the same intrinsic sensitivity in both species. However, employing the same principle for DNA damage seemed to indicate a reduced sensitivity of the DNA in *Ulva* as compared to *Rhizoclonium*. Still, it is important to keep

Table 2

Repair rates and mean CPDs Mb^{-1} ($n = 8, \pm 1 \text{ SD}$) after 1 h UVB exposure, corrected for control CPD sample values (before UVB exposure). CPD repair rates h^{-1} were calculated from curve fitting of the data to the two parametric exponential function $f(x) = a \exp(-bx)$ over 48 h (only 24 h for R1). Coefficients of determination of the resulting regressions are shown.

Experiment	<i>Ulva</i>			<i>Rhizoclonium</i>		
	CPDs Mb^{-1}	Repair rate h^{-1}	r^2	CPDs Mb^{-1}	Repair rate h^{-1}	r^2
R1	731 ± 153	0.024	0.53	313 ± 76	0.053	0.61
R2	884 ± 130	0.036	0.87	252 ± 47	0.049	0.78
R3	759 ± 44	0.017	0.69	531 ± 69	0.032	0.57
R4	786 ± 74	0.043	0.58	866 ± 76	0.021	0.52
R5	–	–	–	650 ± 273	0.0076	0.16
Mean	0.0301 ± 0.012			0.0326 ± 0.019		

in mind that UVB screening has been assessed by a method which estimates the transmittance of incident radiation reaching the chloroplasts. Accordingly, for UV-sensitive structures within the chloroplasts, such as PS II, the fluorescence method provides an accurate measure of stressful UVB as demonstrated by our results (Fig. 5). On the other hand, the nucleus may be arranged differently in the cells such that the screening of chloroplasts may not be identical to the screening of DNA.

Here, one also has to consider the differing ultrastructural morphologies of *Ulva* and *Rhizoclonium*. One even could imagine that the chloroplasts themselves are screening the nucleus in *Ulva*. The siphonocladal organization with multinucleate cells and a reticulate, parietal chloroplast structure of *Rhizoclonium* may result in a similar accessibility of the plastids and the nuclei for radiation [47]. This would be in line with the localization of the screening compounds in the cell walls or in their proximity in *Rhizoclonium* (F. Pescheck, unpublished data) generating a homogenous intracellular light milieu. In contrast, in the genus *Ulva* one large cup-shaped chloroplast per cell may shade the nucleus, as this chloroplast is moving during illumination to face the flat surface of the thalli [51,12,13]. Hence, in *U. clathrata* the plastids would have experienced the full strength of UVB while the remainder of the cytoplasm would have been protected by the absorption of the plastids. A similar explanation for species specific UV resistance among Antarctic diatoms with differing ultrastructural morphologies was suggested by Karentz et al. [37] and described as “chloroplast shielding” or “nuclear hiding”. For this mechanism to function a high repair rate of chloroplast damage is required. However, PAR damage to the chloroplasts leading to bleaching [5] would expose the nucleus to enhanced UVB radiation.

4.2. High repair rate as a UVB resistance strategy in a non-screening alga

Besides UV screening, another important resistance mechanism is the repair of UVB-induced damage. Application of an exponential model to the data allowed to calculate repair rate disclosing considerable differences in the recovery of PS II function after UVB treatment (Figs. 7 and 8). In *Rhizoclonium* repair rate was about a quarter of that observed in *Ulva*. The decrease of F_v/F_m was presumably not influenced by a down regulation of PS II causing non-photochemical quenching (NPQ) of F_m . First, the applied PPFD during the treatment was very low ($15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). This is so low that light-induced NPQ would have been quite small at most. Furthermore, the application of lincomycin, a compound that inhibits plastidic protein synthesis and, hence, repair of damage of PS II, allowed to resolve that part of repair that was dependent on protein synthesis. Clear differences in the lincomycin sensitive repair rate were observed (Fig. 8) pointing to a compromised D1 turnover in *Rhizoclonium*. On the other hand, the hypothesized high repair rate in *Ulva* could be established, which is supposed to be a prerequisite for *Ulva* to use its chloroplasts to screen the nucleus against UVB radiation. The observed repair rate is in the same order of magnitude as that observed in higher plants [1] and cyanobacteria [2].

On the other hand, no significant differences were observed for *Ulva* and *Rhizoclonium* regarding the removal of CPDs (Fig. 6 and Table 2). Since the algae were exposed to PAR of $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ during recovery one may assume that a large fraction of CPD repair was due to photoreactivation by the photolyase [11]. Repair in darkness was considerably slower (less than 20% of the damage repaired after 24 h in both species; data not shown). In general, CPD removal was relatively slow as compared to other reports [61,63]. It is possible that the photoreactivation was limited by low light availability or the low temperature of 9 °C.

4.3. Biological relevance of UVB screening in Cladophorales

We observed up to 90% UVB screening in *Rhizoclonium* and even up to 97% under appropriate cultivation conditions (data not shown). We have demonstrated that this screening capability functions to protect sensitive cellular compounds such as photosystem II and DNA, making this species very UVB-resistant. Consequently, we had to apply high UVB irradiances to reach the same damage level as in *Ulva* in order to compare subsequent recovery rates. Notably, UVB_{BE} irradiances as those applied in these experiments (up to 10 W m^{-2}) exceed those occurring in the natural habitat. This is also true when one considers the cumulative UVB_{BE} dose taking into account that the sun may shine for 12–16 h. In the absence of additional damage by high PAR, it seems that *Rhizoclonium* would be able to survive any solar UVB exposure possible on earth, primarily because of its ability to screen UVB. As confirmed in the repair experiments this species does not employ the strategy of cellular tolerance by potent repair mechanisms but of preventive screening (Figs. 6 and 7). However, the relative importance of UVB screening mechanisms under natural conditions, where UVR is accompanied by high PAR and other stressors, remains to be explored. High UVB resistance for another species from the Cladophorales as compared with the genus *Ulva* has been reported. Bischof et al. [7] observed high UVB resistance of photosynthesis in *Chaetomorpha linum*, whereas in a comparable study *Ulva rotundata* partly disintegrated during analogous treatments after 3 days [5]. This observation is in line with our finding of substantial UVB screening in several species of the order of the Cladophorales, including also *C. linum* [58]. One may speculate that species of the Cladophorales are in general similarly well protected as *Rhizoclonium* while *Ulva* species apparently lack significant protective screening [58].

4.4. Calibration of standard DNA

A prerequisite for the determination of the protective function of the screening was the quantitative measure of CPDs with the help of a calibrated standard. Only two different standard preparations have previously been applied for the quantification of CPDs by an immunoblot assay in plants. One of these originated in the group of Roza et al. [65] who determined the concentration of CPDs Mb⁻¹ in UVC irradiated human fibroblasts by HPLC analysis. In many publications the concentration of CPDs Mb⁻¹ was quantified in direct comparison to this standard preparation (e. g. [8,14,31]). Another standard preparation was introduced by Sinha et al. [72]. This one was generated by total dimerisation of a plasmid with known sequence and subsequent calculation of the maximal concentration of CPDs. This second approach has many advantages as the principle can be applied to any standard DNA with known sequence and does not require an alternative detection system such as HPLC. As we did not have access to one of these standards we generated a new standard following the approach of Sinha et al. [72].

Producing a CPD standard with the described method is relatively easy and very reproducible although in our hands the induction of all possible dimers per plasmid has proven to be more complex than published earlier [72]. Firstly, only during a small window of UVB dosage the maximal amount of DNA damage is present (Fig. S1). Therefore, care has to be taken with respect to UVB irradiance and DNA concentration, as the plasmid like any DNA is not very stable under UVB irradiation [41]. However, the exact DNA concentration is an indispensable factor for the subsequent computations and has to be determined carefully.

Secondly, the computation of the maximal number of CPDs has to take account of (a) the presence of two dimers too close to each other to allow binding of two separate antibodies, (b) the

competitive induction of alternative dimers and (c) the competitive induction of 6,4-photoproducts, all of which are not detected.

Thirdly, as shown here for the first time, a TE-buffer dilution of exposed plasmid DNA increased the H3 binding efficiency compared to the dilution with calf thymus or plasmid DNA. The dilution with calf thymus DNA corresponds to the situation in algal sample DNA, where a large proportion of DNA molecules is undamaged. One reason for the lower signals in DNA-diluted UV-exposed standard DNA could be a masking of damaged nucleotides by the bulk of undamaged ones. A similar observation was reported in Karakoula et al. [36] who proposed a steric hindrance of lesion assessment in a competitive ELISA. This steric hindrance will lead to an underestimation of CPDs in the investigated samples. Therefore the dilution conditions should be as similar as possible in the calibration series and the samples, or a correction for unequal binding has to be included as it was shown in this study (Fig. S2). Another possible reason for the difference of the detection efficiency between the DNA-dilution methods and the TE-buffer dilution might be partial renaturation of the DNA. This is a very important point in the assay as only dimers in single stranded polynucleotides can be detected by the H3 antibody. These are commonly produced by heat denaturation during the immunoassay. As the plasmid DNA has a very different structure from genomic DNA it tends to recombine much easier. This would lower the binding of the antibody [46,18] and is especially prevalent when damaged plasmid is diluted with a solution of unexposed plasmid.

4.5. Magnitude of CPD formation

Using the CPD calibration standard the detected CPD concentrations were in a wide range from close to zero to over 700 CPDs Mb⁻¹ (Fig. 2). Very low CPD concentrations below 20 CPDs Mb⁻¹ were observed in control samples (Fig. 2, y-axis intercept of the regression line at 24–37 CPDs Mb⁻¹), indicating that a false positive detection by the primary antibody was largely lacking. The UVB dose dependent amount of CPDs Mb⁻¹ of the two green macroalgae was in the same order of magnitude as observed in other studies. In temperate red algae van de Poll et al. [61] reported maximal lesion concentrations around 250 CPDs Mb⁻¹. In their study the applied UVB irradiance was higher than in the present study while the exposure time was only 20 min. Gao et al. [24] used lower UVB irradiance but extended the exposure over 8 h and found maximal concentrations of about 450 CPDs Mb⁻¹ in a marine cyanobacterium. Both authors did not report the origin of the standard preparations they used. Lower CPD concentrations, saturating around 25 CPDs Mb⁻¹, were observed in *Prasiola crista* exposed to ambient plus 0.54 W m⁻² enhanced UVB for 10 h [45]. In bacterioplankton even under ambient UVB levels similar photoproduct concentrations as we describe here were reported [35].

In addition to UVB irradiance and exposure duration the amount of induced CPDs may also be affected by thallus characteristics such as thickness, screening compounds, self-shading or repair capacities [5,64]. Taken together, we have demonstrated that the applied method to quantify CPDs yields reliable results in a reasonable range and is well suited for DNA damage detection in macroalgae.

Acknowledgements

For technical support we thank Prof. K. Krupinska and S. Braun. Further thanks to Nele Hein and Caroline Hesse for help with DNA extraction. Prof. C. Wiencke is thanked for the loan of a blotting apparatus and Prof. R. Schmitz-Streit for the loan of a peristaltic pump for seawater filtration.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jphotobiol.2014.02.006>.

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Chapter 4 – Relative sensitivity of DNA and photosystem II in *Ulva intestinalis* Linnaeus (Chlorophyta) to natural solar irradiation

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(Manuscript as prepared for submission to Marine Ecology Progress Series)

Abstract

The concentration of DNA damages and efficiency of photosystem II (PSII) were analyzed in *Ulva intestinalis* in the field. The aim of this study was to directly compare the sensitivity of both targets under natural sunlight on short-term and long-term scales. For PSII a tight linking of the optimal quantum yield (F_V/F_M) to the diel variation of irradiance was confirmed. Although F_V/F_M was extremely reduced during noon a fast recovery followed in the afternoon. As dark adapted ground fluorescence (F_O) of PSII was substantially decreased during the day non-photochemical quenching is suggested to be a key photoprotective strategy in *U. intestinalis*. Over a longer period of high irradiance PSII efficiency at midday and also midnight gradually declined but recovered as soon as daily doses of sunlight decreased. In contrast, for ultraviolet-B (UVB) -induced DNA damages a distinct pattern was found neither in the daily courses nor in the long-term study. We demonstrate that the concentration of DNA lesions was not related to the accumulated UVB dose. Thus, under natural sunlight efficient photoreactivation is supposed to balance the induction of dimers as opposed to experimentally UVB stressed algae. The relative importance of PSII and DNA damages under natural irradiation is discussed.

Key words: cyclobutane pyrimidine dimer, DNA; in situ, photosystem II, photoinhibition, ultraviolet B radiation, *Ulva intestinalis*, sunlight

1. Introduction

The vitality of an organism is the result of the interplay of all its physiological reactions with its complex environmental and developmental situation. In plants, one of the most important life processes is photosynthesis but the integrity and accurate expression of the DNA is crucial to any physiological reaction. If one of these functions is significantly impaired no continual growth and reproductive success is feasible (Jha 2008). Beside its function as the ultimate energy source for plants sunlight can also act as a signaling and regulating factor (Rico and Guiry 1996, Franklin et al. 2005, Jansen and Bornman 2012) and has a damaging potential (e.g. reviews by Powles 1984, Aro et al. 1993, Vass 2012). The damaging action depends on light intensity as well as on the spectral composition and the overlap with the absorption properties of the biomolecules (Peak and Peak 1983). In the case of DNA the absorption increases with decreasing wavelength and photodamages to DNA are mainly generated by ultraviolet-B (UVB, 280 – 315 nm) radiation (Cadet et al. 2012). After absorption of the high-energy UVB photons a covalent bond can be generated between two neighboring cytosines or thymines resulting in cyclobutane-pyrimidine dimers (CPDs) (Setlow and Carrier 1966). These are the most abundant photoproducts in chromatin representing about three quarters of all radiation-induced DNA damages (Mitchell and Nairn 1989, Douki et al. 2000). Already a single dimer may completely stop the progression of DNA and RNA polymerases and thereby inhibits replication and gene expression (Sauerbier et al. 1970).

For photosynthesis, the destruction of the water splitting apparatus of photosystem II (PSII) is one of the starting points of photoinactivation (Vass et al. 1996, Hakala et al. 2005). Damage to this protein complex can also be explained by the direct absorption of UVB radiation (Ohnishi et al. 2005). In the absence of water splitting absorption of photosynthetically active radiation (400 – 700 nm, PAR) by photosynthetic pigments leads to the accumulation of positively charged P680⁺ radicals and the following oxidation of components of PSII. This has been denoted as step two in the inactivation of PSII in the two step theory of photoinhibition (Ohnishi et al. 2005). In addition, the high irradiances of sunlight can also lead to excessive and therefore potentially harmful irradiation as soon as an imbalance of light and dark reactions of photosynthesis sets in. In this situation an uncontrolled transfer of excited electrons on oxygen might occur. Conclusively, UVB radiation as well as PAR can induce the formation of ROS in chloroplasts (Snyrychova et al. 2007). One of the central proteins of PSII, the D1 protein and its turnover are the major targets of these radicals (Jegerschöld et al. 1990, Nishiyama et al. 2001). If the D1 protein is damaged the electron transport chain is inhibited and the quantum yield of PSII drops immediately (Vass et al. 1996, Carr and Björk 2007).

Plants have developed several protective resistance mechanisms against radiation stress. As a tolerance mechanism the fastest and most efficient repair mechanism of CPDs is photoreactivation carried out by the photolyase (Britt 1996). This enzyme splits the dimers after absorption of blue

light and UVA radiation (Dany et al. 2001) which are in sunlight always accompanying the harmful UVB radiation. This emphasizes the importance to evaluate the influence of solar radiation on the vitality of an organism under the polychromatic composition of sunlight. UVB tolerance of PSII mainly is attributed to an active repair cycle of PSII, ensuring the degradation and re-synthesis of the D1 protein (Xiong 2001). The proper function of this repair cycle can be protected by antioxidative substances and enzymes in the chloroplast preventing an inhibition of the repair cycle by ROS (Nishiyama et al. 2001, Hakala-Yatkin et al. 2011). Another high-light acclimation mechanism lowers the production of ROS by quenching of absorbed energy via efficient heat dissipation, the non-photochemical quenching (NPQ) (Müller et al. 2001, Jahns and Holzwarth 2012). This way the effective quantum yield of PSII is reduced as in photoinactivation but in a controlled and regulated photoprotective way (Osmond et al. 1993).

Besides the acute effects of high irradiances and UVB radiation, on longer time scales other aspects, like acclimation and accumulation of damages as a result of the balance between induction and repair have to be considered (Jansen et al. 1998, Allakhverdiev and Murata 2004). To study this acclimation and to reveal the ecological relevance of light-induced damages long-term monitoring under natural solar irradiation is necessary. Up to now some few long-term studies of photosynthetic efficiency in plants or algae have been conducted in the field (Valladares and Pearcy 1997, Altamirano et al. 2000, Michler et al. 2002, Bischof et al. 2002). In contrast to PSII function, for DNA damages there are much less long-term field observations. In south Argentina in *Gunnera magellanica*, an herbaceous plant a positive correlation of increased DNA damage with increased UVB irradiances due to ozone variations was observed (Rousseaux et al. 1999). From the same field experiment it was shown that acclimation to UVB can increase the photoreactivation capacity in *G. magellanica* (Giordano et al. 2003).

The organism of our study, *Ulva intestinalis* Linnaeus is one of the most dominant green macroalgal species in the intertidal zone (Sousa 1979, Nelson et al. 2003, Wang et al. 2012). The genus is well known being highly tolerant towards large variations in terms of salinity (Reed and Russell 1979), temperature and light (Henley et al. 1992, Hanelt et al. 1997) including UVB radiation (Bischof et al. 1998, Gómez et al. 2004). *U. intestinalis* establishes dense populations in the upper eulittoral zone and due to its tubular morphology (Koeman and van den Hoek 1982) the gas filled thalli are often floating on the water surface so that exposure to unattenuated solar radiation happens not only during the time of emergence. Certainly, the species-specific capacity of this alga to tolerate strong variations in the absorption of light is one of the key factors for growth and the competitive success of this species in its habitat. Accordingly, for the relation of photosynthesis and light absorption a wealth of information is available for several *Ulva* species (e.g. Henley et al. 1991, Franklin et al. 1992, Figueroa et al. 2003, Gómez and Huovinen 2011). On the other hand the investigations of UVB-induced DNA damages in macroalgae are only scarce and many of these results have been

gained from laboratory studies (van de Poll et al. 2001, Roleda et al. 2006, Pescheck et al. 2014). In the presented study the effect of sunlight on two major targets, PSII and the integrity of the DNA, on short-term as well as on long-term scales was comparatively analyzed in *U. intestinalis*. Since it possesses no UVB-absorbing pigments (Pescheck et al. 2010) that may interfere with the solar spectrum one may expect that it is well suited to evaluate photo-induced damages. Additionally, this fact provokes the question if effective physiological photoprotective mechanisms might compensate the missing UVB screening under natural sunlight.

2. Material and Methods

2.1 Field experiments

From May 2009 to September 2013 six short-term field studies with *Ulva intestinalis* Linnaeus were conducted (Table 1). The optimal quantum yield of PSII and the concentration of cyclobutane-pyrimidine dimers (CPDs) in the DNA were analyzed. Additionally, the UVB transmittance was determined for a subsample of the analyzed populations. The short-term investigations took place at different locations in the Baltic Sea around Kiel, Germany (Table 1). On the beaches of Meierwik, Dänisch-Nienhof and Surendorf dense populations of *U. intestinalis* are growing on small boulders being visible from April to November. In the long-term study on the lock island in Kiel Holtenau an experimental set-up with mesocosms was installed where the algae could be observed for several weeks under natural light, temperature and salinity conditions. For this experiment stones with a population of *U. intestinalis* were collected in Surendorf and transferred to the lock island in buckets with water. There the stones with the algae were placed in floating plastic boxes (Modell SN 106601 600 x 400 x 400 mm, Schoeller Allibert, Schwerin, Germany) having a 1 cm x 1 cm grid of holes on all sides to allow water exchange with the fjord. Inside the mesocosms the stones were kept in position by holes that were cut into a custom-made floor of PVC where the stones fitted into to about one third. The height of this floor was adjusted so that most of the algae reached the water surface and were horizontally floating due to the gas filled thalli segments. Four parallel mesocosms were tied to the north side of one fender in the entering of the small lock at the fjord side. Buoyancy was provided with the help of two connected air filled plastic pipes (1 m x 15 cm). This experiment was run two times with fresh collected stones and algae (exp. A from the 28th of June 2013 – 24th of July 2013, exp. B from the 25th of July 2013 – 16th of August 2013). During both runs the mesocosms were repeatedly cleaned from epibionts (e. g barnacles, diatoms, green microalgae) with a scrubbing brush. Sometimes also on *U. intestinalis* some epiphytes accumulated which were removed by carefully washing the algae in the open water on the other side of the fender by pulling the stones through water. As this was only done at night and in the mesocosms no waves occurred the thalli were not mixed with each other during the day. Also in Surendorf the water surface was very calm and the investigated thalli remained in stable positions over the whole day.

Table 1. Overview over the daily courses with location, date (year, month, day), daily accumulated PAR and UVB_(280 – 315 nm) dose (weighted after Ghetti et al. 1999), maximal PFD and UVB irradiance, mean water temperature (T_{water}), measurement interval for optimal quantum yield (F_V/F_M) and DNA damages (CPDs), UVB transmittance (T_{UVB}) of macrothalli, n.d. = no data

Location	Date	Daily integral		Maximum irradiation		T_{water} , C°	Measure interval	T_{UVB}
		PAR mol/m ²	UVB _{BE} kJ/m ²	PFD μmol/m ² s	UVB W/m ²			
Meierwik (N54° 50' 8.82", E9° 30' 9.83")	2009-05-07	14.2	1.04	660	0.23	8	04:30 – 22:30, F _V /F _M and CPDs every 4h, n= 5	83 ± 2 %
Dänisch-Nienhof (N54° 28' 51.25", E10° 8' 7.93")	2010-07-16	58.3	5.03	1682	0.67	20	04:30 – 20:30, F _V /F _M every 2h, CPDs every 4h, n= 8	96 ± 8 %
Surendorf (N54° 28' 55.20", E10° 8' 51.82")	2012-07-24	48.7	4.60	1477	0.64	21	04:00 – 04:00, F _V /F _M every 2h, CPDs every 4h, n= 5	79 ± 3 %
Lock Kiel-Holtenau (N54° 22' 7.34", E10° 8' 43.24")	2013-07-12	56.8	5.60	1724	0.76	20	00:00 – 00:00,	n.d.
	2013-07-23	58.2	5.79	1715	0.78	22	F _V /F _M every 2h,	n.d.
	2013-08-06	42.5	4.37	1592	0.68	20	CPDs every 4h, n= 4	79 ± 11 %

2.2 Environmental data

In Meierwik and on the lock in Holtenau a data logger (CR 1000, Campbell Scientific, Logan, USA) was installed recording several environmental parameters. The water temperature was measured with a resistance thermometer PT100 in Meierwik or on the lock island with a temperature probe (107-L, Campbell Scientific) directly under the surface. Approximately 2 m above the water surface UVB and visible radiation were registered with a UVB-sensitive photodiode (in Meierwik: GUVB-T10GD, Roithner Lasertechnik, Vienna, Austria; on the lock island: Tocon E + diffusor, sglux SolGel Technologies GmbH, Berlin, Germany) and a photodiode (G1118, Hamamatsu Photonics, Hamamatsu-City, Japan), respectively. The diodes were incorporated into custom-made sensor devices constructed for cosine-corrected measurements. During the field measurements in Dänisch-Nienhof and Surendorf the UVB radiation was measured with a hand held custom-made UVB-sensor (diode: UVS-1, Vistek, Seefeld, Germany) connected to a voltmeter approximately 1.5 m above the water surface. White light was measured using a LI-COR quantum sensor (LI-190, LI-COR, Lincoln, Nebraska, USA), and water temperature with a standard thermometer. All UVB and white light measuring devices were calibrated against a double monochromator spectroradiometer (DM 150, Bentham Instruments, LTD, Berkshire, UK) and the signals converted into W m⁻² UVB irradiance. The irradiance of UVB was further weighted with the function of Ghetti et al. (1999) using

a correlation factor for unweighted to weighted irradiance from a solar spectrum recorded in April 2009 in Kiel, Germany with the same spectroradiometer.

2.3 Measurements of chlorophyll fluorescence

For all measurements the upper 4 – 6 cm of the thalli were torn off from algae growing attached on the substrate. Immediately the upper 2 cm of these pieces were frozen to liquid nitrogen for subsequent DNA extraction. The rest of the same thalli were used for the determination of the optimal quantum yield of PSII so that it was possible to pair the data of F_V/F_M and CPDs from one individual thallus. The thallus pieces were mounted in leaf clips (Walz GmbH, Effeltrich, Germany) and kept in a water bath at ambient water temperature for the dark adaptation of 20 min (in Surendorf 15 min). Then the levels of F_O and F_M were determined using a Mini-PAM fluorometer (Walz) in Meierwik, Dänisch-Nienhof and Surendorf or a PAM-2100 fluorometer (Walz) on the lock island in Holtenau. In Surendorf and on the lock island after the first saturating pulse the thalli were kept in darkness in the leaf clips and the measurement of F_O and F_M was repeated after 1 h dark adaptation. Afterwards, for each leaf clip the specific signal output of the leaf clip and temperature dependence of the instrument was recorded with a blue fluorescence standard (Walz). From this data the normalized F_O values after 15 or 20 and 60 min of darkness were calculated by dividing the signals from the algae by that obtained with the blue standard. The measurement schedule of the short-term studies was covering complete daily courses starting before dusk. The criteria for the choice of thalli for sampling were vertical floating on the water surface and green thalli. But especially between the years the thalli for the investigations were of different sizes and ages, with differing degrees of gas filled segments.

2.4 DNA damage detection

The frozen samples were transported to the laboratory in liquid nitrogen and stored at -85 °C until analysis. All procedures for the detection of CPDs by immuno-dot-blotting followed the method described in Pescheck et al. (2014). As the damages were lower than after experimental UVB exposure a higher dilution of the calibration standard for CPDs was included on the membranes to be able to cover the right range of damage. The reagent for chemiluminescence was Pierce ECL (Thermo Fisher Scientific, Waltham, USA).

2.5 UVB screening

From the analyzed populations some thalli were chosen with the same criteria as for the other samplings and were brought to the lab and used for the screening measurements directly after the last measurement in the field. The thalli were transported in 50 mL tubes filled with water from the sampling site at ambient temperature. The ground fluorescence excited with blue-green light (420 – 550 nm) and UVB radiation ($\lambda_{max} = 314$ nm, 18 nm half band width) was determined with a Xenon

PAM fluorometer (Walz) as described in Bilger et al. (1997) and Pescheck et al. (2010) and apparent UVB transmittance was calculated as defined there.

2.6 Data analysis

Statistics were calculated and graphs were created in SigmaPlot version 11.0 (Systat Software, Inc., San Jose CA, USA). The diel cycles of F_V/F_M and CPDs Mb^{-1} were analyzed by one way repeated measures ANOVA and if a significant difference was detected the groups were separated with a Tukey post hoc comparison. All data are shown as means ($n= 4 - 8$) with standard deviations. In the long-term study linear regression analysis was performed of F_V/F_M and accumulated CPDs Mb^{-1} against the daily dose of PFD and UVB_{BE} (both integrated until time point of measurement; 13:00). From the light measurements recorded with the data logger mean values of every 15 min were calculated and integrated over 24 h for the accumulated doses.

3. Results

3.1 Environmental data

The environmental data of the daily courses are summarized in Table 1 and in Fig. 1. The irradiance curves of the daily courses measured in air are shown. While on the 7th of May 2009 and the 6th of August 2013 the sky was overcast the rest of the days were clear and the irradiance courses resembled sinusoidal curves. The maximal irradiances were present between 13:15 and 14:00 and accounted for PFDs of 660 – 1700 $\mu mol photons m^{-2} s^{-1}$ (Table 1).

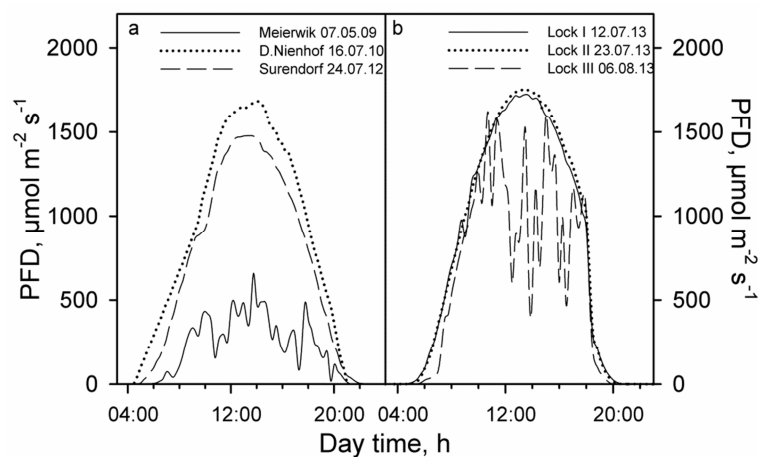


Figure 1. Horizontal photon flux density (PFD) on the daily courses measured 1 – 2 meters above the water surface of (a) the three beach locations, (b) the lock island in Kiel-Holtenau. PFD was measured automatically as 15 min means in Meierwik and on the lock island. In D. Nienhof and Surendorf PFD was measured approximately every 30 min.

3.2 Daily courses

In all the daily courses characteristic diel variations of the optimal quantum yield with irradiance were found (Fig. 2a, b). The highest values of F_V/F_M were always present at night (Meierwik), predawn (Dänisch-Nienhof) or the early morning (Surendorf, Lock I-III, repeated measures ANOVA $p < 0.05$). During noon concomitant with the peak of irradiance a significant reduction of F_V/F_M occurred between 12:00 and 16:00 except for the 7th of May, 2009 in Meierwik and the 6th of August, 2013 on the lock island. Photoinhibition maximally amounted to 55 – 77 % compared to the highest values (Table 2).

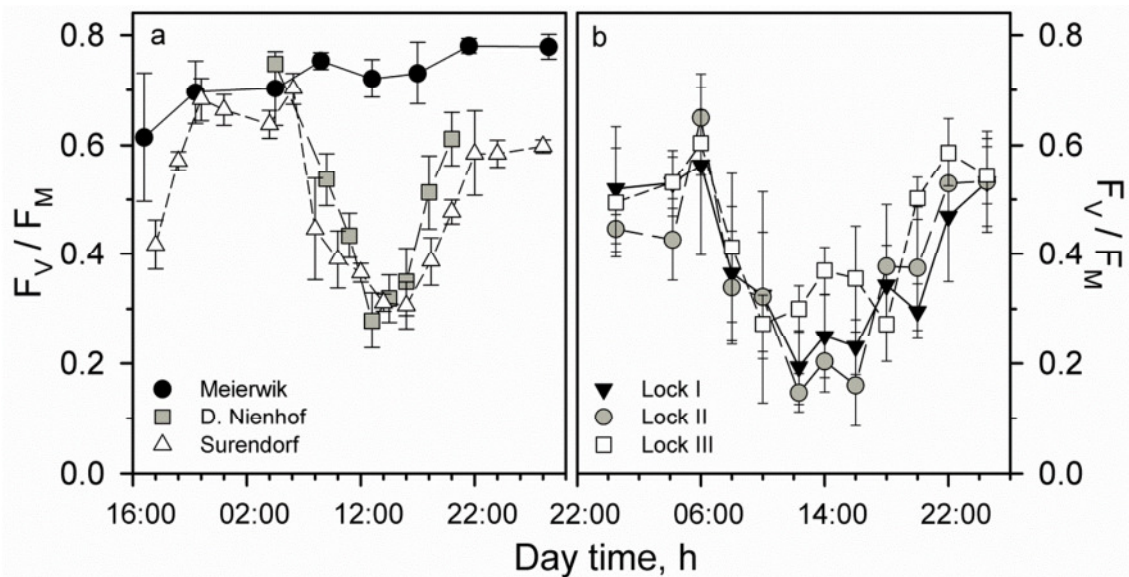


Figure 2. Optimal quantum yield of PSII measured after 15 or 20 min dark incubation on the daily courses; a) *in situ* measurements on the three beaches, b) mesocosms from the lock island. Data are means \pm 1 SD, $n \geq 4$. Notice the different scaling of the day time in a) and b).

In Dänisch-Nienhof PSII recovery started with a significant increase from 16:00 to 18:00 and was finished at 20:00 when the F_V/F_M values were identical to the morning values. Likewise, recovery was completed at 20:00 in Surendorf or 22:00 on Lock I and II. At that day time no statistically significant difference to the maximum F_V/F_M values of the early morning could be found anymore. In contrast to the other daily courses in Meierwik the weather was very cloudy and the PFD was below $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ most of the day (Fig. 1). Accordingly, no statistically significant decrease of F_V/F_M was present throughout the day, except for the late afternoon value of the previous day (Fig. 2a, repeated measures ANOVA, $p < 0.05$). Similarly, on the daily course of Lock III the sky was first clear but around 11:00 clouds appeared (Fig. 1). Consequently, until 10:00 the development of F_V/F_M equaled the ones of the sunny days with a significant decrease in PSII efficiency (Table 2).

Table 2. Maximal reduction of $F_V/F_M = 100 - (F_V/F_M (time\ x) / \max F_V/F_M * 100)$ and maximal concentration of CPDs with time of occurrence during the short-term observations. n.s.: not significantly different from all other day times (repeated measures ANOVA), n.d.: not determined.

Location	Date	Max. reduction of F_V / F_M	Max. CPDs Mb^{-1}
Meierwik	2009-05-07	8 % (13:00) ^{n.s.}	44 ± 24 (04:00) ^{n.s.}
D. Nienhof	2010-07-16	63 % (13:00)	44 ± 38 (13:00) ^{n.s.}
Surendorf	2012-07-24	56 % (16:00)	6 ± 2 (12:00) ^{n.s.}
Lock island	2013-07-12	66 % (12:00)	40 ± 35 (04:00) ^{n.s.}
Kiel-Holtenau	2013-07-23	77 % (12:00)	49 ± 14 (14:00) ^{n.s.}
	2013-08-06	55 % (10:00)	n.d.

On Lock III after 10:00 the drop of the optimal quantum yield stopped and F_V/F_M slightly recovered. Only in the afternoon a significant photoinhibition compared to the early morning and night values was found again ($p < 0.05$, repeated measures ANOVA). The relation between F_V/F_M and the instantaneous photon flux density during the six daily courses is given in Fig. 3. This relation was quite similar for both the morning and the afternoon hours in all the days. During Lock I and II the quantum yield of PSII started to increase again already before the peak in irradiance was reached (Fig. 3b). Moreover, during the 23rd of July (Lock II) the PSII activity was less inhibited during the afternoon hours than during the morning hours at the same PFD.

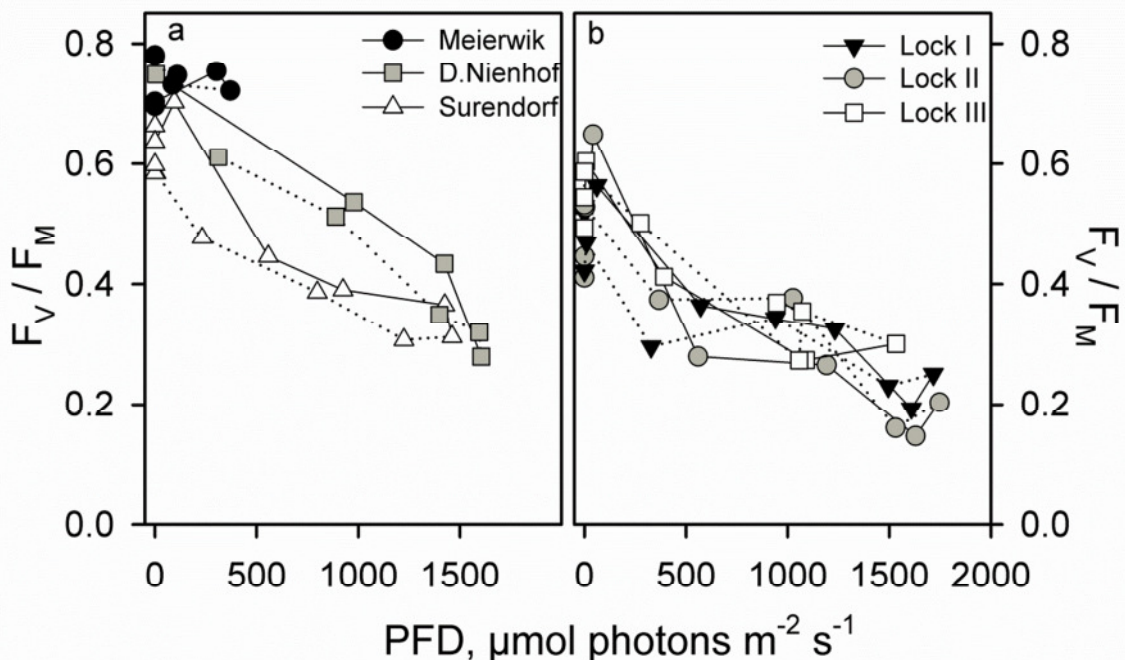


Figure 3. Relation of optimal quantum yield of PSII measured after 15 or 20 min dark incubation and incident PFD during the daily courses. Solid lines connect values from increasing PFDs, dotted lines connect values from decreasing PFDs. Data are from Figs. 1 and 2.

Additionally to the optimal quantum yield, the diel variations of the ground fluorescence (F_0) were determined after 15 or 20 min and 60 min of dark incubation in four of the daily courses (Fig. 4a – d). We assumed that during the night the F_0 levels for the two darkening periods of 15 or 20 min and 60 min were identical and therefore only took the 20 min values.

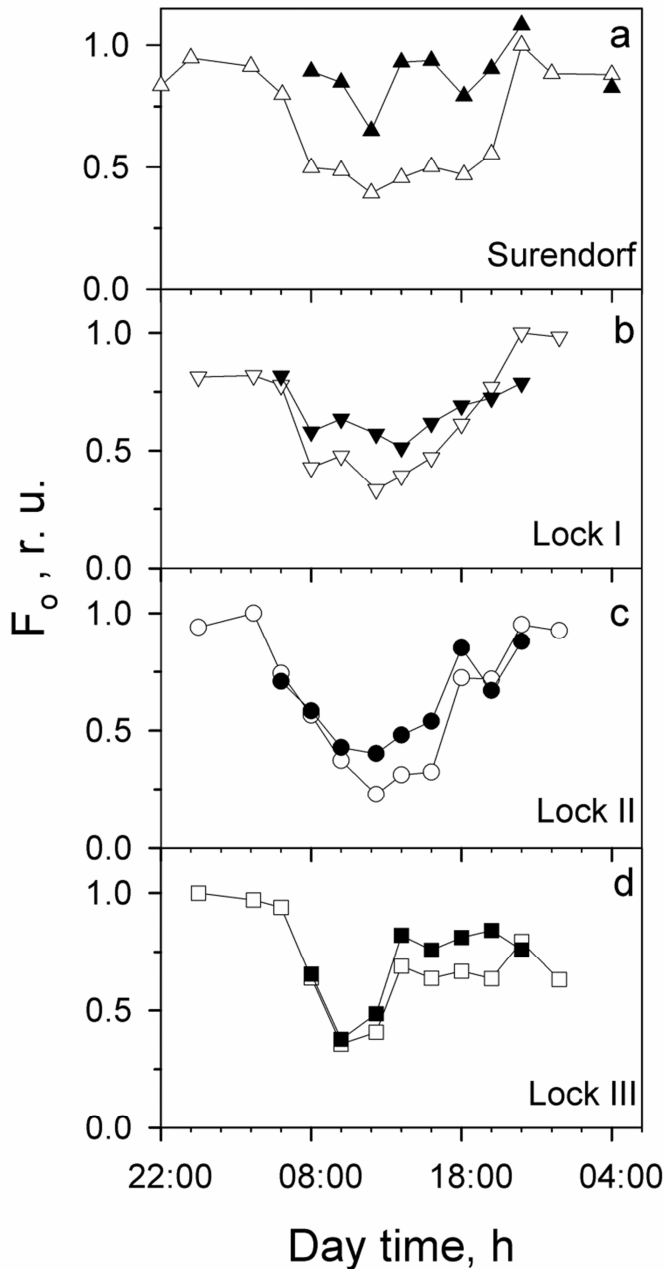


Figure 4. Normalized F_0 - values after 15 or 20 min (open symbols) and 60 min (closed symbols) darkness during the daily courses in Surendorf and on the lock island in Kiel-Holtenuau. Data are means, $n \geq 4$.

At all days a strong decrease of the normalized F_0 signal was found during the day after 15 or 20 min of dark incubation. This was nearly completely reversed after 60 min dark incubation in Surendorf, but not on the first two days on the Lock (Fig. 4b, c). On average over all days at noon around 65 % of F_0 were quenched as measured after 15 or 20 min dark incubation and still almost 50 % after 60 min with respect to the mean early morning and night levels of F_0 . This indicates a faster reversible component of F_0 -quench superimposed on a slower reversible component. The faster component (i.e. the difference of F_0 between 15 or 20 min and 60 min darkening) established during the hours of maximal irradiances concurrent with the strongest decline of F_V/F_M (compare Figs. 2 and 4). It diminished synchronously with the recovery of F_V/F_M during the evening when the level of F_0 was identical after both darkening times. Along with that the slow component of F_0 -quench (i.e. the difference of F_0 after 60 min darkening and F_0 measured during the night and early morning) disappeared again. In contrast, on the 6th of August (Lock III) the difference of F_0 between 20 min and 60 min of darkening was considerably lower and only appeared in the later afternoon, when irradiance levels were more stable (Fig. 4d).

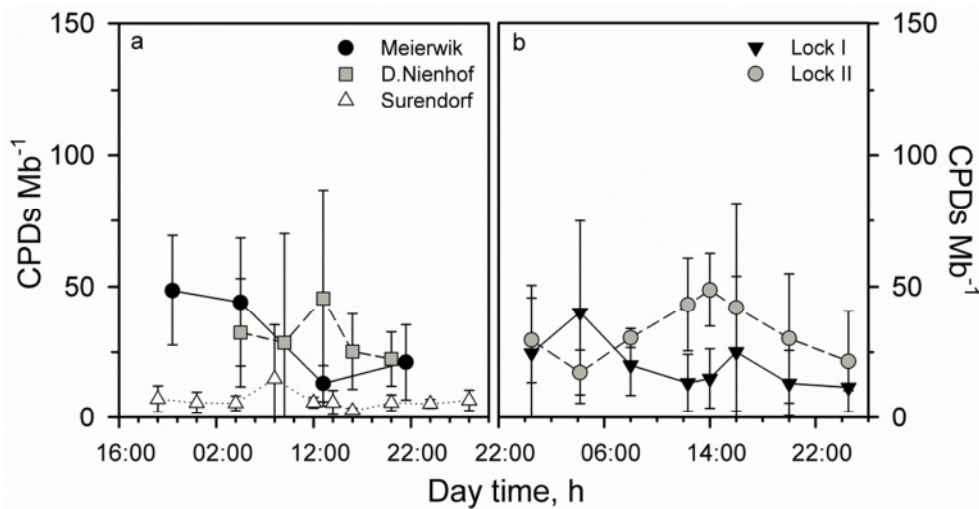


Figure 5. Concentrations of cyclobutane-pyrimidine dimers per megabase (CPDs Mb⁻¹) during the daily courses in *U. intestinalis* in a) the *in situ* measurements on the three beach locations, b) the mesocosms from the lock island. Data are means \pm 1 SD, $n \geq 4$. Notice the different scaling of the day time in a) and b).

With regard to the concentration of DNA damages in none of the daily courses a distinct pattern in the variation of CPDs Mb⁻¹ was found (Fig. 5a, b). Accordingly, no statistically significant differences were detected between day times ($p > 0.05$, one way repeated measures ANOVA, Table 2). There was also no relationship of CPDs Mb⁻¹ level with accumulated UVB dose (Fig. 6). For comparison the dashed line indicates the CPDs induced in *U. intestinalis* under experimental exposure conditions (0 – 5 W m⁻² UVB_{BE} + 15 μ mol photons m⁻² s⁻¹ PAR, for 1 h, at 9°C, results from Pescheck et al. in prep.) (Fig. 6). The y-axis intercept at 36 CPDs Mb⁻¹ of this line is higher than the mean of all field measured CPD concentrations which is 28 CPDs Mb⁻¹. In a linear regression analysis of the field data no statistically significant slope was found ($p = 0.61$, regression not shown).

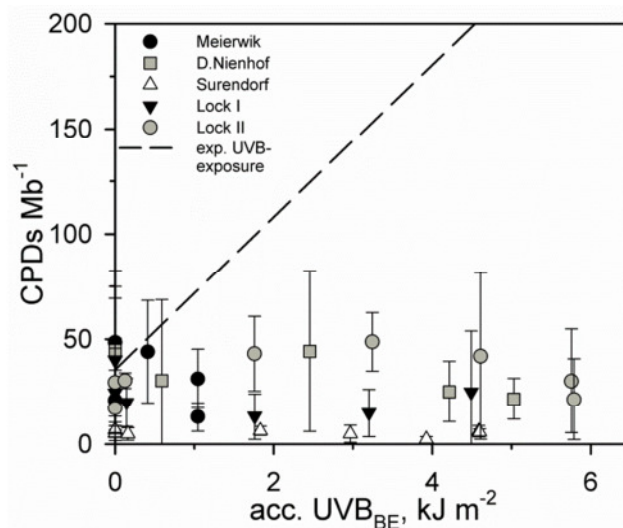


Figure 6. CPD concentrations in *U. intestinalis* in relation to accumulated UVB dose. CPD data are from Fig. 5. For comparison as a dashed line the induction of CPDs Mb⁻¹ in *U. intestinalis* exposed to experimental UVB (1 h at 0 – 5 W m⁻² UVB_{BE} at 9 °C, unpublished data from F.Pescheck, $r^2 = 0.77$) is given.

3.3 Relative sensitivity of DNA and PSII

In a direct sample by sample comparison DNA lesion frequency did not vary with PSII efficiency from the short-term measurements (Fig. 7). While the optimal quantum yield was maximally reduced down to 0.12 the highest concentration of dimers was below 150 CPDs Mb⁻¹. It is clearly demonstrated that both targets are not synchronously reacting and that F_V/F_M showed a much higher sensitivity than DNA integrity towards sunlight. The regression line from the combined field data gave no significant slope ($p=0.411$, $r^2 = 0.064$, regression not shown) confirming that *in situ* no relation existed between DNA damages and optimal quantum yield. As opposed to this, under the artificial UVB spectrum emitted from fluorescent tubes a tight correlation of DNA lesions and optimal quantum yield was found in *U. intestinalis* (Data from Pescheck et al., in prep.) (Fig. 7, open circles).

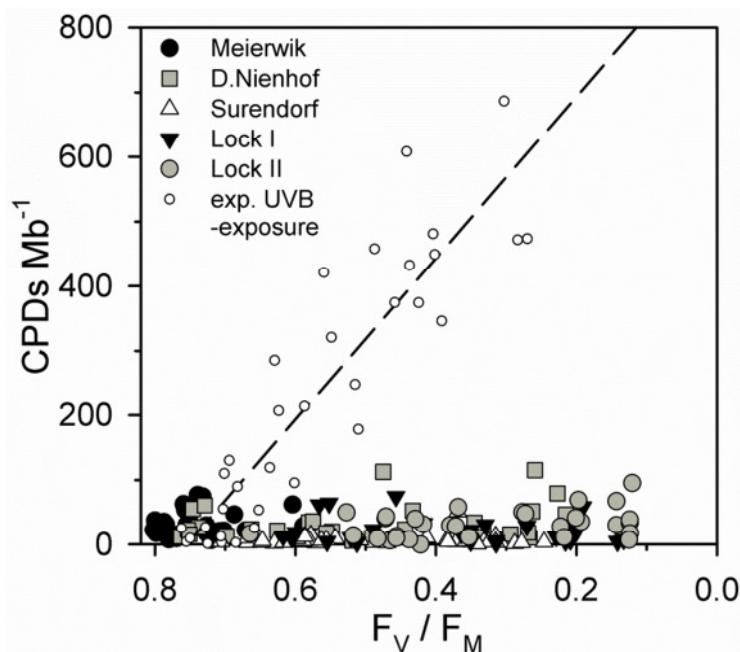


Figure 7. Paired CPD concentrations and levels of F_V/F_M in *U. intestinalis* from the daily courses. Each data point is from the same individual thallus. Additionally, the CPD concentrations and respective F_V/F_M values are shown after experimental UVB exposure as described in the legend of Fig. 6 ($r^2 = 0.82$).

3.4 Long-term study

The long-term development of the optimal quantum yield is shown in Fig. 8. In the beginning of the study period a high optimal quantum yield of nearly 0.70 was observed during the night (Fig. 8a). These high night values kept stable for the first week, during which the daily doses of PFD were mostly between 20 – 30 mol m⁻² (solid line in Fig. 8). When the daily PFD doses suddenly increased at the 5th of July the night and day maximum quantum yields remarkably dropped. The overall minimum of 0.14 was observed on the 20th of July at 12:00 coincident with the highest daily PFD dose. During the second run of the experiment the variation of F_V/F_M with the daily PFD doses

was similar (Fig. 8b). Especially after several consecutive cloudy days the midday and night values of F_V/F_M increased again. Using linear regression analysis a significant relation of the F_V/F_M noon level on the accumulated PFD dose (calculated until 13:00) was identified, with an adjusted R^2 of 0.611 for the first run of the long-term experiment (regression not shown). In the second run probably due to fewer observations no significant regression coefficient was detectable. However, in a combined data set of both runs all values matched one line and the regression was highly significant ($R^2_{adj.} = 0.685$).

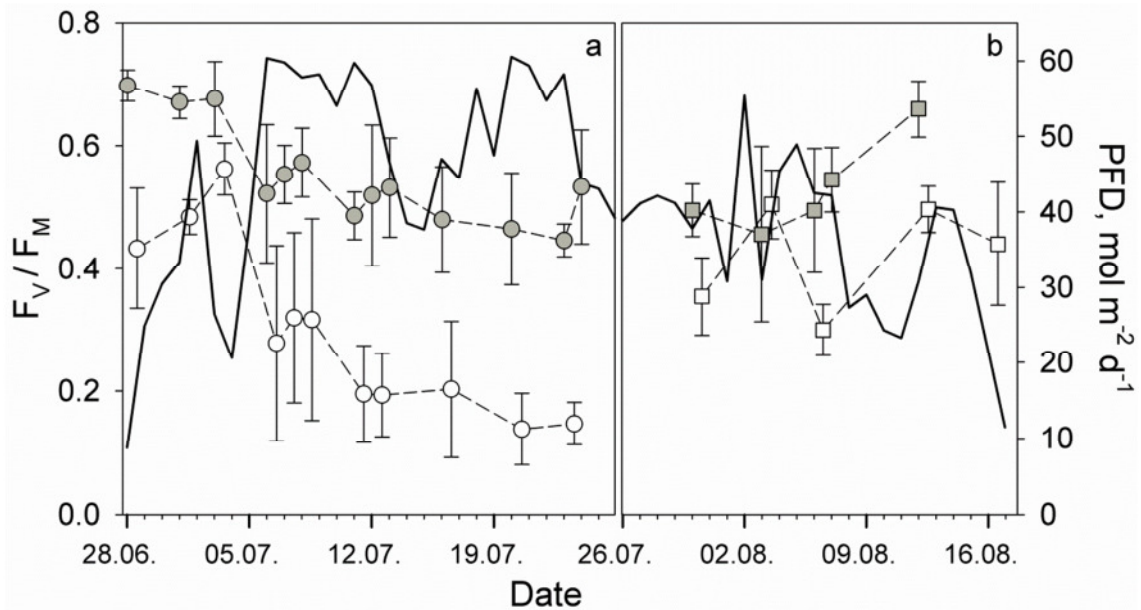


Figure 8. Long-term observation of optimal quantum yield of PSII in *U. intestinalis* from the mesocosms on the lock island, a) first run, b) second run. Data are means \pm 1 SD, $n = 4$. Symbol code: grey symbols: midnight values; open symbols: noon values; solid line: daily dose of PFD.

The variation of CPD concentrations and the associated weighted daily UVB_{BE} doses during the first run of the long-term study are shown in Fig. 9. In strong contrast to PSII activity no differences between night and day concentrations were observed. During the first week the concentrations of CPDs were barely above the detection limit. Even on the 6th of July when the daily UVB dose doubled no strong increase of CPDs Mb^{-1} occurred. No significant relationship of the concentration of CPDs with the daily accumulated dose of UVB_{BE} radiation was present ($p = 0.071$, $R^2_{adj.} = 0.243$, regression not shown). During the second run of the experiment no CPD concentrations were determined.

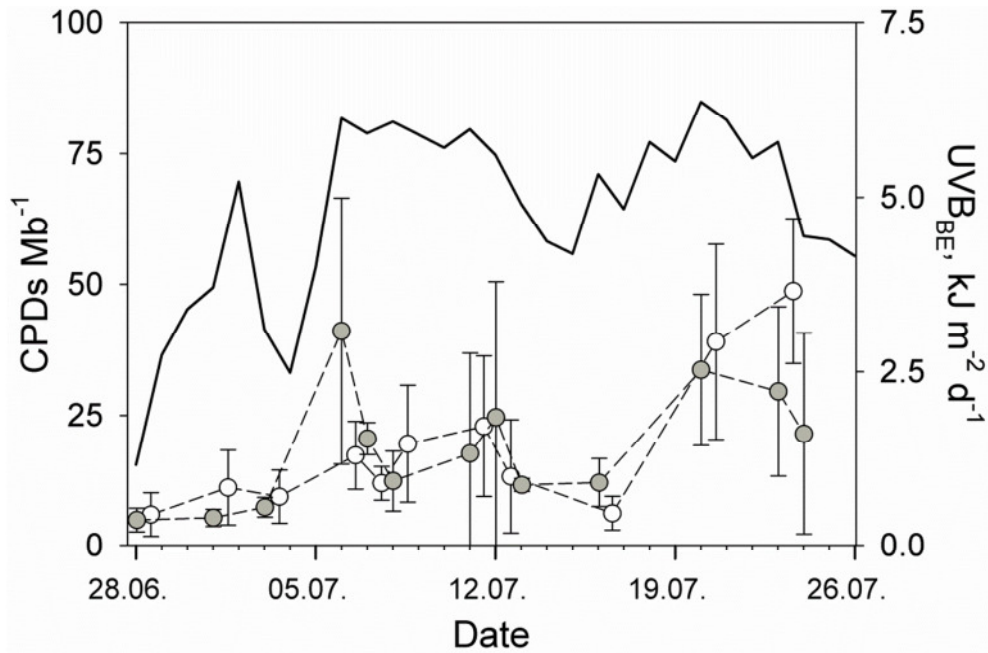


Figure 9. Long-term observation of cyclobutane-pyrimidine dimers per megabase (CPDs Mb⁻¹) in *U. intestinalis* from the first run of the mesocosms experiment on the lock island. Data are means \pm 1 SD, n = 4. Symbol code: grey symbols: midnight values; open symbols: noon values; solid line: daily cumulative dose of weighted UVB.

4. Discussion

4.1 DNA damages and optimal quantum yield

No other study investigated the concentration of DNA damages and the course of the optimal quantum yield of PSII in an intertidal macroalga over such a long period and with that high temporal resolution in the field. Although the presented data are derived from four different populations of *U. intestinalis* and four years all results are in complete agreement. Three of the daily courses were measured *in situ* on the beaches. In the long-term study on the lock island we were able to keep the algae in the mesocosms in nearly undisturbed natural conditions considering the main abiotic parameters light, temperature, nutrient supply and salinity.

Nevertheless, inside the mesocosms the water movement was strongly reduced and the algae were stably positioned. However, also in Surendorf the water surface was very calm and the thalli were not mixed by waves over the whole day. Thus, the light absorption of the thalli was extremely high on this day and during the whole long-term study. Due to the absence of UVB screening in *U. intestinalis* (see Table 1 and Pescheck et al. 2010) no physical shielding lowered the effective radiation and considerations about possible protection by absorbing pigments as they are encountered in field grown higher plants are not necessary (e.g. Takayanagi et al. 1994). In spite of this strong irradiation we observed very low concentrations of CPDs in *U. intestinalis*. There was no statistically correlation of DNA damages with UVB radiation neither on diel nor on long-term scales

(Figs. 5 and 9, Table 2). This proves that *U. intestinalis* did not accumulate relevant frequencies of CPDs over our study period of more than one month. CPD concentrations similar low like ours were described in a study from Antarctica with the green macrophyte *Prasiola stipitata* (Lud et al. 2001). The maximal damage in their study amounted to around 25 CPDs Mb⁻¹ even under artificially elevated UVB irradiances. In contrast to our results, a correlation of CPD concentration with cumulative UVB dose was found in that study. The UVB resistance of the DNA in *U. intestinalis* is also quite remarkable if one compares the data with those obtained from higher plants. E.g. in maize and *Gunnera magellanica* significant levels of CPDs were induced depending on daytime or cumulative UVB dose, respectively (Stapelton et al. 1997, Rousseaux et al. 1999). Unfortunately, no direct quantitative comparison of the CPD concentrations to these data is possible due to methodological differences. The virtual absence of an accumulation of DNA lesions points to a minor influence of UVB radiation on DNA integrity of *U. intestinalis* under field conditions.

In our study the failure of a relation of DNA lesions either with diel variations of UVB irradiance or cumulative UVB dose (Figs. 5 and 6) might be explained by photoreactivation. The highest photoreactivation rates were always reported from plants acclimated to natural sunlight due to an induction of the photolyase (Takayanagi et al. 1994, Kang et al. 1998). Equally important for the activity of the photolyase is irradiation with sufficient UVA radiation or blue light concomitant with the UVB exposure (Takeuchi et al. 1996, Dany et al. 2001). Therefore, we assume that the non-significant levels of CPDs we measured under strong solar irradiation may result from photoreactivation highly efficient due to both factors, induction of high photolyase activity and the presence of sufficient radiation at the appropriate wavelengths.

In contrast to the DNA, a strong reduction of optimal quantum yield of PSII was found *in situ* (Figs. 2 and 8). An explanation for this unexpected disparity might be unequal absorption of UVB radiation by the PSII centers and the DNA. In *Ulva* in each cell one large cup-shaped chloroplast is located at the periphery of the cytoplasm which during illumination orientates towards the outer surface of the thallus and thus might shade the nucleus efficiently from radiation (Britz et al. 1976). In *U. clathrata* this effect is suggested to increase the UVB resistance of the DNA by 50 % in comparison to *Rhizoclonium riparium*, a multinucleated siphonocladal species with a net-like chloroplast structure (Pescheck et al. 2014). The concept of 'chloroplast shielding' in combination with a very efficient repair capacity of PSII in *U. clathrata* was proposed to be of great importance in the UVB resistance of this species as it was also described for arctic diatoms (Karentz et al. 1991). One can speculate that the maintenance of gene expression and DNA replication is of major importance in a growing organism or in reproductive tissues reasoning a preferred protection by scarifying the (repair competent) chloroplasts. In photolyase-deficient mutants of *Synechosystis* the loss of PSII activity was significantly increased and the recovery significantly delayed after UVB exposure (Vass et al. 2013). This means a prevention of DNA damage would be also beneficial for the chloroplasts.

On the other hand it is not clear to what extent the observed changes in F_V/F_M were due to photodamage or photoprotection. Both processes are a “function of PFD..., duration of exposure, and spectral quality” (Henley 1993 and references therein) and have been observed to concur during the day in *Ulva rotundata* (Henley et al. 1992). From the daily cycles (Fig. 2) and the long-term study of F_V/F_M (Fig. 8) we can definitely state that in *U. intestinalis* strong photoinhibition occurred. A large portion of this photoinhibition was reversible within a few hours (Figs. 2 and 3). However, the observed low quantum yields during the day were supposedly not exclusively due to dynamic downregulation of PSII. A strong hint for photodamage at PSII is given by the incomplete recovery during the evenings until the midnight measurements at about 1:00 (Figs. 2 and 8). Theoretically, at night the residual photoinhibition from chronic damage to PSII may account for the divergence of the observed optimal quantum yield from the empirical maximum (close to 0.8 in *U. rotundata* from Henley et al. 1991, and 0.748 as average of *U. intestinalis* from this study, respectively). In fact, the course of the night values indicated a slowly decreasing recovery capacity of F_V/F_M overnight as long as the daily doses of PFD remained high (Fig. 8). These falling F_V/F_M values at midnight might have been caused by an accumulation of PSII damage over several days or by the development of a longer lasting non-photochemical quench, i.e. a down regulation of PSII quantum efficiency which was not reversible overnight. Interestingly, in the daily courses it was observed that not in absolute darkness during the night, but in the early morning hours in dim light the highest optimal quantum efficiencies were present and F_V/F_M recovered to an almost non-inhibited level (Fig. 2). Thus, probably the midnight values from the long-term study cannot be interpreted as persistent photodamage to PSII.

Further, we observed a strong decrease of the ground fluorescence on the daily courses (Fig. 4). A similar diel variation of F_O was observed by Henley et al. (1992) in *Ulva rotundata* acclimated and exposed to full sunlight. As described above in *Ulva* the chloroplasts do not turn to the sides in high-light but in the opposite orientation parallel to the outer thallus surface. Hence, the lowering of F_O is probably not due to chloroplasts movements but the development of a substantial F_O -quench. This F_O -quench indicates efficient non-photochemical quenching (NPQ) during the hours of maximal irradiances (Quick and Stitt 1989). In our study during the exposure to sunlight the F_O -quench may have even been stronger, since a significant dark period (of 15 or 20 min) was applied before the ground fluorescence was determined (Fig. 4, open symbols). During noon F_O was decreased compared to the morning level even after 60 min (Fig. 4, filled symbols). This observation of a slower than 60 min reversible component of F_O -quench emphasizes the probability that also at midnight still some down regulation of PSII was present in *U. intestinalis* leaving less probability of chronic photoinhibition due to damages. The underlying processes of the non-photochemical quenching which was responsible for the observed F_O -quench have not been revealed. But as also shown in *Ulva rotundata* (Franklin et al. 1992) and as reviewed in Jahns and Holzwarth (2012) the

complete reversal of quenching by the xanthophyll cycle can take more than 60 min (which was our longest darkening time) especially after exposure to very high irradiances. This retarded back-reaction of zeaxanthin to violaxanthin in high irradiances would be an acceptable explanation for the behavior of the slower reversible component of the observed F_0 -quench in our study (Fig. 4, filled symbols).

Moreover, in our study the recovery of F_v/F_M already started while irradiances still were oversaturating (Fig. 2a, b). This was also observed in a field study of *U. rotundata* by Henley et al. (1992). The apparent absence of a pronounced hysteresis in Fig. 3 indicates that the recovery process was rather rapid, suggesting either the presence of down-regulation or a well working repair cycle (Allakhverdiev and Murata 2004). Additionally, photodamage to the PSII reaction center normally is mirrored in an increase of F_0 as the consequence of an over-reduced primary electron acceptor Q_A (Franklin et al. 1992, Osmond et al. 1993, Vass et al. 1996). From these arguments it can be concluded that sunlight acclimated *U. intestinalis* possesses a very effective photoprotective potential similar to *U. rotundata* (Henley et al. 1992).

4.2 Relative sensitivity of DNA integrity and PSII

The parallel analysis of two major targets of sunlight in the presented study allows to consider the relative sensitivity of the two processes photosynthesis and gene expression *in situ* (Fig. 7). While PSII efficiency apparently was inhibited by more than 60 % during noon, no significant CPD concentrations were detectable (see Table 2). In laboratory studies, however exposure to experimental UVB (of artificial spectral composition with strongly elevated UVB:PAR ratios) caused linear relationships of PSII inhibition and DNA damage concentration with increasing UVB dose in *U. intestinalis* and two other green macroalgae (dashed line in Fig. 7 and Pescheck et al. 2014).

On the other hand the induction of thymine dimers is highly specific for UVB radiation while PSII can be inactivated by distinct mechanisms either by UVB radiation resulting in donor side photoinhibition or high PAR leading to acceptor-side photoinhibition (Vass 2012). Further, the two spectral regions act contrastingly on the repair rates for both targets. In *Arabidopsis thaliana* photoreactivation of CPDs is driven by UVA radiation (Dany et al. 2001) which in contrast might intensify the damage to PSII by destruction of the manganese cluster (Hakala et al. 2005, Ohnishi et al. 2005). Our data suggest that the balance of CPD induction and photoreactivation is shifted towards dimer splitting under natural sunlight compared to the experimental UVB exposure. Opposed to that, it seems that PSII function is not favored similarly by solar irradiation resulting in substantial photoinhibition. This demonstrates that exposure to natural sunlight is necessary to be able to judge the ecological impact of UVB on DNA integrity and PSII function.

Acknowledgement

During the field work Nele Hein, Cayssa Staggen, Christine Bönninger, Hanna Jämlich, Paul Wieners and Jens Hermann helped a lot. Florian Weinberger gave useful advices for the conduction of the mesocosm experiment. Many thanks to Susanne Wolf for help with DNA extraction and blotting. From Prof. Brendelberger the loan of life jackets is acknowledged. Prof. Krupinska is thanked for the ability to use the dark room. Thanks to the Waterways and Shipping office in Kiel Holtenau for the facilitation and support of the long-term study on the lock island in Kiel Holtenau.

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Chapter 4

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Chapter 4

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Chapter 5 – UVB resistance mechanisms in four green macroalgae with different ecological niches

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(Manuscript as prepared for submission to the Journal of Experimental and Environmental Botany)

Abstract

Most species from the class of green macroalgae have a predominant distribution in the intertidal, an opportunistic life strategy, and a good tolerance towards abiotic stresses. Despite these similarities, this class also encompasses quite different types of life forms regarding morphology and anatomy as well as physiology and ecology. To find out how individual species of green algae respond and how differently they have adapted to ultraviolet-B (UVB, 280 – 315 nm) radiation an extensive experimental study was conducted. The UVB-induced reduction of photosystem II (PS II) efficiency and the accumulation of DNA damages in *Acrosiphonia spec.*, *Bryopsis hypnoides*, *Cladophora sericea* and *Ulva intestinalis* were determined as a quantitative measure of UVB susceptibility. Additionally, the protection by UV screening and the cellular tolerance based on repair rates were analyzed. UVB-induced damage to PS II and the DNA was lowest in the screening alga, *C. sericea* and highest in the more subtidally growing *B. hypnoides*. While recovery of PS II was significantly faster in *U. intestinalis* than in the other species the repair of DNA damages was equal in all of them. Overall the results reveal species-specific UVB resistance strategies which are discussed in relation to the ecological niche of the species.

Key words: *Acrosiphonia*, *Bryopsis hypnoides*, *Cladophora sericea*, cyclobutane pyrimidine dimer, green algae, photosystem II, recovery rate, ultraviolet-B radiation, *Ulva intestinalis*, UV screening, zonation

1. Introduction

Macroalgae are very important components of coastal ecosystems as they give food and shelter to a diverse assemblage of invertebrates, larvae and small fish (Lubchenko 1978, Dodds and Gudder 1992, Råberg and Kautsky 2007). They also substantially contribute to the carbon fixation of marine primary production (Valiela et al. 1997) and can build up massive populations under certain circumstances (Taylor et al. 2001, Nelson et al. 2008). Especially green algae from the family Ulvaceae are regularly dominant during blooms (Nelson et al. 2008, Wang et al. 2012), and this group contains many opportunistic and pioneer species (Sousa 1979, Littler and Littler 1980, Juanes et al. 2008, Fricke et al. 2011). But also species from the Cladophoraceae are possible candidates for mass developments (Dodds and Gudder 1992, Peckol and Rivers 1996, Wahl et al. 2004). Nevertheless, little is known about differences of stress responses within the group of green filamentous uniseriate or branched species, the so called turf algae. In many morpho-functional models this group is not differentiated further (Littler and Littler 1980, Steneck and Dethier 1994). On the other hand, the exact function for the ecosystem is individual from species to species as some species have a higher grazing resistance than others (Lubchenko 1978, Guidone et al. 2010) and have a different morphology, growth rate, and biochemical composition (Nelson et al. 2003). Therefore it is an important question how the dominance pattern among several green algae might be influenced.

Very high abundances of species from the genus *Ulva* and *Cladophora*, and from time to time also other green algae, in the eulittoral zone indicate a good adaptation of this group to the highly variable environmental factors (Davison and Pearson 1996). One of the most striking of the abiotic factors is the sunlight, clearly creating a vertical gradient on the shore. As an intrinsic part of sunlight ultraviolet-B (UVB, 280 – 315 nm) radiation also has the potential to shape the distribution of species along this gradient and to structure the community of macroalgae (reviewed in Bischof et al. 2006a). UVB radiation is strongly attenuated by coastal waters, especially due to high concentrations of colored dissolved organic matter (CDOM) (reviewed by Tedetti and Sempere 2006). For the western Baltic very high diffusive attenuation coefficients above 10 m⁻¹ have been reported (Molis et al. 2003). This means, while the damaging potential of UVB radiation is especially pronounced during emersion periods, it rapidly decreases with growth depth. Although the development of the ozone holes is slowed or even stopped the UVB flux density on the earth surface currently still is substantially increased compared to 1980s levels (Newman et al. 2009). Future predictions tend to prognosticate a small decrease in UV irradiances for mid and high latitudes and an increase for low latitudes (McKenzie et al. 2011).

In algae UVB radiation can reduce growth (Michler et al. 2002, Grobe and Murphy 1998) and photosynthesis as well as protein and pigment content (Bischof et al. 2000). With regard to

photosynthesis the primary target probably is photosystem II (PS II). Absorption of UVB radiation by the manganese cluster of the oxygen evolving complex may result in a reduced electron delivery towards the PS II reaction center (reviewed by Vass et al. 2012). Subsequently the oxidized radical (P680^{•+}) accumulates and in turn may oxidize neighboring compounds, as the D1 protein (Jegerschöld et al. 1990, Ohnishi et al. 2005). Overall these events cause a decreased optimal photochemical quantum yield (F_V/F_M) of PS II. Another important target of UVB radiation is the DNA. Absorption by pyrimidine bases can lead to the formation of a covalent electron bond between two neighboring thymines or cytosines. Thereby mainly cyclobutane-pyrimidine dimers (CPDs) but also pyrimidine(6,4)pyrimidone photoproducts (6,4-PPs) are formed (Mitchell and Nairn 1989, Douki et al. 2000) which inhibit the progression of DNA and RNA polymerases on the damaged DNA molecule (Sauerbier et al. 1970).

Photoautotrophic organisms employ UVB resistance mechanisms to cope with UVB radiative stress. These can be physical by the accumulation of UV-screening compounds or physiological by cellular tolerance mechanisms. Screening serves as a barrier to UVB radiation avoiding the induction of damages, especially if it is localized in the exposed tissue. In contrast, cellular tolerance is a complex system of repair or replacement of damaged structures, diverse enzymatic reactions and the accumulation of defensive molecules, like antioxidants (Murata et al. 2012). For example one major tolerance mechanism is the PS II repair cycle, coordinating a fast turnover of the D1 protein (Xiong 2001, Mulo et al. 2012). Another very important UVB tolerance mechanism in plants is the reversal of dimerisation of pyrimidine bases in the DNA (Britt 2004). Most important is the photoreactivation driven by a specific enzyme, the photolyase. This enzyme uses UVA and blue radiation to split the covalent bond of the DNA dimer. In turn, one has to consider that all cellular tolerance processes depend on gene expression. Thus, inhibition of transcription by UVB-induced DNA damages may intensify the UVB impact on other targets as it was observed for PS II inhibition in a DNA repair-deficient mutant of *Synechocystis* (Vass et al. 2013).

Overall there is a lack of data comparing the UV resistance of green macroalgal species and elucidating the strategies with respect to possible UVB resistance mechanisms within this seemingly homogenous group. Pronounced differences have been observed between species from the Ulvales and Cladophorales with respect to UVB screening (Pescheck et al. 2010). In a detailed laboratory study significant UVB protection of the DNA and PS II could be demonstrated by screening in *Rhizoclonium riparium* in comparison to the non-screening *Ulva clathrata* (Pescheck et al. 2014). In contrast *U. clathrata* displayed a significantly higher repair rate of PS II and additionally a higher tolerance of DNA towards UVB, increasing its cellular UVB tolerance.

In the present study we wanted to analyze UVB resistance mechanisms in four species of green macroalgae living close to each other along a short depth gradient. Samples of algae were collected

from the field of the species *Ulva intestinalis* (Ulvales), *Cladophora sericea* (Cladophorales), *Acrosiphonia spec.* (Ulotrichales) and *Bryopsis hypnoides* (Bryopsidales). The first three species typically occur in the upper eulittoral (Munda 1999, Choo et al. 2005). In contrast *B. hypnoides* is a representative of the upper sublittoral. On the other hand, *Acrosiphonia spec.* stops its growth around June, and after pigment breakdown disappears completely by the beginning of July. We hypothesized that the existing elevational and seasonal patterns, forming the respective ecological niches, would be correlated to the extent of UVB resistance of the four species. This resistance was assessed as UVB screening capacity and the responses of optimal quantum yield of PS II and DNA lesions following exposure to UVB radiation. Furthermore, repair rates were assessed for both targets as a measure of cellular tolerance.

2. Material and Methods

2.1 Plant material

Between April 2009 and September 2012 macrothalli of the four species, *Ulva intestinalis* (Linnaeus), *Acrosiphonia spec.* (J. Agardh), *Cladophora sericea* ((Hudson) Kützinger) and *Bryopsis hypnoides* (J.V. Lamouroux) (hereafter designated as *Ulva*, *Acrosiphonia*, *Cladophora* and *Bryopsis*) were collected at the northern bank of the Kiel Canal close to the lock in Kiel Holtenau (54°22'N, 10°08'E, Kiel, Germany). The sampling site was inside the Canal where no water-level fluctuations occur. The salinity at the sampling station varies between 10 -14 PSU (Gocke et al. 2010). All collected algae were identified on the basis of morphological characters examined under a light microscope (Carl Zeiss, Oberkochen, Germany) in the lab. At this location a dense population of *Ulva* grows almost all year round on some boulders placed for shore stabilization. Many thalli are positioned in the supralittoral zone where they are moistened only by waves from passing ships. We only sampled thalli attached to the rocks but floating on the water surface due to their gas filled tubes. At the same location from about April to the end of June right under the water surface *Acrosiphonia spec.* builds up dense stocks of typical tufts consisting of felted filaments. Sampling was stopped before the thalli turned brownish later in the season. *Cladophora* instead appears around June and lives in between the mats of *Ulva* and other macroalgae like *Fucus spec.* Once day length and solar angle have almost reached their maximum *Bryopsis* can be found in scattered patches markedly below the water surface. We sampled thalli from a depth range of 40 – 60 cm. On each sampling event two species were collected and were used in parallel for the experiments.

2.2 Culture conditions

In the lab the algae were thoroughly cleaned from other algae and small animals and placed in petri dishes with 40 mL nutrient enriched 1:1 diluted sterile filtered seawater. The medium was changed every 2 – 3 days. The algae were kept in a temperature controlled climate cabinet (Rumed, Rubarth

Apparate GmbH, Laatzen, Germany) at 9 °C and illumination was from three fluorescence tubes (F15W, General Electrics, Fairfield, CT, USA) delivering around 10 – 12 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) for 16 h per day. This pre-experimental culture was maintained for 10 – 19 days after sampling to ascertain that no UVB induced damages originating from the field were present in the algae anymore. If sampling coincided with a phytoplankton bloom GeO_2 (1 mg L^{-1}) and/or ampicillin (2 mg L^{-1}) was added to the medium to inhibit growth of diatoms or cyanobacteria for the first seven days. Additionally from all species a larger pool of biomass was kept in 200 mL glass beakers under the same conditions. One dose response experiment per species was conducted with the biomass from this cultured material (experiments marked with crossed symbols in the graphs).

2.3 UVB exposure experiments

In all experiments two species were exposed simultaneously as described in Pescheck et al. (2014). For each species the experiments were at least repeated three times with random pairs of species. In the dose response experiments (numbered as D1 – D6) for 1 h five UVB fluence rates were applied from 0 – 4.30 W m^{-2} (biologically weighted with the function of Ghetti et al. (1999), (Table 1)). As the UVB fluorescence tubes (TL40 / 12RS, Philips, Amsterdam, Netherlands) also emit a small proportion of visible light, this varied synchronous with UVB fluence rate between 2 and 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. An irradiance spectrum of the used set-up can be found in Pescheck et al. (2014). Exposure was conducted in two 50 mL petri dishes per treatment covered with WG295 filters (Schott, Mainz, Germany) to exclude wavelengths below 295 nm. Directly after exposure, from each of the two petri dishes four subsamples were taken for CPD analysis, frozen in liquid nitrogen and stored at -85 °C until DNA extraction. The remaining algae in the dishes were darkened for 20 min and F_V/F_M was determined using an Imaging-PAM chlorophyll fluorometer (Walz, Effeltrich, Germany). In the recovery experiments 12 petri dishes per species (resulting in two independent dishes per sampling time point) were exposed to a moderate intensity of UVB radiation for 1 h and thereafter transferred to continuous low white light of the pre-experimental intensity (numbered as R1 – R5, Table 1). By varying the UVB irradiances it was intended to induce approximately 50 % of PS II inhibition in the different species. At different time points from 0 – 48 h four subsamples per petri dish from the two dishes at each occasion for CPD analysis and F_V/F_M determination have been analyzed. Control concentrations of CPDs and control values of F_V/F_M were measured in eight randomly chosen subsamples from all prepared dishes before the start of the UVB exposure. In experiments R5a – c in *Ulva* three different UVB irradiances were applied and only before, directly after UVB-exposure and after 48 h of recovery F_V/F_M and CPDs were analyzed.

Table 1. UVB_{BE} irradiances (weighted after Ghetti et al. 1999) used for the dose response experiments (D1-D6) and repair experiments (R1-R5). Exposure lasted always 1 h. In experiments 5a – c *Ulva* was exposed to three levels of UVB irradiance and recovery was measured after 48 h.

species	UVB _{BE} , W m ⁻²																			
	<i>Acrosiphonia</i>					<i>Bryopsis</i>					<i>Cladophora</i>					<i>Ulva</i>				
	Experiment	1 /	2 /	3 /	4 /	5	1 /	2 /	3 /	4 /	5	1 /	2 /	3 /	4 /	5	1 /	2 /	3 /	4 /
D1	0 /	0.37 /	0.71 /	1.48 /	3.10	0 /	0.42 /	0.83 /	1.61 /	3.04	0 /	0.41 /	0.78 /	1.54 /	3.29	0 /	0.37 /	0.71 /	1.48 /	3.10
D2	0 /	0.38 /	0.73 /	1.50 /	3.00	0 /	0.40 /	0.91 /	2.09 /	4.24	0 /	0.38 /	0.73 /	1.50 /	3.00	0 /	0.41 /	0.78 /	1.54 /	3.29
D3	0 /	0.82 /	1.66 /	1.92 /	3.47	0 /	0.60 /	1.28 /	2.20 /	4.30	0 /	0.55 /	1.03 /	1.91 /	3.75	0 /	0.55 /	1.03 /	1.91 /	3.75
D4		-									0 /	0.40 /	0.91 /	2.09 /	4.24	0 /	0.82 /	1.66 /	1.92 /	3.47
D5																0 /	0.42 /	0.83 /	1.61 /	3.04
D6																0 /	0.60 /	1.28 /	2.20 /	4.30
R1			3.20					3.46					3.52					3.46		
R2			3.16					1.62					3.41					3.20		
R3			3.23					1.79					3.78					3.16		
R4			3.03					2.08					3.90					3.23		
R5a-c			-					-					-					1.88 /	3.22 /	4.12

2.4 DNA extraction and quantification

The DNA from the eight subsamples per treatment was extracted as described in Pescheck et al. (2014). Determination of DNA concentration was conducted with a fluorometric assay with the QuantiFluor dye (QuantiFluor, Promega, Madison, USA).

2.5 CPD detection

In principle the quantification of cyclobutane-pyrimidine dimers (CPDs) by immuno-blotting followed the protocol as explained in van de Poll et al. (2002) and Pescheck et al. (2014). The only differences were as follows. After blotting 50 ng of heat denatured samples onto the membrane it was incubated over night with the primary antibody (monoclonal anti-thymine dimer antibody, Sigma-Aldrich, St. Louis, USA) at 4 °C. After three washing steps with PBST (PBS, 0.1 % Tween (vol/vol), Roth, Karlsruhe, Germany) the membrane was exposed to the secondary antibody (anti-mouse IgG peroxidase antibody, 1:25000, Sigma-Aldrich) at room temperature for 2 h. Detection was performed by chemiluminescence using an ECL reagent (Pierce ECL western blotting reagent, Fisher Scientific, Schwerte, Germany). On each blot a serial dilution of a CPD standard with known amounts of CPDs Mb⁻¹ as described in Pescheck et al. (2014) was included in duplicate and the greyscale values of this were used to generate a calibration function. With this function the greyscale values of the samples were converted to CPDs Mb⁻¹.

2.6 Apparent UVB transmittance

One day before the UVB exposure experiments the apparent UVB transmittance of the thalli was determined with a Xenon-PAM chlorophyll fluorometer (Walz) after the method described in Pescheck et al. (2010). In short, chlorophyll fluorescence was induced with an UVB measuring beam ($\lambda_{\text{max}} = 314$ nm, 18 nm halfband width) and a blue green measuring beam ($\lambda = 420 - 550$ nm). The ratio of both signals was related to that obtained from unprotected samples, i.e. isolated chloroplasts of the respective species. Thereby a quantitative determination of the amount of UVB radiation passing through the cell wall and cytoplasm into the chloroplasts could be achieved.

2.7 Statistical analysis

In the dose response experiments for each species a linear regression analysis was run using GraphPad Prism 5 (GraphPad Software, San Diego, USA). The reduction of PS II efficiency and CPD accumulation was analyzed with the pooled raw data from all replicate experiments and the resulting regressions are drawn as a solid line in the figures. With the 95 % confidence interval of the slopes of these regressions the significant differences between the species were determined. In the repair experiments the recovery kinetics of PS II efficiency were determined by fitting the data to the model: $(f)x = y_0 + \alpha (1 - \exp(-xb))$ according to Campbell and Tyystjärvi (2012) using SigmaPlot 11 (Systat Software GmbH, Erkrath, Germany). Due to limited sampling from experiment R5a – c in *Ulva*, this experiment could not be analyzed in the repair kinetics. The removal rate for CPDs h^{-1} was calculated as the slope of the linear regression over the log-natural transformed DNA damage data using SigmaPlot 11.

3. Results

3.1 UVB screening

UVB screening was assessed as apparent UVB transmittance. An apparent UVB transmittance of 1.0 corresponds to complete absence of screening substances absorbing in the wavelength region of the UVB excitation beam. In *Acrosiphonia* the UVB transmittance was not different from 1.0 (student's *t*-test, $p=0.39$) (Table 2). *Bryopsis* also showed very high transmittances, resulting in low UVB protection of less than 10 % shielding. In *Ulva* the transmittance varied between 0.931 and 0.732, meaning a maximal screening of 26.8 %. In contrast, in *Cladophora* a much lower mean UVB transmittance of 0.475 was measured, shielding the chloroplasts from more than 50 % of the applied UVB irradiance.

Table 2. Mean values of apparent UVB transmittance ($T_{UVB} \pm 1$ SD, $n=8$) of the algae one day before the respective experiments. Data marked with the asterisk are from experiments with the six weeks cultured biomass pool, for further explanation see Material and Methods section.

species Experiment	T_{UVB}			
	<i>Acrosiphonia</i>	<i>Bryopsis</i>	<i>Cladophora</i>	<i>Ulva</i>
D1	1.090 ± 0.022	0.933 ± 0.028*	0.514 ± 0.027	0.832 ± 0.039
D2	1.048 ± 0.024*	0.862 ± 0.042	0.474 ± 0.016*	0.891 ± 0.041
D3	0.926 ± 0.026	0.932 ± 0.027	0.425 ± 0.041	0.845 ± 0.047
D4	-	-	0.529 ± 0.037	0.931 ± 0.090
D5	-	-	-	0.829 ± 0.072*
D6	-	-	-	0.873 ± 0.018
D7				
R1	1.005 ± 0.033	0.931 ± 0.030	0.451 ± 0.024	0.770 ± 0.035
R2	1.030 ± 0.063	0.894 ± 0.034	0.456 ± 0.047	0.732 ± 0.076
R3	1.025 ± 0.032	0.932 ± 0.018	0.465 ± 0.021	0.749 ± 0.027
R4	0.923 ± 0.034	0.978 ± 0.019	0.489 ± 0.018	0.798 ± 0.032
R5a-c	-	-	-	0.836 ± 0.014

3.2 UVB sensitivity of photosystem II

In Fig. 1 the percentage reduction of the optimal quantum yield of PS II for all experiments is shown compared to non-irradiated control samples in dependency of the applied UVB irradiance. *Acrosiphonia* and *Ulva* displayed a similar UVB sensitivity of PS II whereas in *Bryopsis* PS II was slightly, but significantly more vulnerable. *Cladophora* turned out to be only half as sensitive as the other species. At a fluence rate of $1 \text{ W m}^{-2} \text{ UVB}_{BE}$ in this species no photoinhibition of PS II occurred at all, whereas in *Bryopsis* at this irradiance already 20 % reduction of F_v/F_M was found. Additionally, the reduction of PS II efficiency from the repair experiments is included in the figure (grey squares) but not in the correlation analysis. By multiplication of the applied UVB irradiance with the respective UVB transmittances of the thalli the effective UVB irradiance was calculated (Schultze 2011, Pescheck et al. 2014). The dose response of PS II inactivation in relation to the effective UVB irradiance is given in Fig. 2. Now, the slopes of *Ulva*, *Cladophora*, and *Bryopsis* converged almost completely clearly demonstrating that the intrinsic sensitivity of PS II towards UVB was identical in these species. Only *Acrosiphonia* had a significantly lower dependency. Between the experiments carried out with algal material of 10 – 19 days or six weeks of pre-experimental culture was no difference (crossed circles in Figs. 1 and 2).

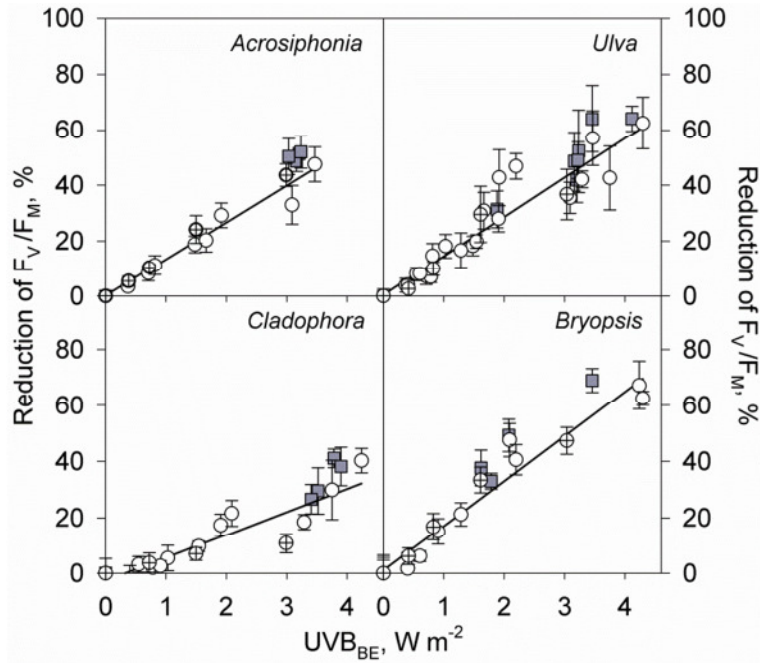


Figure 1. Reduction of optimal photosynthetic efficiency (F_V/F_M) dependent on applied UVB irradiance. % reduction of $F_V/F_M = 100 - (F_V/F_M / F_V/F_M \text{ mean of control} * 100)$, mean \pm 1 SD ($n=8$). Symbol code: open circles, experiments D1 – D6, crossed circles, experiments with algae cultured for six weeks, grey squares, experiments R1- R5. Regression lines were calculated from the pooled raw data excluding the repair experiments: *Acrosiphonia*: $y = 0.2 + 13.2x$, $r^2 = 0.92$ (A); *Ulva*: $y = 0.5 + 14.1x$, $r^2 = 0.84$ (A), *Cladophora*: $y = -2.7 + 8.2x$, $r^2 = 0.78$ (B); *Bryopsis*: $y = 1.1 + 16.0x$ $r^2 = 0.91$ (C). Capital letters in brackets indicate statistically identical slopes of the regressions.

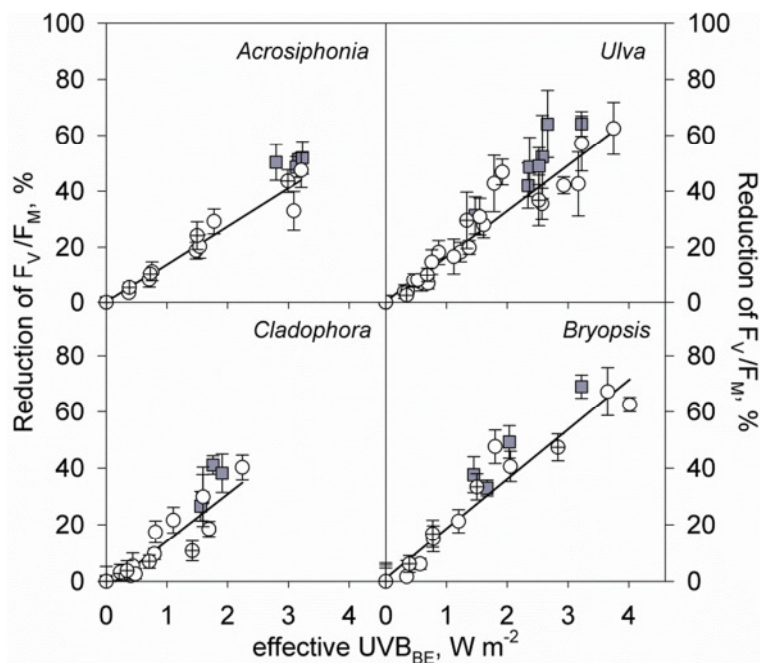


Figure 2. Reduction of optimal photosynthetic efficiency (F_V/F_M) dependent on effective UVB irradiance calculated as $UVB_{BE} \times T_{UVB}$. Symbol code and calculations are as in Fig.1. *Acrosiphonia*: $y = 0.2 + 13.6x$, $r^2 = 0.91$ (A); *Ulva*: $y = 0.4 + 16.3x$, $r^2 = 0.86$ (B); *Cladophora*: $y = -2.5 + 16.7x$, $r^2 = 0.78$ (B); *Bryopsis*: $y = 1.0 + 17.6x$, $r^2 = 0.91$ (B). Capital letters in brackets indicate statistically identical slopes of the regressions.

3.3 UVB sensitivity of the DNA

Induction of cyclobutane-pyrimidine dimers (CPDs) was a linear function of applied UVB irradiance in all four species (Fig. 3). The highest sensitivity of DNA was found in *Bryopsis* and was followed by *Acrosiphonia* being not statistically different from each other. *Ulva* showed an intermediate sensitivity of the DNA. Again, *Cladophora* was less sensitive than the other three species and its DNA was damaged with a significantly reduced magnitude when correlated to the applied UVB.

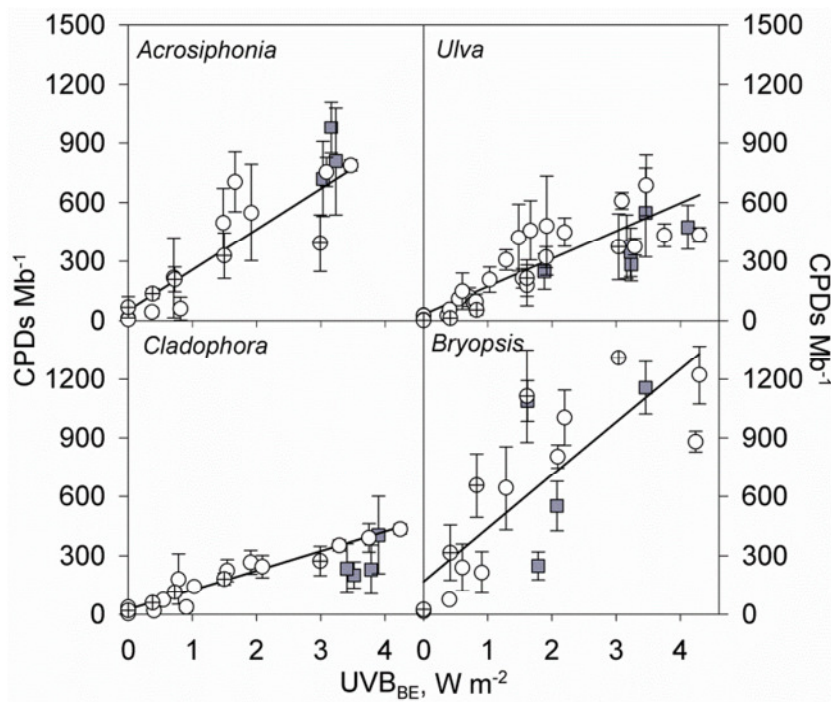


Figure 3. Induction of DNA lesions (CPDs Mb⁻¹) dependent on applied UVB irradiance, mean \pm SD (n=8). Symbol code is as in Fig.1. Regression lines: *Acrosiphonia*: $y = 51.0 + 205.9x$, $r^2 = 0.64$ (A); *Ulva*: $y = 30.7 + 141.3x$, $r^2 = 0.66$ (B); *Cladophora*: $y = 25.7 + 99.1x$, $r^2 = 0.84$ (C); *Bryopsis*: $y = 168.9 + 270.5x$, $r^2 = 0.67$ (A). Capital letters in brackets indicate statistically identical slopes of the regressions.

This picture changes in Fig. 4 where the induction of CPDs is plotted against the effective UVB. Here, *Ulva* had the lowest sensitivity but not significantly different from *Acrosiphonia*. In *Cladophora* the sensitivity was increased and was equal to that of *Acrosiphonia* now. Still, the DNA of *Bryopsis* was outstandingly strongly damaged. As in Figs. 1 and 2 the data from the repair experiments are included in the Figs. 3 and 4 but not in the correlation analysis. Again, as for PS II inhibition, the experiments with the algae from about six weeks culture gave the same results as from the two weeks pre-cultured material (crossed circles in Figs. 3 and 4).

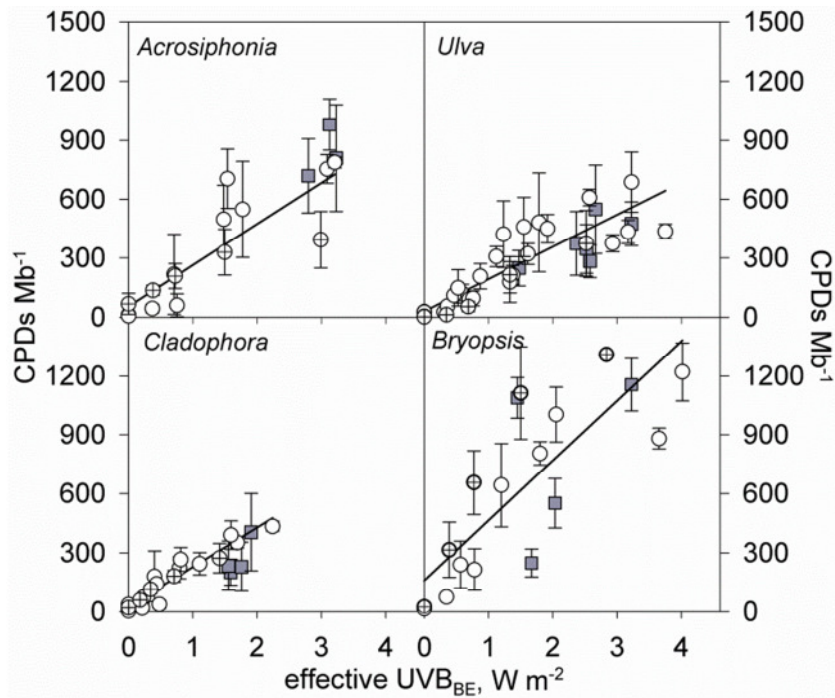


Figure 4. Induction of DNA lesions (CPDs Mb⁻¹) dependent on effective UVB irradiance calculated as $UVB_{BE} \times T_{UVB}$, mean \pm SD (n=8). Symbol code is as in Fig.1. Regression lines: *Acrosiphonia*: $y = 54.3 + 209.5x$, $r^2 = 0.70$ (AB); *Ulva*: $y = 32 + 155.5x$, $r^2 = 0.65$ (A), *Cladophora*: $y = 35 + 173.0x$, $r^2 = 0.69$ (B); *Bryopsis*: $y = 156 + 297.3x$, $r^2 = 0.63$ (C). Different capital letters in brackets indicate statistically different regressions.

3.4 Repair rates of PS II and CPDs

Recovery of PS II efficiency and removal of CPDs was followed over 48 h at 9° C and 10 – 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figs. 5 and 6). The mean control level of F_V/F_M was between 0.70 and 0.73 in all species (Fig. 5, dashed lines). After the UVB exposure this was reduced down to 0.21 (*Bryopsis*, R1) or 0.49 (*Bryopsis*, R3) corresponding to the varied applied UVB irradiances. The recovery kinetics were fitted to a three parametric model $(f)x = y_0 + \alpha (1 - \exp(-xb))$ according to Campbell and Tyystjärvi (2012) with the initial damage after the stress (y_0), the amplitude of recovery (α) and the repair rate (b). For each replicate experiment this fit was done separately and the mean of each parameter was calculated to give the solid line in Fig. 5. In *Bryopsis* and *Cladophora* the model did not converge in one or three of the four experiments, respectively and the drawn line is the based on the parameters derived from the rest of the experiments. However, in these species the repair was very slow and inefficient and only 17 % of the induced reduction of F_V/F_M was repaired after 48 h in *Bryopsis* and 31 % in *Cladophora*. *Ulva* had the highest repair rate ($b = 0.264$) with two thirds of the impairment reversed within the first 6 h. In *Acrosiphonia* the repair rate was only 1/4th of the efficiency of *Ulva*. Despite significant repair activity, 15 – 30 % of F_V/F_M was not restored after 48 h of recovery in *Ulva* or *Acrosiphonia*, respectively.

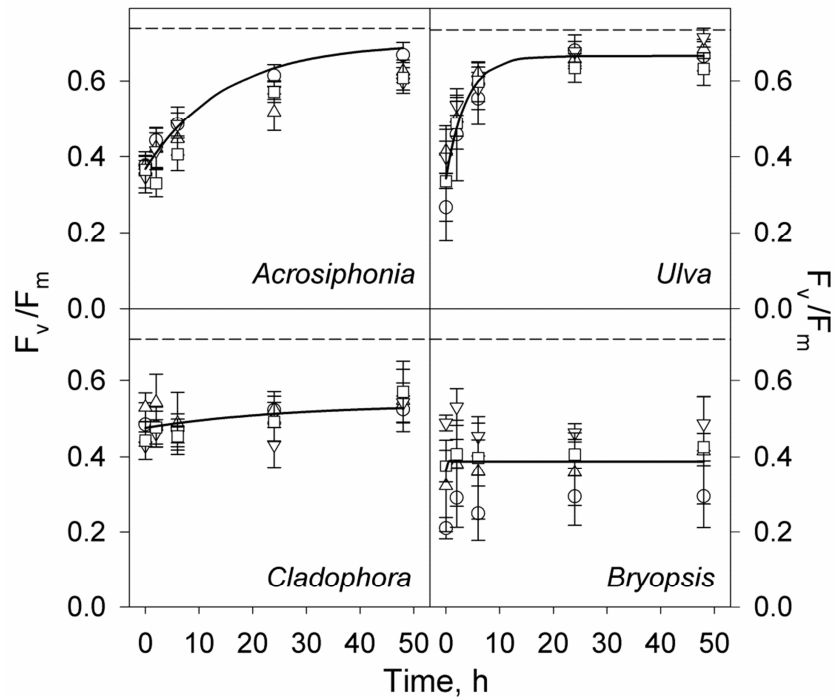


Figure 5. Recovery of optimal photosynthetic efficiency (F_v/F_m) over 48 h after 1 h UVB exposure. Symbol code: circles, R1; triangles up, R2, triangles down, R3; squares, R4; diamonds, R5a; crosses, R5b; hexagons, R5c. Dashed lines indicate (F_v/F_m) of control values before exposure.

For the DNA damages, in all species a decrease of CPDs was detected but the repair was not complete after 48 h (Fig. 6). While in *Acrosiphonia* the repetitive experiments gave quite similar results, in *Ulva*, *Cladophora*, and *Bryopsis* in one experiment no repair was observed (experiments marked with open symbols in Fig. 6 and an asterisk and italics in Table 3). It is not clear why there was no CPD repair in these experiments as the PS II data were normal from these experiments. The remaining DNA damage amounted to 15 % in *Bryopsis*, 17 % in *Acrosiphonia*, 19 % in *Cladophora* and 21 % in *Ulva* excluding the experiments without repair. The repair rates for CPDs were calculated from the linear regressions of the natural-log transformed data after correction for control values (Table 3). Excluding the experiments in which no repair was measurable the mean repair rates of all species were not significantly different (ANOVA, $p=0.228$).

4. Discussion

4.1 UVB resistance

As observed in our study, but also from many other locations, there is a distinct seasonal and elevational community pattern within the chlorophyceae and the ability of these “green tide” algae to grow in one of the most stressful habitats is not identical (Nelson et al. 2003, 2008, Figueroa et al. 2003, Wahl et al. 2004). In the presented study very different degrees of UVB resistance of PS II and the DNA were found in the four investigated species. One major aspect of UVB resistance, the sensitivity of cellular targets was investigated as dose responses of PSII and DNA related to the

applied UVB irradiances (Figs. 1 and 3). Overall the differences were smaller with respect to PS II than DNA susceptibility but resulted in almost the same ranking. In this ranking, *Bryopsis* had by far the highest sensitivity for both targets. *Acrosiphonia* and *Ulva* displayed very similar PS II sensitivities but considering the steeper slope of DNA damage induction in *Acrosiphonia* than in *Ulva* the UVB sensitivity of the former was higher. Clearly, the lowest UVB sensitivity was found in *Cladophora*. This species was the only included species with efficient UVB screening protecting the cells from more than 50 % of the impinged UVB irradiance (Table 2). None of the other species exhibited a UVB screening that provided sufficient UVB protection to yield reduced UVB sensitivity (Figs. 2 and 4, Table 2). As the results of the dose response experiments were reproduced over four years with independently sampled algae the observed dissimilarities seem to represent fixed, i.e. adaptive differences of species-specific UVB sensitivity. Thus, we used the slopes of the regression lines of the dose response experiments as a measure of UVB sensitivity and ordered the species in this sequence of increasing UVB sensitivity: *Cladophora* > *Ulva* > *Acrosiphonia* > *Bryopsis*.

On the other hand, not only the sensitivities of targets are determining the UVB resistance but cellular tolerance processes also might play an important role. In this regard we intended to minimize parallel ongoing repair during the dose response experiments to be able to compare the intrinsic sensitivity of the species towards UVB. The very short exposure to a broad range of relatively strong irradiances accompanied by low PAR was suited for this mechanistic study of UVB sensitivity.

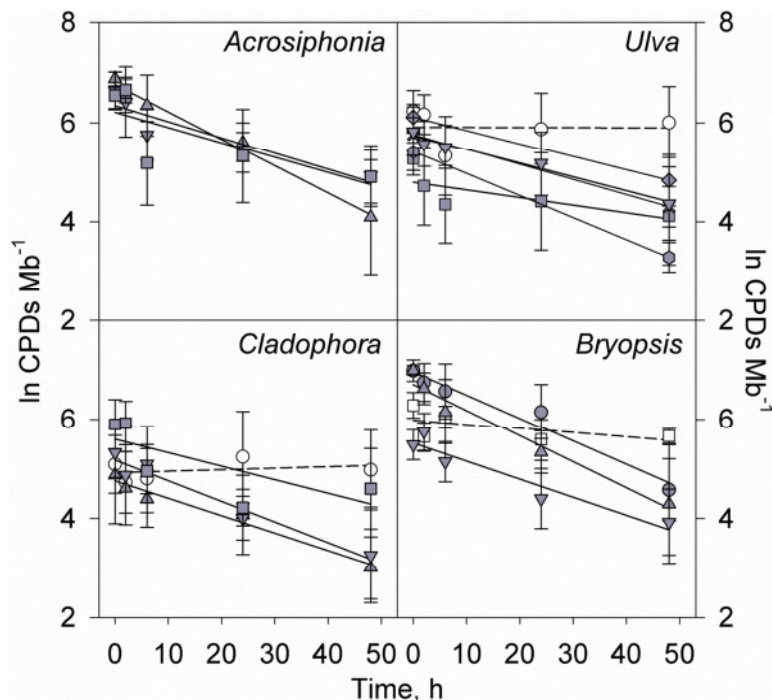


Figure 6. Natural log transformed CPDs (corrected for the CPD concentrations of control samples) over 48 h repair time after 1 h UVB exposure. Symbol code as in Fig. 5. Open symbols mark experiments where no repair was observed.

Table 3. DNA damage (CPD Mb⁻¹, corrected for the CPD concentrations of control samples) after 1 h UVB exposure, repair rates calculated from linear regression of natural-log transformed data, and regression coefficients. In experiments R1 in *Acrosiphonia* and *Ulva* an impure RNase added to the extracted DNA cross reacted with the anti-thymine dimer antibody and no CPD data exist. In experiments marked with italics and asterisk no repair was measurable and these results are not included in the mean calculation.

species	Exp.	CPDs Mb ⁻¹	r ²	Repair rate, h ⁻¹
<i>Acrosiphonia</i>	R1	-	-	-
	R2	981	0.74	0.055
	R3	931	0.52	0.032
	R4	719	0.35	0.031
	Mean ± 1SD			0.039 ± 0.0136
<i>Bryopsis</i>	R1	1155	0.76	0.047
	R2	1088	0.69	0.052
	R3	249	0.62	0.036
	R4	551	<i>0.18</i>	<i>0.009*</i>
	Mean ± 1SD			0.045 ± 0.0082
<i>Cladophora</i>	R1	202	<i>0.01</i>	<i>0.003*</i>
	R2	245	0.41	0.035
	R3	230	0.51	0.041
	R4	404	0.36	0.027
	Mean ± 1SD			0.034 ± 0.007
<i>Ulva</i>	R1	-	-	-
	R2	548	<i>0.00</i>	<i>0.0002*</i>
	R3	374	0.47	0.028
	R4	204	0.10	0.015
	R5a	464	0.76	0.027
	R5b	337	0.82	0.031
	R5c	239	0.92	0.044
	Mean ± 1SD			0.029 ± 0.0104

The second aspect of UVB resistance, cellular tolerance was assessed as recovery kinetics after a UVB challenge. In these experiments we varied the UVB irradiance from species to species in order to produce comparable levels of PS II inhibition and DNA lesions (cf. to Table 1). However, in the recovery experiments PS II and DNA damages were not as congruent as in the dose response experiments. PS II recovery determined as the rate constant from the model of Campbell and Tyystjärvi (2012) was outstandingly fast in *Ulva*, while no and only very minor recovery of PS II was observed in *Bryopsis* and *Cladophora*, respectively (Fig. 5). In contrast, *Acrosiphonia* could restore its optimal quantum yield to the same extent as *Ulva* but four times slower. This finding is confirmed

by earlier studies demonstrating the ability of several *Ulva* species to recover more than 50 % of its PS II efficiency within 3 – 6 h (Carr and Björk 2007, Han et al. 2007, Pescheck et al. 2014). A major part of this recovery was blocked by lincomycin and thus shown to depend on protein biosynthesis underlining the high capacity of the D1 turnover in *U. clathrata* (Pescheck et al. 2014). Similarly, in *U. intestinalis* 60 % of PS II recovery was lost using the same inhibitor (data not shown). This result is analogous to studies in higher plants and green freshwater microalgae where the extent of UVB tolerance of PS II was proven to primarily rely on PS II repair (Park et al. 1995, Xiong 2001).

Unexpectedly, the removal rates of DNA lesions were identical in all studied species (Fig. 6 and Table 3). Repair of CPDs is mainly due to photoreactivation in plants (Britt 2004) and the activity of the photolyase depends on several factors. For example, it is known from *Arabidopsis thaliana* that the action spectrum of some types of photolyase peaks in the UVA radiation range (Dany et al. 2001, Sancar 2003) but we did not provide wavelengths shorter than 400 nm during repair. Hence, photoreactivation might have been light limited. However, we rather wanted to avoid light-induced stress and therefore restricted the recovery conditions to the pre-experimental low-light. Further, the temperature during repair was quite low, which was also shown to inhibit photoreactivation in cucumber and a red macroalga (Takeuchi et al. 1996, Pakker et al. 2000). Finally, in some plants the photolyase activity depended on the growth conditions and was reduced under UV exclusion (Takayanagi et al. 1994, Kang et al. 1998, Giordano et al. 2003). Although this was never studied in green macroalgae, insufficient photolyase induction after the two weeks of pre-experimental low-light culture could also be a reason for the low CPD removal rates of our study. Under the same conditions CPD repair rates were identical in two other green macroalgae to the ones from this study (Pescheck et al. 2014). These arguments suggest that the experimental conditions were limiting the repair of CPDs rather than the absence of adaptive differences in the repair capacities of the studied species. Overall, for the cellular tolerances of PS II (without DNA repair) under the used conditions the following ranking was identified: *Ulva* > *Acrosiphonia* > *Cladophora* > *Bryopsis*. In summary, the combined ranking of UVB resistance can be ordered this way: *Cladophora* ~ *Ulva* > *Acrosiphonia* > *Bryopsis*.

4.2 UVB resistances related to ecological niches

The described different UVB sensitivities and cellular tolerances might be related to the ecological niches of the species. Although at the sampling site the algae can be found within 1 m² the niches of the four studied species are differing in quantitative UVB encounter. The location inside the Kiel Canal has a constant water level and already on this limited area a well-established depth zonation can be found. There, *Ulva* grows directly at the water surface and the sampled thalli were always gas filled and horizontally floating on the water. This demonstrates that *Ulva* regularly absorbed very high UVB irradiances and needs a reliable UVB resistance. *Cladophora* and *Acrosiphonia* can even

grow on the same stones as *Ulva* but are not floating on the water and thus experience slightly attenuated sunlight. Additionally, the thalli of these two species are more upright growing and not that stably horizontally positioned as the floating thalli of *Ulva*, so that the absorption is reduced in *Acrosiphonia* and *Cladophora* compared to *Ulva*. Furthermore, *Acrosiphonia* has its main growth season in spring and early summer. During that time the solar angle is lower and day lengths are shorter and thus less sunlight, including UVB radiation is absorbed as in summer. *Bryopsis* was originating from the deepest growth position of this study at 40 – 60 cm water depth. Due to permanent waves generated by passing ships the water at the study site is quite turbid and the penetration of UVB is very low (10 % depth for $\lambda = 312$ nm in about 50 cm depth corresponding to a vertical attenuation coefficient K_d of 4.7, as calculated from transmission spectra measured in august 2013; personal observation Wolfgang Bilger). Algae growing at this depth experience therefore a strongly reduced UVB stress compared to the water surface. Conclusively we tentatively ordered the niches of the species in a sequence of decreasing UVB impact: *Ulva* > *Cladophora* > *Acrosiphonia* > *Bryopsis*. Comparing this to our ranking of the UVB resistance, UVB radiation might be an accounting factor for the spatial and temporal zonation of these four species.

Nevertheless, also other factors might change on this small gradient: the amount of PAR, the temperature, mechanical stress and the diverse biotic stresses, like grazing and competition. All these factors are also possibly influencing the distribution of the species and we do not know to what extent UVB radiation is contributing to it. On the other hand, a regularly UVB-exposed species needs a reliable UVB resistance whereas a more subtidally growing species probably can save the investment. A similar correlation of growth depth and UV sensitivity was found in different species from red and brown macroalgae from the Skagerrak (Johansson and Snoeijs 2002) and in a set of 21 species from the Antarctic Peninsula (Huovinen and Gómez 2013). Similarly, UVB resistance was related to zonation in several cultured macroalgae originating from the Arctic (Bischof et al. 1998). Additionally, as shown for the brown alga *Desmarestia anceps* and the red alga *Chondrus crispus*, thalli originating from deeper waters had an increased UVB susceptibility (Rautenberger et al. 2013) and slower repair rates of PS II after photoinhibition (Sagert et al. 1997) in comparison to shallower grown ones. This is an example of the escape strategy combined with a regulated cellular tolerance which was however not analyzed the present study.

In particular, the two aspects of UVB resistance, the intrinsic sensitivity and the cellular tolerance were analyzed under controlled laboratory and consequently markedly artificial conditions in this study. A ranking of the UVB resistance on this basis cannot necessarily be transferred to natural field conditions because algae like plants can acclimate substantially to the prevailing conditions (Vergara et al. 1998, Rautenberger et al. 2013). Also, it is known that the interaction of the different parts of the solar spectrum may influence the sensitivity towards UVB radiation (Vass et al. 1999, Sicora et al. 2003, Hanelt et al. 2006). As a next step *in situ* measurements combined with UVB

exclusion experiments could help to elucidate the importance of UVB effects in sunlight on the different species. Especially in view of the ecological role of early successional algae like *Acrosiphonia*, *Ulva* and *Cladophora* the physiological repair capacity and efficiency of screening in the natural habitat would be very interesting. These pioneer species are known to establish UV-tolerant communities forming a canopy under which in turn other, UV-sensitive understory species might germinate (Littler and Littler 1980, Molis and Wahl 2009).

4.3 Different strategies of UVB resistance

Our results clearly show that the species displayed not only different degrees of UVB resistance but also employed different strategies to achieve this. In the four analyzed species we observed three main strategies: Firstly, the damage can be avoided by protective UV-absorbing pigments excluding the harmful radiation from the targets. Secondly, a cellular tolerance can be built up achieving a balance of UVB-induced damage and repair. Thirdly, the plants can escape the UVB stress by growth in deeper waters or outside of the summer season or by mat formation (Cockell and Knowland 1999). All methods have advantages and disadvantages for the plants and can be combined individually.

If a screening is employed, as in *Cladophora* in our study, the substances have to be stored in between the impinging radiation and the targets, like it is realized in the epidermis cells of higher plants, the cell walls of green microalgae or the extracellular sheets of cyanobacteria (Robberecht and Caldwell 1978, Xiong et al. 1997, Garcia-Pichel et al. 1992). The accumulation of these compounds should be temporally synchronized with the encounter of the stress otherwise significant damages might be the consequence. Interestingly, in a survey of low-light cultured green macroalgae a pronounced screening was present in all included Cladophorales, obviously constitutively expressed to a large extent in this group (Pescheck et al. 2010). On the other hand, as with every metabolic product, the plant should not spend energy in a non-needed sink. Accordingly, the potential to shield against UVB radiation is regulated in higher plants, cyanobacteria, red and brown algae (Schmitz-Hoerner and Weissenböck 2003, Garcia-Pichel and Castenholz 1991, Hoyer et al. 2002, Gómez and Huovinen 2010). A similar adjustment seems to be present in the Cladophorales which has to be analyzed in more detail (F.Pescheck, unpublished data). As the repair capacity of PS II and DNA was remarkably low in *Cladophora* (Fig. 5 and 6, Tab. 3) the protective function of screening seems to serve as the predominant mechanism of UVB resistance in this species. A disadvantage of this solely physical strategy might become manifested if a substantial UVB dose accumulates over time which is high enough to harm the cells. Nevertheless, the screening seems to be sufficient to render *Cladophora* and other members of the Cladophorales very UVB resistant. Photosynthesis and related proteins were markedly more UVB resistant in *Chaetomorpha linum*, being as well a member of the Cladophorales, as in *Ulva rotunda*

as investigated in two comparable field studies in southern Spain (Bischof et al. 2002, 2006b). This high UVB resistance of the Cladophorales in comparison to the Ulvales was also observed in *Cladophora glomerata* which displayed much lower levels of oxidative stress than *Ulva procera* after UVB exposure (Choo et al. 2005) and in *Rhizoclonium riparium* with less PS II inhibition than *Ulva clathrata* (Pescheck et al. 2014). Therefore, high screening in this group could be an explanation of the repeatedly observed different UVB resistances of Ulvales and Cladophorales.

Cellular tolerance was of major importance in two of the investigated species, *Ulva* and *Acrosiphonia*. Additionally, the absence of screening emphasizes the importance of the cellular tolerance in these two species. In general, cellular tolerance is a very flexible strategy that might acclimate to varying, sometimes extremely high UVB levels. Similarly, a high UVB tolerance for growth but no UVB protection by absorbing pigments was found in *Ulva expansa* sampled from the field (Grobe and Murphy 1998). On the other hand, Han and Han (2005) observed an unidentified UVB protective pigment in *Ulva pertusa* significantly reducing the UVB-induced photoinhibition of PS II as compared to thalli lacking this pigment. On the other hand, the cellular tolerance mechanisms might be very energy consuming. These costs are only reasonable if the alga or plant receives enough energy, i.e. light and has a sufficient access to nutrients in its habitat. It was shown in *Ulva rotundata* that the high-light protection mechanisms for PS II depended on nitrogen supply and were strongly reduced under nutrient limiting conditions (Henley et al. 1991). In contrast, the escape strategy seems to be of great importance in *Bryopsis*. We did not observe a UVB resistance but rather UVB sensitivity in this species. The complete lack of UV screening and PS II repair in *Bryopsis* seem to be key factors for its zonation in a UVB-depleted habitat.

Acknowledgement

Many thanks go to Caroline Hesse and Susanne Wolf for help with DNA extraction and immunoblotting. From Prof. Schmitz-Streit the loan of a peristaltic pump for seawater filtration and from Prof. Krupinska the possibility to use the dark room is acknowledged.

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Chapter 6 – Discussion

The degree of resistance to the various abiotic stress factors in the intertidal zone is an important factor for the success of a species and the composition of the community (Davison and Pearson 1996). UVB represents one major stress factor that influenced plant evolution from the beginning and evoked the development of defensive mechanisms. In this thesis the adaptation towards UVB radiation in green macroalgae was investigated. The first mechanism examined was avoidance of UV absorption by sensitive molecules through the accumulation of protective screening compounds. This is a common and efficient resistance strategy of phototrophs hitherto not reported for green macroalgae. In this regard a survey of 50 species from six orders of green algae was conducted (chapter 2). Then, if physical screening was detected the protective function for cellular targets had to be proven. For this purpose a mechanistical approach with experimental UVB exposure was followed (chapter 3). In contrast, for non-screening species a high risk of UVB-induced damage accumulation exists and cellular tolerance mechanisms have to compensate this. To evaluate the tolerance potential recovery rates after UVB challenge experiments were determined and compared to the ones from screening species (chapter 3). Further, the kinetics of induction and recovery processes were supposed to be affected by the interaction of UVB radiation with visible radiation. This aspect could also influence the relative sensitivity of different targets of UVB radiation. Therefore the UVB resistance of a non-screening alga, *Ulva intestinalis*, was investigated in its natural habitat under solar irradiation (chapter 4). Finally, UVB resistance and the underlying mechanisms were related to the ecological niches of four selected species (chapter 5).

UV screening in green macroalgae

A very important and new finding of this thesis was the occurrence of efficient UV screening in two orders of green macroalgae, the Valoniales and the Cladophorales (which have been fused recently as the Cladophorales (Cocquyt et al. 2010), chapter 2). Also for the genus *Prasiola* UV screening could be demonstrated. This genus was previously shown to contain UV-absorbing MAAs (Karsten et al. 2005). Also the absence of screening in the remaining orders of studied green algae (Ulvales, Ulotrichales, and Bryopsidales) is in agreement with the existing literature (Karsten et al. 1998). The consistency of the different methodological approaches of this thesis and that study makes the missing screening in these orders a robust fact. Moreover, this agreement underlines the validity of the detected screening in the Cladophorales. In the broad survey of UV screening a clear

phylogenic pattern of the capability to synthesize the UV-absorbing substances was revealed with only minor influence of the climatic origin of the species. From an evolutionary view point this means no adaptation to the respective UVB stress in the different habitats might exist. On the other hand, it is possible that the genetic potential to establish efficient screening was not expressed in low-light acclimated cultures. Field sampled specimens absorbed not significantly more compared to cultured algae for most of the orders. For example, in *Ulva intestinalis* only a very minor though statistically significant seasonal acclimation of UVB screening was found from mid-winter to end-summer levels (Lohbeck 2008). However, from laboratory experiments under different irradiation conditions a strong inducibility of screening by high-light or UVB radiation was observed in *Rhizoclonium riparium* (F.Pescheck, data not shown). Thus, an acclimation potential of this important UVB resistance mechanism is very likely in the Cladophorales. But as these investigations have not been finished until now the results are not included in this thesis. Overall the successful adoption of the *in vivo* method from leaves of higher plants to macroalgae allowed the analysis of a taxonomically wide range of green algae leaving almost no doubts about the UV screening capacity in this group.

However, the nature of the UV-absorbing compound in the Cladophorales remains unresolved. Nevertheless, a few indications about the physico-chemical properties exist which are not included in the foregoing chapters and therefore will be summarized here:

1) The UV-absorbing compounds seem to be chemically very resistant as they were not extractable with conventional (Karsten et al. 1998, and own observations) or even harsher acid hydrolysis methods (Graham et al. 2013). This implies that they might consist of insoluble large polymers which could be tightly bound to the cell walls as e.g. reported for sporopollenins or sporopollenin-like substances from green microalgae (Atkinson 1972, Gorton and Vogelmann 2003, de Leeuw et al. 2006). Unlike as in an investigation of *Ulva pertusa* (Han and Han 2005) no UV absorption over a broad wavelength range could be detected after methanol extraction neither in non-UV screening *Ulva clathrata* nor in UV screening *Cladophora sericea* or *Rhizoclonium riparium*.

2) UVA excitation induced a blue autofluorescence in the cell walls of mature *Cladophora glomerata* cells which was ascribed to phenolic compounds (Graham et al. 2013). This blue autofluorescence is an intrinsic property of some aromatic UV-absorbing compounds (Hutzler et al. 1998). An examination of *R. riparium* with a confocal laser scanning microscope (Leica, TCS SP5, Wetzlar, Germany) and UVA excitation confirmed the cell wall associated origin of a blue autofluorescence. The presumable UV-absorbing compounds were integrated into the inner part of the cell wall and also in an outer layer enclosing the whole filament (Figure 6.1). This localization was also observed in other Cladophorales, like *C. sericea* and *Chaetomorpha tortuosa* but not in *U. clathrata*, *U. intestinalis*, *Acrosiphonia spec.* or *Bryopsis hypnoides*.

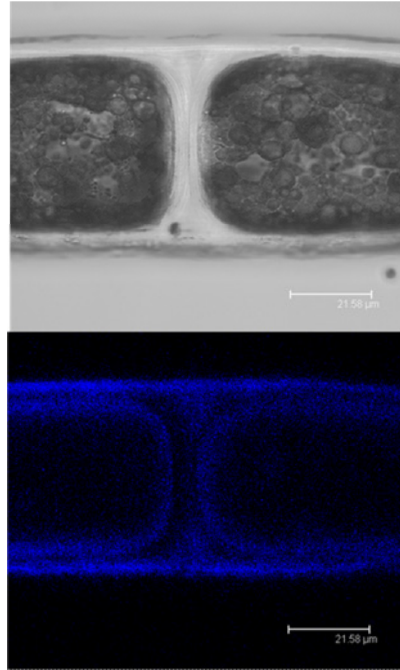


Figure 6.1. Microscopic image of *Rhizoclonium riparium* with a confocal laser scanning microscope. Upper panel: transmission image with blue excitation ($\lambda_{\text{max}} = 480 \text{ nm}$), lower panel: UVA- excited ($\lambda_{\text{max}} = 358 \text{ nm}$) autofluorescence recorded from 420 – 505 nm.

3) As determined by differential fluorescence excitation spectra of whole thalli of *R. riparium* and *C. sericea* with isolated chloroplasts of these species the absorption maximum of the UV-screening substances was around 315 nm. Again, in similar investigations with *U. clathrata*, *U. intestinalis*, *Acrosiphonia spec.* and *B. hypnoides* no such absorption was detected.

In summary, the UV-screening compounds of the Cladophorales seem to be cell wall bound chemically resistant, blue fluorescing molecules with a maximal absorption around 315 nm. These characteristics make them very likely to serve as efficient UVB protection for the intracellular UV-sensitive targets.

Protective function of UV screening in Cladophorales

Comparing the screening species *R. riparium* with the non-screening species *U. clathrata* a significant higher UVB resistance was found in the former. The slopes of linear regression lines of PS II impairment on applied UVB fluence were found to be smaller in *R. riparium* than in *U. clathrata* (chapter 3). Similar results were gained in experiments with other pairs of algae as e.g. the screening *Cladophora sericea* and non-screening *Acrosiphonia spec.* (chapter 5). In UVB challenge experiments always the screening species had lower damages than the other. This gave strong hints to the protective function of the detected screening in the Cladophorales.

To determine to what extend the observed species-specific UVB resistance differences can be ascribed to the screening an analytical approach was employed quantifying the portion of UVB

radiation that actually penetrates into the cells or more specifically the chloroplasts, called the effective UVB (Schultze 2011). This can be achieved by multiplying the applied UVB fluence with the apparent UVB transmittance of the thalli. Doing so, the cellular, i.e. the intrinsic sensitivities of DNA and PS II can be compared if under the used experimental conditions parallel ongoing repair is unlikely. This was ascertained in the mechanistical approach by short exposure times with relatively strong UVB fluences and at low temperatures with no additional white light which is necessary for both, the repair of PS II and thymine dimer splitting (Allakhverdiev and Murata 2004, Takeuchi et al. 1996). It was found that the intrinsic PS II sensitivity was very similar for all studied species with an average damage of 16.2 % (± 1.6) / 1 W m⁻² effective UVB_{BE} (chapter 3 and 5). Thereby the quantitative function of the UVB screening in two species of the Cladophorales was confirmed. In contrast to the constant relation of screening and PS II protection, this was not true for the induction of DNA damages. Supposedly, structural differences of the cells are affecting the accessibility of intracellular targets for UVB radiation. These species-specific optical characteristics are integrated in the chlorophyll fluorescence measurements of the screening for chloroplasts but are probably not valid for the nuclei. Accordingly, the screening as assessed by chlorophyll fluorescence cannot predict the induction of DNA damages per W m⁻² in a species to species comparison.

Cellular UVB tolerance mechanisms in non-screening macroalgae

Knowing that the intrinsic sensitivity of PS II was identical for screening and non-screening species in the UVB challenge experiments it was hypothesized that non-screening algae, like *Ulva spec.* employ effective repair mechanisms to gain UVB resistance. The recovery of PS II efficiency and the removal of DNA lesions were analyzed in *U. clathrata* and *R. riparium* (chapter 3, Table 6.1). Employing an exponential three parametric model (Campbell and Tyystjärvi 2012) a 4x faster PS II recovery rate was calculated for *U. clathrata* in comparison to *R. riparium*. Including another pair of a non-screening and a screening species, *U. intestinalis* and *C. sericea*, into the analysis the difference between the Ulvales and Cladophorales was confirmed (chapter 5). As a major part of the PS II recovery was sensitive to a protein biosynthesis inhibitor in *U. clathrata* and *U. intestinalis* (data not shown), it may well be assumed that especially a high D1 turnover accounted for the fast recovery of PS II. However, two other species with low screening and low repair kinetics of PS II, *Acrosiphonia spec.* and *B. hypnoides* were identified as potentially more UVB susceptible than the other four species. The recovery rates of PS II in *Ulva* from this thesis fit very well with literature values of several *Ulva* species (Carr and Björk 2007, Han et al. 2007).

On the other hand, the repair of CPDs was similar for all species and relatively inefficient (Table 6.1). Clearly, *Ulva* showed no enhanced dimer removal in comparison to any other algae no matter if screening or non-screening. In all species, after 48 h post UVB still detectable frequencies of

dimers were present and the resulting repair rates were about half of that of the only other published one of a marine green alga *Monostroma arcticum* (van de Poll 2002).

The absence of species-specific repair rates for DNA damages in the presented thesis does not necessarily exclude adaptive differences of CPD repair between the studied species. Probably these were just not detected due to the used experimental conditions. In the recovery experiments photoreactivation was studied under low visible light with pre-culture intensity to avoid additional photoinhibition by PAR in UVB-stressed algae. As the photolyase is activated by UVA radiation and blue light the reaction might have been light limited. It was shown that the photoreactivation rate was not saturated at $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in cucumber (Takeuchi et al. 1996). Also the provided spectrum did not contain wavelengths shorter than 400 nm which are especially effective for photoreactivation in the folate type of photolyases (Dany et al. 2001). Furthermore, in the experiments of this thesis the temperature was relatively low during repair, orientated at culture conditions for cold-temperate algae and the annual mean water temperature from the Kiel Canal of approximately 10 °C (Gocke et al. 2010). Photoreactivation rates reduced by more than 30 % at 15 °C compared to 25 °C were reported by Takeuchi et al. (1996) or Pakker et al. (2000). Moreover acclimation to UVB radiation might change the cellular defense capabilities. Plants developing under UV-exclusion have been shown to express reduced amounts of photolyase activity amounting to only half of the photoreactivation rates of field grown plants (Giordano et al. 2003, Takayanagi et al. 1994). This means in field grown algae constant exposure to UVB radiation may lead to an acclimation of photolyase activity. A preliminary experiment with *U. intestinalis* removed directly from the field showed a CPD removal rate 10 times higher than that observed with laboratory grown samples (data not shown). The question remains open if acclimation to sunlight may cause an increase in CPD repair activity. Hence, no final conclusion is possible on the photoreactivation rates of the investigated species under natural conditions.

DNA damages in Ulva intestinalis in the field

Due to the inefficiency of DNA repair in *U. intestinalis* combined with the absence of UV screening a high risk to accumulate DNA lesions might be expected for this species in its natural habitat. By following the frequencies of CPDs during daily cycles the impact of natural sunlight on DNA integrity was analyzed. In strong contrast to the expectations almost no CPDs could be detected and no meaningful diel variations were observed (chapter 4). Even over longer periods of very high solar irradiances no significant correlations of dimer frequency and cumulative UVB dose were present. A possible explanation for this is a substantially enhanced photoreactivation under natural irradiation conditions. Probably as already discussed, the results from the laboratory experiments cannot predict the impact of UVB radiation under solar irradiation but are only usable for direct comparisons among species. *In situ* the conditions and prerequisites for photoreactivation were undoubtedly

much better met. The spectrum was ideal for photoreactivation, the temperature was twice as high as in the laboratory experiments and the algae were acclimated to UVB radiation.

Thus, it is suggested that photoreactivation might have been responsible for the prevention of CPD accumulation in *U. intestinalis in situ*. Altogether UVB-induced DNA damages do not represent a significant threat in *Ulva* under field conditions.

Relative sensitivity of DNA and PS II in situ

An interesting open question was which cellular target of sunlight might be of greater ecological importance in *U. intestinalis*. This was approached by parallel determinations of PS II impairment and DNA lesion frequency in thallus segments located directly adjacent to each other. The very low frequencies of CPDs combined with substantial photoinhibition of PS II imply a higher sensitivity of PS II than that of DNA towards natural sunlight in *U. intestinalis* (chapter 4). A direct comparison of both targets revealed no correlation in contrast to results from experimental UVB exposure. An explanation for the observed UVB resistance of the DNA *in situ* is rapid photoreactivation as proposed above. The study of variations in the optimal quantum yield of PS II lead to the following conclusions: Aside from dynamic photoinhibition also chronic photodamage occurred at PS II dependent on the daily doses of photon flux density (PFD). On the other hand the relation of optimal quantum yield and PFD was quite constant over the day without a delay in recovery during the afternoon. This might be interpreted as dynamic photoinhibition possibly combined with efficient PS II turnover, like it was demonstrated in the closely related species *U. clathrata* and *U. intestinalis* (data not shown) in laboratory experiments. Nevertheless, a quantification of the D1 protein would be necessary to elucidate the impact of chronic photoinhibition and the potential of the PS II repair cycle. Furthermore, a remarkable strong quench of ground fluorescence at noon points to very efficient non-photochemical energy quenching (NPQ). NPQ lowers or even prevents the induction of photoinactivation (Lambrev et al. 2012). This competence was already described earlier by Henley et al. (1992) for *Ulva rotundata*.

UVB resistance strategies of green macroalgae

UVB resistance can be achieved by several mechanisms, which have been introduced as avoidance, cellular tolerance or escape. As outlined by Cockell and Knowland (1999) the combination of these mechanisms results in the species-specific UVB resistance. In the following paragraph the strategies of six species will be summarized which have been studied in greater detail (chapter 3 and 5).

In all of the experiments of this thesis predominantly adaptive differences were studied. The potential for acclimation was not addressed although it might play an important role in nature. The

algae were either low-light grown with UV-exclusion or field sampled but afterwards also kept in pre-experimental culture for 10 – 19 days to allow for recovery of all sunlight-induced damages. It may well be that after the pre-experimental low-light culture previous acclimation was largely diminished. Two hints exist that exclude a major effect of unequal acclimation:

First, experiments produced identical results conducted with longer cultured (about 6 weeks) biomass and after 10 – 19 days pre-culture (data shown in **chapter 5**, crossed symbols in Figure 1 – 4). This indicates that eventual acclimation to field conditions may have been already lost in 10 days of culture. Second, both species of the genus *Ulva* behaved relatively similar in all experiments although *U. clathrata* was exclusively analyzed from cultures and *U. intestinalis* exclusively from field samples (**Table 6.1**). This similarity of UVB resistance of these two species from the same genus which also occupy similar ecological niches might be attributed to their close genetic relationship and hence an adaptive character. This is agreement with results from Hanelt et al. (1997a) who demonstrated that the species-specific capability of dynamic photoinhibition, i.e. an important component of cellular tolerance remained constant during prolonged periods of low-light culture indicating the presence of adaptive differences in high-light tolerance between several marine macroalgae.

A summary of resistance parameters of the six species is presented in **Table 6.1**. As all analysis were carried out under the same experimental conditions a quantitative comparison can be done. For the sensitivities of PS II and DNA the slopes are given of the dose responses to the applied UVB irradiances. The repair rates for PS II were calculated using a three parametric model (after Campbell and Tyystjärvi 2012) in which the exponent b is the rate constant of repair. The other two parameters, y_0 as the minimal level of F_v/F_M , and α as the maximal amplitude of recovery, may also influence the repair, although there was no correlation of y_0 and b in *U. clathrata* and *R. riparium* (data not shown). By varying the irradiances in the UVB challenge experiments a relatively similar level of y_0 was achieved which was between 0.49 in *Rhizoclonium* and 0.34 in *Bryopsis*. The values of α were in the same range for all species with an average of 0.26 except for *Bryopsis* and *Cladophora* having α data of only $1/10^{\text{th}}$ or $1/4^{\text{th}}$ of the other species, respectively. The removal of CPDs is based on the negative slopes of either exponential decay functions (data for *U. clathrata* and *R. riparium*) or from linear regressions after natural-log transformation (*Acrosiphonia*, *Cladophora*, *Bryopsis* and *U. intestinalis*).

Table 6.1. Overview of resistance to experimental UVB and underlying mechanisms. Data are compiled from the chapters 3 and 5, calculations as explained therein. Origin: C = culture (15 – 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR, 9°C), F = field (and 10-19 days pre-experimental culture equal to culture). Experimental conditions for dose response experiments: 1 h UVB exposure (fluence rates between 0 – 10 W m^{-2} UVB_{BE}, 2 – 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) at 9 °C; repair experiments: 1 h UVB exposure (fluence rates between 1.6 – 8.5 W m^{-2} depending on the species to induce approximately 30 – 40 % PS II damage in *Rhizoclonium* or *Bryopsis* respectively, 2 – 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) at 9 °C, followed by recovery at 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR and 9 °C.

STRATEGY	RESISTANCE		AVOIDANCE	TOLERANCE	
	Induction / 1 W m^{-2} UVB _{BE}		Screening, %	PS II repair rate ^a	CPD removal rate
SPECIES/ORIGIN	PS II damage, %	CPDs Mb ⁻¹			
<i>Rhizoclonium</i> /C	3	58	90	0.05	0.033
<i>Cladophora</i> /F	8	99	58	0.04	0.034
<i>U. intestinalis</i> /F	14	141	27	0.26	0.029
<i>U. clathrata</i> /C	16	170	16	0.22	0.030
<i>Bryopsis</i> /F	16	270	14	0.7 ^b	0.045
<i>Acrosiphonia</i> /F	13	206	8	0.07	0.039

^a parameter b calculated with the function: $(f)x = y_0 + \alpha (1 - \exp(-xb))$, from Campbell and Tyystjärvi (2012)

^b equation gave 0.7, but the α value (interpreted as the amplitude of recovery) was only 0.024.

As can be seen from Table 6.1 the two species with high screening, *Rhizoclonium riparium* and *Cladophora sericea* showed the lowest inductions of PS II damages and DNA lesions. In this regard it is interesting that the less protected species *Cladophora* showed also a higher sensitivity of intracellular targets. In contrast, the PS II repair rates of these two species were the slowest of this study, while CPDs were removed with an identical rate as in the other species under the used conditions. This means the cellular tolerance made not an important contribution to the UVB resistance of *Rhizoclonium* or *Cladophora*. Conclusively, both species are typical representatives of the avoidance strategy rendering *Rhizoclonium* the most and *Cladophora* the second most UVB resistant species of this study. The habitat of both species is the upper eulittoral with according high insolation where a reliable UVB resistance is required. A disadvantage of this exclusively physical strategy might become manifested if over time the effective UVB irradiance accumulates to a substantial dose high enough to harm PS II. At this moment a very slow recovery of PS II might be lethal. This aspect definitely deserves further investigations in the field.

Ulva intestinalis was under special examination in this study as it is a very abundant, cosmopolitan and therefore ecologically important species. The morphology and life strategy of *U. intestinalis* allow the conclusion that this alga experiences the maximum UVB stress in the intertidal. It grows at very high shore levels where regular emergence is programmed thereby exposing the thalli to

unattenuated sunlight. Furthermore, the tubular thalli which are often gas filled are floating on the water surface leading to the exposure to direct sunlight beyond the emersion periods. One can assume that all phototrophs orientate themselves in a way that they perceive an amount of light energy they are adapted to. Therefore an advantage may exist in the way living on top of the water in full sunlight which compensates for the high risk of damage and the costs connected to high light resistance.

In high light the photosynthetic electron flow is saturated as soon as the rate of energy consuming carbon fixation reaches its maximum which is enzymatically restricted. This restriction is known to be promoted by CO₂ limitation as it impairs the activity of Rubisco and therefore the whole dark reactions (Levavasseur et al. 1991). Thus, the favoring fact for *U. intestinalis* when swimming on the water might be direct contact to air. In water the diffusive speed of CO₂ is 1000 times smaller than in air and the availability of CO₂ is low, especially in dense stands of phototrophs. To overcome this limitation macroalgae have the ability to utilize alternative inorganic carbon sources, as e.g. HCO₃⁻, but this mechanism is relatively ineffective in *U. intestinalis* (Andría et al. 2001). Therefore in *U. intestinalis* with an increased access to CO₂ a higher maximal photosynthesis rate can be achieved. On one hand this supports growth but it also delivers energy for repair processes. In addition, the higher photosynthetic rate lowers the portion of sunlight that is excessive and therefore is coupled to a reduced risk of acceptor side inhibition (Osmond et al. 1993). On the other hand this high photosynthetic rate requires high concentrations of enzymes and is therefore depending on sufficient nutrient supply (Henley et al. 1991b). In fact, *U. intestinalis* was observed to build up blooms under eutrophication conditions (Nelson et al. 2003b, Worm and Lotze 2006). Furthermore, the described non-photochemical quenching and proposed efficient PS II repair detected in the field study and the experimental UVB exposure study (chapters 3, 4 and 5) contribute to the high light adaptation of *U. intestinalis*. So far these considerations indicate a possible strategy how *U. intestinalis* is coping with high irradiances of visible sunlight but not with the associated high UVB exposures.

As in the UVB resistance experiments the two species from the genus *Ulva*, *U. intestinalis* and *U. clathrata*, gave very conform results and seem to follow the same UVB resistance strategy in the following both species will be discussed side by side. After UVB exposure PS II efficiency was affected with 14 – 16 % / W m⁻² and DNA dimers were induced with 141 – 170 CPDs Mb⁻¹ / W m⁻² (Table 6.1). Due to the absence of efficient physical UV-shielding *Ulva* has to rely primarily on cellular tolerance mechanisms to achieve UVB resistance.

Indeed after experimental UVB exposure, both species displayed PS II recovery rates which were by a factor of 4 – 5 higher than those of the other species. Again for DNA dimers the removal rates of CPDs were identical to the other species as discussed above. However, with respect to DNA

damages, *U. clathrata* and *U. intestinalis* had a 50 – 90 % increased stability in comparison to *R. riparium*, after correcting for the screening in *R. riparium* (chapter 3). Compared to *B. hypnoides* this difference was similar. Thus, an alternative cellular mechanism seems to protect the DNA from UVB-induced dimerisation in *Ulva*. Following the hypothesis of Karentz et al. (1991) the idea of ‘nuclear hiding’ or ‘chloroplast shielding’ was considered to be this additional protective mechanism. In the genus *Ulva* each cell harbors one large cup-shaped chloroplast sometimes occupying the whole cell surface. A careful observation of Britz and Briggs (1976) revealed a circadian rhythm of chloroplast movements with the plastids in face (= parallel to the outer surface) position during the day and in profile position during the night. High-light promoted this orientation locating the chloroplasts in face position. As chlorophyll in the thylakoid membranes effectively absorbs UVB radiation the relatively smaller nucleus might be shaded from exposure to UVB. For this strategy a competent plastidal recovery would be essential. This efficient PS II repair has been shown in both *Ulva* species under experimental UVB and under natural sunlight (chapter 3, 4 and 5, Table 6.1). Hence, a sacrifice of the chloroplasts to protect the nucleus might be advantageous. The importance of maintaining an intact DNA, allowing unhampered gene expression for the function of other physiological tolerance processes was recently demonstrated by Vass et al. (2013). Overall, *U. intestinalis* has a remarkable cellular UVB tolerance preventing the accumulation of photodamages even in the absence of screening. *Ulva clathrata* was almost as UVB resistant as *U. intestinalis*. The slight difference for all studied parameters might be attributed either to the species-level or to the fact, that *U. intestinalis* was always sampled from the field.

With the presented data *Bryopsis hypnoides* can be categorized as a UVB-sensitive species. It suffered the highest DNA damages of all studied species (chapter 5, Table 6.1). Although the sensitivity of its photosynthetic efficiency was only slightly higher than in the other species, once damage was induced *Bryopsis* had no chance to repair it. This was recognized from the exceptional low amplitude of PS II recovery (α -value of 0.024 in the model) meaning a repair capacity of only around 3 % (chapter 5). This low amplitude nullifies the high value of b which is given in Table 6.1 as α determines the actual amount of recovery. *Bryopsis* also lacked UV screening and exhibited the lowest UVB resistance of all studied species. It is distributed in the upper sublittoral where emergence never happens and light becomes a limiting instead of an excessive resource. Accordingly, the pigment composition of the light harvesting complexes of *Bryopsis* include the accessory pigment siphonaxanthin, which enables the species of the Siphonales to absorb more efficiently in the green spectral region than algae without this pigment (Anderson 1985, Wang et al. 2013). The water transparency for UVB radiation was also very low at the sampling site with only 10 % of surface irradiance at 50 cm water depth for $\lambda = 312$ nm (cf. to Figure 1.1). Thus the water column effectively filters out UVB radiation. This attenuation decreased with increasing wavelength and at the same depth still 60 % or 85 % of surface irradiance were present at 400 nm and 600 nm,

respectively (data from spectroradiometer measurements (RAMSES, All-UV-VIS, Trios Optical Sensors, Oldenburg, Germany) in the fjord at L2 from **Figure 1.2**). Therefore, the habitat can be characterized as UVB depleted in comparison to PAR. This might be a key factor leading to the growth of *B. hypnoides* at this depth and can be interpreted as a pronounced UVB escape mechanism.

Acrosiphonia spec. had an intermediate resistance to experimental UVB radiation (**Table 6.1**). It showed the lowest screening and a relatively slow but complete repair with the highest α value of this study. This is in agreement with a study from Bischof et al. (1998) in which *Acrosiphonia arcta* recovered completely but slowly within 48 h. It is a typical spring species (Kornmann and Sahling 1977) and the solar irradiances are comparably low during this season. Accordingly, the strategy of *Acrosiphonia* can be identified as escape mechanism combined with a limited cellular tolerance.

Conclusion

In two species from the Cladophorales, *R. riparium* and *C. sericea*, avoidance by screening was shown to yield the highest resistance to experimental UVB exposure of all analyzed species. The efficiency of screening determined the degree of resistance of the DNA and PS II demonstrating the functional protection by a physical UV shield. On the other hand, this strategy is probably a costly and relatively inflexible defense. Firstly, the synthesis of screening compounds is an extra sink of energy, and depending on the chemical nature maybe also nutrients. Secondly, even if the level of screening may be varied along with e.g. seasonal changes in UVB dose this will probably not be the case on shorter time scales, especially in view of the proposed localization of the unknown screening compounds linked to the cell wall and their biochemical resistance towards degradation. This means a fine temporal adjustment of screening is not very likely in the Cladophorales. Thus, the investment in this screening strategy may be advantageous under high UVB radiation conditions but cannot easily be allocated into other sinks under lower stress conditions. Thirdly, the screening has a very specialized function: it shields the cells from UVB. Defense against other stresses that possibly induce similar physiological impairments, like high irradiances of PAR, nutrient limitations, or desiccation stress and others, that for example all may harm the photosynthetic machinery will not benefit from the UVB screening. Lastly, if as here reported the alternative cellular tolerance is relatively inefficient the screening should ascertain that no damages are induced; otherwise photosynthesis and DNA integrity may be impaired and growth inhibited. Thus, a UVB resistance solely based on UVB screening seems questionable and possibly the two species, *R. riparium* and *C. sericea* have other tolerance mechanisms that were not studied in this thesis. Also as shown for *U. intestinalis* laboratory results are no good predictors of field situations opening the chance for *R. riparium* and *C. sericea* to display an increased cellular tolerance *in situ*.

The alternative situation with high cellular UVB tolerance was demonstrated for *Ulva*. The two studied species, *U. intestinalis* and *U. clathrata* showed no efficient UVB screening and the induction of UVB damages was considerable higher than in the two cladophoracean species under experimental UVB exposure. But, they exhibited a fast recovery of PS II after UVB challenges. This might be explained by a high turnover of the D1 protein although this was not measured directly. However, experiments with addition of a protein biosynthesis inhibitor demonstrated a major contribution of D1 turnover to the recovery capacity in *U. clathrata* and *U. intestinalis*. Additionally, in the field another cellular tolerance mechanism of photosynthesis, the light-dependent development of non-photochemical quenching, was observed. D1 turnover and NPQ can combine to yield a tolerance of PS II towards UVB but also high PAR that is adjustable on short times scales to the current conditions. Conclusively, these physiological defenses render *Ulva* tolerant towards the

exposure to direct sunlight as observed in the presented field study. The costs for enzymatic driven repair may be equally high or even higher as in screening species but the flexibility of this strategy may represent an unrivaled advantage. As discussed in the previous section *Ulva intestinalis* is probably not light limited and is able to use a high portion of this light energy for these cellular tolerance mechanisms.

No UVB resistance was found in *B. hypnoides* from the upper sublittoral. Exposure to experimental UVB induced severe PS II impairment and very high concentrations of DNA damages. Defenses as screening or physiological repair processes were absent under the used experimental conditions. Thus, exposure to UVB radiation inevitably seems to lead to irreversible damages and leaves escape from this stress as the only opportunity for *B. hypnoides* to survive. By far not that pronounced but also a high UVB sensitivity was found in *Acrosiphonia spec.* which exhibited only a limited cellular tolerance and also no UV screening. The main growth season of this species is in spring when the solar angles are lower and day length are shorter and therefore sunlight absorption is reduced compared to the summer. Thus the two latter species receive less energy but also less light stress.

Chapter 7 – Summary

Within the research of this thesis green macroalgae grown under controlled laboratory conditions as well as field sampled algae were analyzed in experiments with artificial UVB exposure or *in situ* in the field. By this approach as much as possible insights into the different mechanisms of UVB resistance have been achieved.

Adaptive differences of UV screening and cellular UVB tolerance were found between two ecologically important orders of green algae, the Ulvales and Cladophorales. While the former lacked significant UV screening they exhibited very high PS II recovery rates. Otherwise the latter group displayed pronounced UVA and UVB screening. The lowest apparent UVB transmittance of 10 % was measured in a *Rhizoclonium riparium* culture. This screening served quantitatively to reduce PS II damage in short-term UVB challenge experiments as shown in *R. riparium* and *Cladophora sericea*. Explicitly, it was necessary to increase the experimental UVB irradiance to levels 5 times higher than ever possible on ground to induce 20 % PS II damage in *R. riparium*. Conclusively, algae with this efficient UVB screening will probably not suffer from UVB-induced damages under field conditions and can save expenses on cellular tolerance mechanisms.

In contrast the non-screening alga *Ulva intestinalis* was suspected to accumulate PS II and DNA damages under field conditions. This was not confirmed in short- or long-term monitoring. At noon PS II was temporarily inhibited *in situ* but this could be ascribed to regulated dynamic photoinhibition to a large extent. The responsible mechanism seems to be a high capacity of non-photochemical quenching, as manifested in strong reductions of F_0 . Additional, a competent PS II repair cycle assists the cellular tolerance of PS II in *Ulva*. This was also observed after experimental UVB exposure in the two studied *Ulva* species. Interestingly, only trace concentrations of UVB-induced DNA dimers were present in *U. intestinalis* in the field and these were not dependent on UVB irradiance or cumulative UVB dose. Here, it is speculated that efficient photoreactivation accounts for an outbalanced rate of dimer induction and splitting.

Alternatively, two other non-screening species, *Acrosiphonia spec.* and *Bryopsis hypnoides* were identified as moderately to highly UVB-sensitive, respectively. *Acrosiphonia* exhibited an intermediate cellular tolerance while *B. hypnoides* was not capable to restore its PS II function once it was damaged. *Acrosiphonia* is growing primarily in spring whereas *B. hypnoides* occupies a more subtidal habitat with attenuated UVB irradiances. Thus the UVB resistance could tentatively be related to the ecological niche of the species.

Zusammenfassung

Innerhalb der Untersuchungen dieser Arbeit wurden grüne Makroalgen, die entweder unter kontrollierten Laborbedingungen aufgezogen oder aus dem Freiland gesammelt worden waren in Experimenten sowohl mit künstlicher UVB-Bestrahlung als auch *in situ* untersucht. Durch diesen Ansatz sollten so viele Einblicke wie möglich in die unterschiedlichen UVB-Schutzmechanismen erlangt werden.

Es wurden adaptive Unterschiede der UV-Abschirmung und der zellulären UVB-Toleranz zwischen zwei ökologisch wichtigen Ordnungen von Grünalgen, den Ulvales und Cladophorales gefunden. Während die Ersten eine signifikante UV-Abschirmung ermangelten, zeigten sie sehr effiziente Erholungsraten des PS II. Die Zweiten hingegen wiesen eine ausgeprägte UVA- und UVB-Abschirmung auf. So wurde die niedrigste apparente UVB-Transmission von 10 % in einer Kultur von *Rhizoclonium riparium* gemessen. Die quantitative Wirksamkeit dieser Abschirmung in der Reduktion von UVB-induzierten PS II-Schäden wurde in Kurzzeitversuchen mit *R. riparium* und *Cladophora sericea* gezeigt. Tatsächlich war es nötig die UVB-Bestrahlungsstärke fünfmal höher als auf der Erdoberfläche möglich einzustellen, um einen 20%igen Schaden an PS II in *R. riparium* zu erzeugen. Demzufolge sollten Algen mit einer derartigen effizienten UVB-Abschirmung wahrscheinlich keine UVB-induzierten Schäden unter natürlichen Freilandbedingungen erleiden und können auf zelluläre Toleranzmechanismen verzichten.

Im Gegensatz dazu wurde angenommen, dass die nicht-abschirmende Alge *Ulva intestinalis* PS II- und DNA-Schäden im Freiland akkumulieren würde. Dies war weder in Kurzzeit- noch in Langzeituntersuchungen der Fall. Zwar war über Mittag PS II vorübergehend inhibiert, jedoch konnte dies größtenteils dynamischer Photoinhibition zugeschrieben werden. Eine hohe Kapazität der nicht-photochemischen Energielöschung scheint dabei der verantwortliche Mechanismus zu sein, der durch eine starke Absenkung des F_0 -Signals deutlich wurde. Zusätzlich unterstützt eine kompetente PS II-Reparatur die zelluläre Toleranz des PS II in *Ulva*. Dies wurde nach experimenteller UVB-Bestrahlung für die beiden untersuchten *Ulva*-Arten bestätigt. Interessanterweise waren nur sehr geringe Konzentrationen von DNA-Schäden in *U. intestinalis* im Freiland vorhanden, die auch nicht von der UVB-Photonenflussrate oder der akkumulierten UVB-Dosis abhingen.

Andererseits wurden zwei andere nicht-abschirmende Arten, *Acrosiphonia spec.* und *Bryopsis hypnoides* als mäßig beziehungsweise hoch UVB-sensitiv identifiziert. *Acrosiphonia* wies eine

mittlere zelluläre Toleranz auf, während *B. hypnoides* einen einmal entstandenen Schaden an PS II nicht mehr reparieren konnte. *Acrosiphonia* wächst überwiegend im Frühjahr, während das Habitat von *B. hypnoides* mehr im oberen Sublitoral mit schon deutlich abgeschwächten UVB-Strahlungsstärken liegt. Die UVB-Resistenz konnte damit tendenziell in Relation zu den ökologischen Nischen der Arten gebracht werden.

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Ich versichere an Eides statt, dass ich bis zum heutigen Tag weder an der Christian-Albrechts-Universität Kiel noch an einer anderen Hochschule ein Promotionsverfahren endgültig nicht bestanden habe oder mich in einem entsprechenden Verfahren befinde.

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Die Arbeit entstand unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft.

Kiel, den

(Frauke Pescheck)

Danksagung

Besonderer Dank geht an Prof. Wolfgang Bilger für die Überlassung des Themas. Während der Entstehung dieser Arbeit leistete er mit viel Geduld, Interesse und Ideen einen wesentlichen Beitrag zum Gelingen. Andererseits schenkte er viel Vertrauen und ermöglichte damit einen sehr großen Freiraum bei der Planung der Experimente und Vorhaben. Mit dieser Mischung aus Unterstützung und Freiheit hat er meine Begeisterung und mein Verantwortungsgefühl für diese Arbeit sehr gefördert. Vielen herzlichen Dank für deine Betreuung!

Ebenfalls besonderer Dank geht an Prof. Kai Bischof, der mir den Einstieg in die Arbeit mit marinen Makroalgen ermöglicht hat und mein Interesse an diesen faszinierenden Pflanzen geweckt hat. Seine Begeisterung für diese Organismen war ansteckend!

Weiterhin möchte ich sehr herzlich Dr. Michael Roleda danken, der mir die Methode der Bestimmung von DNA Schäden beigebracht hat. In diesem Zusammenhang gilt auch mein herzlicher Dank Prof. Christian Wiencke für die großzügige Überlassung der „Erstausrüstung“ für die CPD-Bestimmung und für die Bereitstellung der Stammkulturen der Grünalgen für den Artenvergleich der UV-Abschirmung.

Besonderer Dank gebührt auch Dr. Kai Lohbeck, der zu Beginn der Arbeiten an den DNA-Schäden mit mir zusammen an der Entwicklung des CPD-Standards gearbeitet hat und dessen Berechnungen immer eine sichere Sache waren und sind!

Außerdem vielen herzlichen Dank an Prof. Karin Krupinska für die Bereitschaft zur Begutachtung dieser Arbeit und die Mitglieder ihrer Arbeitsgruppe für die Unterstützung und Beratung bei molekularbiologischen Fragen und für die ungezählten Male der Dunkelkammerbenutzung. Dr. Christine Desel möchte ich herzlich für die Unterstützung bei der Fluoreszenzmikroskopie danken.

Großer Dank gilt Herrn Koch und den Mitarbeitern vom Wasser- und Schifffahrtsamt Kiel Holtenau für die Ermöglichung der Langzeituntersuchung auf dem Gelände der kleinen Schleuse. Entsprechend möchte ich der Werkstatt des Biozentrums für die schnelle und haltbare Konstruktion der Algenkisten für dieses Freilandexperiment danken.

Bei den unterschiedlichen Freilanduntersuchungen haben viele Menschen mitgeholfen, ob bei Regen oder Hitze, den ganzen Tag über oder mitten in der Nacht. Diesen möchte noch einmal ausdrücklich für ihre verlässliche Hilfe danken: Nele Hein, Cayssa Staggen, Christine Bönninger, Hanna Jämlich und Hanna Campen. Ebenso wichtig für diese Untersuchungen war die freundliche Ausleihe der Schwimmwesten von Prof. Heinz Brendelberger.

Selbstverständlich danke ich der ganzen Arbeitsgruppe der Ökophysiologie der Pflanzen, die definitiv mehr zu dieser Arbeit beigetragen haben als sie selber annehmen. Danke für die vielfältige Hilfe!

Ich danke meiner Familie und allen, die mir zur Seite stehen!

Schlussendlich möchte ich der Christian-Albrechts-Universität Kiel für die finanzielle Förderung durch ein Promotionsstipendium über zwei Jahre danken.