# EFFECTS OF PAR AND HIGH UVR ON ENZYMES AND OTHER PROTEINS INVOLVED IN THE FUNCTION AND PROTECTION OF PHOTOSYNTHETIC APPARATUS OF MARINE MACROALGAE

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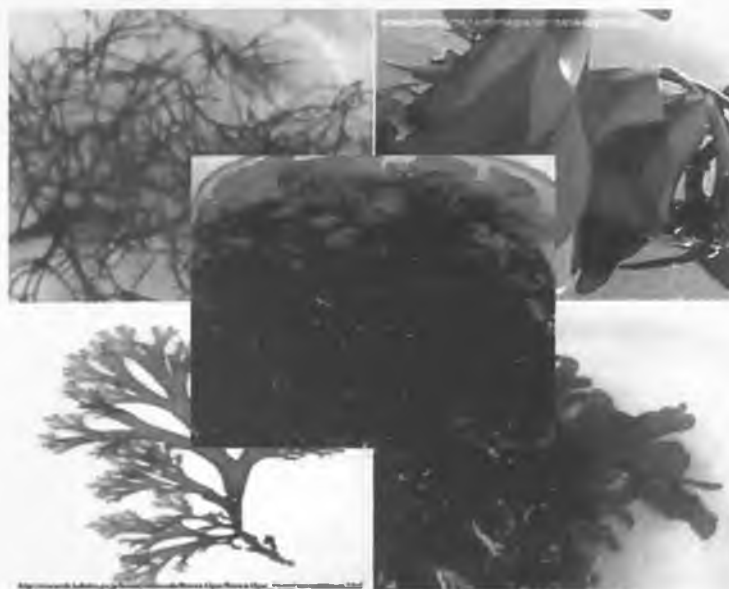
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TABLE OF CONTENTS

Contents

LIST OF ABBREVIATIONS

λ	wavelength
^1Chl, ^3Chl	singlet chlorophyll, triplet chlorophyll
$^1O_2$, O_2^-	singlet oxygen, superoxide radical
A, V, X, Z	anteraxanthin, violaxanthin, xanthophyll, zeaxanthin
AL	actinic light
AP	alkaline phosphatase
APX	ascorbate peroxidase
AsA	ascorbic acid
CAT	catalase
Chl	chlorophyll
$CuSO_4$	copper sulphate
Ddx, Dtx	diadinoxanthin, diatoxanthin
dH_2O	distilled water
DHA	dehydroascorbate
DMF	dimethylformamide
DMSO	dimethylsulphoxide
ETC	electron transport chain
FI	fluorescence induction
FR	far-red light
FW	fresh weight
GAP	glyceraldehyde-3-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GR	glutathione reductase
GSH, GSSG	glutathione, oxidized glutathione
H&L	heavy and light chains of antibodies
H_2O_2	hydrogen peroxide
HRP	horse radish peroxidase
HSP	heat shock protein
IC	induction curve
K_2CrO_4	potassium chromate
KNO_3	potassium nitrate
LHC	light harvesting complex

LSD	least significance difference
LSU, SSU	large subunit and small subunit of RuBisCO
Lut, Lx	lutein, lutein epoxide
ML	measuring light
MAA	mycosporine-like amino acid
PAM	pulse amplitude modulation
PAR	photosynthetically active radiation (400-700 nm)
PAR+UVR	PAR+UVA and PAR+UVA+UVB
PFR	photon fluence rate
PGA	phosphoglycerate
P-I	photosynthesis-irradiance curve
PPFD	photosynthesis photon flux density
PSI, PSII	photosystem I, photosystem II
PSU	photosynthetic unit
PVPP	polyvinylpolypyrrolidone
RC	recovery curve
rETR	relative electron transport rate
RLC	rapid light curve
ROS	reactive oxygen species
R-PC	R-phycoerythrin
R-PE	R-phycocyanin
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	ribulose bisphosphate
SNK	Student-Neumann-Keuls test
SOD	superoxide dismutase
SP	saturation pulse
Trp	tryptophan
TSP	total soluble proteins
U	enzyme unit
UVA	ultraviolet A (315-400 nm)
UVB	ultraviolet B (280-315 nm)
UVC	ultraviolet C (100-280 nm)
UVR	ultraviolet radiation. UVA and/or UVB
VIS	visible light

LIST OF TERMINOLOGIES

$(1-qP)/NPQ$	susceptibility of PSII to light stress index
$\Delta F/F_m$' or $\Phi PSII$ or Y	effective quantum yield of PSII $[(F_m\text{'}-F)/F_m\text{'}]$
$1-qP$	excitation pressure of PSII
F or F_t	steady-state fluorescence yield
F_m, F_m'	dark-adapted maximal fluorescence yield; pre-illuminated maximal fluorescence yield
F_o, F_o'	dark-adapted minimal fluorescence yield; pre-illuminated minimal fluorescence yield
F_v	variable fluorescence yield, steady-state fluorescence yield
F_v/F_m	maximal quantum yield $[(F_m-F_o)/F_m]$
I_k	light saturation parameter
NPQ	Stern-Volmer's non-photochemical quenching parameter $[(F_m - F_m\text{'})/F_m\text{'}]$
qE	non-photochemical energy-dependent quenching parameter
qI	non-photochemical inhibitory quenching parameter
qN	non-photochemical quenching parameter $[1- ((F_m\text{'} - F_o\text{'})/(F_m - F_o))]$
qP	photochemical quenching parameter $[(F_m\text{'} - F)/(F_m\text{'} - F_o\text{'})]$
qT	non-photochemical state transitions quenching parameter
$rETR_{max}$	maximal relative electron transport rate
vide infra, vide supra	refer text below, refer text above
α	photosynthetic efficiency parameter $[rETR_{max}/I_k]$

SUMMARY

-vii-

The changes in the quality and quantity of the solar radiation may affect photosynthetic organisms. An increase in irradiation of UVB (290-320 nm) of the solar radiation that reaches the earth's surface due to thinning of the ozone layer, for instance, can cause destructive consequences to these photoautotrophs. Thus, like any other photoautotrophs, macroalgae are deemed to be affected and loss of these important biomass producers of the aquatic ecosystem may disrupt the primary productivity and the whole ecosystem integrity. However, the macroalgae have somehow developed protective mechanisms to ensure their survivality in the extreme environment.

In this study, short-term responses of five marine macroalgae, *Solieria chordalis*, an intertidal red alga; *Palmaria palmata*, an intertidal or upper sublittoral red alga; *Laminaria digitata*, an upper to middle sublittoral brown alga; *Dictyota dichotoma*, an upper sublittoral brown alga; and, *Ulva lactuca*, an intertidal or upper sublittoral green alga, to ultraviolet radiation (UVR) were investigated. The algae were originally collected from the North Sea islands of Sylt and Helgoland and were further cultivated in a temperature-controlled laboratory under ~32 μmol m^{-2} s^{-1} of white light at 12.5±0.5°C. The algae were irradiated for 5 h to a high UVR in combination with either low or high background photosynthetically active radiation (PAR, 400-700 nm) emitted by fluorescent lamps or a sun simulator, respectively. Four light regimes were created using cut-off filters: PAR, PAR+UVA (UVA, 320-400 nm), PAR+UVA+UVB or UVA+UVB. Recovery kinetics were also determined by incubating the irradiated algae in dim light for 18 h. Responses were evaluated on the basis of photosynthetic performance (i.e. F_v/F_m, $rETR_{max}$, α and I_k), photodamage or photoinactivation (i.e. *via* pigments analysis, total soluble proteins content, RuBisCo activity and its large subunit (LSU) composition, GAPDH activity and D1 protein content), and photoprotective mechanisms (i.e. *via* antioxidative enzymes activity, presence of stress proteins and non-photochemical quenching).

Higher reductions in F_v/F_m were observed with high UV and high background PAR than under low PAR and additional UVB caused the highest reduction. Most of the algae showed high F_v/F_m with UV alone. Low PAR alone had weak or no effect on the algae. Recovery was the fastest with PAR alone but was slow with additional UVB, indicating permanent damage may have occurred in the PSII reaction centres. Some algae showed a delay in recovery with UV alone. All affected algae showed signs of recovery from the stress with one exception. UV strongly reduced the convexity of the rETR vs. irradiance plot resulting in a lower $rETR_{max}$, a lower α and a higher I_k compared to the controls indicating damage or inactivation to the reaction centres. It is probable that UV- or high PAR-induced inhibition in all species caused accumulation of reactive oxygen species (ROS) especially H_2O_2 as indicated by increase in the antioxidative enzymes. The production of ROS may be triggered by the photosynthetic pigments (i.e. chlorophylls and phycobiliproteins) which were subsequently damaged through absorption with UV and high PAR, hence, a reduction in the pigments content was also observed. In addition, accumulation of ROS may also result from the photorespiratory pathway indicated by high induction of catalase. As a consequence, low content of D1 protein, loss of total soluble proteins, low activity of RuBisCO and its LSU composition, and low activity of GAPDH were observed during the inhibitory phase. High content of antioxidative enzymes and stress proteins, HSP60 and HSP70, detected in the irradiated algae indicated the trigger of photoprotective mechanisms functioning in the repair and recovery processes; hence, all species were able to recover from stress. However, rate of inhibition and recovery differed between the light treatments indicating different mechanisms of inactivation and protection which were induced by each of the different spectral wavelength ranges.

UV and high PAR significantly reduced the fluorescence yield signal (i.e. ΔF, $F_m'-F_o'$), the effective quantum yield (Y) and photochemical quenching parameter (qP) of the irradiated algae. In addition, non-photochemical quenching parameters, qN and NPQ of the irradiated algae were induced indicating high ability to dissipate excess energy as heat. Dark relaxation kinetics revealed that in the irradiated algae, most of the NPQ were made up of photoinhibitory quenching (qI). Furthermore, Y showed a steady but slow recovery during the dark phase as well indicating that damage may have occurred in the reaction

centres. Higher 1-qP and lower (1-qP)/NPQ indices were measured in irradiated algae compared to the controls. Reduced Q_A accumulated in the irradiated algae as indicated by the complete quenching of the second rise of the fluorescence signal of the rapid induction curve which can also be due to the strong qI quenching. Within the ratio of PAR:UVA:UVB close to the natural conditions, the algae were more tolerant to high UVB/low UVA than low UVB/high UVA with high background PAR. In contrast, most of the algae showed low inhibition at low UVB flux in combination with low background PAR.

In addition to the above observations, it was interesting to note that in some species, UVB showed an ameliorating effect on the recovery of the algae as indicated by faster recovery with PAR+UVA+UVB than PAR+UVA. This effect was observed in *D. dichotoma* irradiated at high UV/high PAR and *P. palmata* irradiated at high UV/low PAR. This supporting role of UVB was reflected in most of the parameters analyzed. It is probable that UVB might induce the transcription of *PsbA* genes of D1 protein leading to a faster recovery in PAR+UVA+UVB than PAR+UVA. Significant delay in recovery of PAR+UVA compared to PAR+UVA+UVB was also observed in *U. lactuca* while similar trend was detected in *S. chordalis* and *L. digitata* irradiated at low UV/high PAR. Comparatively, some of the species were shown to respond more to UVA than UVB and this could be an ecological importance as well.

The results obtained showed that the algae responded according to the zonation pattern of their natural habitats. For instance, the sublittoral *L. digitata* was the most inhibited by the high UV/high PAR stresses. Several dissimilarities in the behaviour between the two brown algae, *L. digitata* and *D. dichotoma*, in counteracting the damaging effect of UVR could be observed. *L. digitata* was strongly affected by UVB but in *D. dichotoma*, UVB showed an ameliorating effect. UV alone caused chronic photoinhibition in *L. digitata* but dynamic photoinhibition in *D. dichotoma*. At high UV/low PAR irradiance, *L. digitata* was less affected than *D. dichotoma*. An increase in $rETR_{max}$ was apparent in *L. digitata* parallel to a decrease in α and an increase in I_k at high UV/low PAR irradiance. This characteristic of *L. digitata* was not displayed by any other species which was also observable at low UV/low PAR. *D. dichotoma* exhibited

high fucoxanthin content in response to high PAR and high UVA but not when supplemented with UVB. Catalase activity at high UV/low PAR increased two-fold in *L. digitata* in comparison to *D. dichotoma*. Responses of both brown algae which were different from other algal classes include a decrease in 1-qP and an increase in NPQ after irradiation but with a slower onset of qN.

The intertidal *S. chordalis* and the upper sublittoral *P. palmata* were more sensitive to the high light stress than the green alga. Upon irradiation, higher antioxidative enzymes were observed in these algae than the other two classes indicating higher oxidative stress conditions. Furthermore, maximal 1-qP was measured in the irradiated algae and the ability to induce qN and NPQ was much lower than the other classes. Thus, the red algae generally showed a slower recovery and adaptation than the other species. In addition, *S. chordalis* was more affected by the low PAR alone in comparison to the other species. Comparatively, the intertidal or upper sublittoral *U. lactuca* was the least affected among the species. Most obvious response shown by *U. lactuca* was the rapid recovery of most of the parameters with all light treatments excluding UV alone. Therefore, *U. lactuca* was said to be well-prepared and well-adapted with the high light stress in comparison to the other species. Indeed, this alga was more inhibited under a low UVB flux and showed an ameliorating UVB effect at this ratio. The highest induction of stress proteins was observed in *U. lactuca* as well. The activity of the antioxidative enzymes was generally low in *U. lactuca*, indicating low oxidative stress in the cells.

In conclusion, all of the algae examined were strongly inhibited by the high UV and high PAR. High tolerance to UVB was displayed by the algae at much lower UVB fluxes. Even though UVB generally caused damaging effects, some of the algae responded positively to UVB. Whilst the brown algae *L. digitata* and *D. dichotoma* collectively showed the highest inhibition, the green alga *U. lactuca* was the least affected and was well-prepared and well-adapted to the high light effect. The red algae *S chordalis* and *P. palmata* were also strongly inhibited but recovered more slowly than the rest of the algal classes.