

Photosynthetic performance of marine macroalgae measured in patagonia on site

Donat-P. Häder¹, Michael Lebert¹ and E. Walter Helbling²

¹ Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudstr. 5, D-91058 Erlangen, Germany

² Estación de Fotobiología Playa Unión, Casilla de Correo N° 153 (9100) Trelew, Chubut, Argentina & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

ABSTRACT

The photosynthetic performance was analyzed in common macroalgae on site and under seminatural conditions in Patagonia, Argentina, using a portable PAM instrument. Solar radiation was monitored in parallel with a filter dosimeter, ELDONET (Real Time Computer, Möhrendorf, Germany), in three wavelength ranges, UV-A, UV-B and PAR. Fluence rate-response curves indicated that the species is a typical shade plant which developed profound non-photochemical quenching at intermediate and higher irradiances. Fast induction and relaxation kinetics indicated a fast adaptation of the photosynthetic apparatus to the changing light conditions by fast adjustment of the photochemical and non-photochemical quenching. Upon exposure to solar radiation the algae responded with photoinhibition but recovered in the subsequent shade conditions within several hours. When the thalli were allowed to free float in the water photoinhibition was less profound and recovery was faster than when the algae were confined in a fixed position. While most of the photoinhibition was due to visible radiation, the UV wavelength range, and especially UV-B, considerably enhanced the decline in photosynthetic quantum yield.

Abbreviations and Symbols

F₀: initial chlorophyll fluorescence in dark-adapted plants, all reaction centers are open
F_m: maximal fluorescence in dark-adapted plants, all reaction centers are closed
F_v: variable fluorescence = F_m - F₀
F₀', F_m' and F_v': the same for the light-adapted state

F_t: current fluorescence of light-adapted plants
LHC: light harvesting complex
PAM: pulse amplitude modulated fluorometer
PAR: photosynthetic active radiation
PS II: photosystem II
qE: energy quenching
qN: non-photochemical quenching
qP: photochemical quenching

INTRODUCTION

The vertical distribution of macroalgae on the coast is controlled mainly by the light gradient in the water column [1,2]. The photoprotective capabilities of a species define its upper growth limit. In the supralittoral and eulittoral, macroalgae are exposed to excessive solar PAR (photosynthetic active radiation, 400 – 700 nm) as well as to UV radiation [3-5]. Algae, being photosynthetic organisms, show various degrees of photoinhibition when exposed to high solar radiation or even irreversible photodamage like terrestrial plants [6-11]. The molecular mechanism of chronic photoinhibition is the light-dependent photodegradation of the D1 protein in photosystem II (PS II) which leads to a decrease in the photosynthetic electron transport [12-14]. As a result, this adaptive mechanism protects the photosynthetic apparatus from excessive radiation energy. In contrast, dynamic photoinhibition is based on the xanthophyll cycle identified in higher plants and in many macroalgae with the exception of the Rhodophyta [15,16].

When exposed to excessive solar radiation, the quantum yield of PS II decreases and the excitation energy is

dissipated thermally. Upon short term exposure this process may be reversible within minutes or hours, while long term exposure may cause irreversible damage. These adaptive processes of the photosynthetic apparatus in algae have been summarized in recent reviews [16,17]. Using pulse amplitude modulated (PAM) fluorescence, a number of fluorescence parameters can be determined from which the photosynthetic quantum yield can be calculated without the previous knowledge of the dark fluorescence parameters F_0 and F_m [18,19]. This approach has been validated by parallel gas exchange measurements [20]. The measured PAM fluorescence signals can also be used for the quenching analysis: In photochemical quenching (qP) the absorbed radiation energy is utilized in the photosynthetic apparatus while non-photochemical quenching (qN) is caused by the built-up of a pH gradient across the thylakoid membrane [9,21]. In many eulittoral macroalgae a pronounced decrease in the photosynthetic yield is observed between noon and early afternoon caused by dynamic photoinhibition during excessive radiation exposure [22-24]. This pattern is overlaid by the tidal rhythm in marine algae. Highest radiation stress occurs when low tides and high solar angles coincide [22,23,25], leading to complicated patterns of photosynthetic activity further modulated by a changing cloud cover. Photoinhibition was found in macroalgae from the tropics and the temperate zone [3,26,27] as well as in Arctic and Antarctic macroalgae [22,28-30].

Most of the observed photoinhibition is due to the visible radiation because of its high proportion in solar radiation. In addition, a significant share of photoinhibition is caused by UV-B (and less so by UV-A) radiation [3,16,31] even though UV-B contributes only 1 % of the radiation energy reaching the Earth's surface. Due to limited penetration of short wavelength radiation, UV related photoinhibition is highest in algae located at or above the water surface during low tide. This contribution characterizes the photosynthetic performance of widespread brown macroalgae under natural conditions at their growth site on the Patagonian coast, Argentina.

MEASUREMENT OF SOLAR RADIATION

Solar radiation was continuously monitored from before dawn to after dusk with a three-channel filter instrument (ELDONET, Real Time Computer, Möhrendorf, Germany,) [32] during the whole measurement period in three wavelength bands (UV-B, 280 - 315 nm; UV-

A, 315 - 400 nm; PAR, 400 - 700 nm). The irradiance in each channel is averaged over 1-min intervals and recorded on a host computer. All data are transferred to the central server of the ELDONET network [33] where they are available to the public (www.power.ib.pi.cnr.it/eldonet/). Figure 1 shows the solar irradiance on a representative day in autumn (18 March 2000). With the exception of some scattered clouds over noon and in the late afternoon the sky was blue throughout the day with maximal irradiances of about 350 W m^{-2} in the PAR region, 45 W m^{-2} in the UV-A and about 1.6 W m^{-2} in the UV-B region.

PULSE AMPLITUDE MODULATED FLUORESCENCE MEASUREMENTS

Photosynthetic performance can be determined by oxygen measurements [34] or using a pulse amplitude modulation (PAM) fluorometer [35]. The latter technique was employed to quantify *in vivo* chlorophyll fluorescence parameters of photosystem II non-invasively with a portable pulse amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany). In dark-adapted organisms the background chlorophyll fluorescence signal F_0 is induced by a weak red light source, so that the PS II reaction centers remain mostly open. Then a saturating white light pulse is flashed which induces maximal fluorescence, F_m , during which the PS II reaction centers are closed. In light-adapted organisms the maximal fluorescence (F_m') is usually lower while F_0' is usually higher. The effective photosynthetic quantum yield Y_{eff} was calculated using the following equation [18].

$$Y_{\text{eff}} = (F_m' - F_t)/F_m' = F_v'/F_m'$$

where F_m' is the maximal fluorescence in a light-adapted plant and F_t denotes the current steady state fluorescence. The optimal quantum yield Y_{opt} was measured in dark-adapted thalli

$$Y_{\text{opt}} = (F_m - F_0)/F_m = F_v/F_m$$

where F_m is the maximal fluorescence and F_0 the ground fluorescence induced by the weak red background light. From these parameters the photochemical quenching (qP) can be determined:

$$qP = (F_m' - F_t)/(F_m' - F_0')$$

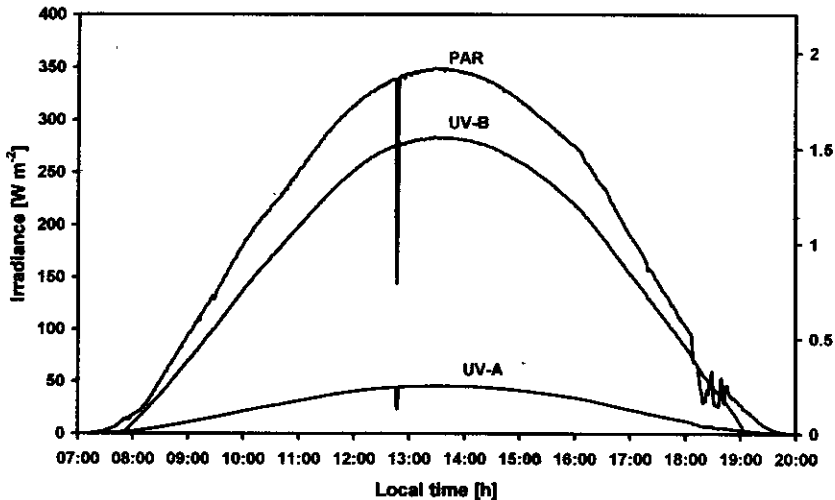


Figure 1. Representative solar radiation for the measurement period in the PAR (400 – 700 nm), UV-A (315 – 400 nm) and UV-B (280 – 315 nm, right ordinate) wavelength ranges measured on 18 March 2000 in Playa Unión, Rawson, Argentina.

as well as the non-photochemical quenching:

$$qN = 1 - (F_m' - F_o') / (F_m - F_o).$$

Specimens of common macroalgae were harvested from tidal pools on east facing rocky shores in Patagonia (Argentina) during low tide. The PAM fluorometer can be programmed to determine the photosynthetic parameters in dependence of the actinic irradiance. Fig. 2 shows an example for the brown alga *Dictyota*. These irradiance-response curves show a slow and steady decline of the F_m' value from about 0.6 to about 0.35. The photochemical quenching decreases from an initial value of close to 1 to 0.35 at 79 W m^{-2} . Also the photosynthetic yield declines from 0.66 for low irradiances to 0.1 at the maximal irradiance. F_t does not vary significantly while the non-photochemical quenching increases to 0.86 indicating a high degree of photoinhibition.

The PAM instrument can also be used to automatically determine induction curves for several fluorescence parameters with on-line quenching analysis. At the beginning of the experiment a saturation pulse is applied to measure F_o and F_m . Subsequently, the fluorescence yield is recorded at 10 ms sampling rate and a sequence

of saturating pulses is elicited at 20-s intervals. After each pulse the quantum yield, and the photochemical and non-photochemical quenching are calculated. In *Dictyota* both the fluorescence yield and qP remained at very low values while F_m and F_t decreased in parallel. In contrast, qN increased from almost zero to 0.86 (Fig. 3).

The relaxation kinetics of qN was determined at a sampling rate of 30 ms immediately following the induction curve after the thallus had developed a considerable non-photochemical fluorescence quenching. During the experiment the algae were exposed to the actinic light to determine F_o and F_m . Two saturating light pulses from the built-in halogen lamp were elicited to measure the quenching parameters in the light-adapted state, and then the actinic light was turned off. Further saturating light pulses were applied with increasing time intervals between pulses following the function $t = 10 \times 1.2^{n-1}$. The results show that the brown macroalga *Dictyota* adapts within a very short period of time to bring up its photochemical quenching to close to 1. The non-photochemical quenching drops only slightly and F_m slightly increases in an antiparallel fashion. The quantum yield increases to above 0.4.

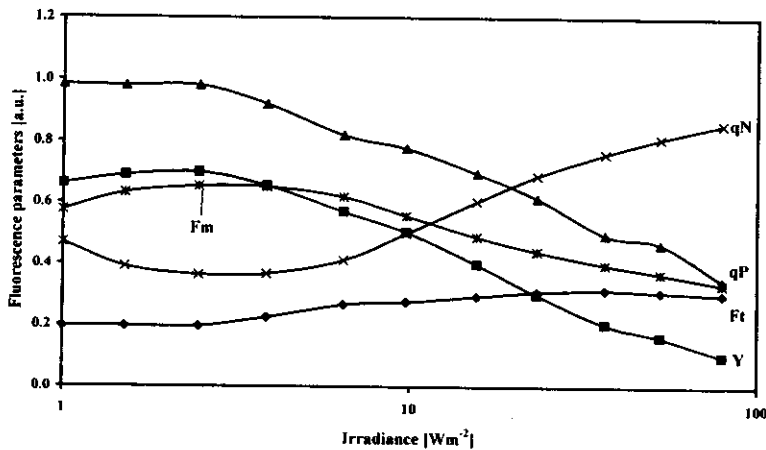


Figure 2. Fluorescence parameters measured in *D. dichotoma* in dependence of the fluence rate of the actinic red light. Before the experiments the thallus was adapted to an intermediate fluence rate of 23 W m^{-2} for 10 min and then exposed to increasing irradiances for periods of 6.5 min each. At the end of each period the fluorescence parameters were determined.

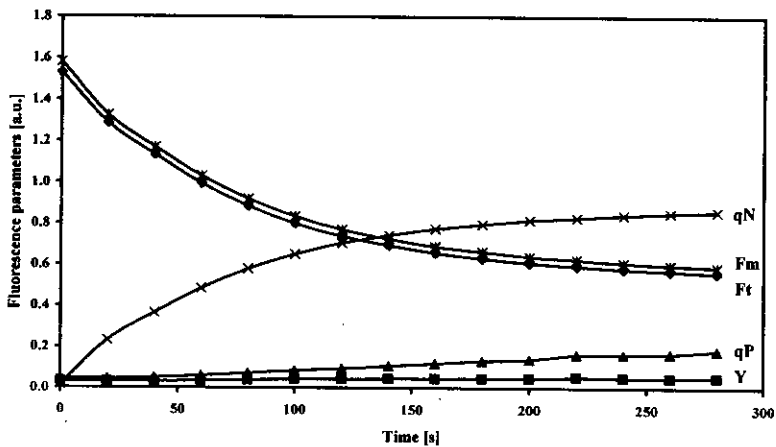


Figure 3. Induction curve with quenching analysis of *D. dichotoma* at 10 ms sampling rate.

PHOTOINHIBITION AND RECOVERY

In order to determine the photosynthetic performance of the macroalgae under natural conditions specimens were collected immediately before the experiment and

mounted in custom-made UV transparent Plexiglas holders (GS 2458, Röhm and Haas, Darmstadt, Germany) with open sides which allowed sea water to flow through the holders. Alternatively, the thalli were

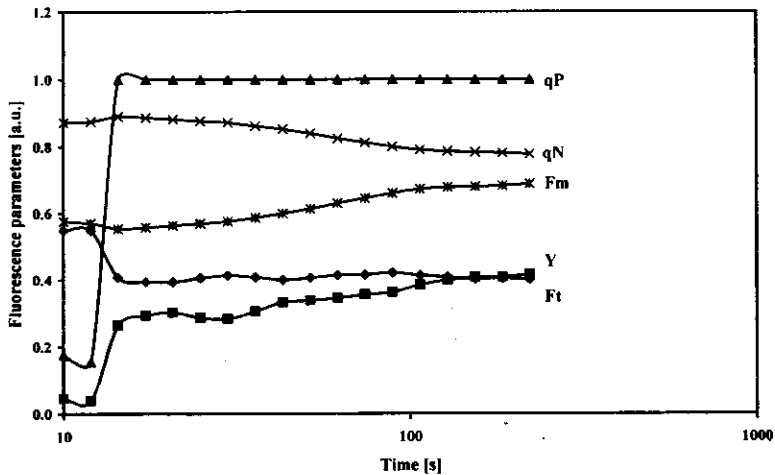


Figure 4. Relaxation kinetics of *D. dichotoma* at 10 ms sampling rate.

allowed to free float in a large volume of sea water in open containers floating on a reservoir of water to keep the temperature constant. UV cut-off filter foils were used to exclude short wavelength radiation below 320 nm (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany) or below 395 nm (Ultraplan UV Opak, Digefra, Munich, Germany). The transmission spectra of these filter foils have been published [36]. The dark-adapted samples showed a quantum yield of about 0.65. After 15 min exposure to solar radiation the yield had dropped to 0.15 (Fig. 5a) and recovered subsequently when the algae were kept in dim light afterwards, but never reached the original values before exposure. Control specimens treated the same way as the exposed samples except for the exposure period showed a similar yield as the dark-adapted plants before exposure indicating that the experimental conditions did not affect the yield; therefore it is safe to conclude that the effect is only due to exposure to solar radiation. When UV-B radiation was excluded, the yield values were higher both during exposure and recovery, and this tendency was even more pronounced when the total UV range was filtered out. In this experiment the thalli were confined in the same position within the Plexiglas holder. When they were allowed to float freely in the water the inhibition was less dramatic even after 30 min exposure, and the yield recovered almost completely within a few hours (Fig. 5b).

In the final experiment photosynthetic the quantum yield was followed in macroalgae on site without removing the thalli from their growth site. For this purpose thalli were studied using non-invasive PAM fluorescence measurements in rock pools in Bahía Bustamante (Patagonia, Argentina) located 250 km south of Rawson. These rock pools were accessible only during low tide and completely covered by 1 - 2 m water during high tide. The first measurement was at 11 h local time shortly after the retreat of the water under clear sky. Algae growing in the shade had a far higher quantum yield than those exposed to solar radiation (Fig. 6).

CONCLUSIONS

Macroalgae are exposed to drastically changing exposure conditions in their habitat and need to accomplish positive net photosynthesis at low and intermediate irradiances early in the morning or late in the afternoon or during high tide. During periods of excessive irradiation they need effective preventive mechanisms to protect themselves from damage. The regulatory mechanisms to reduce light stress include adjustment of antenna size, thermal dissipation of excess excitation energy, antioxidant enzymes and a fast repair of photooxidative damage [37]. The photosynthetic apparatus is protected by down regulation of the electron transport chain (photoinhibition) and

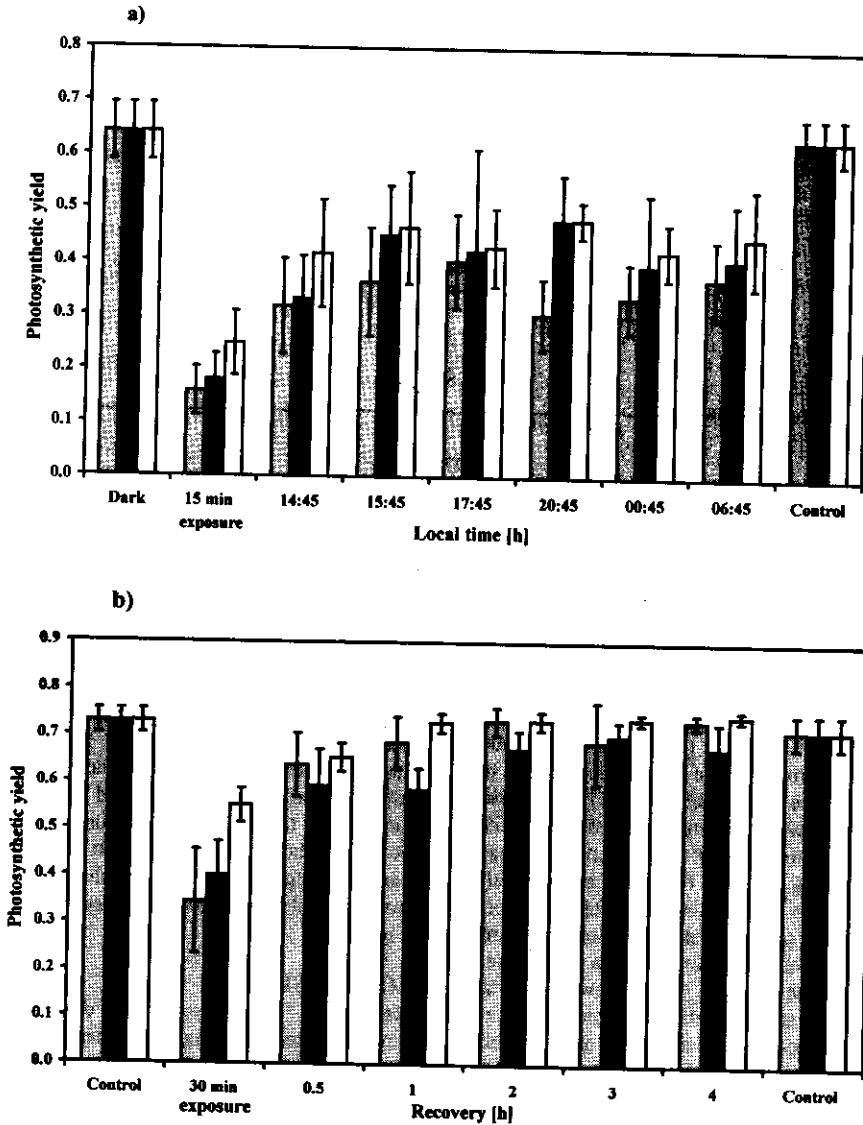


Figure 5. Photosynthetic quantum yield of *D. dichotoma* measured after 15 min of exposure to solar radiation, in Plexiglas holders (a) or 30 min free floating (b) and after increasing recovery times (details in the text). The specimens were exposed either to unfiltered solar radiation (gray bars), radiation filtered through a 320 nm cut-off filter foil (black bars) or filtered through a 395 nm cut-off filter foil (white bars). Independent controls were subjected to the same treatment except solar exposure and measured after the recovery period. For each data point at least eight measurements were averaged and the standard deviation calculated.

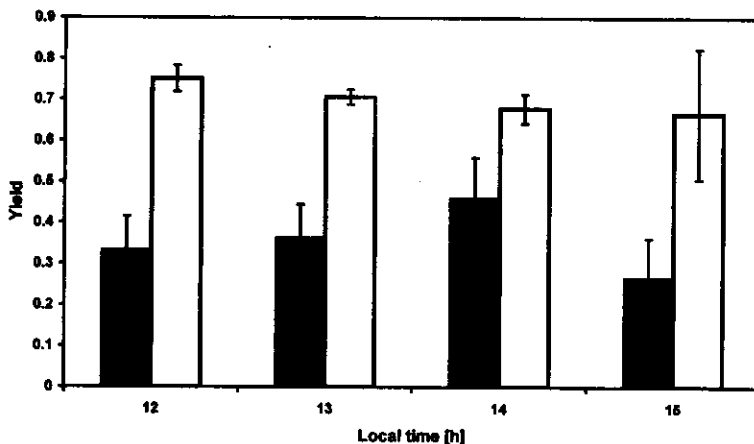


Figure 6. Photosynthetic quantum yield of *D. dichotoma* in its natural environment in a rock pool in Bahía Bustamante measured at hourly intervals during low tide in the shade (open bars) and in the sun (dark bars).

involvement of the violaxanthin cycle [14]: at low and intermediate irradiances zeaxanthin is converted to antheraxanthin and finally to violaxanthin, while the reversed sequence is found during exposure to excessive irradiation. Zeaxanthin is located close to the excited chlorophyll to dissipate the excess excitation energy thermally before the chlorophyll can undergo intersystem crossing to the triplet state from which the energy may be transferred to oxygen to produce the highly photooxidative singlet oxygen [37].

The fluence rate-response curves indicate that the macroalgae possess effective adaptation mechanisms to adjust to the prevailing light conditions. Non-photochemical quenching commences and photochemical quenching declines even at low fluence rates indicating that the algae are typical shade plants adapted to effectively harvest and utilize light energy at low fluence rates. The induction and relaxation kinetics of qN demonstrate that the algae can adapt within minutes to the changing light conditions. During exposure to unfiltered solar radiation photoinhibition decreases the availability of radiation energy for photosynthesis and thus reduces the quantum yield as the energy relaxes by thermal dissipation. This observation is in line with results found in several eulittoral Mediterranean and Atlantic species [16]. It was found that surface-adapted algae recover much

faster from exposure to unfiltered solar radiation than algae adapted to deeper water [38,39]. Ecologically even more important is the observation that the algae suffer photoinhibition at their natural growth site during low tide, indicating that supralittoral and subtidal macroalgae follow a complicated pattern of the photosynthetic yield to adapt to the changing light conditions. A remarkable finding is the fact that solar UV has a significant share in photoinhibition under natural conditions even though the incident energy represents only a small fraction in solar radiation.

ACKNOWLEDGMENTS

This work was financially supported by the European Community (ENV4-CT97-0580 and EV5V-CT94-0425; DG XII, Environmental Programme) to D.-P. H. and the Fundación Antorchas, Argentina (A-13669/1-3) to E. W. H. This is contribution N° 30 of Estación de Fotobiología Playa Unión.

REFERENCES

- [1] Lüning, K. 1985, Meeresbotanik, Thieme, Stuttgart, New York.
- [2] Bischof, K., Hanelt, D., and Wiencke, C. 1998, Mar. Biol., 131, 597.
- [3] Dring, M. J., Makarov, V., Schoshina, E., Lorenz, M., and Lüning, K. 1996, Mar. Biol., 126, 183.

- [4] Larkum, A. W. D., Wood, W. F. 1993, *Photosynth. Res.*, 36, 17.
- [5] Hanelt, D., Melchersmann, B., Wiencke, C., and Nultsch, W. 1997, *Mar. Ecol. Prog. Ser.*, 149, 255.
- [6] Hanelt, D., Huppertz, K., and Nultsch, W. 1993, *Mar. Ecol. Prog. Ser.*, 97, 31.
- [7] Hanelt, D., Huppertz, K., and Nultsch, W. 1992, *Bot. Acta*, 105, 278.
- [8] Häder, D.-P., Worrest, R. C., Kumar, H. D., and Smith, R. C. 1994, *UNEP Environmental Effects Panel Report*, Nairobi, 65.
- [9] Krause, G. H., and Weis, E. 1991, *Annual Reviews Plant Physiology Plant Molecular Biology*, 313.
- [10] Andersson, B., Salter, A. H., Virgin, I., Vass, I., and Styring, S. 1992, *J. Photochem. Photobiol. B: Biol.*, 15, 15.
- [11] Öquist, G., Anderson, J. M., McCaffery, S., and Chow, W. S. 1992, *Planta*, 188, 422.
- [12] Renger, G., Völker, M., Eckert, H. J., Fromme, R., Hohm-Veit, S., and Gräber, P. 1989, *Photochem. Photobiol.*, 49, 97.
- [13] Trebst, A. 1991, *Zeitschr. Naturforsch.*, 46, 557.
- [14] Osmund, C. B./1994, in: N. R. B. Baker, J.R. (Eds.), *Photoinhibition of Photosynthesis, from the Molecular Mechanisms to the Field*, 1.
- [15] Demmig-Adams, B., and Adams III, W. 1992, *Ann Rev. Plant Physiol. Plant Mol. Bio.*, 43, 599.
- [16] Häder, D.-P., and Figueroa, F. L. 1997, *Photochem. Photobiol.*, 66, 1.
- [17] Franklin, L. A., and Forster, R. M. 1997, *Europ. J. Phycol.*, 32, 207.
- [18] Genty, B. E., Briantais, J. M., and Baker, N. R. 1989, *Plant Physiol. Biochem.*, 28, 1.
- [19] Weis, E., and Berry, J. 1987, *Biochim. Biophys. Acta*, 894, 198.
- [20] Schreiber, U., and Bilger, W. 1993, in: U. Lüttge, Ziegler, H. (Eds.), *Progr. Bot.*, 151.
- [21] Schreiber, U., Endo, T., Mi, H., and Asada, K. 1995, *Plant Cell Physiol.*, 36, 873.
- [22] Hanelt, D., Jaramillo, J. M., Nultsch, W., Senger, H., and S. Westermeier, R. 1994, *Ser. Cient. Inst. Antarct. Chil.*, 44, 67.
- [23] Hanelt, D. 1998, *Mar. Biol.*, 131, 361.
- [24] Henley, W. J., Lindley, S. T., Levavasseur, G., and Osmond, C. R. 1992, *Oecologia*, 89, 516.
- [25] Häder, D.-P., Porst, M., and Lebert, M. 2000, *J. Photochem. Photobiol. B: Biol.*, 57, 159.
- [26] Franklin, L. A., Seaton, G. G. R., Lovelock, C. E., and Larkum, A. W. D. 1996, *Plant, Cell Environment*, 19, 825.
- [27] Wood, W. F. 1989, *Aquatic Botany*, 33, 41.
- [28] Hanelt, D., and Nultsch, W. 1995, *J. Plant Physiol.*, 145, 31.
- [29] Gomez, I., Wiencke, C., and Weykam, G. 1995, *Mar. Biol.*, 123, 167.
- [30] Gomez, I., Thomas, D. N., and Wiencke, C. 1995, *Bot. Mar.*, 38, 157.
- [31] Häder, D.-P., Porst, M., and Santas, R. 1998, *Plant Ecol.*, 139, 167.
- [32] Häder, D.-P., Lebert, M., Marangoni, R., and Colombetti, G. 1999, *J. Photochem. Photobiol. B: Biol.*, 52, 51.
- [33] Marangoni, R., Barsella, A., Gioffré, D., Colombetti, G., Lebert, M., and Häder, D.-P. 2001, *J. Photochem. Photobiol. B: Biol.*, 58, 178.
- [34] Häder, D.-P., Porst, M., Herrmann, H., Schäfer, J., and Santas, R. 1996, *Photochem. Photobiol.*, 64, 428.
- [35] Schreiber, U., Schliwa, U., and Bilger, W. 1986, *Photosynthesis Research*, 10, 51.
- [36] Figueroa, F. L., Jiménez, C., Lubián, L. M., Montero, O., Lebert, M., and Häder, D.-P. 1997, *J. Plant Phys.*, 151, 6.
- [37] Niyogi, K. K. 1999, *Ann. Rev. Plant Physiol.*, 50, 333.
- [38] Häder, D.-P., Porst, M., Herrmann, H., and Santas, R. 1996, *Photochem. Photobiol.*, 64, 428.
- [39] Herrmann, H., Ghatti, F., Scheuerlein, R., and Häder, D.-P. 1995, *J. Plant Physiol.*, 145, 221.