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Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field

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ABSTRACT: Photoinhibition of photosynthesis and its recovery was investigated in the laboratory and in the field with fluorescence and oxygen measuring devices. Photosynthetic efficiency measured at non-saturating fluence rates and the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) showed an approximately inverse course compared to the fluence rate of daylight measured continuously during the day. In the morning photosynthetic efficiency was high, but decreased with increasing fluence rate. Maximal photoinhibition of photosynthesis occurred around noon or in the early afternoon. During the afternoon photosynthetic efficiency increased again and full recovery was reached in the evening. These kinetics of recovery differ from those obtained in the laboratory under artificial conditions, where the red algae required up to 48 h to recover from a strong photoinhibition. Different species showed different sensitivity to photoinhibition and different capability for recovery. The red alga *Porphyra* spp., living in the upper eulittoral, was able to cope with the high fluence rates at the water surface. The red alga *Delesseria sanguinea*, living in the subtidal zone, shows the highest sensitivity to photoinhibition. Thus, a relation between photoinhibitory sensitivity and the zonation of the algae in the littoral exists.

INTRODUCTION

At high fluence rates, when photosynthesis is saturated, an excess of absorbed energy can damage the photosynthetic apparatus (Huppertz et al. 1990). Therefore, several photoprotective mechanisms have been developed in plants. One of them is photoinhibition, which protects the photosynthetic apparatus by impairing photosystem II (PS II) so that the absorbed energy is rendered harmless by thermal dissipation (cf. Krause & Weis 1991, Demmig-Adams & Adams 1992). Depending on weather conditions marine macrophytes can be exposed to considerable diurnal changes of the impinging solar fluence rates due to tidal changes in sea level. In particular, if low tide coincides with high fluence rates at noon, many algal species absorb many more photons than they require to drive photosynthesis. Thus, dissipation of excess absorbed energy is a prerequisite for their survival in the field.

Some marine brown algae are able to displace their chromatophores and, hence, to actively decrease their absorption cross section (Nultsch & Pfau 1979, Hanelt & Nultsch 1990). As shown in the brown alga *Dictyota dichotoma* the active displacement of the chromatophores protects the photosynthetic pigments against photodamage (Hanelt & Nultsch 1991). The extent of photoinhibition may be also diminished by decreasing the number of photons absorbed by the strong light arrangement of the chloroplasts. In red algae, however, chromatophore displacements were not observed (Nultsch & Pfau 1979).

Photoinhibition of photosynthesis in red algae and its recovery has been investigated recently by Hanelt et al. (1992). Photoinhibition caused a decrease of the photosynthetic efficiency at higher fluence rates, even before reaching the saturation level. A linear relation between the fluorescence ratio F_v/F_m and gross photosynthesis at different levels of photoinhibition was demonstrated. Thus, both fluorescence and oxygen measurements are suitable methods for studying photoinhibition in marine algae. Laboratory experiments have shown that in red algae about 48 h are

required for full recovery of photosynthesis. This is surprising, because a fast recovery during the day should be a prerequisite for a light-adaptation mechanism protecting the algae in their natural environment. However, in field experiments Huppertz et al. (1990), Henley et al. (1991) and Hanelt (1992) showed with brown algae and with the green alga *Ulva rotundata* that photoinhibition occurred at excessive fluence rates and recovery of photosynthesis commenced during the afternoon. Full recovery was mostly reached in the evening.

Thus, the question arose as to what extent photosynthesis of red algae is photoinhibited and how fast it recovers in the natural environment.

MATERIAL AND METHODS

The experiments were carried out with the red algae *Delesseria sanguinea* (Huds.) (growing in the middle sublittoral), *Chondrus crispus* (Stackh.) (from the lower eulittoral), *Porphyra* spp. (from the middle and upper eulittoral) and the brown alga *Petalonia fascia* (Kuntze) (from the lower eulittoral). All algae were collected in spring on the rocky shore of Helgoland, Germany, in the SE North Sea. According to the Jerlov system of optical water types (Jerlov 1951) the water at the site of experiments was classified as coastal water type no. 7 (maximal transmittance at 558 nm, Huppertz et al. 1990). The water temperature in the field during the time of our experiments was ca 8 to 9°C. The temperature of the water in the measuring cuvettes was adjusted to that measured in the field. Fluence rate-response curves were measured in the laboratory at constant temperatures of 7 or 17°C. To investigate diurnal changes in photosynthesis and photoinhibition the algae were collected on the shore and fastened with a thin thread or a special device (described by Hanelt & Nultsch 1990) to a pontoon near the harbour. Thus it was ensured that the algae floated at a constant water depth independent of the tide level. The algae were adapted to the new environment during the night and measurements were started in the early morning. For laboratory experiments the algae were adapted in a constant seawater flow at dim white light (10 µmol m^{-2} s^{-1}) for ca 24 h in the laboratory before experiments were started. Photosynthesis and the degree of photoinhibition were measured by means of an oxygen electrode and a chlorophyll fluorometer. To continuously measure the fluence rate during the course of the day, a calibrated photodetector with a cosine factor was mounted on the roof of the Biologische Anstalt, Helgoland (cf. Hanelt & Nultsch 1990).

Oxygen measurements. Relative oxygen production (i.e. O_2 production rate in an open system recorded as voltage-amplitude of dark and light signal) was measured with a Clark electrode (Hydro Bios, Kiel, Germany) connected to a flow-through system at constant temperature (Nultsch et al. 1990). Discs of 7 mm in diameter were cut out of the thalli, transported in a black vessel to the laboratory nearby and the photosynthetic efficiency of the algae was determined at the constant fluence rate of 11 µmol m^{-2} s^{-1} red light (672 nm) as long as the O_2 production rate was maximal (ca 5 to 10 min). Red light was used to suppress photorespiration. A projector (Prado, Leitz, Germany) equipped with a halogen lamp (Xenophot, Osram, Germany) was used as the light source for the photosynthetic measurements and for photoinhibitory treatment. Different fluence rates were set by inserting neutral density filters and monochromatic light by inserting an SFK-Filter (Schott, Germany) into the light beam. Photoinhibition is expressed as the oxygen production of the control (100 %) minus the percentage of the oxygen production after photoinhibitory treatment. In measurements of the daily course of photosynthesis the maximal O_2 production of a non-photoinhibited piece of the thallus was defined as the 100 % standard.

Fluorescence measurements. *In vivo* chlorophyll fluorescence was measured with a pulse amplitude modulation fluorometer (PAM, Walz, Germany), as devised by Schreiber et al. (1986). Discs of algae (11 mm diameter) were cut out of the thalli and fastened at the end of the fiber optics of the fluorometer. The samples were darkened for 15 min in a special seawater cuvette equipped with a cooling jacket. First F_0 (initial fluorescence) was measured at a very low fluence rate of the red measuring light (≈ 0.244 µmol m^{-2} s^{-1}, 650 nm). F_m (maximal fluorescence) was achieved by application of a saturating white light pulse (≈ 3700 µmol m^{-2} s^{-1}) of 700 ms. The ratio F_v/F_m (F_v = variable fluorescence = $F_m - F_0$) was calculated to determine the degree of photoinhibition of photosynthesis (cf. Krause & Weis 1991). The transportation of the algal disc to the measuring device required several minutes. Therefore, the algal samples were transported in a black bottle, because in darkness the degree of photoinhibition does not significantly change (Hanelt et al. 1992). As mentioned above, oxygen and fluorescence measurements give comparable results. Thus both methods were used for the investigations of photoinhibition presented in this paper.

RESULTS

Red algae irradiated with increasing fluence rates of white light showed an increasing degree of photoinhibition of photosynthesis. However, in different species the same fluence rate caused photoinhibition to different extents. As shown in Fig. 1, *Delesseria*

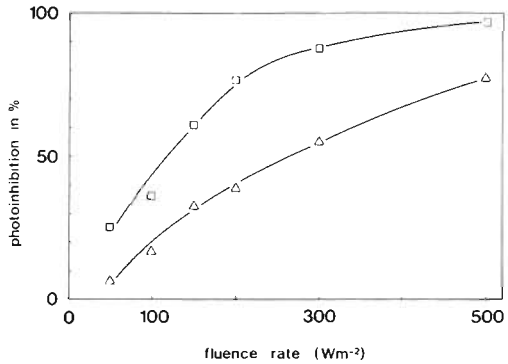

Fig. 1. *Delesseria sanguinea, Porphyra* spp. Fluence rate response curves of photoinhibition in *D. sanguinea* (□) and *Porphyra* spp. (△) in white light at 7 °C. Time of pre-irradiation was 1 h. Following the photoinhibitory pretreatment O_2 production was measured at 11 µmol m^{-2} s^{-1} red light

sanguinea growing in the sublittoral is more sensitive to strong white light than *Porphyra* spp. After a 1 h irradiation at 500 W m^{-2} (≈ 2500 µmol m^{-2} s^{-1}) white light in the laboratory, no significant O_2 production was detectable in *D. sanguinea*, i.e. photoinhibition was 100 %, whereas photosynthesis of *Porphyra* sp. growing in the upper eulittoral was photoinhibited by only 75 %. The samples of both algal species were cut out of the phylloid which consists of only 1 cell layer in both species. In multilayered thalli the photoinhibitory effect is decreased because of the self-screening effect (Table 1). In the monolayered thallus of *P. purpurea* the fluence of 1 h at 1500 µmol m^{-2} s^{-1} white light caused ca 51 % photoinhibition. When this sample was covered with 1 or 2 additional monolayered thalli, photoinhibition was reduced to 20 and 8 %, respectively. Thus, in emerged overlapping *P. purpurea* thalli or also multilayered thalli self-screening attenuates the radiation which impinges on cells covered by one or more cell layers. In this way it reduces the overall extent of photoinhibition of the thallus.

In laboratory experiments the full recovery of photosynthesis requires up to 48 h in several red algal species growing on the shore of Helgoland (Hanelt et al. 1992). For example, the intertidal red alga *Chondrus crispus* requires about 25 h for full recovery (Fig. 2). This is surprising, as this species is for some time uncovered during low tide and, hence, shows a strong photoinhibition on sunny days. Moreover, for recovery dim light is necessary, while in darkness photosynthesis recovers only to a small extent (Fig. 2). In comparison to *C. crispus* the sublittoral alga *Delesseria sanguinea* shows a higher degree of photoinhibition after irradiation with the same fluence rate, and the kinetics of recovery are much slower (Fig. 3). Moreover, *D. sanguinea* does not recover in darkness.

In the natural environment the benthic algae are often exposed to high fluence rates during the day. To investigate how the algae can cope with the natural light conditions, field experiments are indispensable.

Fig. 4A shows the relative oxygen production of *Chondrus crispus* floating near the water surface in the course of a day. At sunrise the efficiency was high. As the sky was clear, the bright sun caused a 77 % decrease in the O_2 production by 12:00 h. In the early afternoon the efficiency increased slightly, and after

Table 1. *Porphyra purpurea.* Reduction of photosynthetic O_2 evolution after 1 h irradiation with 1500 µmol m^{-2} s^{-1} white light at 17 °C. Samples were measured with and without covering thalli. Mean number = 5; SD: standard deviation

Layers	Photoinhibition (%)	SD (%)
1 monolayered thallus	50.7	8.9
Thallus covered with 1 monolayered thallus	19.5	8.7
Thallus covered with 2 monolayered thalli	8.0	4.4

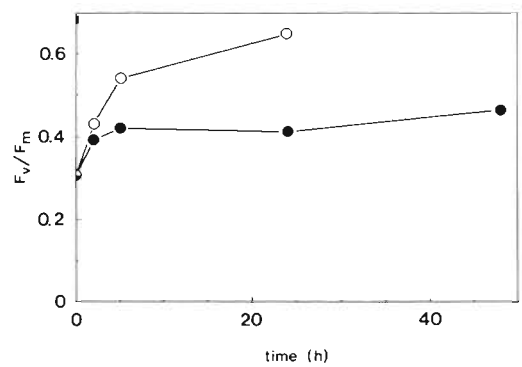

Fig. 2. *Chondrus crispus.* Recovery of photosynthesis in dim white light (○, 10 µmol m^{-2} s^{-1}) or in darkness (●) at 7 °C. Photosynthesis was photoinhibited for 1 h by 1000 µmol m^{-2} s^{-1} white light. (▮) Control measured before photoinhibitory treatment

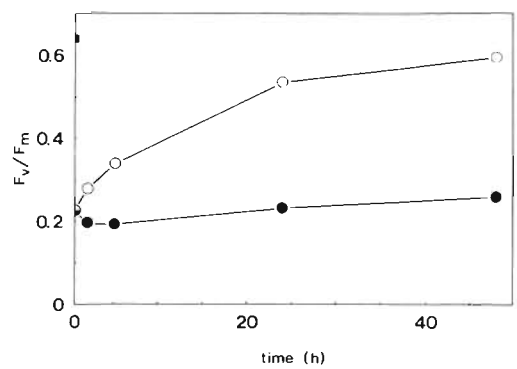

Fig. 3. As in Fig. 2 but for *Delesseria sanguinea*

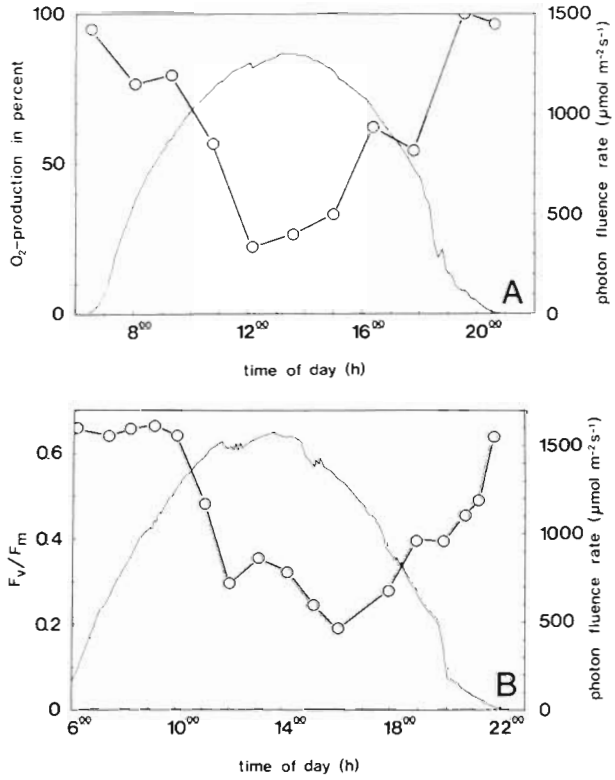

Fig. 4. *Chondrus crispus*. (A) Photosynthetic O_2 evolution at 11 µmol m^{-2} s^{-1} of *C. crispus* floating near the water surface in the course of a day. (B) Photosynthetic efficiency measured by fluorescence at 1 m water depth during the course of a day. Thin lines show the photon fluence rate of daylight measured in air

15:00 h very rapidly. At sunset (19:00 h) the photosynthetic O_2 production had fully recovered. Fig. 4B shows the same experiment carried out 1 yr later. In this case however, the F_v/F_m ratio, measured with the PAM-fluorometer, was used as a parameter, and the algae floated at a water depth of ca 1 m. Due to the turbidity of the water body the light transmittance at 1 m was only ca 60 %. At the beginning of the experiment the algae were shaded by the pier. Consequently, the F_v/F_m ratio was high from sunrise to 10:00 h, indicating that photoinhibition occurs later than in the experiment carried out the year before. Then the algae were exposed to the full sunlight. The F_v/F_m ratio decreased and reached the lowest value at 16:00 h, indicating that photoinhibition of photosynthesis was maximal. During the late afternoon and the early evening the ratio increased as a result of recovery of photosynthesis. At 22:00 h photosynthesis had fully recovered. Even with turbid water, midday sunlight at 1 m depth was still sufficient to cause substantial photoinhibition. Recovery commenced later in the afternoon and, hence, full recovery was reached also later in the evening.

Two daily courses of oxygen production of *Delesseria sanguinea* floating at a depth of 1.5 m measured on different days are shown in Fig. 5A, B. In Fig. 5A the irregular course of irradiance shows that it was a sunny day with intermittent clouds. Although only about 20 % of the light measured on the surface was transmitted into a depth of 1.5 m, *D. sanguinea* was photoinhibited by 35 to 50 % from 11:00 to 19:00 h. Full recovery occurred very late in the evening at 20:30 h. Thus, this sublittoral alga is apparently very sensitive to light. On another day (Fig. 5B) the sky was cloudy, but cleared up temporarily in the afternoon. The course of photoinhibition corresponds very well with this peculiar course of the fluence rate of daylight, but oxygen production decreased maximally by 30 % in the afternoon. Recovery commenced in the early evening and advanced very rapidly. Less than 10 % of the surface irradiance had reached a depth of 3 m. Therefore, at 3 m the photosynthetic efficiency decreased by only 20 %, although the day was sunny and clouds appeared only temporarily (data not shown).

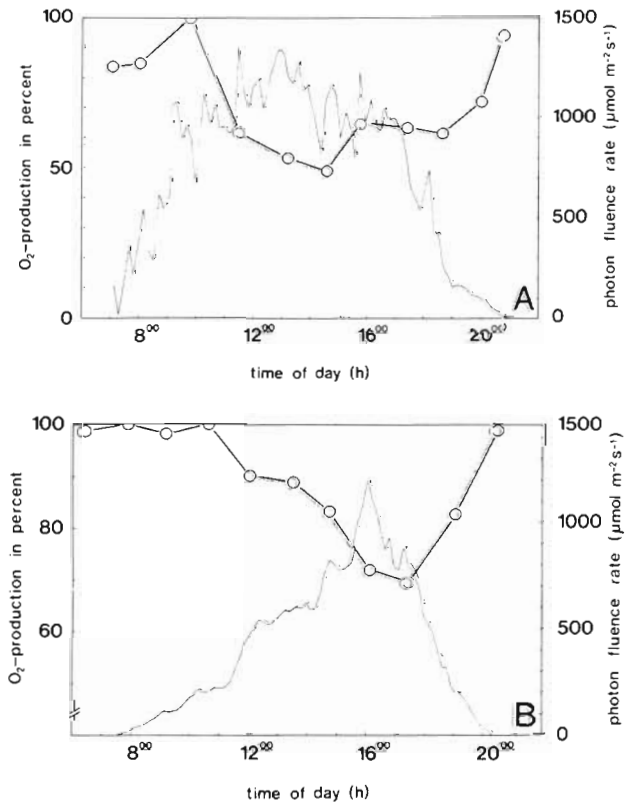

Fig. 5. *Delesseria sanguinea*. Photosynthetic O_2 evolution at 11 µmol m^{-2} s^{-1} of thalli floating at a water depth of 1.5 m during the course of 2 d: (A) a sunny day with some clouds; (B) a cloudy day with brief sunshine beginning at 16:30 h (note difference in O_2 production scales). Thin lines show the photon fluence rate of daylight measured in air

Porphyra sp. grows in the upper eulittoral and, hence, is often irradiated by direct sunlight for a long period of time, especially during low tide. In the experiment shown in Fig. 6 the alga floated on the water surface and was exposed to full sunlight. Morning cloudiness decreased the fluence rate so strongly that photosynthetic efficiency remained at a high level until 10:00 h. After 10:30 h the cloudiness decreased and the fluence rate correspondingly increased, diminishing the photosynthetic efficiency to a minimum of about 55% at 14:00 h. Recovery commenced in the afternoon and was completed at 18:00 h.

The brown alga *Petalonia fascia*, growing in the lower eulittoral, was investigated on a clear sunny day at 2 different depths (Fig. 7). About 60% of surface light penetrated to 0.5 m depth. In the morning photosynthetic efficiency remained at high levels, but at 13:00 h it decreased so rapidly that almost no oxygen production could be measured. At 16:00 h the alga became shaded by the pontoon and, later, by the pier. In the shadow the irradiation was diminished by about 75%. This cannot be recognized in the irradiance curve, because the photosensor was mounted on the roof of the institute. The sudden decrease in the irradiance was accompanied by a very fast recovery of photosynthesis so that the efficiency had already reached the maximum values in the early afternoon. Only about 20% of surface irradiance penetrated 1.5 m water depth. The photosynthetic efficiency decreased only to 30% of the maximal O_2 production. Recovery also commenced in the early afternoon (15:00 h) as at 0.5 m. It progressed during the late afternoon and was complete by the evening. Thus, the brown alga *P. fascia* showed a strong photoinhibition and the kinetics of photoinhibition as well as of recovery were very fast.

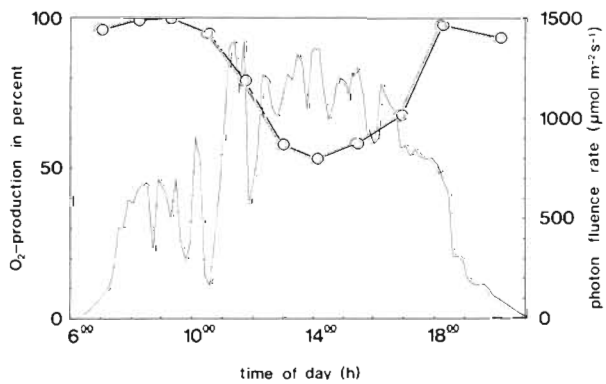

Fig. 6. *Porphyra* spp. Photosynthetic O_2 evolution at 11 µmol m^{-2} s^{-1} of a thallus floating at the water surface during the course of a day. Thin line shows the photon fluence rate of daylight measured in air

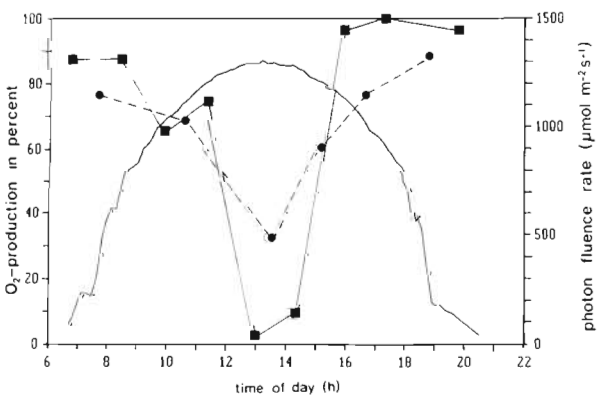

Fig. 7. *Petalonia fascia*. Photosynthetic O_2 evolution at 11 µmol m^{-2} s^{-1} of thalli floating at a water depth of (■) 0.5 m and (●) 1.5 m during the course of a day. Thin line shows the photon fluence rate of daylight measured in air

DISCUSSION

Photoinhibition of photosynthesis in marine algae was investigated using 2 different methods, one measuring oxygen production and the other measuring changes in the variable fluorescence. As shown by Hanelt et al. (1992), both methods yield comparable results. Those authors found differences only in recovery experiments with *Polyneura hilliae* and *Phyllophora truncata*.

Whereas the irradiance above the water surface depends only on the position of the sun and the weather conditions (e.g. clouds and rain), the irradiance below the surface depends in addition on the transmittance of the water body and reflection at the water surface. If the transmittance is low, as is often the case in the coastal water around Helgoland, the depth of the water column covering the algae considerably influences the fluence rate impinging on the algae. This in turn depends on the tide level and, hence, affects the extent of photoinhibition (Hanelt 1992, Henley et al. 1992). In order to exclude tidal influence on these field experiments the algae were exposed to the sun at a constant water depth by tethering to a floating device.

The results indicate that in the natural environment photoinhibition in *Delesseria sanguinea* may also occur at low tide, i.e. in the sublittoral, where it grows between 1.5 and 10 m (Lüning 1970). In uncovered *Chondrus crispus* and *Porphyra* sp. photoinhibition and recovery of photosynthesis appear as one should expect. Thus, the opinon of Kirk (1983) that photoinhibition in marine macrophytes is of less ecological significance is wrong. At low tide many macrophytes of the eulittoral and upper sublittoral showed photoinhibition in the field (Huppertz et al. 1990, Hanelt 1992, Henley et al. 1992).

In laboratory experiments with marine red algae, full recovery of photosynthesis after a strong photoinhibition required about 2 d (Hanelt et al. 1992). Thus, if the results obtained in the laboratory are transferred to the

field uncritically, the red algae should remain photoinhibited during the night. If so, photoinhibition would not be a proper photoprotective mechanism, as the red algae would still be photoinhibited in the morning. Though in the field high fluence rates caused a strong photoinhibition around noon, a full recovery of photosynthesis was, however, already observed in the evening. The reason for these contradictory results is not yet clear. As dim light conditions were found to be necessary for the recovery of photosynthesis and different spectral ranges showed different effects (Hanelt et al. 1992), the difference in the spectral composition of the daylight and the light of the lamps used in the laboratory could be responsible at least in part. Moreover, the different nutrient supply of the seawater in the field and in the laboratory cultures may also be of importance. In any case, the different responses of the algae in the field and in the laboratory demonstrate the importance of field experiments to study the ecophysiology of photoinhibition.

The investigations show clearly that different species differ in their sensitivity to strong light. Moreover, a correlation between photoinhibitory effect and algal zonation was found. The subtidal *Delesseria sanguinea* showed the highest sensitivity to photoinhibition and the slowest recovery of photosynthesis. It was followed by *Chondrus crispus* growing in the lower eulittoral and upper sublittoral which reacted much faster and withstood the high fluence rates at the water surface better. *Porphyra* sp., however, which grows in the upper eulittoral has the best capability to cope with strong light. It shows the least degree of photoinhibition and the fastest recovery which is an adaptation to its natural environment. This is consistent with the finding that the subtidal *P. nereocystis* is faster and more strongly photoinhibited than *P. perforata* growing in the eulittoral (Herbert & Waaland 1988). Henley et al. (1991) even observed different responses of individuals of the same species, *Ulva rotundata*, if they were adapted to different light conditions. Sun-adapted thalli exhibited a greater capability for protection than shade-adapted ones.

In *Ulva rotundata* high temperature and fluence rates act together in decreasing photosynthetic efficiency and capacity (Henley et al. 1992). In our experiments temperature effects can be practically excluded. The algae were submersed in an open water body whose temperature did not change significantly during the day. Moreover, the temperature of the water supplied to the measuring devices were adjusted to the temperature of the water outside.

The brown alga *Petalonia fascia* shows strong and fast reactions. This is in accordance with the results of field experiments with other brown algae (Huppertz et al. 1990, Hanelt 1992 and unpubl. data). However, in contrast to red algae many brown algae, including *P. fascia*, are able to displace their chromatophores in response to changing fluence rates in order to reduce (or increase) the light absorption by the photosynthetic apparatus (Nultsch & Pfau 1979, Nultsch et al. 1981). The occurrence of chromatophore displacement in the field (Hanelt & Nultsch 1990) may also have an effect on the extent of photoinhibition and, hence, modify the photoinhibitory reaction during the course of the day.

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