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In situ photosynthetic performance of *Laminaria digitata* (Phaeophyceae) during spring tides in Northern Brittany

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Abstract: The ability of *Laminaria digitata* (Hudson) J.V. Lamouroux to cope with rapid and drastic changes in light was studied in the field by measuring photosystem II fluorescence, net oxygen production and pigment content. Experiments were conducted during two spring tides of late spring in Northern Brittany where low spring tides occur around noon. Daily patterns of the photosynthetic performance of *Laminaria digitata* were observed in relation to changes in incident underwater light. Photoinhibitory light exposure induced a sharp decrease of the optimal quantum yield (F_v/F_m), a decline of the net oxygen production in the first of the two spring tides investigated and a concomitant increase in the de-epoxidation ratio of the Violaxanthin pool. Photoinhibition persisted at a lower extent at the end of the day and complete recovery was achieved during the night. The implications of the photoinhibition of photosynthesis of *Laminaria digitata* are discussed in relation to the natural ambient conditions experienced in the field.

Résumé : *Performances photosynthétiques* in situ *de* Laminaria digitata *(Phaeophyceae) au cours des marées de vives-eaux en Bretagne Nord*. La capacité de *Laminaria digitata* (Hudson) J.V. Lamouroux à faire face aux variations rapides et prononcées de lumière a été étudiée *in situ* en mesurant la fluorescence du photosystème II, la production nette d'oxygène et les contenus pigmentaires. Les expériences ont été réalisées au cours de deux marées de vives-eaux en fin de printemps en Bretagne Nord, où les basses mers ont lieu au zénith. Des changements journaliers des performances photosynthétiques ont été observés en relation avec les éclairements perçus au cours de la journée. L'exposition à des éclairements photoinhibants a entraîné une forte diminution du rendement quantique optimal (F_v/F_m), une diminution de la production nette d'oxygène au cours du premier des deux cycles de marées étudiés, en lien avec une augmentation du taux de déépoxydation de la Violaxanthine. La photoinhibition a persisté en fin de journée et la restauration complète a été atteinte au cours de la nuit. Les implications du phénomène de photoinhibition de la photosynthèse de *Laminaria digitata* sont discutées en lien avec les conditions du milieu.

Keywords: Kelp ● Photoinhibition ● Net oxygen production ● Chlorophyll fluorescence ● Xanthophyll cycle

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Introduction

Kelp beds are a major feature of temperate coastal ecosystems. They constitute dense populations inhabiting rocky shores from the low intertidal to the uppermost part of subtidal areas. By their productivity (Mann, 1973) and structure, they largely contribute to the functioning of the ecosystem. They bring basal source of marine food web through bacterial-mediated degradation and provide shelter for a large diversity of organisms such as macroalgae and macrofauna. Moreover, some kelp species, like *Laminaria digitata* (Hudson) J.V. Lamouroux, are of particular interest in the view of the alginate industry. Due to the recent reduction in abundances of *Laminaria digitata* along the French coast of the English Channel (Gevaert et al., 2008) many questions are arising on the consequences of a rapid environmental change on *Laminaria digitata*. As a photo-autotroph, interactions of *Laminaria digitata* with light are crucial in all aspects of the life cycle. Light will drive species composition, abundance and productivity (Gerard, 1984) and thereby the vertical distribution of macroalgae (Markager & Sand-Jensen, 1992; Häder & Figueroa, 1997; Roleda et al., 2006). Incident underwater light arriving on macroalgae is highly variable on many different time scales as it depends on the combination of the solar elevation, the weather, the optical properties of seawater (scattering and absorption processes) and the tidal elevation which plays a major role in coastal areas. Semi-diurnal tides drive complex schemes of tidal elevation throughout the year with the alternation of spring/neap tide cycles. In Northern Brittany, where low spring tides occur around noon, *Laminaria digitata* experiences drastic and rapid changes of light exposure. The questions that are arising are: how does *Laminaria digitata* cope with these rapid changes in light and what are the incidences of those exposures on its photosynthetic performance?

The ability to withstand high light exposure and to recover from it is crucial for preventing the formation of oxygen radicals and maintaining a sufficient photosynthetic performance. This ability could be one of the major factors regulating the upper depth limit of a species (Hanelt, 1998; Harker et al., 1999). Exposure to high light at noon can lead to a reduction in photosynthesis efficiency of macroalgae, a process called photoinhibition (Long et al., 1994) and commonly observed in the field (Henley et al., 1991; Hanelt et al., 1993; Hanelt, 1998; Gevaert et al., 2003, Hanelt & Roleda, 2009). These authors pointed out the occurrence of a diurnal pattern of photoinhibition and photosynthetic performances in macroalgae in relation to the diurnal changes of incident irradiances. Photoinihibition mainly corresponds to an active decrease in photosystem II (PSII) efficiency (Huppertz et al., 1990) which protects the photosynthetic apparatus from excess of light. This down-regulation of PSII efficiency is associated with the setting up of photoprotective mechanisms and is generally separated in two processes. The first part which is called "dynamic photoinhibition" corresponds to the dissipation of excess energy from the PSII reaction centres as heat *via* the completely reversible xanthophyll cycle (XC) in the Light Harvesting Complexes (LHC) of the PSII (Harker et al., 1999). The second part is called "chronic photoinhibition" and is associated with the turn-over of the D1 protein, a subunit of the PSII core which is essential for the electron transfer. The degradation of the protein D1 subunit inactivates the PSII allowing the wastage of the excess photons. The reversibility of the chronic part of photoinhibition depends on the balance between the rate of degradation and repair of the D1 subunit (Critchley & Russell, 1994; Carr & Björk, 2007) and hence is slower than the dynamic phase.

Photoinhibition was studied in large kelp species (Hanelt et al., 1997; Gevaert et al., 2002; Altamirano et al., 2004) mostly with Pulse-Amplitude-Modulated (PAM) fluorescence, which is recognized as a reliable and powerful tool. *In situ* measurements of oxygen production are scarcer due to the technical issues associated with the deployment of large chamber with enough power supply for mixing and renewal of the media for a complete tidal cycle. Very few studies report accurate rates of primary productivity of macroalgae in the field during a complete tidal cycle or a complete day (see Fairhead & Cheshire, 2004 and references in). PAM fluorescence and oxygen production measurements provide different but highly complementary results. When PAM fluorescence provides insight on the PSII efficiency and the rate of electron transfer through PSII on a small part of the thallus, oxygen production provides insight into the whole photosynthetic processes including the activity of the Calvin cycle as well as the total respiration of the whole entire sporophyte.

In our study, we propose to combine these two complementary techniques in order to bring a complete and accurate description of the photosynthetic activity of *Laminaria digitata* throughout a whole tidal cycle. We studied the daily photosynthetic activity of *Laminaria digitata* under the light conditions of two spring tides of late spring using PAM fluorescence, oxygen production measurements and pigment analyses. The aim of the study was to elucidate (1) if photoinhibition of photosynthesis occurs in the field and (2) how *Laminaria digitata* copes with it. This study was a part of the French program ANR ECOKELP and was used to determine if *Laminaria digitata* present sufficient mechanisms to face the rapid changing light conditions in the field in order to further predict its response to rapid environmental changes in coastal areas.

Materials and Methods

Study site

The photosynthetic performance of *Laminaria digitata* was investigated *in situ* on the kelp bed of Roscoff (France, 48°5'N-3°6'W) the 6th of May and 6th of June 2008. This period was chosen as maximal values of incident light in Roscoff typically occurred in June and because days are the longest of the year. All the experiments were investigated at a depth between 0.2 to 0.5 m below chart datum in order to assess the photosynthetic activity only under immersed conditions. Tidal elevation varied from 0.9 m to 9.1 m during the experiments. The lowest tidal elevation occurred around noon giving maximum exposure to light under clear sunny days.

Light and temperature measurements

Underwater Photosynthetic Photon Flux Fluence Rate (PPFFR, µmolphotons.$m^{-2}.s^{-1}$) or quantum scalar irradiance was measured at 1-min intervals throughout the experiment with an underwater spherical quantum sensor (MKV/L, MDS, Alec electronics Co Japan). Measurements integrated the underwater light incoming from all directions and thereby values are larger than what is expected with more conventionally used cosine corrected quantum sensor. PPFFR was averaged for incubations with the benthic chamber while instantaneous measurements were preferred for the relative electron transport rate (rETR) measurements as they refer to instantaneous photosynthetic activity. Water temperature was measured inside the chamber (Multi-parameter TROLL 9000, In-Situ Inc.).

Fluorescence measurements

In vivo chlorophyll *a* (chl *a*) fluorescence of *Laminaria digitata* was measured underwater using a Diving PAM (Heinz Walz, Effeltrich, Germany). Three large adult sporophytes of an approximate size of 150 to 180 cm were randomly chosen and were tagged in the field. Fluorescence parameters were measured (on the mid part of the frond) three times a day on the same tagged sporophytes by scuba divers; one measurement in the morning (around 11:00, local time), one in the afternoon (around 15:00) and one in the early evening (around 19:00). The effective quantum yield (ϕ_{PSII}) (efficiency of the photosystem II photochemistry) was measured under natural ambient light. A home-made designed clip was used to ensure a constant distance and a constant angle of 60° between the end of the optic fibre and the sample. The effective quantum yield was calculated according to Genty et al. (1989):

$$\phi_{PSII} = (F_m' - F_t) / F_m' \quad (1)$$

where F_t is the steady-state level of the fluorescence under ambient light and F_m' the maximal level of fluorescence obtained using a 0.8 s saturating pulse (2500 µmol.$m^{-2}.s^{-1}$). ϕ_{PSII} is used to calculate the rETR which is an indication of overall photosynthesis (Maxwell & Johnson, 2000). The rETR was preferred to the real ETR as it was not possible to measure the chl *a* absorption coefficient in the field. rETR was calculated as follows:

$$rETR\ (\mu mol\ e^-.m^{-2}.s^{-1}) = \phi_{PSII} \times PPFFR \times 0.5 \quad (2)$$

where 0.5 is the assumption that incident energy is equally partitioned between photosystem I and the PSII as the transport of one electron required at least two photons (Maxwell & Johnson, 2000; Longstaff et al., 2002). At the same time, the optimal quantum yield (F_v/F_m) of PSII (Genty et al., 1989) was calculated to assess the extent of photoinhibition (Krause & Weis, 1991):

$$F_v/F_m = (F_m - F_0) / F_m \quad (3)$$

where F_v is the variable fluorescence, F_0 is the basic fluorescence signal measured under non actinic red light of samples darkened for 10 min using a leaf clip, and F_m is the maximal fluorescence during the application of a saturating pulse of white light on these samples. Percentage of decrease and recovery of F_v/F_m were calculated:

$$\%\ decrease = (F_v/F_{m(morning)} - F_V/F_{m(afternoon)}) / (F_v/F_{m(morning)}) \times 100 \quad (4)$$

$$\%\ recovery = (F_v/F_{m(early\ evening)} / (F_v/F_{m(morning)}) \times 100 \quad (5)$$

Oxygen production measurements

Net oxygen production of a single entire sporophyte (of the same approximate size than for fluorescence measurement, see above) cleared out of its epiphytes was measured using an automated benthic chamber placed at the collection site and thereby at the same depth. The system is made of a closed Perspex dome transparent to visible light radiations but not to ultraviolet radiations (UVR), tightly sealed on the polyvinylchloride (PVC) base of the chamber and encloses a volume of 35.3 L. An electronic management system controlled three external pumps; two pumps ensured the rapid and constant homogenization of the media while the third one ensures the renewal of the media by flushing ambient seawater between two consecutive incubations. Oxygen concentration (µmol.L^{-1}) was measured with an oxygen sensor based on life-time optical fluorescence measurements (Multi-parameter TROLL 9000, In-Situ Inc.) and monitored every 10 s. All incubations were performed in ambient light conditions except from the last incubation of the day performed after the chamber has been covered with a dark and opaque tarp in order to assess the dark respiration rate (R_d). Net oxygen production rates (µmol.$g_{FW}^{-1}.h^{-1}$) were calculated using the slope of the

linear regression of oxygen concentration against time, the volume of the enclosure and the fresh weight (FW) of the sporophyte. Photosynthesis versus PPFFR (P-E) curves were constructed by plotting calculated oxygen fluxes against the corresponding average PPFFRs and fitting the model of Eilers & Peeters (1998) using the simplex technique.

Pigment analysis

Two discs (8 mm diameter) were taken from each thallus (on the mid part of the frond) with a cork borer and were immediately darkened and frozen in liquid nitrogen. Thallus discs were then first gently wiped in order to remove epiphytes, and pigments were extracted by grinding them in a cold mortar with methanol and small drops of methylene chloride under dim light. Extracts were centrifuged (5 min, 13 000 rpm) and supernatants were collected and filtered on polytetrafluoroethylene membranes (0.45 µm) and dry-evaporated under nitrogen. Salt contents of the extract were removed from the pigment solution in a methylene chloride:distilled water mixture (50:50, v/v) (salts stay in the aqueous phase while pigments are found in the organic phase). The organic phase was then evaporated with nitrogen and redissolved in 40 µL methanol for injection. Pigment analysis was performed by high performance liquid chromatography (HPLC) (Beckman, system Gold, 126) with a reverse-phase column (C 18 Allure, Restek). 20 µl were injected and separation was made with a solvent delivery profile adapted from Arsalane et al (1994). Pigment contents were normalized to the chl *a* content of the sample. The conversion of Violaxanthin (V), a pigment with no photoprotective properties into Antheraxanthin (A) and Zeaxanthin (Z) which are involved in the dissipation process of energy into heat (Bilger & Björkman, 1990), was estimated by calculating the de-epoxidation ratio (DR):

$$DR = (A + Z) / (V + A + Z) \quad (6)$$

Statistical analyses

Within a single day, the significant differences in fluorescence and DR measurements to varying sample time were tested using the non parametric one-tailed Wilcoxon signed ranks test for paired data (T). Paired data were tested between the groups of the morning and the afternoon, and between the groups of the morning and the early evening for F_v/F_m and DR. The probability value $p = 0.125$ for $T = 6$ indicated a complete separation of the groups of 3 replicates. Comparison of the afternoon values of DR and the molar content of xanthophylls between May and June were done using the non parametric Mann-Whitney test (U).

Results

Light and temperature measurements

Underwater PPFFR and depth are given in Fig. 1 for the spring tides of May (A) and June (E). Low tides occurred respectively at 13:46 (local time) with 1.4 m depth in May and 15:06 with 1.7 m depth in June. Instantaneous underwater PPFFR measurement reached 2538 $\mu mol.m^{-2}.s^{-1}$ in May and 2092 $\mu mol.m^{-2}.s^{-1}$ in June. Seawater temperature varied during tidal cycles from 12.3 to 13.9°C in May and from 14.3 to 15.6°C in June. Highest temperatures were recorded around 1 h after low tide.

Photosynthetic activity of Laminaria digitata

Changes in PPFFR throughout the day resulted in diurnal patterns of the optimal quantum yield (F_v/F_m) of *Laminaria digitata* in May and June (Fig. 1 B, F). Maximum values of F_v/F_m were measured in the morning. Values well above 0.7 indicated the good physiological status of the investigated algae at the start of the experiment. With the ebb tide and under increasing surface PAR, the F_v/F_m declined in May ($T = 6$, $p = 0.125$) indicating that photoinhibition of photosynthesis occurred in the afternoon. In June, this decline was less pronounced and there was no significant difference with the morning. Average reduction of the photosynthetic efficiency was of about 50% in May and of 28% in June. The decrease in incident light in the afternoon (rising tide and decrease in surface PAR), allowed an increase of F_v/F_m but not a complete recovery at the time of measurement. F_v/F_m at the end of the day was significantly lower than in the morning ($T = 6$, $p = 0.125$) and represented an average recovery of 91% in May and 94% in June.

The effective quantum yield of the photosystem II (ϕ_{PSII}) presented the same diurnal patterns as the F_v/F_m, with distinct trends during the ebb and the rising tide in relation to ambient PPFFR. The relative electron transport rate (rETR) maximal values were measured in the morning in May (50 ± 3 $\mu mol\ e^-.m^{-2}.s^{-1}$, Fig. 1C) and in the late afternoon in June (58 ± 9 $\mu mol\ e^-.m^{-2}.s^{-1}$, Fig. 1G).

The increase of net oxygen production with increasing PPFFR in the morning was obvious in May (Fig. 1D). Maximal net oxygen production value was 18.7 $\mu mol\ O_2.g_{FW}^{-1}.h^{-1}$ for an average PPFFR of 645 $\mu mol.m^{-2}.s^{-1}$. After the afternoon depression, net oxygen production seemed to recover values similar to the morning but unfortunately data for the rising tide are missing due to a sudden shutdown of the benthic chamber. In June, a maximal value of 11.8 $\mu mol\ O_2.g_{FW}^{-1}.h^{-1}$ was observed for an average underwater PPFFR of 1042 $\mu mol.m^{-2}.s^{-1}$ (Fig. 1F). Cloud passing in the early afternoon reduced the PPFFR (average

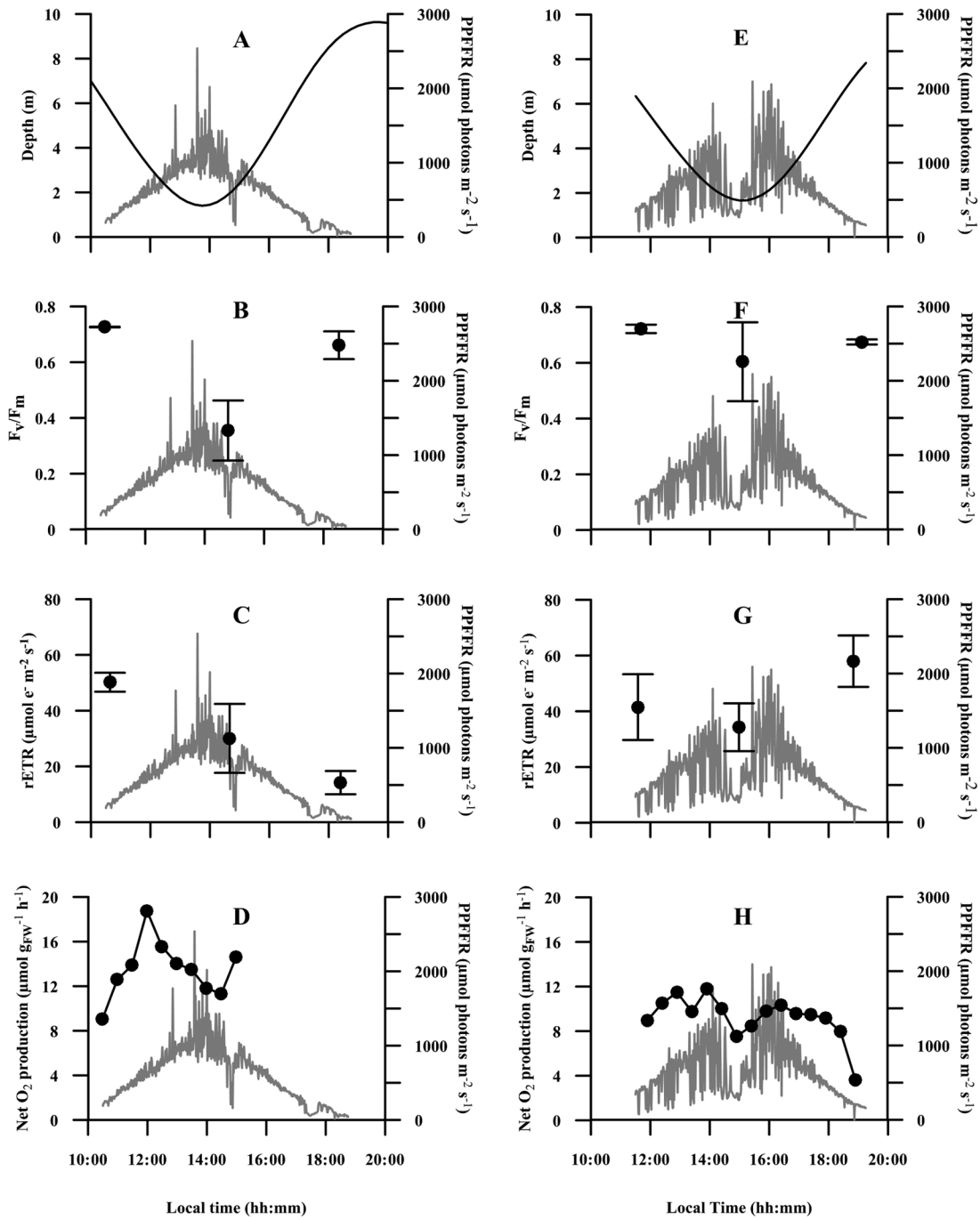

Figure 1. *Laminaria digitata.* Underwater light availability and diurnal photosynthetic performance measured during the two spring tides of the 6th May (from **A** to **D**) and the 6th June 2008 (from **E** to **H**) in Northern Brittany (Roscoff). (**A, E**) Quantum scalar irradiance (PPFFR, expressed in µmol photons.m^{-2}.s^{-1}) and depth (m). (**B**, **F**) Optimal quantum yield (F_v/F_m) of 3 sporophytes means ± standard deviation (SD). (**C**, **G**) Relative Electron Transport Rate of the PSII (rETR, µmol e$^-$.m^{-2}.s^{-1}) of 3 sporophytes means ± SD. (**D**, **H**) Net oxygen production (µmol O_2.g_{FW}^{-1}.h^{-1}) of one sporophyte.

Figure 1. *Laminaria digitata.* Lumière et performances photosynthétiques journalières mesurées au cours de deux marées de vives-eaux, les 6 mai (de **A** à **D**) et 6 juin 2008 de **E** à **H**) en Bretagne Nord (Roscoff). (**A, E**) Lumière (PPFFR, exprimé en µmolphotons.m^{-2}. s^{-1}) et profondeur (m). (**B**, **F**) Rendement quantique maximal (F_v/F_m) moyen de 3 sporophytes ± écart type. (**C**, **G**) Taux de transfert relatif des électrons au niveau du PSII (rETR, µmol e$^-$.m^{-2}.s^{-1}) moyen de 3 sporophytes ± écart type. (**D**, **H**) Production nette d'oxygène (en µmol O_2.g_{FW}^{-1}.h^{-1}) d'un sporophyte.

358 µmol.m^{-2}.s^{-1}) and led to a decline of the net oxygen production (Fig. 1H). Net oxygen production of *Laminaria digitata* as a function of light was higher in May than in June (Fig. 2). The saturation onset irradiance I_k was 306 µmol.m^{-2}.s^{-1} in May and 178 µmol.m^{-2}.s^{-1} in June. Photoinhibition was observed in May for irradiances above 971 µmol.m^{-2}.s^{-1}.

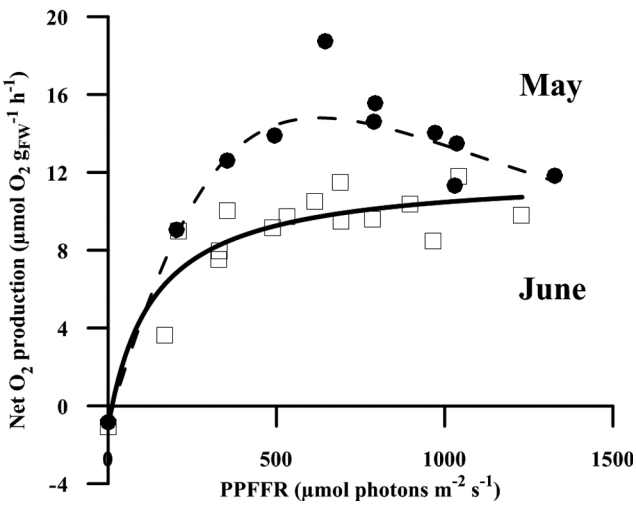

Figure 2. *Laminaria digitata.* Photosynthesis (expressed as µmol $O_2.g_{FW}^{-1}.h^{-1}$) versus quantum scalar irradiance (PPFFR) during the two spring tides of the 6th May (filled circles) and the 6th June (open squares) in Northern Brittany (Roscoff). Data were fitted according to the model of Eilers & Peeters (1988).

Figure 2. *Laminaria digitata.* Courbe reliant la photosynthèse (exprimée par la production nette d'oxygène en µmol $O_2.g_{FW}^{-1}.h^{-1}$) aux éclairements perçus (PPFFR) au cours des 2 marées de vives-eaux du 6 mai (cercles noirs) et du 6 juin (carrés blancs) en Bretagne Nord (Roscoff). Les données ont été ajustées à l'aide du modèle décrit par Eilers & Peters (1988).

Pigment analyses

No significant differences occurred in the total molar content of the xanthophylls pool size relative to chl *a* between May and June, with 12.4 ± 0.9 moles per 100 moles of chl *a* in May and 11.4 ± 1.5 moles per 100 moles of chl *a* in June (n = 3). When exposed to increasing light in May, V was progressively conversed into A and Z with the afternoon value of DR significantly higher than the morning value (T = 6, p = 0.125) (Fig. 3A). Re-conversion of Z and A to V was completely achieved in the early evening. Relatively high average DR was found in the morning in June (0.19 ± 0.02) and no significant difference were observed throughout the day (Fig. 3B). DR values in the afternoon in May were significantly higher than DR values in the afternoon in June (U = 0, p = 0.049).

Discussion

As low spring tides occur around noon in Northern Brittany, *Laminaria digitata* can be exposed to saturating and over-saturating irradiances. Optimizing the harvesting of light under low light conditions exposes *Laminaria digitata* to the potential adverse effects of excessive light exposure. When incident light exceeded its potential use in the photosynthetic electron transfer, photoinhibition is observed. It is measured as a decline of the optimal quantum yield, which is considered as an accurate indicator of PSII efficiency (Bruhn & Gerard, 1996; Maxwell & Johnson, 2000), indicating the development of non-photochemical quenching. The occurrence of this photoinhibition process was demonstrated *in situ* for many macroalgae (Huppertz et al., 1990; Hanelt et al., 1993; Longstaff et al., 2002; Gévaert et al., 2003; Hanelt & Roleda, 2009). The present study was performed during one of the most harmful periods for *Laminaria digitata* as it was performed under maximum tidal range and sunny weather. In May, the photosynthetic efficiency of *Laminaria digitata* was reduced by about 50% and this decline was related to an increase in DR. The xanthophyll cycle is known to be a major photoprotective mechanism among brown algae (Uhrmacher et al., 1995; Harker et al., 1999) as direct relation exists between DR and F_v/F_m as well as between DR and the non-photochemical quenching process. Photoprotective mechanisms efficiently dissipated the excess of light mainly by heat (dynamic photoinhibition) through the completely reversible enzymatic-mediated conversion of V into A and Z in the Light Harvesting Complexe of the PSII (Bruhn & Gerard, 1996; Gevaert et al., 2002). This photoinhibitory process affected the photosynthetic activity of *Laminaria digitata* in May resulting in a decline of both rETR and the net oxygen production in the afternoon in relation to the de-activation of the PSII (Harker et al., 1999). In June, *Laminaria digitata* displayed higher values of F_v/F_m and lower DR values than in May. Those differences could be mainly attributed to the cloud passing in the afternoon that considerably reduced the incoming light level, whereas in May the measurements were done just before the very brief cloud passing. The differences in photoinhibition levels are related to instantaneous incoming light levels but also to the previous light history. The light dose received by *Laminaria digitata* during the hour before the afternoon fluorescence measurement was of 4064 mmol.m^{-2} in May and only 2544 mmol.m^{-2} in June and the average irradiance just before the measurement was 1034 µmol.m^{-2}.s^{-1} in May and only 321 µmol.m^{-2}.s^{-1} in June. The re-conversion of Z and A into V is of the minute-time magnitude (Ralph et al., 2002), and hence *Laminaria digitata* may have recovered for the high irradiance exposure of the late morning.

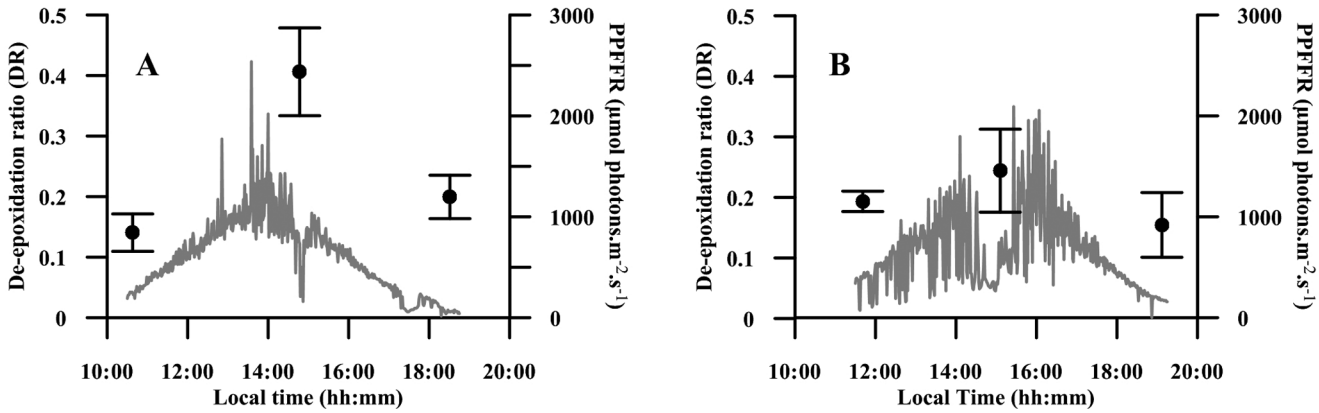

Figure 3. *Laminaria digitata.* Diurnal changes in De-epoxidation ratio (DR) of the Violaxanthin (V) determined on 3 sporophytes and underwater ambient quantum scalar irradiance (PPFFR) of the 6th May (**A**) and the 6th June (**B**) in Northern Brittany (Roscoff). Data are means ± SD.

Figure 3. *Laminaria digitata.* Changements journaliers du taux de dé-époxydation (DR) de la Violaxanthine (V) de 3 sporophytes et de la lumière (PPFFR) du 6 mai (**A**) et du 6 juin (**B**) en Bretagne Nord (Roscoff). Les données sont sous la forme moyenne ± écart type.

Despite this cloud passing, evidence of some differences in photosynthetic performance was found as shown in the oxygen photosynthetic parameters, with lower net oxygen production rates in June but no photoinhibition of the oxygen production at high irradiances. Photoprotective mechanisms as well as photosynthetic performances change through seasonal acclimation processes in relation to the ambient light conditions but also the other environmental conditions such as temperature and nutrient status encountered in the field by macroalgae (Long et al., 1994; Gevaert et al., 2002; Fairhead & Cheschire, 2004). The molar content of the xanthophyll pool relative to chl *a* was the same between the two months investigated. *Saccharina latissima* is able to accumulate more pigments involved in the xanthophyll cycle in relation to the seasonal increase in irradiance in order to reduce photoinhibition (Gevaert et al., 2002). The changes of incident irradiance during the month of May in this study were probably not sufficient to induce this pigment accumulation in *Laminaria digitata*. At the end of the day, measurements showed that both in May and in June, total recovery was not completely reached. Complete recovery is generally achieved before the sunset in macroalgae (Hanelt et al., 1993) but in the present study, the last measurements were made while significant amounts of light were still present. Recovery is mainly governed by the reconversion of Z and A to V, which is generally considered as the first component of the recovery process (Franklin et al., 1992) and by the de *novo*-synthesis of a major component of the PSII, the protein D1 (chronic photoinhibition), which is disrupted under high light in order to inactivate the PSII (Bruhn & Gerard, 1996). The following morning, values of F_v/F_m above 0.7 were measured, indicating that total recovery was achieved just before the sunset or during the night thus avoiding the cumulative effects of a new stress period coming from the following low tide at noon. This is crucial for maintaining an appropriate photosynthetic activity and for preventing irreversible photodamages of the photosynthetic apparatus that could further affect the seaweed growth.

Henley et al. (1991) demonstrated that differences occur between measurements of PAM fluorescence and oxygen production under high light, saturation of electron transfer through PSII being reached with higher irradiances than for net oxygen production. A direct comparison of rETR and net oxygen value was not possible as rETR values were measured on a small part of the thallus while oxygen measurements included the whole sporophyte. It would have been possible to compare the two measurements if actual light interception area as well as actual thallus absorbance of the sporophyte in the benthic chamber was known. Measurements did also not investigate light on the same scale as instantaneous measurements of PPFFR are used for fluorescence while oxygen measurements are correlated with average PPFFR for the time of the incubation. Saturation was reached for *Laminaria digitata* in May and June for the major part of the tidal cycle. Photoinhibition of oxygen production was measured in May resulting in a reduction of the net oxygen production of about 25% from the maximal oxygen production. Previous studies on *Dictyota dichotoma* (Uhrmacher et al., 1995) highlighted the reduction of oxygen production under high light with a concomitant increase in zeaxanthin content. The down-regulation of photosynthesis as well as a consecutive increase in respiration rate could be responsible for the decline of net oxygen production despite the increase of irradiances (Henley et al., 1991). In June,

oxygen production did not seem to be photoinhibited but a slight decrease was observed between oxygen production before and after maximal light was measured.

Study of photoinhibition in the field is of particular interest as many factors will synergistically affect the degree of photoinhibition of photosynthesis and recovery (Hanelt et al., 1993). In dense canopy-forming algal stand, light exhibits short-term variability due to the constantly wave-induced moving canopy (Gerard, 1984). This high temporal light variability influences the photosynthetic activity of tissues as reflected by the variability in ϕ_{PSII} measurements and thus the small temporal variations of oxygen production. Spectral properties of light reaching the benthic macroalgae also vary during the tidal cycle and can affect the extent of photoinhibition of photosynthesis (Nultsch et al., 1987). The photosynthetic measurements were performed under the full natural sunlight (PAR but also UVR) that they experienced *in situ*. *Laminaria digitata* thus encountered UVR exposure, especially around low tides due to the high water transparency. UVR are known to affect photosynthetic activity by reducing the efficiency of photoprotection and delaying the kinetic of recovery (Hanelt et al., 1997; Bischof et al., 2002; Hanelt & Roleda, 2009), but also to affect growth and morphology of young sporophytes (Roleda et al., 2004). Hence, UVR must have acted on the photoinhibition level and the speed of recovery measured with PAM fluorescence. On the contrary, oxygen production was not affected by UVR and the photoinhibition level in the benthic chamber was most probably underestimated since the dome was absorbing UVR.

The study was intentionally performed during immersion, working on the mid-belt of *Laminaria digitata* in order to get the most representative results of its *in situ* physiology. As previously studied, depth zonation is related to physiological performance of macroalgae (Lüning, 1981; Markager & Sand-Jensen, 1992). The uppermost part of the population is exposed to stronger and longer exposure to light as well as to a desiccation period. By the use of a simple calculation, it was possible to show that the uppermost submerged part of the belt received twice the total amount of light received in the lowest part during the experiment carried out in June. However, previous studies on photoprotection efficiency along the *Saccharina latissima* belt (Audresselles, France) showed that there were slight differences in photoprotective efficiency between the lowest and the uppermost part of the bed (Gevaert et al., 2002) reflecting the existence of inter-individual variability in photoprotection process. Moreover the uppermost part of the belt may have to cope with water stress, nutrient depletion and rapid changes in temperature when emerged. This desiccation stress is known to lower the photoprotection efficiency in Phaeophyta (Harker et al., 1999). Temperature may also have a harmful effect on the response of *Laminaria digitata* to light. As many photoprotective processes are driven in an enzymatical way, temperature will affect the response of *Laminaria digitata* to light by enhancing the photoprotective mechanisms but disrupting repair processes (Bruhn & Gerard, 1996). Seasonal acclimation processes optimize protective mechanisms as for the response to light throughout the year and protect the photosynthetic apparatus from small changes of temperature. The constant submersion of the thallus protects *Laminaria digitata* from wider changes of temperature and from the desiccation process and the changes could be more drastic when considering the emerged part of the belt, especially under clear sunny days. Synergistic effects of abiotic stress could further result in a partial greening of the frond as it was observed in the field for emerged sporophytes of *Laminaria digitata*. By affecting photosynthetic efficiency on a cellular and individual scale, high light exposure could affect the productivity of kelp stands and increase their overall sensitivity to other abiotic or biotic stress especially considering the uppermost part of the stand. This will be particularly true when the number of low spring tides is maximal during the year (in relation to the moon cycle) or during extreme heat wave as it was the case in 2003.

Photophysiology of macroalgae in the field are of particular interest in understanding the pattern of abundance and species distributions, as photosynthesis is the major metabolic process in benthic macroalgae. The complex interactions between the timing of extreme spring tides and the abiotic factors define the occurrence of the hardest abiotic conditions experienced by the macroalgae throughout the year. It has to be taken into account in the view of the impact of rapid changes of the environmental conditions in coastal areas, as the pattern of response of benthic macroalgae may be closely linked to the occurrence of these extreme events and their ability to cope with it.

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