Photo-inhibition and seasonal photosynthetic performance of the seaweed *Laminaria saccharina* during a simulated tidal cycle: chlorophyll fluorescence measurements and pigment analysis

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ABSTRACT

Laminaria saccharina **(Lamouroux) form the largest, most abundant and conspicuous seaweed populations along the French coast of the eastern English Channel. As they are located in the intertidal zone, they are exposed to considerable irradiance variations, mainly related to tidal cycles. The response of these macro-algae to light variations over a simulated daily tidal cycle was investigated in the laboratory during spring, autumn and winter using chlorophyll fluorescence and pigment analysis. The maximum quantum yield of photosystem II (PSII) photochemistry (F_v/F_m) and the operating PSII efficiency (Φ_{PSII}) showed clear daily cycles according to the irradiance variation throughout the 12 h simulated tidal cycle, whereas the pattern of the relative photosynthetic electron transport rate (rETR) was not so obvious. The algae reacted to the light increase by developing photoprotective mechanisms able to dissipate the excess energy reaching PSII by the de-epoxidation of violaxanthin into zeaxanthin. Because of their better acclimation to strong irradiance, spring populations were less affected by this light treatment than were winter populations. In particular,** *L. saccharina* **showed more pigments of the xanthophyll cycle in spring to cope with strong irradiance exposure. Alternatively, they developed their antenna complexes in winter to harvest a maximum of light.**

Key-words: *Laminaria saccharina*; daily and seasonal variations; light stress; pulse amplitude modulated (PAM); photoprotection; xanthophylls.

INTRODUCTION

Light is one of the main abiotic factors that regulate benthic seaweed abundance and distribution in the marine habitat, as well as its depth limit zonation or seasonal growth pattern (Gerard 1988; Hanelt, Huppertz & Nultsch 1993; Hanelt *et al*. 1997b). The light intensity that reaches sea-weeds, and the intertidal algae *Laminaria saccharina* in particular, depends on several abiotic factors and is subject to considerable variations during the course of the day (Hanelt *et al*. 1997b). First at all, as a consequence of the tidal cycle, the algae are gradually covered during flood tide by an increasingly thick layer of water. Therefore, the amount of incident irradiance decreases gradually due to absorption by water. This attenuation is aggravated by coastal water turbidity attributable to strong tidal currents and continental inputs (Gentilhomme & Lizon 1998). *Laminaria saccharina* thalli have to harvest maximum light in these low photon flux density conditions. Alternatively, when exposed at low tide to higher irradiance, *L. saccharina* have to prevent inhibition of photosynthesis and the degradation of its photosynthetic apparatus. In particular, a reduction in the photosynthetic activity of several species of algae, caused by photo-inhibition, was observed during periods of intense sunlight (Ramus & Rosenberg 1980; Coutinho & Zingmark 1987; Huppertz, Hanelt & Nultsch 1990; Henley *et al*. 1991a, 1992; Hanelt *et al*. 1993), followed by a slow recovery. In addition, the illumination of these kelps also depends on the position of the sun during the day, as well as the season.

Studies of photosynthesis and photo-inhibition, determined from oxygen evolution or variable fluorescence, have been carried out with several macro-algae (e.g. Huppertz *et al*. 1990; Henley *et al*. 1991b; Hanelt *et al*. 1993; Dring & Lüning 1994; Häder *et al*. 1997; Sagert *et al*. 1997; Cabello-Pasini, Aguirre-von-Wobeser & Figueroa 2000; Ensminger, Hagen & Braune 2000; Rodrigues *et al*. 2000). Their biochemical and physiological mechanisms are now well documented but few studies have been performed in the framework of shade-adapted and sun-adapted plants (e.g. Henley *et al*. 1991a; Sagert *et al*. 1997; Harker *et al*. 1999; Ensminger *et al*. 2000; Rodrigues *et al*. 2000) as well as seasonal variations (Ensminger *et al*. 2000).

In this study, a daily cycle for different seasons was simulated by a progressive fluctuation of irradiance in an indoor system using pulse amplitude modulated (PAM) fluorometry methods. This investigation has been devoted to the analysis of potential effects of variations in photon flux

density on *L. saccharina* physiology and their photosynthetic performance. Indeed, irradiance variations could have measurable consequences on photosynthesis for example, *via* photo-inhibition processes, and therefore on the settlement, productivity or survival of plants at different depths in the intertidal zone.

MATERIALS AND METHODS

Living material collection and acclimation

Young sporophytes of *Laminaria saccharina* Lamour. (Heterokontophyta, Phaeophyceae, Laminariaceae), with blades about 30 cm long, were collected from the kelp community of the eastern English Channel (50°49′ N, 1°35′ E) between May 1998 and January 2000. Sampling was carried out at low tide on the rocky areas to which *Laminaria* communities are restricted. The studied site is characterized by a semidiurnal tide with an average amplitude of 6·30 m and by strong tidal currents that are typical of the eastern English Channel. These parallel and alternating currents, with a speed faster than 3 knots, occur at mean spring tide (Seuront, Gentilhomme & Lagadeuc 2001) and result in rather harsh living conditions for seaweeds. Moreover, they increase the coastal water turbidity originating from the neighbouring cretaceous rocks and cliffs, the sand deposited between rocky areas and the ooze and terrigenous continental inputs. The *L. saccharina* thalli were collected the night before the experiment and transferred in dark bags to the laboratory. They were laid out in large tanks, continuously fed by running natural seawater directly pumped out of the sea (and consequently with the same temperature and nutrient content), and maintained in their new environment during the night. Samples of two different shore levels corresponding to various bathymetric zones were thus collected: the thalli from the infralittoral fringe were called 'low levels' (LL) and those coming from the lower midlittoral zone were called 'high levels' (HL). The LL are usually exposed to very low irradiances, whereas the HL are exposed more often, and for longer periods, to higher photon flux density during low tides and are subjected to larger fluctuations in incident irradiance.

Simulation of a light tidal cycle

The whole sporophyte, laid out in the tank, was exposed to various incident photosynthetically active radiations (PAR) by changing the distance between the actinic light source and the surface of the seawater tank. The sporophyte was submerged and then maintained about 3 cm under the surface to prevent desiccation and temperature stress. The spectrum of light from artificial sources was similar to that of visible natural sunlight (Tungsten lamp 1000 W, Aquastar 36 W; Sylvania, Canton, MA, USA and Triton 36 W, Triphosphor lamp; Interpet, Dorking, Surrey, UK). Incident PAR was measured close to the algal sample with an underwater quantum sensor Li-192 SA connected to a Li-cor Data Logger LI 1400 (Li-Cor, Lincoln, NB, USA).

In order to choose the range of irradiance to be used in our tidal cycle, preliminary *in situ* measurements of light intensity were performed using the underwater quantum sensor on a sunny summer day. The maximum irradiance range measured close to the algae never exceeded 750 μmol m^{-2} s^{-1} at low tide and could reach 0 μmol m^{-2} s^{-1} at high tide. Ebb tide was thus simulated by increasing the irradiance for 6 h (with successive steps of 1 h) from darkness to 696 μmol m^{-2} s^{-1}. Similarly, decreasing the irradiance from 696 μmol m^{-2} s^{-1} to darkness simulated rising tide. At simulated low tide, sporophytes were submitted to the maximal photon flux density. This study also simulated a tidal cycle during which *L. saccharina* was always submerged and, as a consequence, not exposed to desiccation. During one simulation, a total of 13 PAM fluorescence measurements were thus performed on the same spot on the thallus, and a last measurement was carried out after 12 h in complete darkness. Such simulations were carried out throughout the year: for chlorophyll fluorescence measurements, 10 different simulations during winter (January and February), 12 during spring (May and June) and six during autumn (October and November); for pigment analysis, five different simulations during winter, three during spring and three during autumn. Data were divided into different groups according to light conditions (LL or HL) and the season of the simulation (spring and winter).

Chlorophyll fluorescence parameters

In vivo chlorophyll fluorescence was measured by a modulated (1·6 kHz) and low-intensity beam from light-emitting diodes (excitation wavelength at 655 nm, detection above 700 nm) using a portable pulse-amplitude-modulated fluorometer (PAM 2000; Walz, Effeltrich, Germany) as described by Schreiber, Schliwa & Bilger (1986). In the early morning, the minimum fluorescence yield F_0 of the sporophyte, adapted to darkness throughout the night, was determined under weak red modulated light. The mid-part of the frond was held in the leaf clip of the fluorometer at a standard distance from the optic fibre probe and a weak 5 s far-red (735 nm) pulse was sent to fully oxidize the electron transport chain. The maximum fluorescence yield F_m of the dark-adapted sporophyte was reached by exposing photosystems to a saturating pulse (0·8 s) of white light. The difference between F_m and F_0 gave the variable fluorescence F_v.

The maximum quantum yield of PSII photochemistry was calculated as the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) and represents the efficiency of open PSII. Under optimal conditions and at the beginning of the experiments, *L. saccharina* reached maximum values of F_v/F_m of around 0·75, a value usually obtained for macroalgae (Büchel & Wilhelm 1993; Dring *et al*. 1996).

After these dark measurements, the sample was exposed to actinic light. At the end of each illumination period, the operating PSII efficiency (Φ_{PSII}), the relative photosynthetic electron transport rate (rETR) and the non-photochemical quenching (NPQ) were determined.

The value of Φ_{PSII} was determined as a measure of the efficiency of closed PSII units (Eqn 1) (Genty, Briantais & Baker 1989):

$$\Phi_{PSII} = \Delta F/F'_m = (F'_m - F_t)/F'_m \qquad (1)$$

with measurements under actinic light of the steady state fluorescence yield F_t and of the maximum fluorescence yield F'_m obtained using a 0·8 s saturating pulse.

The rETR was calculated according to Eqn 2 (Genty *et al.* 1989)

$$\text{rETR} = \Phi_{PSII} \times \text{PAR} \times 0·5 \qquad (2)$$

NPQ measured the efficiency of thermal energy dissipation (Maxwell & Johnson 2000) and thus the amount of energy not used in photochemistry. It was used to assess photoprotection (Müller, Li & Niyogi 2001) and was calculated according to Eqn 3 using the initial F_m measured after the long darkness period and using the F'_m measured after the light exposure (Bilger & Björkman 1990):

$$\text{NPQ} = (F_m - F'_m)/F'_m \qquad (3)$$

After measuring these parameters, the thallus was then transferred to complete darkness for 5 min. This period was estimated to be long enough to allow complete re-oxidation of the PSII reaction centres (determined when the fluorescence level F_0 reached a stable value) without changing the efficiency of non-photochemical quenching. The F_v/F_m ratio was recalculated. The sample was then exposed to another irradiance and measurements under actinic light were again taken.

Light-saturation curves were established using the rETR experimental values obtained when increasing irradiance during the simulated tidal cycle. The data were fitted using the model of Eilers & Peeters (1988) for each simulation in order to obtain the characteristic photosynthetic parameters:

1. the maximum relative photosynthetic electron transport rate (rETR_{max}) or light-saturated photosynthetic rate,
2. the initial slope of the non-saturated photosynthetic rate (α_I),
3. the optimum light intensity (I_k), also called the light saturation parameter and widely used to indicate the beginning of light-saturated photosynthesis:

$$I_k = \text{rETR}_{max}/\alpha_I \qquad (4)$$

4. the saturating light intensity (I_m), obtained when rETR is maximum (Coutinho & Zingmark 1987; Henley 1993).

Pigment extraction and characterization

The early state of photo-inhibition, corresponding to the occurrence of photoprotective mechanisms, takes part in the non-photochemical energy dissipation, as a result of the conversion of energy not used in photochemistry into harmless thermal radiation (Krause & Weis 1991). The fluorescence measurement of the non-radiative energy dissipation could be linked to the concentration in photoprotective

pigment zeaxanthin in the PSII antenna as demonstrated by Benet *et al.* (1994), Uhrmacher, Hanelt & Nultsch (1995) or Harker *et al.* (1999). To investigate this specific relation, a second sporophyte was submitted in parallel to the same irradiance conditions to analyse the light dependence of de-epoxidation of the xanthophyll cycle components. At the end of each illumination period, thallus discs (8 mm in diameter) from the middle of the frond were immediately frozen in liquid nitrogen. They were then ground in a mortar with methanol/methylene chloride (20/1, v/v) in a cold room under dim light. The extracts were centrifuged at $15\,000 \times g$ for 5 min. Supernatants of these pigment extracts were filtered with PTFE membranes (0·45 μM, 13 mm; Millipore, St Quentin Yvelines, France) and dry evaporated under a stream of nitrogen. Pigments were redissolved with a mixture of methylene chloride/distilled water (50/50, v/v) and after decantation, the aqueous phase was discarded with the aim of removing the salt, before the injection. The pigment phase was then evaporated under a stream of nitrogen and redissolved with 100 μl methanol before injection. Sample volumes of 20 μl were injected into a high-performance liquid chromatography (HPLC) reverse phase column (Zorbax; Agilent Technologies France, Massy, France) and separated following the method of Arsalane, Rousseau & Duval (1994). Pigments were detected and quantified close to their absorption maxima by peak area calculations using the integrator of the Waters 991 photodiode array detector. A preliminary calibration of the apparatus was made by injecting known amounts of standards, estimated with the molar extinction coefficient cited by Berkaloff, Caron & Rousseau (1990). The de-epoxidation ratio (DR) was calculated as:

$$\text{DR} = (A + Z)/(V + A + Z) \qquad (5)$$

where V = violaxanthin, A = antheraxanthin and Z = zeaxanthin amounts.

NPQ and DR were projected on a 0–100 scale (nX) as a reference of comparison between the different simulations, where X_{max} was the highest value of the variable among the 13 light conditions applied during the simulation of the tidal cycle (Eqn 6):

$$nX = 100 - [100(X_{max} - X)/X_{max}] = 100X/X_{max} \qquad (6)$$

where X is NPQ or DR during exposure.

RESULTS

Main patterns of chlorophyll fluorescence

Chlorophyll fluorescence parameters measured on *L. saccharina* throughout the 12 h simulated tidal cycle showed clear daily cycles according to the irradiance variation. For pooled data that covered the whole sampling period, the maximum quantum yield of PSII photochemistry (F_v/F_m) decreased when irradiance increased (simulated ebb tide) and increased when irradiance decreased (simulated rising tide) (Fig. 1). The highest values of F_v/F_m were observed for low irradiances at the beginning of the simulated tidal cycle
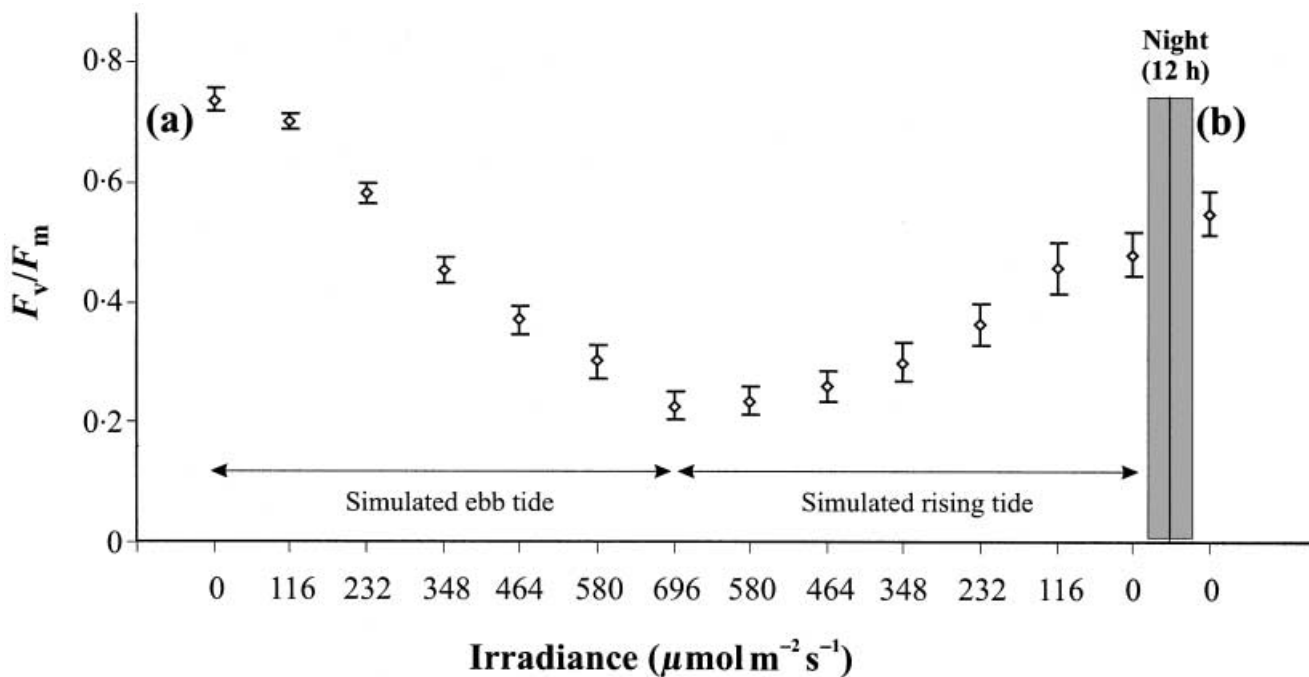
Figure 1. (a) Variation of the maximum quantum yield of PSII photochemistry (F_v/F_m) for *Laminaria saccharina* thalli exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated tidal cycle of 12 h (b) Measurement after a 12 h dark period [means ($\pm$ 95% CI), $n = 28$].

(simulated high tide). This initial quantum yield [mean $\pm$ 95% confidence interval (CI)] of $0 \cdot 74 \pm 0 \cdot 02$ dropped dramatically to $0 \cdot 23 \pm 0 \cdot 02$ (or 31% of the initial quantum yield) during simulated low tide when irradiance was at its maximum (696 μmol m^{-2} s^{-1}) and then slowly recovered. However, the last high tide values did not reach the rates observed for the first high tide, leading to a recovery of only 65% of the initial value ($0 \cdot 48 \pm 0 \cdot 04$; significantly different from the initial value, *t*-test: $P < 0 \cdot 001$). Even after a 12 h dark period, recovery only reached 75% of the initial value ($0 \cdot 55 \pm 0 \cdot 04$; significantly different from the initial value, *t*-test: $P < 0 \cdot 001$), even if values were significantly higher than those from the previous evening (*t*-test: $P < 0 \cdot 05$).

The operating PSII efficiency (Φ_{PSII}) followed a pattern similar to that of F_v/F_m (Fig. 2). The value of Φ_{PSII} regularly decreased from $0 \cdot 63 \pm 0 \cdot 02$ (100% at 116 μmol m^{-2} s^{-1}) to $0 \cdot 11 \pm 0 \cdot 02$ (18% at 696 μmol m^{-2} s^{-1}) during simulated ebb tide. It increased as soon as irradiance decreased and reached $0 \cdot 42 \pm 0 \cdot 04$ (only 67% at 116 μmol m^{-2} s^{-1}) at the end of the simulated rising tide, which was significantly different from the initial value ($P < 0 \cdot 001$).

The relative electron transport rate (rETR), derived from values of operating PSII efficiency, also varied according to the irradiance (Fig. 3). The initial value of $36 \cdot 79 \pm 1 \cdot 44$ rose to $51 \cdot 97 \pm 4 \cdot 09$ at 232 μmol m^{-2} s^{-1} in the simulated early ebb tide, slowly decreased until low tide to remain at about 38 (not significantly different from the initial value) during the simulated rising tide and finally decreased following the later and weaker irradiances to reach $24 \cdot 61 \pm 2 \cdot 44$ (significantly lower than the initial value, $P < 0 \cdot 001$).

The value of NPQ followed the irradiance variation (Pearson correlation: R = $0 \cdot 343$, $n = 308$, $P < 0 \cdot 001$), gradually increasing to $6 \cdot 19 \pm 1 \cdot 79$ at first and then decreasing (Fig. 4a). This pattern was consistently observed for different scales, due to individual and seasonal variability. The evolution was therefore better represented by the normalized NPQ (nNPQ). Its variation was also highly correlated with the irradiance (R = $0 \cdot 769$, $n = 308$, $P < 0 \cdot 001$) (Fig. 4b). It was still significantly higher for the last exposure to 116 μmol m^{-2} s^{-1} than for the first one ($P < 0 \cdot 001$).

Main patterns of pigment content

Pigment compositions before the simulated tidal cycle (dark incubated), during the maximum irradiance (696 μmol m^{-2} s^{-1}) and at the end of the cycle (after the last obscurity period) are shown in Table 1. Large amounts of molecules of the antennary pigments fucoxanthin and chlorophyll *c* per 100 molecules of total pigments were observed. Whereas amounts of chlorophyll *c*, fucoxanthin, chlorophyll *a* and β carotene remained fixed during this cycle, the amounts of pigments involved in the xanthophyll cycle fluctuated. The xanthophyll cycle was functional, as shown by the de-epoxidation of violaxanthin into antheraxanthin and zeaxanthin. The initial amount of violaxanthin significantly decreased as the irradiance increased from 0 to 696 μmol m^{-2} s^{-1} (*t*-test: $P < 0 \cdot 01$), whereas both antheraxantin and zeaxanthin simultaneously increased during the same interval ($P < 0 \cdot 001$). The reverse reaction also
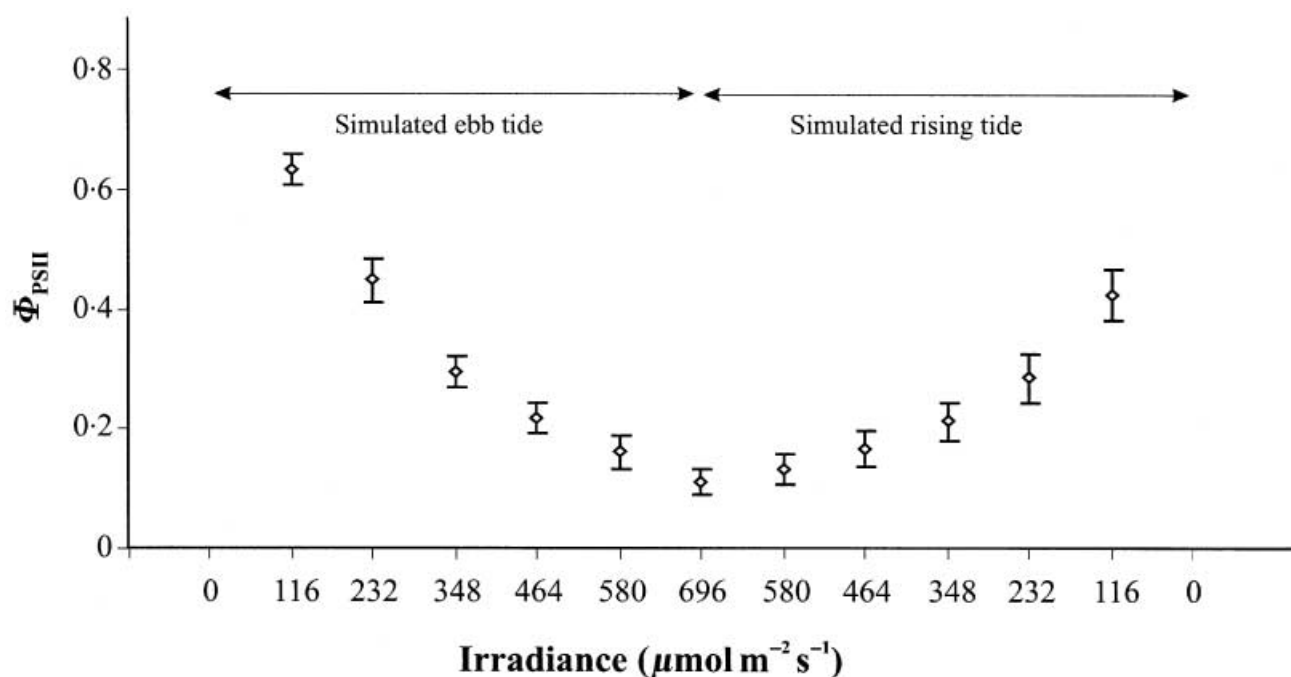
Figure 2. Variation of the operating PSII efficiency (Φ_{PSII}) for *Laminaria saccharina* thalli exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated tidal cycle of 12 h [means ($\pm$ 95% CI), $n = 28$].

occurred from 696 to 0 μmol m^{-2} s^{-1}. The amount of zeaxanthin was significantly lower at the end of the simulated tidal cycle whereas violaxanthin was higher ($P < 0.005$ and $P < 0.01$, respectively). The recovery was not totally achieved because the amounts of antheraxanthin and zeaxanthin were still higher at the end of the tidal cycle than at the beginning ($P < 0.001$).

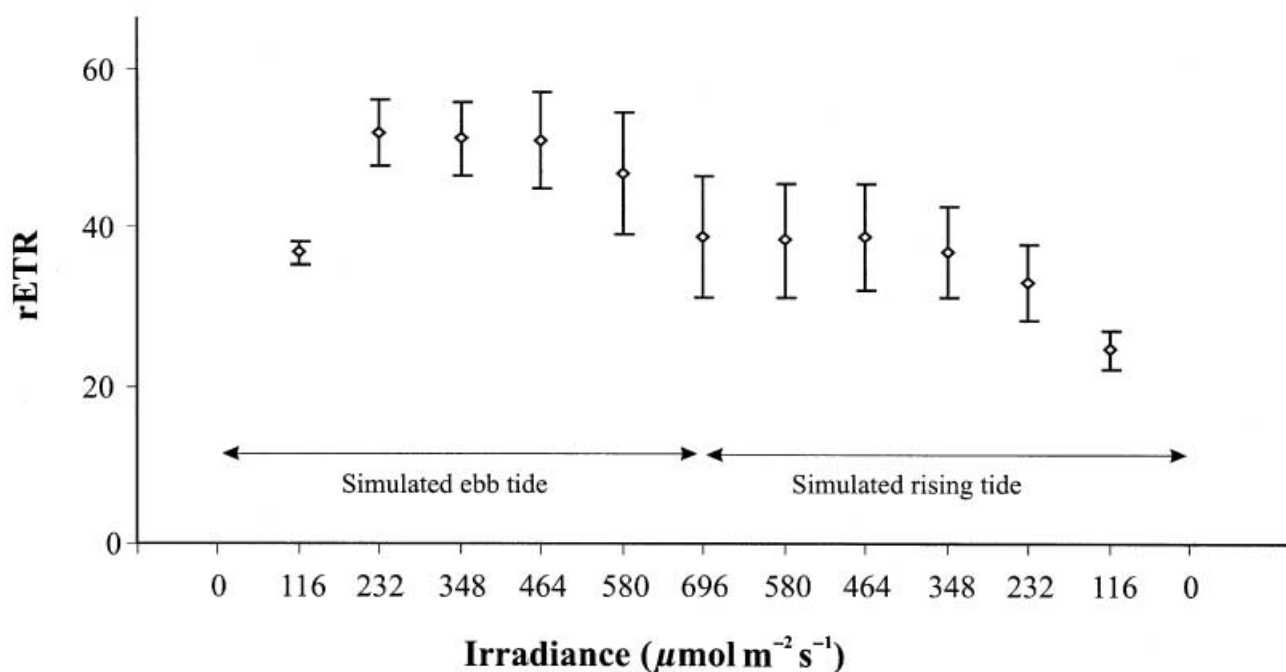The course of the de-epoxidation ratio (DR) (Fig. 5),

Figure 3. Variation of the relative electron transport rate (rETR) for *Laminaria saccharina* thalli exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated tidal cycle of 12 h [means ($\pm$ 95% CI), $n = 28$].
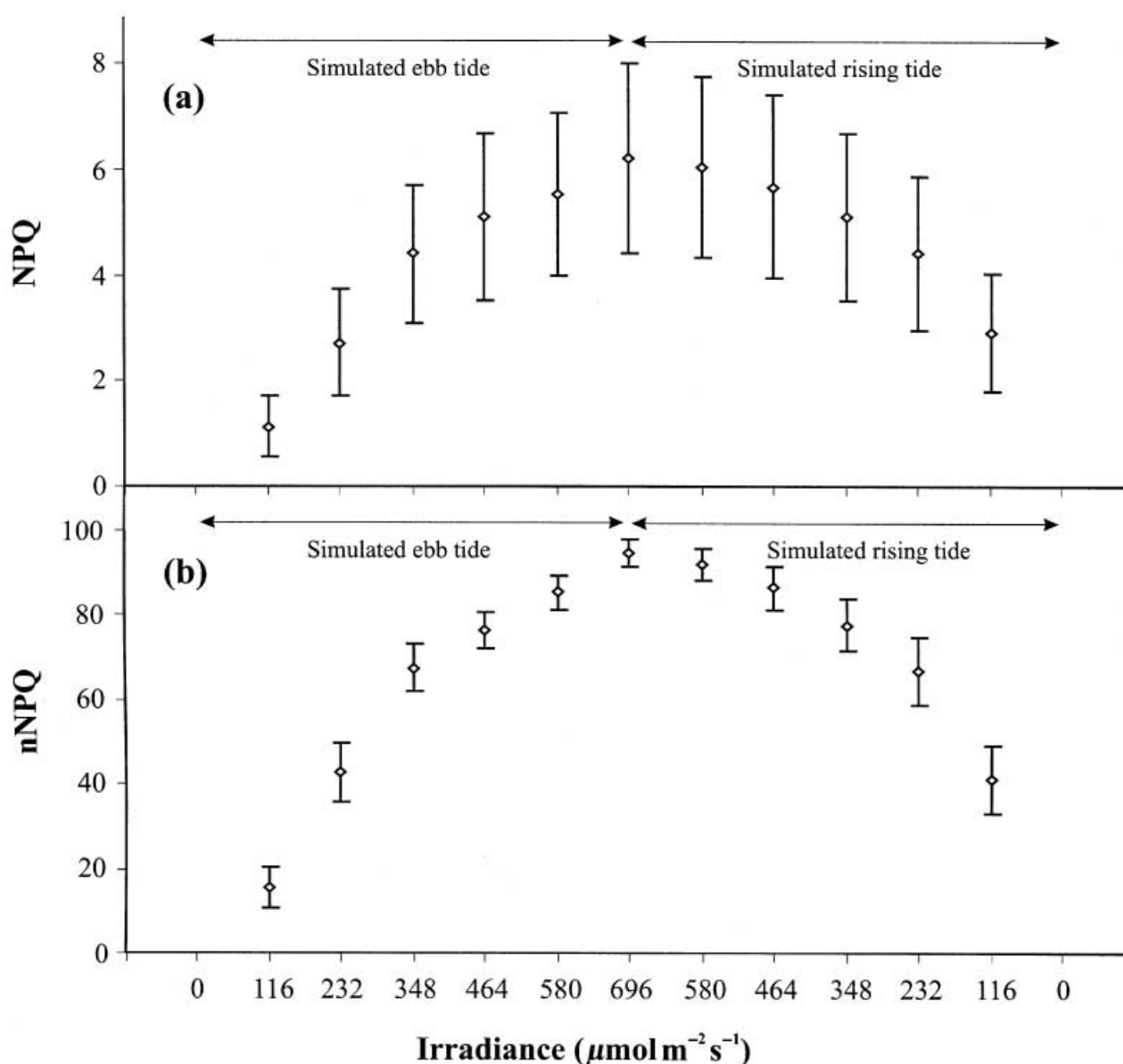
Figure 4. Variation of the non-photochemical quenching (NPQ) for *Laminaria saccharina* thalli exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated tidal cycle of 12 h [(a) non-photochemical quenching (NPQ); (b) non-photochemical quenching on a 0–100 scale (nNPQ); means ($\pm$ 95% CI), $n = 28$].

Table 1. Pigment composition (moles per 100 mol of total pigment) in *Laminaria saccharina* before, during and after light exposure of the tidal cycle (means $\pm$ 95% CI)

|	Beginning of the simulation, after the dark incubation, $n = 11$	Simulated low tide, $n = 11$	End of the simulation, after the last dark period, $n = 11$
Chlorophyll *c*	5·91 ± 1·29	5·38 ± 1·45	5·74 ± 1·22
Fucoxanthin	31·41 ± 1·65	31·97 ± 2·51	32·55 ± 2·39
Violaxanthin	4·18 ± 0·94	2·64 ± 0·72	4·93 ± 1·48
Antheraxanthin	0·15 ± 0·08	0·79 ± 0·26	0·68 ± 0·13
Zeaxanthin	0·07 ± 0·05	1·58 ± 0·52	0·59 ± 0·27
Chlorophyll *a*	55·99 ± 1·85	55·64 ± 2·80	52·96 ± 1·97
β carotene	2·29 ± 0·98	2·00 ± 0·98	2·55 ± 1·29
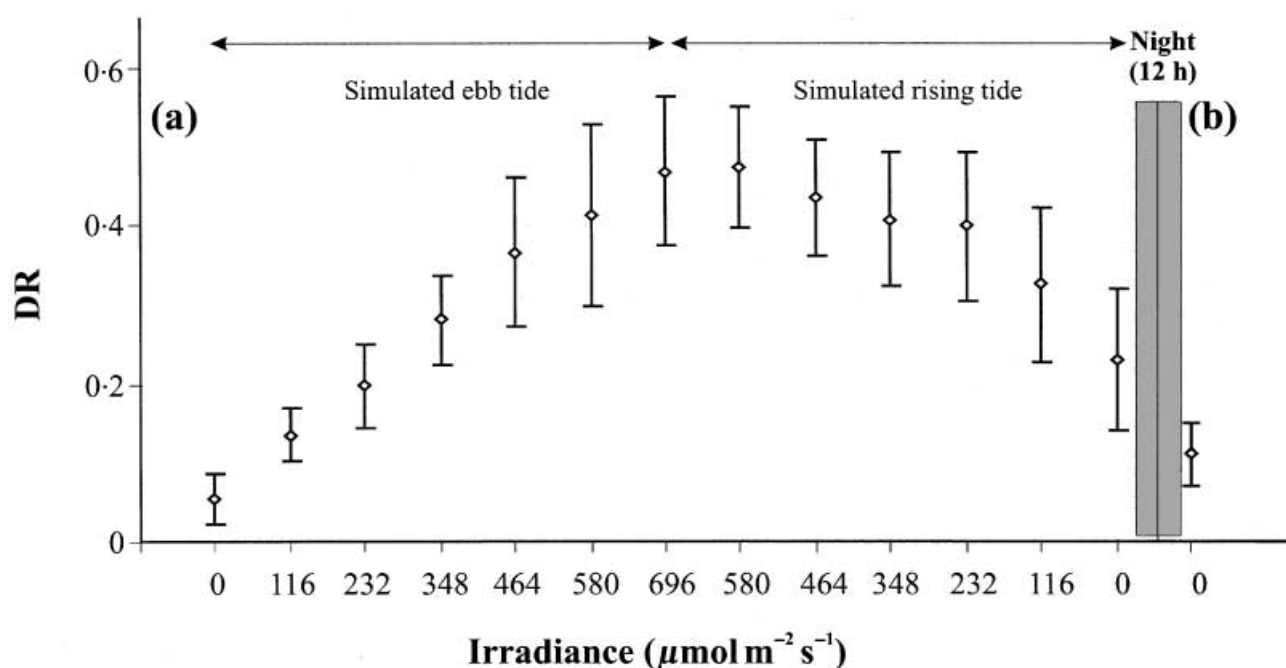
Figure 5. (a) Variation of the de-epoxidation ratio (DR) of violaxanthin into antheraxanthin and zeaxanthin for *Laminaria saccharina* thalli exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated tidal cycle of 12 h (b) Measurement after a 12 h dark period [means ($\pm$ 95% CI), $n = 11$].

which represents the proportion of de-epoxided xanthophylls involved in photoprotection, was significantly correlated with the irradiance ($R = 0.612, n = 143, P < 0.001$). DR showed a pattern similar to that of NPQ, as demonstrated by their significant correlation ($R = 0.355$, $n = 242$, $P < 0.001$) and the correlation between nDR and nNPQ ($R = 0.722, n = 242, P < 0.001$). *Laminaria saccharina* reacted to light stress by developing photoprotective mechanisms involving the xanthophyll cycle. The DR of 0.23 ± 0.09 measured at the end of the simulation was significantly higher than the initial value of 0.05 ± 0.03 ($P < 0.001$). This measure was still higher after a 12 h night with a value of 0.11 ± 0.04 ($P < 0.05$).

Seasonal and bathymetrical patterns of chlorophyll fluorescence

The course of the relative electron transport rate (rETR) during simulated tidal cycles in spring and winter, as well as for LL and HL, is presented in Fig. 6. Whatever the irradiance, rETR was higher in spring than in winter for both HL and LL. The values of rETR exhibited different patterns in spring and winter with an earlier decrease in winter even for smaller irradiances. In spring, photosynthesis of *L. saccharina* is highly stimulated by the strong light, when winter seaweeds are disturbed by it. We know that electron transport rate was affected by these light treatments since the last value remained lower than the initial one. Light-saturation curves were established for the previous data subsets (Fig. 7) and differences in their characteristics were

analysed (Table 2). Although no significant difference in the initial quantum use efficiency (i.e. α_I) was observed in *L. saccharina* from both low and high levels of winter and spring, values of rETR at higher irradiances were significantly higher in spring than in winter for both HL and LL ($P < 0.005$ and $P < 0.001$, respectively). The values reached by rETR$_{max}$ were 63.6 for HL and 70.8 for LL in spring and 48.5 and 36.0, respectively, in winter. In contrast to the higher levels, low level seaweeds showed a significantly higher value in the saturating light intensity I_m ($P < 0.05$) in spring (576 μmol m^{-2} s^{-1}) than in winter (230 μmol m^{-2} s^{-1}). The only significant difference between LL and HL is the maximum value reached by rETR in winter with a higher value for HL (48.5) than for LL (36.0) ($P < 0.05$). LL are less able to devote energy to electron transfer in the photochemical reactions than are HL. The optimum light intensity I_k did not significantly differ between winter and spring and between LL and HL (Table 2).

The operating PSII efficiency (Φ_{PSII}) for LL and HL in spring and winter was also assessed (Fig. 8). No significant difference was observed between LL and HL in spring. Alternatively, the yield measured in winter was always significantly higher for HL than for LL ($P < 0.005$ in the beginning of the cycle, $P < 0.05$ afterwards at 696 μmol m^{-2} s^{-1} and at the end of the cycle). With the exception of HL in the early cycle, differences in the Φ_{PSII} values between spring and winter were significant, with higher values in spring for HL ($P < 0.005$ in the middle of the cycle and $P < 0.01$ at the end) and for LL ($P < 0.01$, $P < 0.005$ and $P < 0.001$ successively). Recovery was slower in winter and did not reach
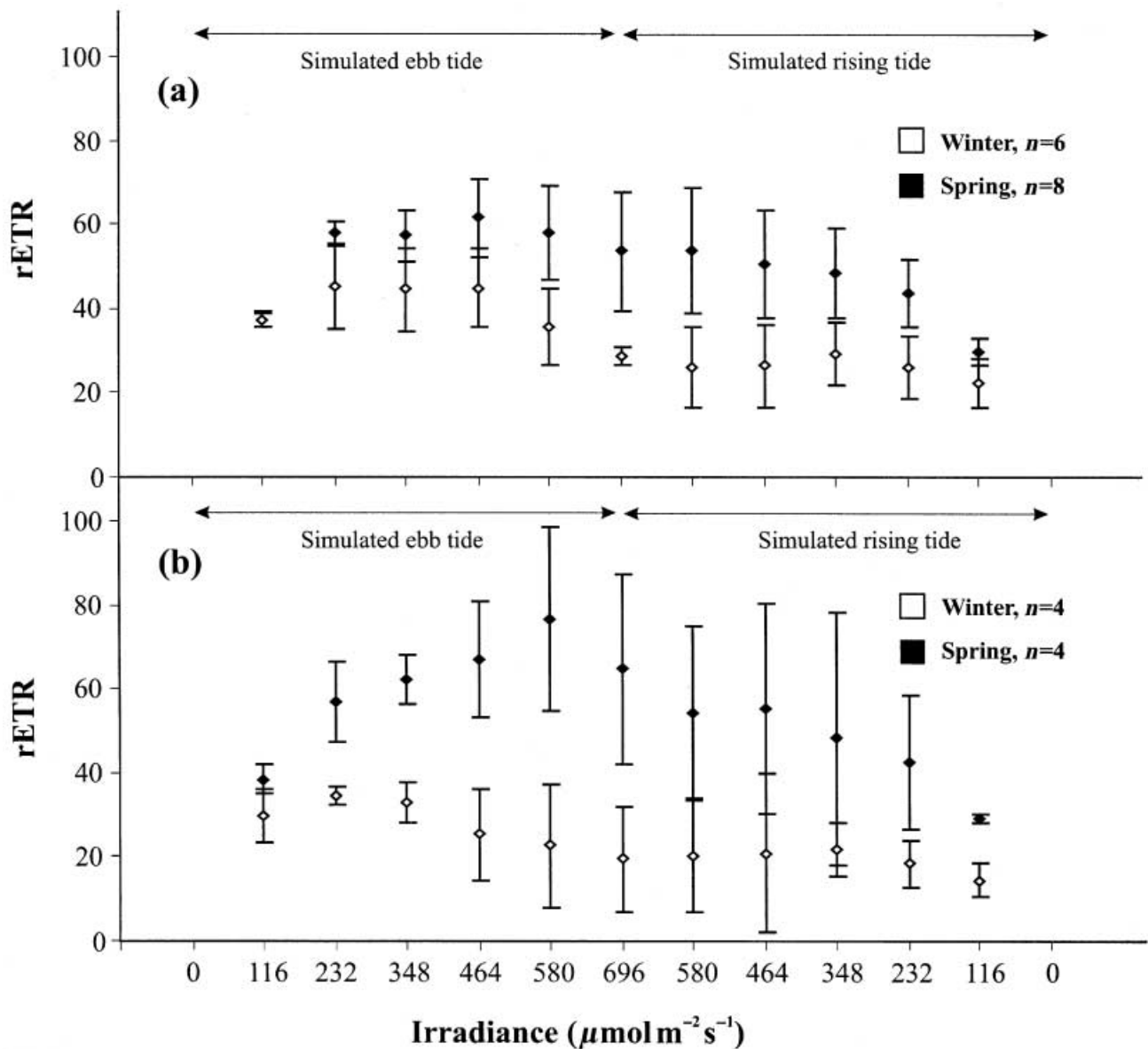
Figure 6. Seasonal variation of the relative electron transport rate (rETR) of *Laminaria saccharina* thalli (means ± 95% CI) exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated tidal cycle of 12 h [(a) high level thalli; (b) low level thalli].

the initial value implying that *L. saccharina* recovered less efficiently in winter than in spring and was more affected by this artificial treatment.

The non-photochemical quenching expressed using a 0–100 scale (nNPQ) (Fig. 9) showed significantly higher values in winter than in spring, after the first and the last irradiance levels for HL ($P < 0.001$ for the two light levels) and only after the last irradiance for LL ($P < 0.001$). Although

Table 2. Mean values of the characteristic parameters of light-saturation curves of *Laminaria saccharina* (model of Eilers & Peeters 1988)

		α_I	rETR$_{max}$	I_k (μmol m^{-2} s^{-1})	I_m (μmol m^{-2} s^{-1})	n	r^2
Spring	High levels	0·43	63·6	163	521	8	0·991
	Low levels	0·41	70·8	186	576	4	0·993
Winter	High levels	0·53	48·5	127	354	6	0·989
	Low levels	0·38	36·0	127	230	4	0·994

α_I, initial slope of the photosynthetic rate; rETR$_{max}$, maximum relative electron transport rate; I_k, optimum light intensity; I_m, saturating light intensity; n, number of samples.
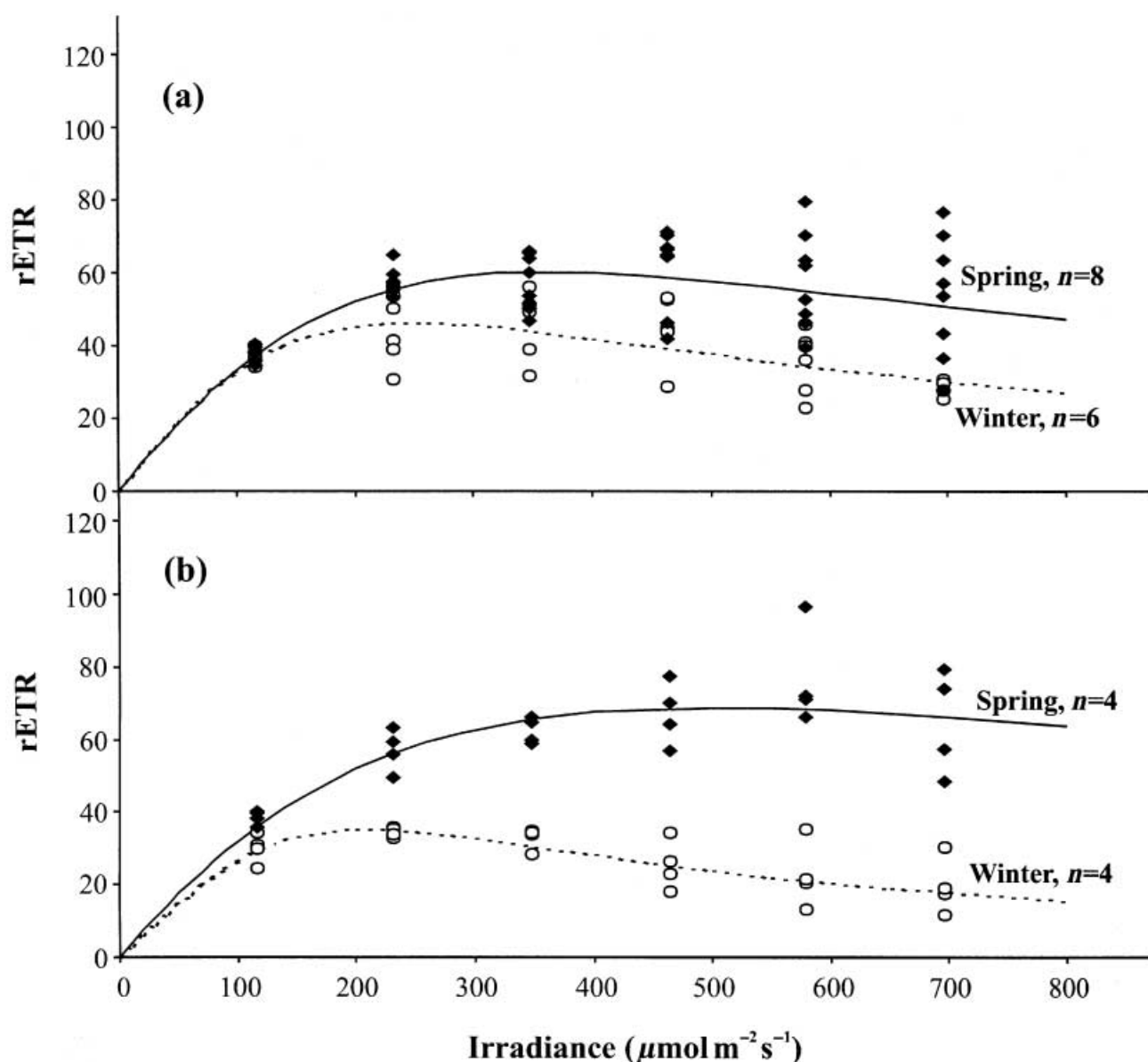
Figure 7. Light-saturation curves for *Laminaria saccharina* thalli exposed to irradiances ranging from 0 to 696 μmol m^{-2} s^{-1} during a simulated ebb tide of 6 h [(a) high levels; (b) low levels].

no significant difference was observed between LL and HL in spring and in winter at the first irradiance and at the maximum irradiance (Fig. 9), nNPQ was always significantly lower for HL than for LL at the end of the simulated tidal cycle both in spring and in winter ($P < 0.005$ and $P < 0.05$), indicating different recovering abilities.

Seasonal and bathymetrical patterns of pigment content

The de-epoxidation ratio DR showed no significant difference between seasons (comparison between spring and winter for HL) or bathymetric levels (comparison between HL and LL in winter).

The photosynthetic pigment content of *L. saccharina* just before the tidal cycle was compared between spring and winter (Table 3). Pigment composition was constant except for chlorophyll *c* (i.e. a light harvesting pigment), which was higher in winter than in spring ($P < 0.005$), chlorophyll *a* and violaxanthin, which were higher in spring ($P < 0.05$). Although no significant difference was observed in the concentrations of total xanthophylls and carotenoids for all the analysed thalli, pigments related to the xanthophyll cycle (violaxanthin + antheraxanthin + zeaxanthin) were more concentrated in spring than in winter ($P < 0.05$). On the other hand, the antenna pigments (fucoxanthin + chlorophyll *c*) were more abundant in winter (38.98 ± 1.46, mean $\pm$ 95% CI) than in spring (32.90 ± 8.52, mean $\pm$ 95%
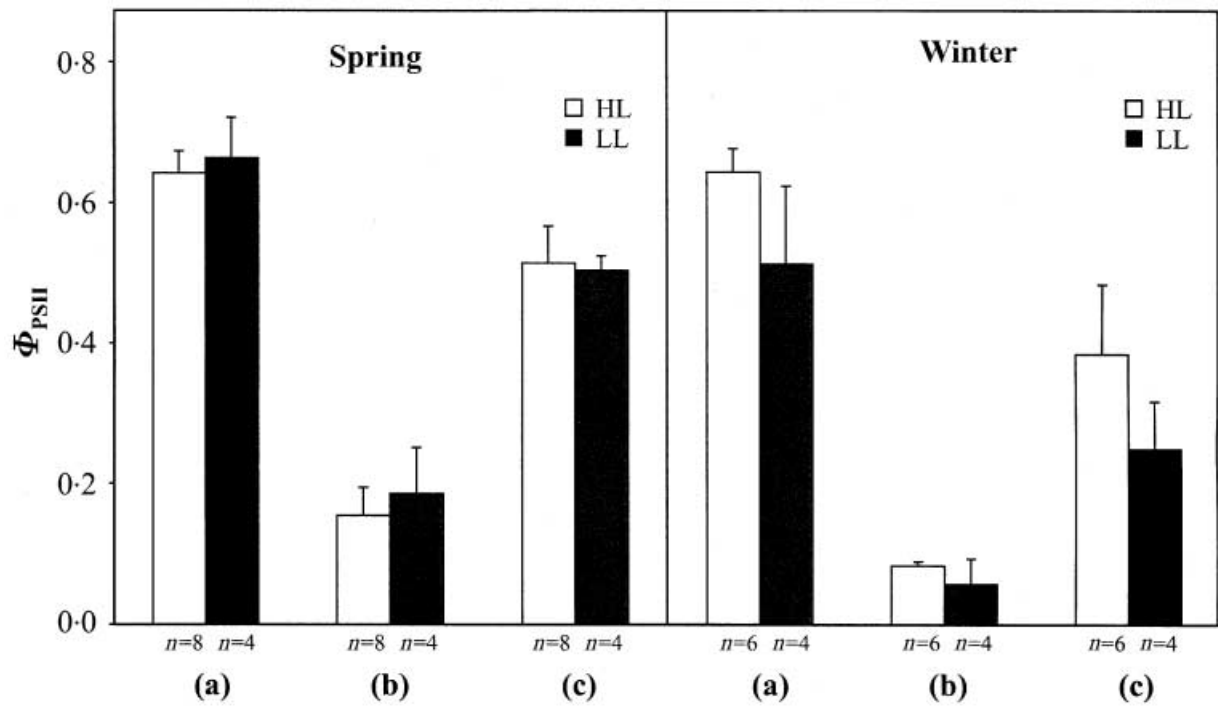
Figure 8. Operating PSII efficiency (Φ_{PSII}) in *Laminaria saccharina* from high levels (HL) and from low levels (LL) after different light exposures during spring and winter (means ± 95% CI) [(a) first irradiance; (b) maximal irradiance; (c) last irradiance of the tidal cycle].
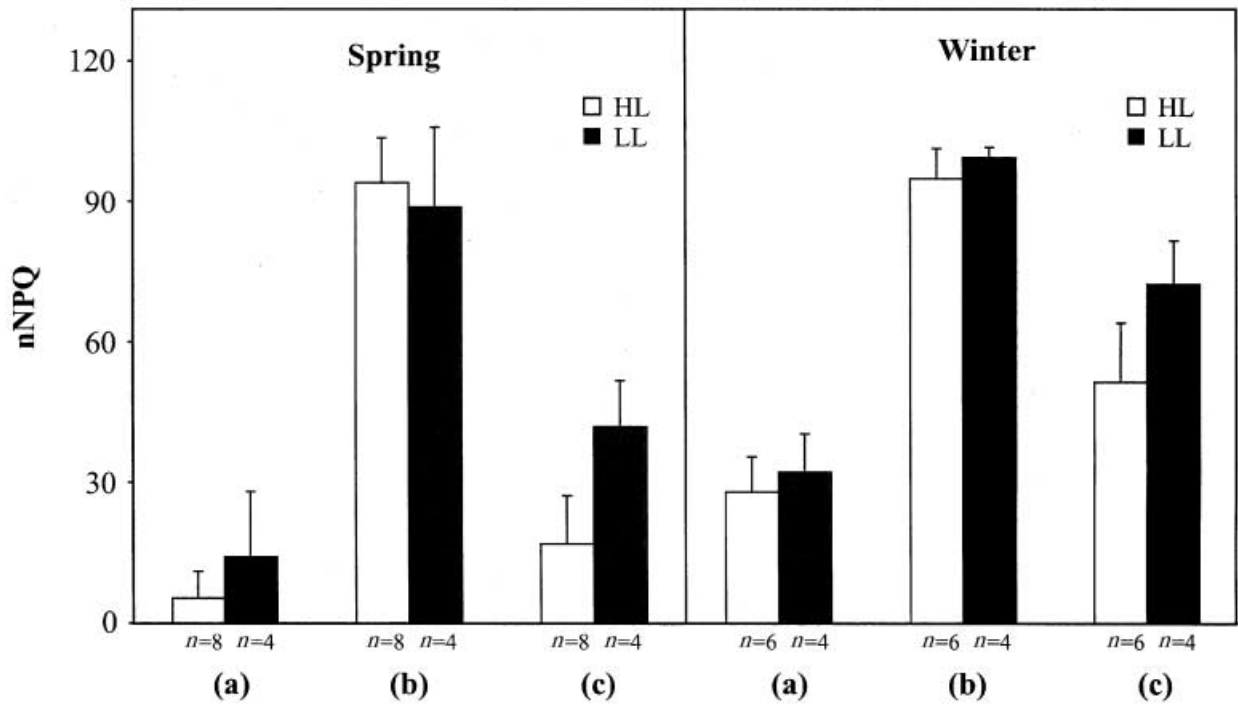

Figure 9. Non-photochemical quenching on a 0–100 scale (nNPQ) in *Laminaria saccharina* from high levels (HL) and from low levels (LL) after different light exposures during spring and winter (means ± 95% CI) [(a) first irradiance; (b) maximal irradiance; (c) last irradiance of the tidal cycle].

Table 3. Seasonal changes in pigment composition (moles per 100 mol of total pigment) and in pigment ratio in *Laminaria saccharina* (means ± 95% CI) just before the simulated tidal cycle for the whole LL and HL

	Spring (*n* = 3)	Winter (*n* = 5)
Chlorophyll *c*	3·20 ± 3·51	6·70 ± 0·70
Fucoxanthin	29·70 ± 10·12	32·28 ± 1·54
Violaxanthin	5·87 ± 2·72	3·50 ± 1·38
Antheraxanthin	0·17 ± 0·04	0·14 ± 0·19
Zeaxanthin	0·07 ± 0·10	0·05 ± 0·06
Chlorophyll *a*	59·19 ± 3·94	54·57 ± 2·71
β carotene	1·81 ± 2·38	2·76 ± 1·82
Total xanthophylls	35·81 ± 7·61	35·97 ± 2·60
Carotenoids	37·61 ± 5·23	38·73 ± 2·32
Fuco + Chl *c*	32·90 ± 8·52	38·98 ± 1·46
V + A + Z	6·11 ± 2·62	3·69 ± 1·44
(V + A + Z)/(Fuco + Chl *c*)	0·19 ± 0·12	0·09 ± 0·04
Chl *a*/Chl *c*	21·11 ± 23·27	8·21 ± 1·16

CI) ($P < 0·01$). The ratio (violaxanthin + antheraxanthin + zeaxanthin) per (chlorophyll *c* + fucoxanthin) was thus higher in spring than in winter ($P < 0·05$). The ratio of chlorophyll *a* to chlorophyll *c* was also significantly higher in spring than in winter for the whole LL and HL ($P < 0·05$). The pigment composition of HL and LL was not significantly different.

DISCUSSION

Photo-inhibition and photoprotection dynamics in *L. saccharina*

The maximum quantum yield of PSII photochemistry (F_v/F_m) is widely used as a sensitive indicator of plant and algae photosynthetic performance (Maxwell & Johnson 2000). In our experiments, this ratio was affected by changes in light conditions during the 12 h simulated tidal cycles. Its fast decrease during the simulated ebb tide showed that photo-inhibition occurred at a relatively low irradiance level (116 μmol m^{-2} s^{-1}). Photo-inhibition, reducing photosynthetic efficiency with light increase, involves either or both of two processes: (i) photoprotection (also referred to as 'dynamic photo-inhibition') mainly *via* thermal energy dissipation and (ii) photodamage to the PSII reaction centre complex (photo-inhibition *sensu stricto*) (Krause 1988; Henley *et al.* 1991a, 1991b; Demmig-Adams & Adams 1992; Osmond *et al.* 1993; Hanelt *et al.* 1997b; Werner *et al.* 2001). Photo-inhibition, estimated by a reduction of the F_v/F_m, was also observed in *L. saccharina* by Hanelt (1998) and Hanelt *et al.* (1997a) but in a more static way by exposing sporophytes to 500 μmol m^{-2} s^{-1} for 2 h. The decrease of this ratio is due to a change in the efficiency of NPQ, resulting from an exposure to environmental stress (Maxwell & Johnson 2000) and involves photoprotective down-regulation of the PSII efficiency.

Our results clearly showed the involvement of the xan-

thophyll cycle in the initiation of mechanisms protecting the frond from light-induced damage when irradiance increased during the simulated ebb tide. Moreover, *L. saccharina*, when subjected to higher spring irradiances, has a large xanthophyll cycle pool of pigments to cope with light stress. Many investigations carried out on algae (e.g. Schubert, Kroon & Matthijs 1994; Rmiki *et al.* 1996; Vershinin & Kamnev 1996; De Martino *et al.* 1997; Harker *et al.* 1999) have already shown the role of the xanthophyll cycle in the development of photoprotective mechanisms but none of these studies were conducted during an entire tidal cycle. Although photoprotective mechanisms in which zeaxanthin plays a role have been discussed, they are thought to occur in the light-harvesting antenna of PSII (Horton, Ruban & Walters 1996; Young & Frank 1996).

During simulated rising tide, recovery from high-light treatment occurred but took longer than the duration of the simulated rising tide and was not complete at the following high tide. Moreover, the complete darkness of the night did not allow *L. saccharina* to fully recover from this tidal cycle (F_v/F_m measured on the following day did not reach the initial value). The above evidence implies that these seaweeds may be ill-adapted to the light treatment applied. This could result in the deactivation of some PSII centres and eventual photodamage. Dim light conditions would also perhaps be better suited for a more complete recovery, as they are known to improve some reactions such as a faster reversion of the xanthophyll cycle under weak illumination conditions rather than in complete darkness (Rmiki, Schoefs & Lemoine 1998).

Photoprotective mechanisms allowed *L. saccharina* to maintain its photosynthetic electron transport rate at a sufficient level even when reduction in photochemical activity occurred. rETR increased rapidly during the first ebb tide showing the need to improve the efficiency of photon capture. It decreased during low tide for the highest irradiance applied, then saturated and finally declined slowly during the late rising tide.

Seasonal differences in the photosynthetic performance of *L. saccharina*

The operating PSII efficiency variations (Fig. 8) indicated different degrees of photosynthetic performance between seasons. In winter, a strong inhibition of photosynthesis and a weak recovery were most probably due to an ill-adaptation to the high-light regime. Nevertheless, it could also be linked to the physiological state of seaweeds and their morphology, as well as their development stages. Moreover, seawater temperature is another abiotic factor that could influence the metabolic reactions rate (Davison 1987; Davison 1991; Huner *et al.* 1995). In particular, low temperatures (8 °C in January as opposed to 14 °C in May) could slow down the xanthophyll cycle and explain the heterogeneity of the values observed during winter. For HL, which are exposed to greater thermal variations than are LL, the non-photochemical energy dissipation into thermal radiation was higher in winter than in spring both at the

beginning and at the end of the tidal simulation, implying some stress. Low temperatures resulted in a greater stress (Bruhn & Gerard 1996) by combination with high light treatment and conducted to a slower recovery (Fig. 8) due to a reduced metabolism. Changes in photosynthetic efficiency were concomitant with changes in pigment composition. *Laminaria saccharina* showed acclimation to low-light environments in winter by developing more efficient light-harvesting complexes, revealed by larger amounts of chlorophyll *c*. These modifications increased the absorption efficiency of the photosystems. On the contrary, seaweeds minimized photo-inhibition and destruction of their photosynthetic apparatus during the strong irradiance periods of spring by accumulating more pigments involved in the xanthophyll cycle. They also down-regulated their light-capture efficiency in this hostile environment. The light-saturation curves showed the acclimation to seasonal changes in environmental conditions (Fig. 7). The rETR versus irradiance relationship differed between the two analysed seasons (Fig. 7, Table 2), with higher values of the ratio in spring than in winter. It thus illustrated the acclimation of algae to high photon flux densities with a more efficient use of light for photochemistry. Photo-inhibition, detected by the model used to calculate the light-saturation curves as a negative slope at over-saturating irradiance levels, occurred for lower irradiance levels in winter than in spring. Photo-inhibition could be seen as time dependent and changes in the curves could result from photoprotection rather than photodamage of the reaction centres (Krause & Weis 1991; Henley 1993; Ensminger *et al.* 2000). At saturating irradiance, the electron transport rate exceeded carbon fixation capacity by carboxylation. Thereafter, at over-saturating irradiances, some PSII centres were deactivated and the resulting photosynthetic rate was then limited by PSII electron transport (Henley 1993; Ensminger *et al.* 2000). This could explain the observed decline of the rETR after it reaches a saturating irradiance level (Fig. 7).

Comparison of photosynthetic parameters between *L. saccharina* collected at high and low levels

Even if the two bathymetric levels studied here were not so far apart, some differences in *L. saccharina* from low and high levels were observed. HL seemed to be better adapted to the applied light regime than was LL. They were more efficient in using the energy in the electron transfer and they converted less of the absorbed energy (i.e. not used in photochemistry) into thermal radiation.

The slight differences observed between bathymetric levels are essentially related to the different morphology of the harvested thalli, which were thicker at low levels and certainly more resistant and older. Indeed, it was difficult to find old sporophytes from high levels, because they were often washed away during the autumn storms and were particularly sensitive to UV radiation in the summer (Dring *et al.* 1996). UV radiation could also play a role in determining the spatial distribution of the species, as reported by Lüning

(1980), who observed that gametophytes of *Laminaria saccharina* could survive under longer exposure to winter sunlight than could *Laminaria digitata* or *hyperborea*; this could explain the appearance of young sporophytes of *L. saccharina* relatively high on the shore in early spring. Moreover, the low level samples we studied were collected during spring ebb tides when they had emerged. Lower levels could have been reached by harvesting samples by scuba diving or performing *in situ* measurements using a diving-PAM. Such an approach would allow us to assess the effects of both the quantity and the quality of light whose spectrum is also modified by the water column. However, the advantage in simulating this tidal cycle in the laboratory was the ability to control parameters and to avoid the practical inconvenience of diving during a 12 h period, especially in cold winter water.

Conclusion

This laboratory ecophysiological study provides information on the variation of energy conversion by PSII during tidal cycles and on the extent of reaction centres damage by excess light. *In situ*, the photon flux density reaching the seaweeds and influenced by the thickness of the water column obviously has a great effect on their photosynthesis. *Laminaria saccharina* have been shown to acclimate to various seasonal conditions by modifying their pigment composition and being able to tolerate light stress thanks to their xanthophyll cycle. Their spatial zonation on the shore certainly reflects both their adaptation to the abiotic environment and their ability to acclimate to temporary changes in photon flux density by photoprotective mechanisms.

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