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Abstract

The boreal kelp *Laminaria digitata* dominates the low intertidal and upper subtidal zones of

moderately exposed rocky shores in north-western Europe. Due to ocean warming, this foundation

species is predicted to disappear from French coasts in the near future. Photosynthesis of *L. digitata*

sporophytes was surveyed *in situ* during spring and summer tidal cycles with emersion periods

around midday during hot, sunny weather. The net production (NP) of whole individuals (i.e. the

difference between their gross primary production and respiration) was assessed by measuring

carbon fluxes inside a closed chamber. Photosynthetic performance of thalli was assessed using

pulse-amplitude modulated (PAM) fluorescence parameters, the effective (Φ_{PSII}) and optimal (F_v/F_m) quantum yields of photosystem II. Content in pigments involved in the xanthophyll cycle, a photoprotective mechanism, was measured in thalli to evaluate the de-epoxidation ratio (DR). NP shifted from positive values (reaching 140 µmol C g_{DW}^{-1} h^{-1}) during morning immersion to negative values during emersion (reaching -37 µmol C g_{DW}^{-1} h^{-1}), and did not return to positive values during the following immersion period when respiration was exacerbated. Φ_{PSII} decreased during emersion (down to 0.01), but recovered during afternoon immersion. F_v/F_m decreased during emersion (down to 0.18) indicating severe photoinhibition. High DR values (up to 0.70) showed the effectiveness of the photoprotective mechanism, which appeared nevertheless insufficient to prevent photodamage during emersion stress. Among the environmental factors contributing to this emersion stress, repeated heat shocks over consecutive tide cycles likely play a leading role. These repeated heat shocks appear to further exacerbate the detrimental effects of warming events on this marginal population of *L. digitata*.

Key words

Kelp; Carbon production; Chlorophyll fluorescence; Photoinhibition; Xanthophyll cycle; *in situ*; English Channel

Introduction

Kelps (i.e. brown macroalgae belonging to the order Laminariales) form dense populations on rocky shores from the low intertidal to the upper subtidal zones of polar and temperate waters. Such populations constitute highly productive systems, viewed as the marine equivalent of the terrestrial rain forest (Mann 1973). However, recent reports indicate a decline in kelp beds worldwide (Araujo et al. 2016; Wernberg et al. 2016). Species living in shallow nearshore and intertidal habitats

experience an extremely variable environment and are particularly exposed to the impacts of climate change (Pereira et al. 2015). As foundation species, kelps can help to mitigate the negative effects of climate change (e.g. by providing a damp, cool refuge at low tide, Burnaford 2004) and therefore contribute to the maintenance of ecosystem function (Wernberg et al. 2010). To help preserve their role as foundation species, it is essential to understand how kelps respond to multiple concurrent stresses.

The physiological characteristics of kelps have received much attention, especially with respect to environmental factors, to explain their biogeography and zonation patterns, and the ecophysiology of the most common Laminariales in the northern hemisphere has been largely investigated (reviewed by Bartsch et al. 2008). Although earlier studies mainly addressed the performance of sporophytes through growth (estimated by weight, length or area increase), more recent studies often use photosynthetic activity as an indicator of physiological performance. Nevertheless, the photosynthetic performance of sporophytes has been mainly investigated under controlled laboratory conditions, examining the response to different environmental factors separately. Generalization to field conditions thus remains hazardous, particularly in the context of global change, which causes changes in the frequency and intensity of many factors.

The boreal kelp *Laminaria digitata* dominates the low intertidal and upper subtidal of moderately exposed rocky shores in north-western Europe, but some populations have dramatically declined over the past few years (Raybaud et al. 2013; Araujo et al. 2016). Although multiple causes may have contributed to this decline, ocean warming appears to be an important factor. Modeling of the ecological niche of *L. digitata* and predicted projections of its distribution along the European coasts for the 21st century show a northward retreat of its current southern limit and therefore its disappearance from French coasts (Raybaud et al. 2013). The disappearance of this species can have catastrophic consequences for its associated communities and ecosystems.

In the south western English Channel, where *L. digitata* approaches the southern edge of its geographical range, low spring tides occur around noon and thalli can be exposed to over-saturating

irradiances on clear sunny days. To understand how *L. digitata* copes with this exposure, the variation in its photosynthetic performance with the tidal cycle was surveyed *in situ.* A previous study showed that *L. digitata* exhibits photoinhibition at low tide but, owing to the development of a photoprotective mechanism, totally recovers photosynthetic performance during the following flood tide (Delebecq et al. 2011). This pattern was observed in the mid part of the kelp zone (which remains immersed even at low tide) and may be very different in the upper part of the zone (which emerges at low tide) where *L. digitata* is likely to be exposed to the most pronounced physical stress (Hanelt 1996). During immersion, the water column protects the thalli by decreasing the intensity of irradiance; in contrast, during emersion, thalli are likely to be exposed to stronger light stress. Furthermore, during emersion, thalli can be exposed to multiple concurrent stresses due to rapid changes in light and temperature, as well as to desiccation and nutrient depletion. For example, high temperatures have been shown to alter photosynthetic performance and induce photoinhibition in *L. digitata* gametophytes (Delebecq et al. 2016) and other temperate Laminariales sporophytes (Terada et al. 2016; Borlongan et al. 2018), and disrupt repair processes (Bruhn and Gerard 1996). To date, the response to high temperatures during emersion has not been evaluated *in situ* but bleaching -a symptom of desiccation or temperature stress- has been observed in intertidal *L. digitata* populations during periods of high temperatures (Hargrave et al. 2017).

The present study aimed to describe the *in situ* dynamics of the photosynthetic performance of *L. digitata* sporophytes at their upper intertidal range limit, throughout a whole tidal cycle including an emersion period. The photosynthetic activity of thalli was measured at different tidal stages (during immersion and emersion) using pulse-amplitude modulated (PAM) fluorescence. Underwater and aerial productivity of an entire mature sporophyte was assessed by measuring carbon fluxes inside a closed chamber over successive incubations. Content in pigments (violaxanthin, antheraxanthin and zeaxanthin) involved in the xanthophyll cycle, the main photoprotection mechanism in this species (Rodrigues et al. 2002), was also assessed at different tidal stages. Hot, sunny days were selected in the middle of spring-tide periods in late spring and summer to follow the response of *L. digitata* to

extreme environmental changes. It was hypothesized that the development of the photoprotective mechanism highlighted for sporophytes in the mid part of the kelp zone are insufficient under the more drastic tidal conditions encountered by sporophytes in the upper part of the kelp zone and that irreversible photoinhibition, i.e. photodamages of the photosynthetic apparatus, may occur in *L. digitata*.

Materials and methods

Study site

The study was performed in the kelp zone of Roscoff (Western English Channel, Brittany, France, 48°43'53''N-3°59'16''W) dominated by *Laminaria digitata*. The mid part of this zone (about 0.5 m below chart datum) has previously been investigated (Delebecq et al. 2011). The present study focused on the upper part of the zone which emerges during spring tides (about 2 m above chart datum).

Environmental conditions

Bottom light and temperature conditions as well as the predicted water depth over the periods of measurements are given in Figure 1. The photosynthetically available radiation (PAR in μmol photons $m^{-2} s^{-1}$) and temperature were measured underwater and in air and recorded every minute using a WinCQ flat sensor (Alec Electronics) and an MDS MkV-T sensor (Alec Electronics) respectively. Water depth was calculated according to the tide level predictions obtained from the French Naval Hydrographic and Oceanographic Service (*Service Hydrographique et Océanographique de la Marine*; SHOM, data available online at http://maree.shom.fr), using the level of 2 m above chart datum as reference.

Net carbon production

Net carbon production (NP) of *L. digitata* was measured on an entire adult sporophyte isolated in a benthic chamber. A sporophyte (frond length of about 1 m) was carefully removed from the substrate rock and divers placed it inside a benthic chamber at the same shore level in the kelp zone to measure its net carbon production (i.e. the difference between gross primary production and respiration) *in situ*. The system for measurements under immersion has been fully described in Gévaert et al. (2011). The benthic chamber is made of a transparent closed Perspex dome tightly sealed on a polyvinylchloride (PVC) base (37 cm diameter) and encloses a volume of 35.3 L. An electronic management system controls three external pumps; two pumps ensuring the rapid and constant homogenization of the medium (with a flow of about 12 L min^{-1}) and the third ensuring the renewal of the medium by flushing ambient seawater between two consecutive incubations. pH was measured with a WTW sentix 41 probe (Multi 350i, WTW) and monitored every minute during 10 min incubations. At the beginning and at the end of incubations, seawater samples were collected from inside the benthic chamber using a 100 mL syringe, then passed through cellulose acetate membrane filters (0.8 μm) and spiked with $HgCl_2$. In the laboratory, total alkalinity of each sample was determined on three 20 mL subsamples using 0.01 N HCl potentiometric titration (Millero et al. 1993). The dissolved inorganic carbon (DIC) concentration of seawater was calculated from the pH, total alkalinity (TA), temperature and salinity according to Strickland & Parsons (1972) and using the formula given in Oviatt et al. (1986). The DIC flux (μmol h^{-1}) was calculated as the difference between the final and the initial concentrations, after checking the linearity of the pH change (corrected from temperature change). For measurements during emersion, the benthic chamber was connected to a closed air circuit (with a flow of about 2 L min^{-1}) for CO_2 analysis as described in Migné et al. (2002). Changes in air CO_2 concentration (ppm) were measured in the chamber with a infrared CO_2 gas analyzer (LiCor Li-6251) and recorded with a data logger (LiCor Li-1400) at 15 s intervals during incubations of about 10 min duration. The CO_2 flux (μmol h^{-1}) was calculated from the slope of CO_2 concentration against time assuming a molar volume of 22.4 L at standard temperature and pressure. Incubations were sufficiently short to avoid feedback of temperature and gas

concentration changes on metabolic rates and the benthic chamber was opened between two consecutive incubations to renew the ambient air. At the beginning and the end of a series of measurements and between consecutive incubations, the sporophyte was weighed to assess its water loss during the emersion period. Brought back to the laboratory, the sporophyte was rehydrated over night to assess its fresh weight (FW) and then dried for 48 h at 60°C to assess its dry weight (DW). DIC and CO_2 fluxes were then expressed as $\mu mol\ C\ g_{DW}^{-1}\ h^{-1}$. The relative water tissue content at the end of emersion was calculated as the percent of total water content [(W-DW)/(FW-DW)]x100 (where W represents the weight measured at the end of emersion).

Fluorescence properties

In vivo chlorophyll *a* fluorescence properties were measured *in situ* on the fronds of three other *L. digitata* sporophytes (frond length of about 1 m) haphazardly selected in the kelp bed at the same shore level and marked. The fluorescence signal was always taken from the middle of the frond at the same place using a submersible PAM fluorometer (Diving PAM, Walz). The effective quantum yield of photosystem II (Φ_{PSII}) was measured under ambient light. The optical fibers were mounted in a home-made transparent Plexiglas holder applied to one side of the thallus so that the distance between the fiber optics and the algal tissue was constant and standard. The optical fibers formed a 60° angle with the sample, avoiding shading or darkening. Φ_{PSII} was calculated as $(F_m'-F_t)/F_m'$ (Genty et al. 1989), where F_m' is the maximal fluorescence level measured during a single saturating light pulse (0.8 s) for light-adapted samples, and F_t is the fluorescence steady-state level immediately prior to the flash. Φ_{PSII} estimates the electron transport rate (ETR in $\mu mol\ electrons\ m^{-2}\ s^{-1}$, hereafter referred to as $\mu mol\ e^-\ m^{-2}\ s^{-1}$) as $\Phi_{PSII} \times PAR \times 0.5\ x\ A$, where PAR is the photosynthetically available radiation (in $\mu mol\ photons\ m^{-2}\ s^{-1}$); 0.5 is a correction factor based on the assumption that the incident photons are absorbed equally by the pigments of the two photosystems and A (= 0.96) is the absorption coefficient determined in the laboratory using an integrating sphere (ISR-240A, Shimadzu). The optimal quantum yield of PSII was calculated as $F_v/F_m = (F_m - F_0)/F_m$ (Genty et al.

1989), 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 (0.8 s). F_v/F_m assesses the extent of photoinhibition (Maxwell and Johnson 2000).

Pigment analysis

Thallus disc samples were taken from the marked *L. digitata* sporophytes for pigment analysis. Two discs (8 mm diameter) were sampled (with a cork borer) from the middle part of the frond and were immediately placed in the dark and frozen in liquid nitrogen until further analysis. Thallus discs were then first gently wiped to remove epiphytes, and pigments were extracted by grinding the discs 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 and pigments are found in the organic phase). The organic phase was then evaporated with nitrogen and dissolved again in 40 µL methanol for injection. Pigment analysis was performed using high performance liquid chromatography (HPLC) (Beckman, system Gold, 126) with a reverse-phase column (C 18 Allure, Restek). Briefly, 20 µL were injected and separation was carried out 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 of energy into heat (Bilger and Bjorkman 1990), was estimated by calculating the de-epoxidation ratio: DR = (A + Z) / (V + A + Z).

Investigation schedule

The photosynthesis of *L. digitata* was investigated *in situ* during alternations of immersion and emersion in spring (May) and summer (August, Table 1). On 18 May 2011, net production and chl *a* fluorescence were measured during immersion (during the ebb tide) and during emersion; thallus samples were taken at these two tidal stages for pigment analysis. On 11 August 2010, NP and fluorescence were measured during immersion (during ebb and flood tides) and during emersion; thallus samples were taken at these three tidal stages. Sporophytes were left lying in the field overnight to be used for subsequent measurements the following day. On 12 August 2010, NP and fluorescence were measured during immersion (during ebb and flood tides, respectively); thallus samples were taken at flood tide.

Results

Net carbon production

A decrease in carbon within the benthic chamber containing a *Laminaria digitata* sporophyte was observed during immersion occurring during the morning ebb tides on 18 May 2011 and 11 August 2010 (Figure 2 A & B), indicating carbon uptake (i.e. gross primary production greater than respiration). The increase in carbon, observed during emersion, indicated carbon release (i.e. gross primary production lower than respiration) which reached 2 µmol g_{DW}^{-1} h^{-1} on 18 May 2011 and 37 µmol g_{DW}^{-1} h^{-1} on 11 August 2010. On 11 August 2010, C-release continued to increase at re-immersion reaching 69 µmol g_{DW}^{-1} h^{-1} and then decreased during the flood tide, reaching 20 µmol g_{DW}^{-1} h^{-1} at the end of the day. The C-release observed at the beginning of the ebb tide the following morning decreased with increasing irradiance, then C-uptake was observed at noon (Figure 2C). The net production of the sporophyte increased with increasing light during the morning ebb tides on 18 May 2011 and 11 August 2010 but not on 12 August (Figure 3). The water tissue content of the sporophyte at the end of the emersion period was 89 and 88% of total water content in May and August, respectively. In August, bleaching was observed.

Fluorescence properties

The effective and the optimal quantum yields of photosystem II measured on three *L. digitata* sporophytes decreased at emersion (Figure 4 A & B) and recovered at re-immersion (Figure 4 B & C). The mean (± se) effective and optimal quantum yield reached values as low as 0.012 ± 0.002 and 0.175 ± 0.028, respectively, during emersion on 11 August 2010. The electron transport rate also decreased at emersion, and although it increased at the beginning of re-immersion, it did not recover during the flood tide due to low light conditions (Table 2).

De-epoxidation ratio

The de-epoxidation ratio (DR) increased at emersion, indicating that violaxanthin was converted into antheraxanthin and zeaxanthin, and decreased during the flood tide, antheraxanthin and zeaxanthin being reconverted into violaxanthin (Figure 5). The mean (± se) DR at emersion reached 0.72 ± 0.02 on 18 May 2011 and 0.69 ± 0.08 on 11 August 2010.

Discussion

A previous survey (Delebecq et al. 2011), performed in the mid part of this *Laminaria digitata* kelp zone, related the tidal pattern of photosynthetic performance to underwater light changes. The aim of the present survey, performed in the upper part of the zone, was to relate kelp photosynthetic performance to environmental changes occurring during the immersion/emersion cycle. In the mid part of the kelp zone, the photosynthetic performance decreases at low tide due to high light stress. A more drastic decrease was expected in the upper part of the zone due to emersion stress. However, results varied with the scale of observation (Table 3). At the cellular scale, the decrease in the electron transport rate (ETR) between the morning ebb tide and low tide was of the same order of magnitude (about 40%) in emerged sporophytes and in sporophytes that remained immersed in spring. In summer, the decrease was much greater (about 90%), but ETR remained relatively high (about 17 μmol e$^-$ m^{-2} s^{-1}) during emersion. At the individual sporophyte scale, NP was negative (i.e.

respiration was greater than gross primary production) at low tide in emerged sporophytes, but in sporophytes that remained immersed, NP was positive, decreasing only slightly (about 12% decrease) between the morning ebb tide and low tide. Furthermore, a relative recovery of ETR was observed at re-immersion in summer, whereas sporophyte NP remained negative during the flood tide and was positive, but very low, during the ebb tide of the following day. Such contrasting results in photosynthetic performance of macroalgae using either fluorescence (cellular scale) or carbon flux (sporophyte scale) have been already highlighted and discussed (Migné et al. 2015 and references therein). Fluorescence signals were measured in the middle of the frond, known to be the most photosynthetically efficient part of the sporophyte (Nitschke et al. 2011), and therefore do not represent the photosynthetic performance of the entire sporophyte. Furthermore, ETR is an appropriate measure of the rate of photosynthesis, as long as environmental stresses do not impose restrictions on photosynthetic carbon fixation. The critical values (< 0.1) of effective quantum yield of photosystem II measured on *L. digitata* at low tide prevented ETR from being used as a measure of photosynthetic rate (Beer and Axelsson 2004). Carbon flux measurements demonstrated that *L. digitata* sporophyte exposure to low tide during summer spring tides induces severe and chronic photoinhibition. C-release was particularly high at re-immersion, much higher than that measured at the end of the day or the following morning under very low light. This high negative value of NP indicates that the decrease in photosynthetic capacity comes with an increased respiration rate. This respiration likely reflects, at least in part, the energetic costs of photoprotection and photodamage repair.

The optimal quantum yield of photosystem II (F_v/F_m) is commonly used to assess stress, its decrease indicating a photoinhibition mechanism (Maxwell and Johnson 2000). In spring, the thalli were in a good physiological state in the morning, despite exposure to high light at low tide the previous days, as indicated by F_v/F_m values above 0.70 (Table 3). At low tide, and particularly under emersion, thalli were photoinhibited as indicated by the decrease in F_v/F_m. In summer, the morning value of 0.60

suggested a breakdown of resilience (see Gévaert et al. 2003) and the decrease under emersion was more severe, with F_v/F_m reaching a value as low as 0.18. Emerged individuals from the upper part of the *L. digitata* zone were not only exposed to high light, but also to a number of environmental factors contributing to emersion stress. Among these factors, temperature likely plays a leading role, as suggested by the increased respiration rate in summer. C-release reached a particularly high level at the onset of re-immersion and then decreased with decreasing water temperature during immersion with the afternoon flood tide (Spearman correlation: $r_s = 1$, n = 5, p < 0.01). High temperatures are known to exacerbate deleterious effects of high light and other Laminariales have shown enhanced photoinhibition at high temperatures (Bruhn and Gerard 1996; Terada et al. 2016; Borlongan et al. 2018). On 18 May 2011, sporophytes underwent a 7°C temperature increase at the onset of emersion and bottom temperature reached 25°C during the emersion period. On 11 August 2010, sporophytes underwent a 10°C temperature increase at the onset of emersion and bottom temperature reached 30.5°C during the emersion period (Figure 1). Furthermore, these dates were respectively the fourth and the third day of the spring-tide period during which the 2 m shore level emerged around midday and sporophytes likely experienced sudden temperature increases. Repeated photoinhibition on a daily basis may have had a cumulative effect. Laboratory experiments have shown that heat shocks, repeated over consecutive tide cycles, impair the photosystem II function of young individuals of *Laminaria ochroleuca* (Pereira et al. 2015) -a species co-occurring in the study kelp stand- as well as in excised discs of tissue from *L. ochroleuca* and *L. digitata* (King et al. 2018). Simulating heat shocks during a summer spring tide cycle, King et al. (2018) suggested a decrease in resistance and resilience of *L. digitata* photophysiology to consecutive low tides (in samples exposed to air for 1 h at 32°C for four days, F_v/F_m decreased by 60% and did not recover in the following days). Here, our survey confirms that, under the field conditions experienced by entire sporophytes, *L. digitata* exhibits low tolerance to consecutive aerial exposures during a period of summer spring tides in an area where low tide coincides with noon.

L. digitata is known to respond to photoinhibitory treatments by displaying violaxanthin de-epoxidation (via the xanthophyll cycle) as a mechanism preventing photodamage both at sporophyte (Rodrigues et al. 2002) and gametophyte stages (Delebecq et al. 2016). Because violaxanthin de-epoxidation can be activated within minutes, this protection mechanism of the photosynthetic apparatus may be particularly important during abrupt changes giving rise to potentially stressful conditions (Koch et al. 2016), which typically occur in the intertidal zone. In the present survey, particularly high DR values were measured on emerged *L. digitata* sporophytes. They reached 0.70 (but were limited to 0.40 on immersed sporophytes at low tide, Delebecq et al. 2011), indicating the effectiveness of this mechanism. The slight decrease observed during the emersion period in May can be attributed to the loss of about 7% of sporophyte water content between the two measurements. A decline in DR has indeed been reported during desiccation of *Saccharina latissima* sporophytes exposed to air in laboratory experiments (Harker et al. 1999). Nevertheless, this mechanism appeared to be insufficient to prevent photodamage because NP remained negative at re-immersion and because bleaching of sporophytes was observed in summer. Such damage is not likely to result from the exposure to ultraviolet radiation, because *L. digitata* adult thalli are expected to be protected by their thickness and optical properties (Roleda et al. 2006; Gruber et al. 2011). The mechanism of photoinhibition and recovery of photosynthesis involves enzymatic steps and, thus, depends on temperature; high temperatures encountered during emersion may enhance the protective mechanism, but disrupt repair processes, as experimentally demonstrated on young *S. latissima* sporophytes (Bruhn and Gerard 1996).

Given that the present survey was performed *in situ* and combined complementary techniques, results provided an integrated overview of the photosynthetic response of a macroalga to extreme changes inherent to the intertidal environment (Tait et al. 2017). Such knowledge is of particular importance for foundation species, which greatly influence communities and ecological processes. We clearly demonstrated that *L. digitata* has low tolerance to consecutive aerial exposure during

high summer temperatures. Such cumulative effects of aerial exposure encountered during spring tidal cycles have been largely ignored in intertidal stress studies, but should be factored into causal models built to predict the impact of warming in species distributions (King et al. 2018). The decline in the spatial extent of *L. digitata* along the coast of France in recent years has been mainly attributed to the increasing sea temperatures and the species is predicted to disappear from the English Channel and the southern part of the North Sea in the near future (Raybaud et al. 2013). In this range limit of the species, the negative impact of high summer temperatures has already been shown on its reproduction (Bartsch et al. 2013) and growth (Hargrave et al. 2017). The present study highlights the adverse effect of high summer temperatures on its photophysiology, further confirming the forecasted detrimental effects of warming events on this marginal population. Local extinction of a foundation species is likely to alter the whole ecosystem, unless a similar species fulfils its function. The decline of the perennial kelp *L. digitata* may benefit the expansion of the annual kelp *Sacchoriza polyschides*, its main competitor in the study area (Engelen et al. 2011; Araujo et al. 2016). This difference in life cycle may promote shift from a stable community to an unstable community. The loss of this key ecosystem engineer has the potential to significantly affect the functioning of rocky shores in the English Channel and North Sea.

Conflict of interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article.

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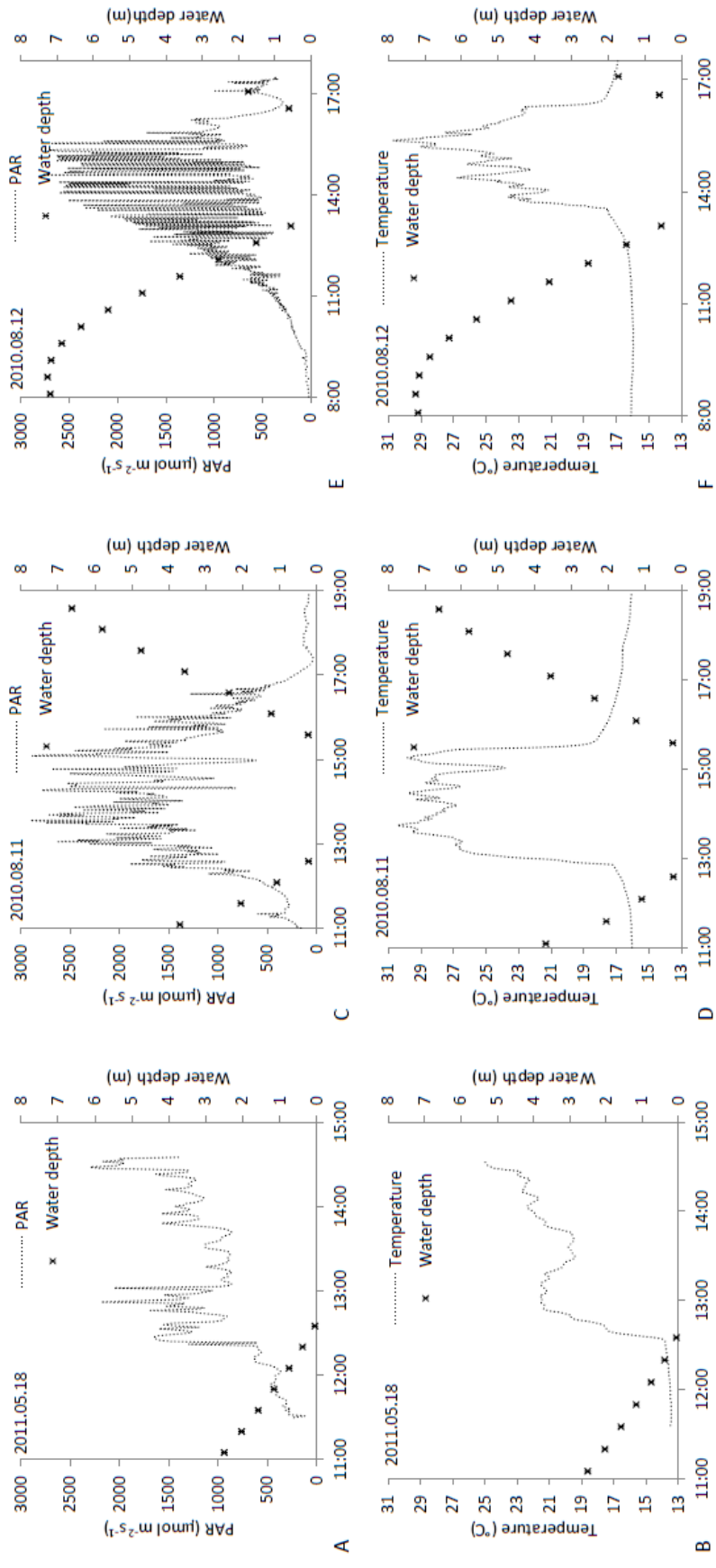
Figure 1: Time course (local time) of bottom light (as photosynthetically available radiation, PAR, A, C & E), temperature (B, D & F) and water depth (crosses) in the upper part of the *Laminaria digitata* zone of Roscoff during the tides occurring on 18 May 2011 (A & B), 11 August 2010 (C & D) and 12 August 2010 (E & F)
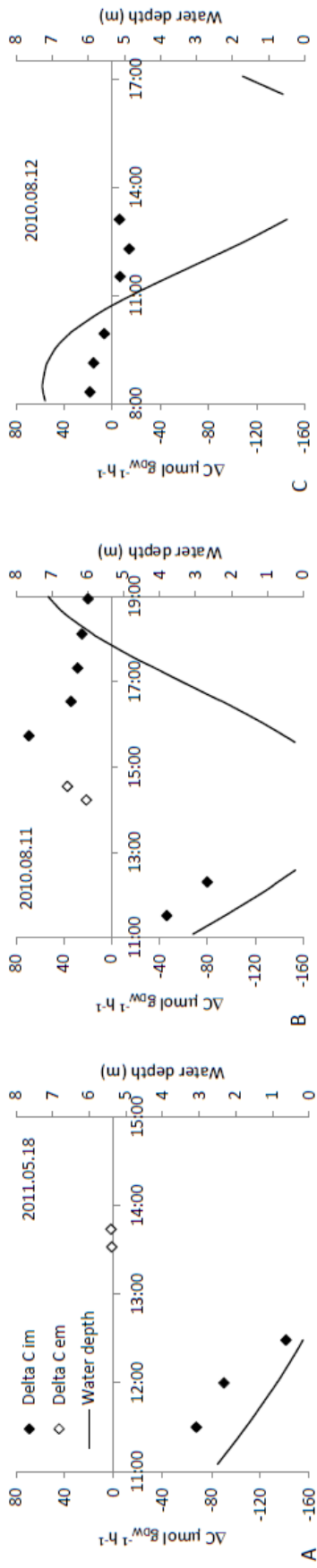
Figure 2: Time course (local time) of carbon flux (Delta C) in the benthic chamber containing an entire sporophyte either during immersion (im) or emersion (em) and water depth in the upper part of the *Laminaria digitata* zone of Roscoff during the tides occurring on 18 May 2011 (A), 11 August 2010 (B) and 12 August 2010 (C). Negative values of ΔC indicate C-uptake by the sporophyte, i.e. gross primary production is greater than respiration. Positive values of ΔC indicate C-release by the sporophyte, i.e. gross primary production is lower than respiration.
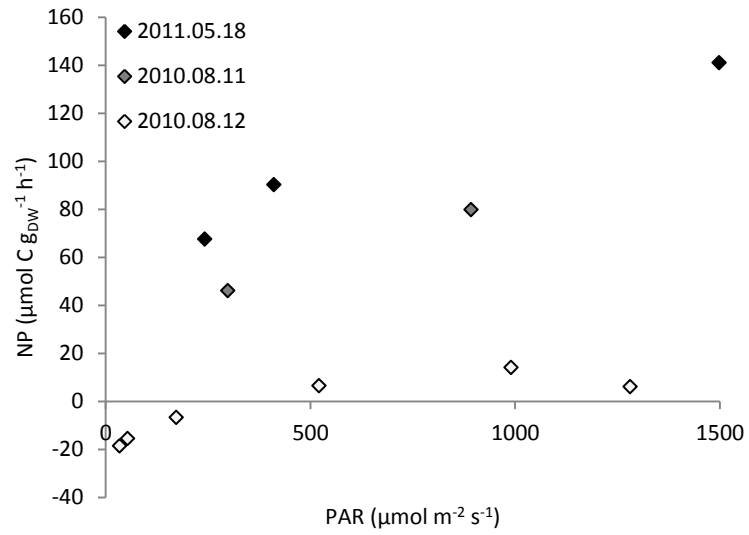
Figure 3: Net production (NP) of a sporophyte in the upper part of the *Laminaria digitata* zone of

Roscoff according to the bottom light (PAR) during the morning ebb tides occurring on 18 May 2011,
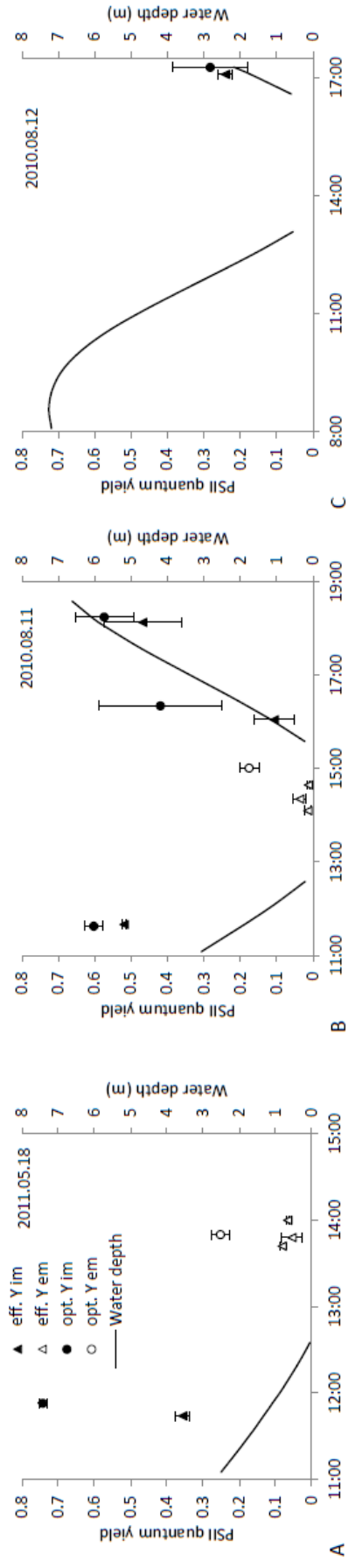
11 August 2010 and 12 August 2010.

Figure 4: Time course (local time) of mean (± se) effective (eff.) and optimal (opt.) quantum yields of photosystem II (Y) measured on three sporophytes either during immersion (im) or emersion (em) and water depth in the upper part of the *Laminaria digitata* zone of Roscoff during the tides occurring on 18 May 2011 (A), 11 August 2010 (B) and 12 August 2010 (C).

Figure 5: Time course (local time) of the mean (± se) de-epoxidation ratio (DR) measured on three sporophytes either during immersion (im) or emersion (em) and water depth (WD) in the upper part of the *Laminaria digitata* zone of Roscoff during the tides occurring on 18 May 2011 (A), 11 August 2010 (B) and 12 August 2010 (C).
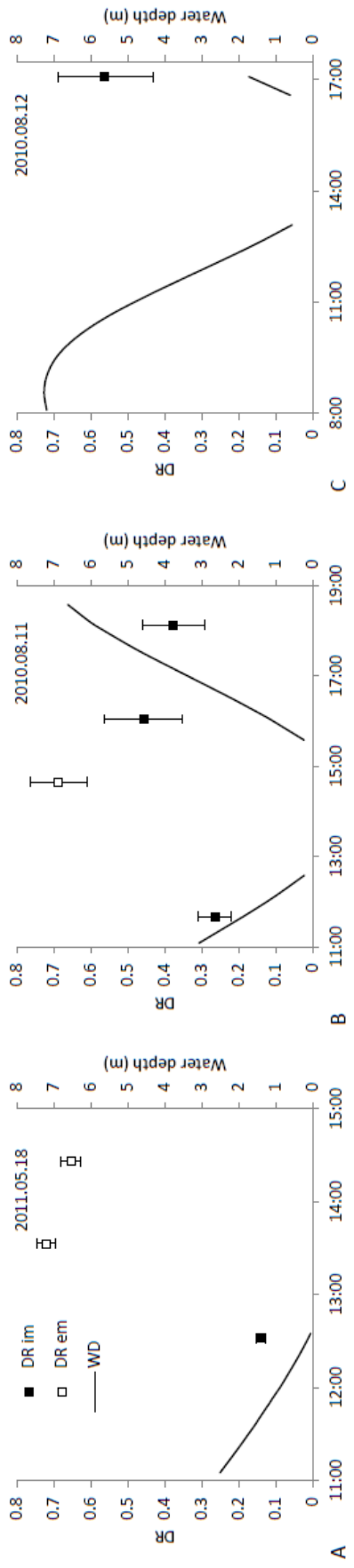
Table 1: Investigation schedule of the photosynthesis of sporophytes in the upper part of the *Laminaria digitata* zone of Roscoff, giving the periods of net production (NP) and fluorescence (Fluo) measurements and sampling for pigment analysis (Pig) during the tidal cycle on each date (local time)

Date		2011.05.18			2010.08.11			2010.08.12	
Low tide time		13:48			14:07			14:52	
Low tide height (m) above chart datum		1.22			0.84			0.87	
2 m emersion period		12:40-14:45			12:45-15:25			13:25-16:15	
Measurement period		11:00-15:00			11:00-19:00			8:00-17:30	
	NP	Fluo	Pig	NP	Fluo	Pig	NP	Fluo	Pig
Ebb tide	x	x	x	x	x	x	x		
Low tide (emersion)	x	x	x	x	x	x			
Flood time				x	x	x		x	x

Table 2: Mean (± se) electron transport rate (ETR) in µmol e$^-$ m^{-2} s^{-1} calculated from the effective quantum yields measured on three sporophytes at different tidal stages (see Figure 4), as well as corresponding water depth (WD in m) and bottom light (photosynthetically available radiation, PAR, in µmol m^{-2} s^{-1}), in the upper part of the *Laminaria digitata* zone of Roscoff on 18 May 2011, 11 August 2010 and 12 August 2010.

	18 May 2011			11 August 2010			12 August 2010		
	WD	PAR	ETR	WD	PAR	ETR	WD	PAR	ETR
Ebb tide	1.3	360	61.5 ± 3.4	1.9	675	168.4 ± 11.5			
Low tide (emersion)	0.0	870	33.7 ± 2.9	0.0	1680	10.7 ± 6.7			
	0.0	1575	37.7 ± 19.0	0.0	1935	24.1 ± 5.1			
	0.0	1440	45.6 ± 3.5	0.0	2870	17.0 ± 3.3			
Flood tide				1.2	965	49.7 ± 25.9	1.7	475	55.0 ± 5.5
				6.0	110	24.3 ± 5.6			

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Table 3: Water depth (WD in m), bottom light (photosynthetically available radiation, PAR, in $\mu mol\ m^{-2}\ s^{-1}$), temperature (T in °C), electron transport rate (ETR in $\mu mol\ e^-\ m^{-2}\ s^{-1}$), net primary production (NP in $\mu mol\ O_2\ g_{DW}^{-1}\ h^{-1}$ or $\mu mol\ C\ g_{DW}^{-1}\ h^{-1}$) and optimal quantum yield of PSII (F_v/F_m) measured on *Laminaria digitata* sporophytes at different tidal stages (morning ebb tide MET, midday low tide MdLT, afternoon flood tide AFT) in a previous survey (06 May 2008, Delebecq et al 2011) and in the present survey (18 May 2011 and 11 August 2010) using the same measurement devices. The PAR value is the one used for ETR calculation, data from Delebecq et al 2011 were converted using the relationship obtained from the present survey ($PAR_{\text{flat sensor}} = 0.80\ PAR_{\text{spherical sensor}}$). Net primary production was measured as $\mu mol\ O_2\ g_{FW}^{-1}\ h^{-1}$ in the Delebecq et al 2011 study and was expressed as $\mu mol\ O_2\ g_{DW}^{-1}\ h^{-1}$ considering that DW = 0.16 FW as observed in the present study.

| | 2008.05.06 (Delebecq et al 2011) | | | | | | 2011.05.18 | | | | | | 2010.08.11 | | | | | |
	WD	PAR	T	ETR	NP (O₂)	F_v/F_m	WD	PAR	T	ETR	NP (C)	F_v/F_m	WD	PAR	T	ETR	NP (C)	F_v/F_m
MET	5.5	280	12.3	38	78	0.73	1.3	360	13.5	62	90	0.74	1.9	676	16.1	168	46	0.60
MdLT	1.9	800	13.9	23	69	0.35	0.0	1575	20.5	38	-2	0.25	0.0	2870	27.4	17	-37	0.18
AFT	8.9	80	12.3	10		0.65							1.2	963	17.5	50	-34	0.42
													6.0	110	16.3	24	-25	0.57