Photosynthesis of Laminaria digitata over the immersion and emersion alternation of spring tides under sunny and hot weather

Aline Migné, Gaspard Delebecq, Dominique Davoult, Nicolas Spilmont, Dominique Menu, Marie-Andrée Janquin, François Gevaert

Photosynthesis of *Laminaria digitata* over the immersion and emersion alternation of spring tides under sunny and hot weather

Aline Migné[1,*], Gaspard Delebecq[2], Dominique Davoult[1], Nicolas Spilmont[2,3], Dominique Menu[2], Marie-Andrée Janquin[2] and François Gévaert[2]

[1]Sorbonne Université, CNRS, UMR ADMM, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France

[2]Univ. Lille, Univ. Littoral Côte d'Opale, CNRS, UMR LOG, Laboratoire d'Océanologie et de Géosciences, 62930 Wimereux, France

[3]Environmental Futures Research Institute, Griffith University, Gold Coast Campus, QLD 4222, Australia

*Corresponding author (migne@sb-roscoff.fr)

Abstract

Photosynthesis of *Laminaria digitata* sporophytes was surveyed *in situ* throughout spring and summer tidal cycles including emersion periods around midday. Photosynthetic performance of thalli was assessed through pulse-amplitude modulated (PAM) fluorescence parameters, the effective (Φ_{PSII}) and optimal (F_v/F_m) quantum yields of photosystem II. Net production (NP) of entire individuals, the balance between their gross primary production and respiration, was assessed by measuring the carbon fluxes inside a closed chamber. Thalli content in pigments involved in the xanthophyll cycle, a photoprotective mechanism, was measured to evaluate the de-epoxidation ratio (DR). Φ_{PSII} decreased at emersion (down to 0.01) and recovered under afternoon immersion. NP shifted from positive values (reaching 140 µmol C g_{DW}^{-1} h^{-1}) under morning immersion to negative values under emersion (reaching -37 µmol C g_{DW}^{-1} h^{-1}) but did not recover under following immersion

when respiration was exacerbated. F_v/F_m decreased at emersion (down to 0.18) indicating a severe photoinhibition. High DR (up to 0.70) showed the effectiveness of the photoprotective mechanism which appeared nevertheless insufficient to prevent photodamages under emersion stress. Among environmental factors contributing to this emersion stress, heat shocks repeated over consecutive tide cycles were suspected to play a leading role. This reinforces the expectation of detrimental effect 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 algae of 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 to terrestrial rain forest (Mann 1973). The physiological characteristics of kelps have received substantial attention, especially with respect to the role of environmental factors to explain their biogeography and zonation patterns, and the ecophysiology of the most common Laminariales in the northern Hemisphere has been widely investigated (reviewed by Bartsch et al. 2008). While earlier studies mainly addressed performance of sporophytes by weight, length or area increase and thus growth, more recent studies have often used photosynthetic activity as an indicator of physiological performance. Nevertheless, the photosynthetic performance of sporophytes has been mainly studied under controlled laboratory conditions and multifactorial experiments have rarely been conducted. Generalization to field conditions remained thus hazardous. The development of field fluorimeter has allowed the *in situ*

survey of photosynthetic performance of macroalgae in response to the combined effects of all environmental parameters. For example, a tidal pattern of photosynthetic performance was shown in *Saccharina latissima* growing in the upper subtidal in the south-western English Channel (Gévaert et al. 2003).

In the south-western English Channel, the low intertidal and very upper subtidal of moderately exposed shores are dominated by *Laminaria digitata*. This boreal species approaches there its trailing edge which is expected to shift northward in the context of the global warming (Raybaud et al. 2013). To understand how *L. digitata* copes with the highly variable environmental conditions that characterize its habitat, exploring its physiological behaviour *in situ* appears crucial. In the south-western English Channel, low spring tides occur around noon and *L. digitata* can then be exposed to over-saturating irradiances. A previous survey allowed to relate the pattern of photosynthetic performance of *L. digitata* sporophytes to changes in underwater light during spring tides in the mid part of the kelp belt which remained underwater at low tide (Delebecq et al. 2011). *L. digitata* exhibited photoinhibition at low tide but, owing to the development of a photoprotective mechanism, totally recovered photosynthetic performance during the following flood tide. Patterns of photosynthetic performance could however be very different in the uppermost part of the belt which emerges at low tide (Hanelt 1996). Indeed, during emersion periods, sporophytes are likely to be exposed to stronger light stress than during immersion when the water column protects them by decreasing the intensity of irradiance. The water column also changes the spectra of irradiance, absorbing ultraviolet radiation (UVR) notably, which can influence the photophysiology of algae. For example, *L. digitata* germlings exposed to UVR (supplemented in the middle of the light phase) exhibited a reduced growth and DNA damage (Roleda et al. 2006). Furthermore, at emersion light stress combines to water and nutrient depletion as well as to rapid changes in temperature. High temperatures have been shown to alter the photosynthetic performance and to induce photoinhibition in *L. digitata* gametophytes (Delebecq et al. 2016) and in other temperate Laminariale sporophytes (Terada et al. 2016; Borlongan et al. 2018) and also to disrupt repair

processes in *S. latissima* sporophytes (Bruhn and Gerard 1996). To date, this has not been evaluated *in situ* under emersion but bleaching, a symptom of desiccation or temperature stress, has been notified in intertidal *L. digitata* populations during periods of high temperatures (Hargrave et al. 2017).

The present study aimed at describing the *in situ* dynamic of the photosynthetic performance of *Laminaria digitata* sporophytes, throughout a whole tidal cycle including an emersion period. To obtain a full picture of the response of *L. digitata* to its environment, the survey of the photosynthetic activity of thalli using pulse-amplitude modulated (PAM) fluorescence was combined with carbon exchange measurements. The underwater and aerial productivity of an entire mature sporophyte was assessed by measuring the carbon fluxes inside a closed chamber over successive incubations. Pigments involved in the xanthophyll cycle, the main mechanism of photoprotection of this species (Rodrigues et al. 2002), were also measured out. Sunny and hot days were selected in the middle of spring-tide periods of late spring and summer to follow the response of *L. digitata* to very sharp environmental changes. It was hypothesized that the development of the photoprotective mechanism highlighted for sporophytes in the mid part of the kelp belt could be insufficient under the more drastic tidal changes encountered by sporophytes in the upper part of the kelp belt and that irreversible photoinhibition, i.e. photodamages of the photosynthetic apparatus, could occur in *L. digitata*.

Materials and methods

Study site and measurement schedule

The photosynthesis of *Laminaria digitata* was investigated *in situ* in the upper part of the kelp belt (about 2 m above chart datum) of Roscoff (Western English Channel, Brittany, France, 48°43’53’’N-3°59’16’’W) over alternations of immersion and emersion in spring and summer. Net carbon production (NP) was measured on an entire sporophyte isolated in a benthic chamber while *in vivo*

chlorophyll *a* fluorescence properties were measured on the fronds of three other sporophytes at different tidal stages. Thallus disc samples were also taken at these tidal stages for pigment analysis. On the 18th of May 2011 (low tide: 1.22 m at 1:48pm local time), the 2 m level emerged from 0:40pm to 2:45pm and measurements were performed from 11am to 3pm; NP was measured during 5 successive incubations (3 under immersion and 2 under emersion), Chl*a* fluorescence was measured and thallus samples were taken at two tidal stages (under immersion at about mid-level of ebb tide and under emersion). On the 11th of August 2010 (low tide: 0.84 m at 2:07pm), the 2 m level emerged from 0:45pm to 3:25pm and measurements were performed from 11am to 7pm; NCP was measured during 9 successive incubations (2 under immersion, 2 under emersion and 5 under immersion), Chl*a* fluorescence was measured and thallus samples were taken at four tidal stages (under immersion at about mid-level of the ebb tide, under emersion, at the beginning of re-immersion and at about mid-level of the flood tide). Sporophytes were left standing in the field over night to be investigated again the following day. On the 12th of August 2010 (low tide: 0.67 m at 2:52pm), the 2m level emerged from 1:25pm to 4:15pm and measurements were performed from 8am to 5:30pm; NP was measured during 6 successive incubations over the ebbing tide (under immersion), Chl*a* fluorescence was measured and thallus samples were taken at the beginning of re-immersion.

Environmental conditions

Bottom light and temperature conditions as well as the predicted tide level over the periods of measurements are given in figure 1. The photosynthetically available radiation (PAR in µmol photons m^{-2} s^{-1}) and the temperature were measured underwater and in air and recorded every minute using a WinCQ flat sensor (Alec Electronics) and a MDS MkV-T sensor (Alec Electronics) respectively. Tide level predictions were obtained from the Service Hydrographique et Océanographique de la Marine (SHOM, data available online at http://maree.shom.fr).

Net carbon production

A *Laminaria digitata* adult sporophyte (frond length of about 1 m) from the upper part of the kelp belt was placed by divers inside a benthic chamber, on the shore at the collection site, to measure its net carbon production (i.e. the balance between gross primary production and respiration). The benthic chamber was made of a transparent closed Perspex dome tightly sealed on a polyvinylchloride (PVC) base (37 cm diameter) and enclosed a volume of 35.3 L. During immersion periods, an electronic management system controlled three external pumps; two pumps ensured the rapid and constant homogenization of the media while the third one ensured the renewal of the media by flushing ambient seawater between two consecutive incubations (Gévaert et al. 2011). 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 the linearity of the pH change (corrected from temperature change) has been checked. During emersion, inorganic carbon fluxes were measured in the benthic chamber using a closed air circuit for CO_2 analysis (Migné et al. 2002). Changes in air CO_2 concentration (ppm) were measured in the chamber with a CO_2 infrared gas analyser (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. The benthic chamber was opened between two consecutive incubations to renew the ambient air. The sporophyte was weighted between two consecutive incubations and at the beginning and end of a series of measurements to assess its water loss during the emersion period. Taken back to the laboratory, it

was rehydrated for one 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 µmol 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)]*100 (where W represents the weight measured at the end of emersion).

Fluorescence properties

In vivo chlorophyll fluorescence properties were measured using a submersible PAM fluorometer (Diving PAM, Walz) on three *Laminaria digitata* sporophytes (frond length of about 1 m) haphazardly-selected and marked. The fluorescence signal was always taken from the middle of the frond in the same place. The effective quantum yield of photosystem II (Φ_{PSII}) was measured under ambient light. The fiberoptics were mounted in a home-made transparent Plexiglas holder applied to one side of the thallus in such a way that the distance between the fiberoptics and the algal tissue was constant and standard. The fiberoptics form 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} allows to estimate the electron transport rate (ETR in µmol electrons m^{-2} s^{-1}, here after referred to as µmol e^- m^{-2} s^{-1}) as Φ_{PSII} × PAR × 0.5 x A, where PAR is the photosynthetically available radiation (in µmol 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 allows to assess the extent of photoinhibition (Maxwell and Johnson 2000).

Pigment analysis

Two discs (8 mm diameter) were taken (with a cork borer) from the mid part of the frond of each *Laminaria digitata* sporophyte used for fluorescence measurements and were immediately darkened and frozen in liquid nitrogen until further analysis. 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 dissolved again 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 and Bjorkman 1990), was estimated by calculating the De-epoxidation Ratio: DR = (A + Z) / (V + A + Z).

Results

Net carbon production

The carbon flux inside the benthic chamber containing a *Laminaria digitata* sporophyte was negative, indicating a carbon uptake (i.e. gross primary production greater than respiration), under immersion during morning ebb tides of the 18th May 2011 and of the 11th August 2010 (Figure 2 A & B). The C uptake increased with increasing light, reaching 140 µmol g_{DW}^{-1} h^{-1} for a mean PAR during incubation

of 1498 µmol m^{-2} s^{-1} the 18th of May 2011 and 80 µmol g_{DW}^{-1} h^{-1} for a mean PAR of 905 µmol m^{-2} s^{-1} the 11th of August 2010. Under emersion, the carbon flux was positive, indicating a carbon release (i.e. respiration greater than gross primary production). The C release reached 2 µmol g_{DW}^{-1} h^{-1} the 18th of May 2011 and 37 µmol g_{DW}^{-1} h^{-1} the 11th of August 2010. At this date it continued to increase at re-immersion reaching 69 µmol g_{DW}^{-1} h^{-1} and then decreased over flooding reaching 20 µmol g_{DW}^{-1} h^{-1} at the end of the day. The carbon flux remained positive the following morning (Figure 2C), decreased with increasing irradiance and became negative but C uptake reached only 14 µmol g_{DW}^{-1} h^{-1} for a mean PAR of 990 µmol m^{-2} s^{-1} at noon. 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 *Laminaria digitata* sporophytes decreased at emersion (Figure 3 A & B) and recovered at re- immersion (Figure 3 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 the emersion of the 11th of August 2010. The electron transport rate also decreased at emersion, re-increased at the beginning of re-immersion, but did not recover during flood tide due to low light conditions (Table 1).

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 4). The mean (± se) DR at emersion reached 0.72 ± 0.02 the 18th of May 2011 and 0.69 ± 0.08 the 11th of August 2010.

Discussion

The previous survey, performed in the mid part of the kelp belt of *Laminaria digitata*, allowed to relate the tidal pattern of the kelp photosynthetic performance to underwater light changes (Delebecq et al. 2011). The present survey, performed in the uppermost part of the belt, should allow relating the kelp photosynthetic performance to environmental changes occurring over the immersion and emersion alternation. In the mid part of the kelp belt, the photosynthetic performance was shown to decrease at low tide due to high light stress. A more drastic decrease was expected in the upper part of the belt due to emersion stress. Contrasting results were nevertheless obtained according to the scale of observation (Table 2). At the cellular scale, the decrease in electron transport rate between morning ebb tide and low tide was of the same order of magnitude (about 40%) in sporophytes that emerged and in sporophytes that remained immersed in spring. In summer, the decrease was much greater (about 90%) but ETR remained to a relatively high value (about 17 µmol e^{-} m^{-2} s^{-1}) under emersion. At the sporophyte scale, the net primary production was negative (i.e. respiration was greater than gross primary production) at low tide in sporophytes that emerged while it was positive, and only slightly reduced (about 12 % decrease) as compared to the morning ebb tide, in sporophytes that remained immersed. Furthermore, a relative recovery of ETR was observed at re-immersion in summer while the net production of the sporophyte remained negative over the flooding 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 did not indicate an integrated status of the photosynthetic performance of the entire sporophyte. Furthermore, the electron transport rate is a relevant 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 prevent ETR to be used as a measure of photosynthetic rate (Beer and Axelsson

2004). Carbon flux measurements demonstrated that low tide exposures experienced by *L. digitata* sporophytes during summer spring tides induced severe and chronic photoinhibition. A particularly high C-release was measured at re-immersion. It was much higher than the one measured at the end of the day or the following morning under very low light. This indicates that the decrease of photosynthetic capacity was related to an increased respiration rate. The increased respiration rate should reflect, at least in part, the energetic costs of photoprotection and of repair of photodamages.

The optimal quantum yield of photosystem II (F_v/F_m) is commonly used to assess stress, its decrease indicating the phenomenon of photoinhibition (Maxwell and Johnson 2000). The morning values above 0.70 in spring (Table 2) indicated the good physiological states of the thalli, despite exposure to high light at low tide the previous days, while the morning value of 0.60 in summer suggested a breakdown of resilience (Gévaert et al. 2003). F_v/F_m decreased at low tide, reaching a value as low as 0.18 under summer emersion. At low tide, emerged individuals from the upper part of the *L. digitata* belt were not only exposed to high light but to a number of environmental factors contributing to emersion stress. Among these factors, the temperature was likely to play a leading role as suggested by the increased respiration rate in summer. The C release reached a particularly high level at the beginning of re-immersion and then decreased with decreasing water temperature over afternoon immersion (Spearman correlation: $r_s = 1$, $n = 5$, $p < 0.01$). High temperatures are known to exacerbate deleterious effects of high light and enhancement of photoinhibition at high temperatures has been evidenced for other Laminariales (Bruhn and Gerard 1996; Terada et al. 2016; Borlongan et al. 2018). On the 18th of May 2011, the sporophytes underwent a 7°C temperature increase at the onset of emersion and the bottom temperature reached 25°C during the emersion period. On the 11th of August 2010, the sporophytes underwent a 10°C temperature increase at the onset of emersion and the bottom temperature reached 30.5°C during the emersion period (Figure 1). Furthermore, it was respectively the fourth and the third day of the spring-tide period during which the 2 m shore level emerged around midday and during which sporophytes should have experienced such a sudden

temperature increase. Photoinhibition repeated on a daily basis might have had a cumulative effect. Laboratory experiments have shown that heat shocks, repeated over consecutive tide cycles, impaired the photosystem II function of young individuals of *Laminaria ochroleuca,* a species co-occurring in the study kelp stand, (Pereira et al. 2015) and of excised discs of tissue of *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 of resistance and of resilience of *L. digitata* photophysiology to consecutive low tides (in samples exposed to air for 1h at 32°C for four days, F_v/F_m decreased of 60% and did not recover in the following days). The present 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.

Laminaria 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 stage (Rodrigues et al. 2002) and gametophyte stage (Delebecq et al. 2016). Since violaxanthin de-epoxidation can be activated within minutes, this protection mechanism of the photosynthetic apparatus might be especially important during abrupt changes to potentially stressful conditions (Koch et al. 2016), which occur in the intertidal. In the present survey, particularly high de-epoxidation ratios were measured on emerged *L. digitata* sporophytes. They reached 0.70 (while they were limited to 0.40 on immerged sporophytes at low tide, Delebecq et al. 2011) indicating the effectiveness of this mechanism. The slight decrease observed over the emersion of May could be explained by the loss of about 7% water content of sporophytes between the two measurements. A decline in de-epoxidation ratio has indeed been shown upon desiccation of *Saccharina latissima* sporophytes exposed to air in laboratory experiments (Harker et al. 1999). This mechanism appeared to be nevertheless insufficient to prevent photodamage as net production remained negative at re-immersion and bleaching of sporophytes was observed in summer. Such damage was not likely to result from the exposure to ultraviolet radiations as *Laminaria 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 could have enhance protective mechanism but disrupt repair processes, as experimentally evidenced on young *S. latissima* sporophytes (Bruhn and Gerard 1996).

The decline in spatial extent of *Laminaria digitata* along the coast of France in recent years has been attributed to the increasing sea temperatures and the species is predicted to disappear from the English Channel and the south part of the North Sea in the near future (Raybaud et al. 2013). In this trailing edge of the species, negative impact of 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 summer high temperatures on its photophysiology reinforcing the expectation of detrimental effect of warming events on this marginal population.

Acknowledgements

This work was funded by the French program EC2CO/INSU. Thanks are due to Renaud Michel and Lucie Camañez for assistance in the field. We also thank the Service Mer & Observation from the Station Biologique de Roscoff, and particularly François Le Ven, for valuable support.

Conflict of interest disclosure

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

References

Arsalane W, Rousseau B, Duval JC (1994) Influence of the pool size of the xantophyll cycle on the effect of light stress in a Diatom: competition between photoprotection and photoinhibition. Photochemistry and Photobiology 60: 237-243 10.1111/j.1751-1097.1994.tb05097.x

Bartsch I, Vogt J, Pehlke C, Hanelt D (2013) Prevailing sea surface temperatures inhibit summer reproduction of the kelp *Laminaria digitata* at Helgoland (North Sea). Journal of Phycology 49: 1061-1073 10.1111/jpy.12125

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: recent insights and developments. European Journal of Phycology 43: 1-86 10.1080/09670260701711376

Beer S, Axelsson L (2004) Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. European Journal of Phycology 39: 1-7

Bilger W, Bjorkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurement in the light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynthesis Research 25: 173-185 10.1007/bf00033159

Borlongan IA, Matsumoto K, Nakazaki Y, Shimada N, Kozono J, Nishihara GN, Shimada S, Watanabe Y, Terada R (2018) Photosynthetic activity of two life history stages of *Costaria costata* (Laminariales, Phaeophyceae) in response to PAR and temperature gradient. Phycologia 57: 159-168 10.2216/17-70.1

Bruhn J, Gerard VA (1996) Photoinhibition and recovery of the kelp *Laminaria saccharina* at optimal and superoptimal temperatures. Marine Biology 125: 639-648 10.1007/bf00349245

Delebecq G, Davoult D, Janquin MA, Oppliger LV, Menu D, Dauvin JC, Gevaert F (2016) Photosynthetic response to light and temperature in *Laminaria digitata* gametophytes from two French populations. European Journal of Phycology 51: 71-82 10.1080/09670262.2015.1104556

Delebecq G, Davoult D, Menu D, Janquin M-A, Migné A, Dauvin J-C, Gévaert F (2011) *In situ* photosynthetic performance of *Laminaria digitata* (Phaeophyceae) during spring tides in Northern Brittany. Cahiers de Biologie Marine 52: 405-414

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 990: 87-92

Gévaert F, Créach A, Davoult D, Migné A, Levavasseur G, Arzel P, Holl A-C, Lemoine Y (2003) *Laminaria saccharina* photosynthesis measured *in situ*: photoinhibition and xanthophyll cycle during a tidal cycle. Marine Ecology Progress Series 247: 43-50

Gévaert F, Delebecq G, Menu D, Brutier L (2011) A fully automated system for measurements of photosynthetic oxygen exchange under immersed conditions: an example of its use in *Laminaria digitata* (Heterokontophyta: Phaeophyceae). Limnology and Oceanography-Methods 9: 361-379 10.4319/lom.2011.9.361

Gruber A, Roleda MY, Bartsch I, Hanelt D, Wiencke C (2011) Sporogenesis under ultraviolet radiation in *Laminaria digitata* (Phaeophyceae) reveals protection of photosensitive meiospores within soral tissue: physiological and anatomical evidence. Journal of Phycology 47: 603-614 10.1111/j.1529-8817.2011.00998.x

Hanelt D (1996) Photoinhibition of photosynthesis in marine macroalgae. Scientia Marina 60: 243-248

Hargrave MS, Foggo A, Pessarrodona A, Smale DA (2017) The effects of warming on the ecophysiology of two co-existing kelp species with contrasting distributions. Oecologia 183: 531-543 10.1007/s00442-016-3776-1

Harker M, Berkaloff C, Lemoine Y, Britton G, Young AJ, Duval JC, Rmiki NE, Rousseau B (1999) Effects of high light and desiccation on the operation of the xanthophyll cycle in two marine brown algae. European Journal of Phycology 34: 35-42 10.1017/s0967026299001924

King NG, Wilcockson DC, Webster R, Smale DA, Hoelters LS, Moore PJ (2018) Cumulative stress restricts niche filling potential of habitat-forming kelps in a future climate. Functional Ecology 32: 288-299 10.1111/1365-2435.12977

Koch K, Thiel M, Hagen W, Graeve M, Gomez I, Jofre D, Hofmann LC, Tala F, Bischof K (2016) Short- and long-term acclimation patterns of the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae) along a depth gradient. Journal of Phycology 52: 260-273 10.1111/jpy.12394

Mann KH (1973) Seaweeds: their productivity and strategy for growth. Science 182: 975-981 10.1126/science.182.4116.975

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51: 659-668 10.1093/jexbot/51.345.659

Migné A, Davoult D, Spilmont N, Menu D, Boucher G, Gattuso J-P, Rybarczyk H (2002) A closed-chamber CO_2-flux method for estimating intertidal primary production and respiration under emersed conditions. Marine Biology 140: 865-869 10.1007/s00227-001-0741-1

Migné A, Delebecq G, Davoult D, Spilmont N, Menu D, Gévaert F (2015) Photosynthetic activity and productivity of intertidal macroalgae: In situ measurements, from thallus to community scale. Aquatic Botany 123: 6-12 10.1016/j.aquabot.2015.01.005

Millero FJ, Zhang J-Z, Lee K, Campbell DM (1993) Titration alkalinity of seawater. Marine Chemistery 44: 153-165 10.1016/0304-4203(93)90200-8

Nitschke U, Ruth AA, Dixneuf S, Stengel DB (2011) Molecular iodine emission rates and photosynthetic performance of different thallus parts of *Laminaria digitata* (Phaeophyceae) during emersion. Planta 233: 737-748 10.1007/s00425-010-1334-3

Oviatt CA, Rudnick DT, Keller AA, Sampou PA, Almquist GT (1986) A comparison of system (O_2 and CO_2) and C-14 measurements of metabolism in estuarine mesocosms. Marine Ecology Progress Series 28: 57-67 10.3354/meps028057

Pereira TR, Engelen AH, Pearson GA, Valero M, Serrao EA (2015) Response of kelps from different latitudes to consecutive heat shock. Journal of Experimental Marine Biology and Ecology 463: 57-62 10.1016/j.jembe.2014.10.022

Raybaud V, Beaugrand G, Goberville E, Delebecq G, Destombe C, Valero M, Davoult D, Morin P, Gevaert F (2013) Decline in Kelp in West Europe and Climate. PloS one 8 e66044 10.1371/journal.pone.0066044

Rodrigues MA, dos Santos CP, Young AJ, Strbac D, Hall DO (2002) A smaller and impaired xanthophyll cycle makes the deep sea macroalgae *Laminaria abyssalis* (Phaeophyceae) highly sensitive to daylight when compared with shallow water *Laminaria digitata*. Journal of Phycology 38: 939-947 10.1046/j.1529-8817.2002.t01-1-01231.x

Roleda MY, Hanelt D, Wiencke C (2006) Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea). Marine Biology 148: 1201-1211 10.1007/s00227-005-0169-0

Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. Bulletin of the Fisheries Research Board of Canada 167: 1-311

Terada R, Shikada S, Watanabe Y, Nakazaki Y, Matsumoto K, Kozono J, Saino N, Nishihara GN (2016) Effect of PAR and temperature on the photosynthesis of the Japanese alga, *Ecklonia radicosa* (Laminariales), based on field and laboratory measurements. Phycologia 55: 178-186 10.2216/15-97.1

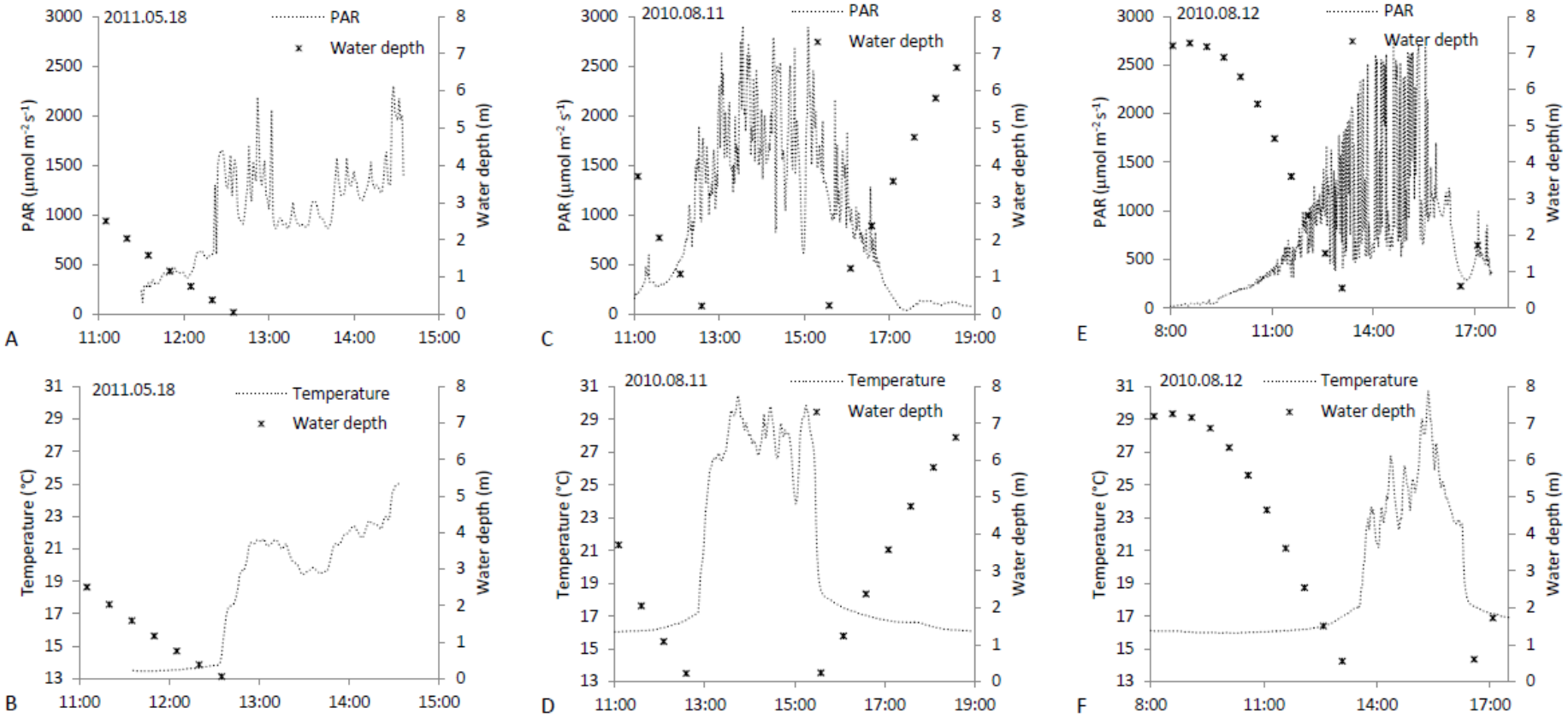

Figure 1: Time course (local time) of bottom light (as Photosynthetically Available Radiation, PAR, A, C & E), temperature (B, D & F) and water depth in the upper part of the *Laminaria digitata* belt of Roscoff over the tides of the 18th May 2011 (A & B), of the 11th August 2010 (C & D) and of the 12th August 2010 (E & F)

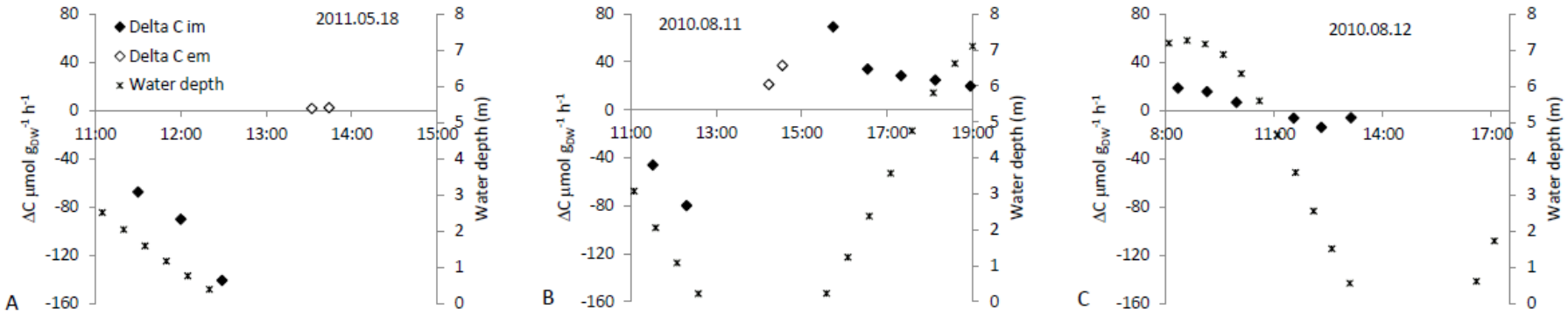

Figure 2: Time course (local time) of carbon flux (Delta C) in the benthic chamber containing an entire sporophyte either under immersion (im) or emersion (em) and water depth in the upper part of the *Laminaria digitata* belt of Roscoff over the tides of the 18th May 2011 (A), of the 11th August 2010 (B) and of the 12th August 2010 (C)

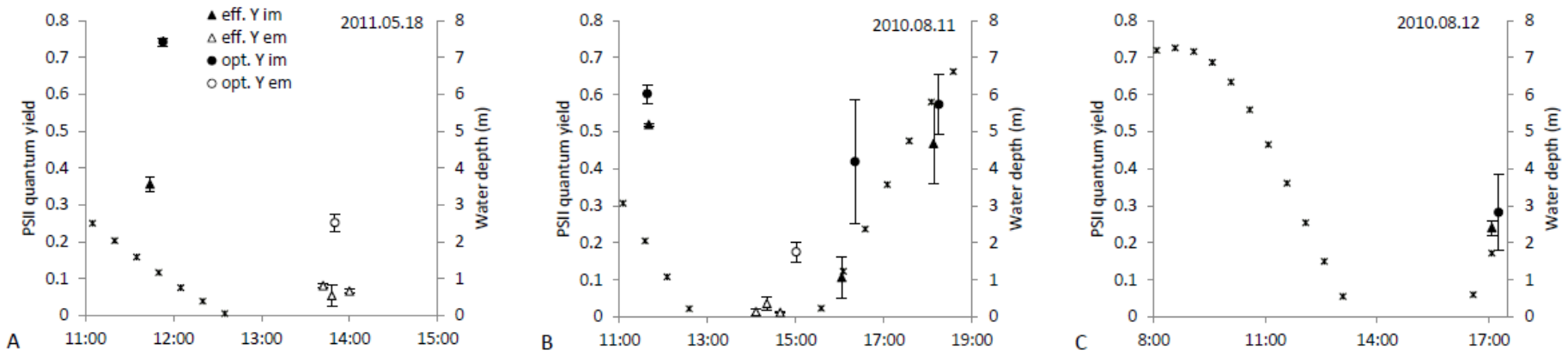

Figure 3: Time course (local time) of mean (± se) effective (eff.) and optimal (opt.) quantum yield of photosystem II (Y) measured on three sporophytes either under immersion (im) or emersion (em) and water depth (crosses) in the upper part of the *Laminaria digitata* belt of Roscoff over the tides of the 18th May 2011 (A), of the 11th August 2010 (B) and of the 12th August 2010 (C)

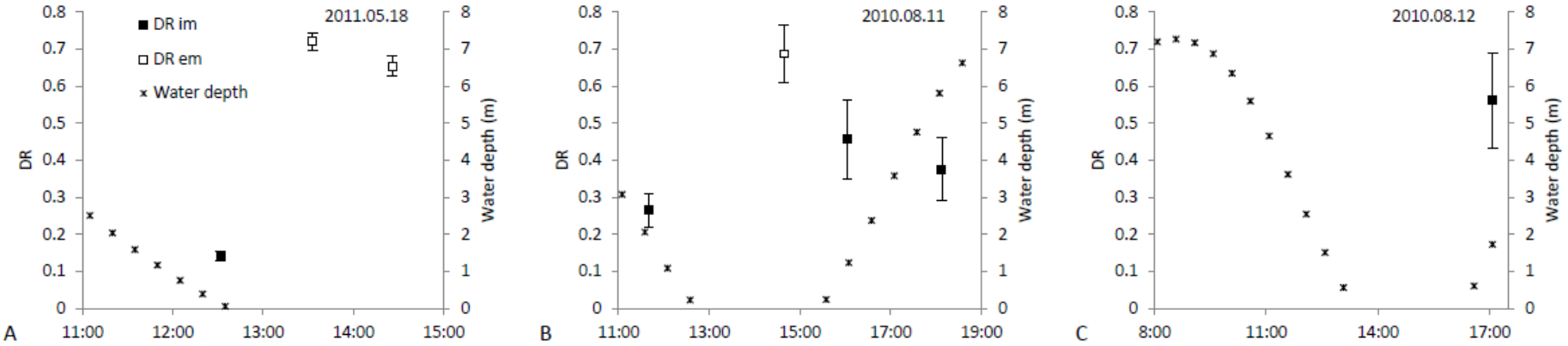

Figure 4: Time course (local time) of mean (± se) de-epoxidation ratio (DR) measured on three sporophytes either under immersion (im) or emersion (em) and water depth in the upper part of the *Laminaria digitata* belt of Roscoff over the tides of the 18th May 2011 (A), of the 11th August 2010 (B) and of the 12th August 2010 (C)

Table 1: Mean electron transport rate (± se) in µmol e^- m^{-2} s^{-1} measured on three sporophytes at different tidal stages in the upper part of the *Laminaria digitata* belt of Roscoff the 18th May 2011, the 11th August 2010 and the 12th August 2010

	May 18th, 2011	August 11th, 2010	August 12th, 2010
Mid ebb-tide	61.5 ± 3.4	168.4 ± 11.5	
Low tide (emersion)	33.7 ± 2.9	10.7 ± 6.7	
	37.7 ± 19.0	24.1 ± 5.1	
	45.6 ± 3.5	17.0 ± 3.3	
Re-immersion		49.7 ± 25.9	55.0 ± 5.5
Mid flood-tide		24.3 ± 5.6	

Table 2: Water depth (WD in m), bottom light (PAR in µmol m^{-2} s^{-1}), temperature (T in °C), electron transport rate (ETR in µmol e^- m^{-2} s^{-1}), net primary production (NP in µmol O_2 g_{DW}^{-1} h^{-1} or µmol 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) the 06th May 2008, the 18th May 2011 and the 11th August 2010. 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 ($PAR_{flat\ sensor}$ = 0.80 $PAR_{spherical\ sensor}$). Net primary production was measured as µmol O_2 g_{FW}^{-1} h^{-1} in the Delebecq et al 2011 study and was expressed as µmol 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 (O_2)	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	305	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	2370	27.4	17	-37	0.18
AFT	8.9	80	12.3	10		0.65							1.2	1080	17.5	50	-34	0.42
													6.0	105	16.3	24	-25	0.57