



Photosynthetic response to light and temperature in *Laminaria digitata* gametophytes from two French populations

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- 1 **Photosynthetic response to light and temperature in *Laminaria digitata***
- 2 **gametophytes from two French populations**
- 3 Running head: Stress response in *Laminaria digitata* gametophytes
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Summary

Given the growing body of evidence on the general decline of kelp beds worldwide, it is crucial to understand the physiological response of kelp gametophyte stages to environmental parameters. We investigated the physiological response of gametophytes to light and temperature in two populations of *Laminaria digitata* occurring in two contrasting environments along the French coast of the English Channel. Results indicate that gametophytes of both populations were highly tolerant to high light through an efficient down-regulation of photosynthesis triggered by the activation of the xanthophyll cycle. Temperature increases promoted photosynthesis and the photosystem II showed high resistance to short-term exposure to high temperatures currently encountered in the field. Gametophytes from the two sites displayed some differences in their pigment contents and photosynthetic characteristics, but low replication size and difference in time of sampling did not permit to confirm a potential local adaptation to the light conditions at each site, as observed in previously published results on adult sporophytes. Gametophytes of *L. digitata* appeared to be resistant to irradiation and temperature conditions currently experienced in the field, confirming their role in persistence of kelp species under stressful environmental conditions.

Keywords: Phaeophyceae, Photosynthesis, Xanthophyll Cycle, Stress, Phenotypic Plasticity, English Channel

49 **Introduction**

50 Kelps are major structural components of the cold temperate and boreal coastal
51 communities (Lüning, 1990). They form a highly productive habitat (Mann, 1973) that
52 harbours a rich biodiversity (Christie *et al.*, 2003). The ecological role of kelp is
53 threatened by their expected vulnerability to changes in the physical environment
54 caused by rapid climate change (Wernberg *et al.*, 2011a; Raybaud *et al.*, 2013) or
55 extreme events (Wernberg *et al.*, 2013), especially near range edges, where populations
56 are at the front line of environmental change (Wernberg *et al.*, 2011b).

57 The persistence of kelp forests mainly depends on the recruitment, growth, competition
58 and the physiological tolerance to environmental factors of the microscopic stages
59 (Reed & Foster, 1984; Ladah & Zertruche-Gonzales, 2007; Matson & Edwards, 2007),
60 which are the crucial phases of the complex heteromorphic life cycle of kelp
61 (Sauvageau, 1915). Gametophytes and embryonic sporophytes can delay their
62 development and reproduction for several months (Carney, 2011) until favourable
63 conditions occur and therefore promote rapid recruitment in the best conditions
64 (Edwards, 2000).

65 Kelp microscopic stages are subject to environmental conditions that differ from those
66 experienced by the macroscopic sporophytes (Reed & Foster, 1984; Martinez &
67 Santelices, 1998) and can therefore have different physiological optima and tolerance
68 levels (Hanelt *et al.*, 1997; Altamirano *et al.*, 2004; Matson & Edwards, 2007). Even
69 under similar environmental conditions, the response of haploid stages differs from
70 those of diploid stages among various taxa of macroalgae (Roleda *et al.*, 2008; Wang *et*
71 *al.*, 2011). Hence, in the context of environmental change, enhancing the understanding

of the physiological tolerance of kelp must incorporate the study of their multiple life-stages, including their microscopic phases (Harley *et al.*, 2012; Wernberg *et al.*, 2012).

Among the potentially adverse environmental conditions, light and temperature can greatly affect the development and survival of microscopic stages and their vulnerability generally determines the ecological success of the species (Bartsch *et al.*, 2008).

Extremely high light has been shown to be adverse for the development and viability of post-settlement stages (gametophytes and embryonic sporophytes) (Lüning & Neushul, 1978; Fetjek *et al.*, 2011). Likewise, extreme high temperature events (such as El Niño events) have been shown to affect the reproductive success of gametophytes (Ladah & Zertruche-Gonzales, 2007; Oppliger *et al.*, 2012).

The photosynthetic apparatus is one of the main targets of these abiotic stressors (Walters, 2005). Rapid fluctuation of light and temperature can cause disruptive stresses (Davison & Pearson, 1996) and affect the survival of kelp gametophytes. Drastic and rapid changes in light exposure and temperature are frequently experienced within a single day (Gevaert *et al.*, 2003; Delebecq *et al.*, 2011) and especially during sunny spring tides.

Therefore, the ability to withstand stressful conditions and to recover from them is crucial for preventing damage to the photosynthetic apparatus and maintaining sufficient photosynthetic performance. After the onset of stressful conditions, the regulation of energy absorption and utilization is essential (Raven & Geider, 2003).

Photoinhibition is the down-regulation of photosynthesis, whose extent is determined by the balance between the rate of photodamage and the rate of repair of photosystem II (PSII) (Takahashi & Murata, 2008). Photoinhibition has been observed in the field on

macroscopic sporophyte stages (Gevaert *et al.*, 2003; Delebecq *et al.*, 2011), in the laboratory on zoospores (Roleda, 2009) and in the gametophytic and embryonic sporophyte stages of kelp (Hanelt *et al.*, 1997; Altamirano *et al.*, 2004). Factors other than light, such as temperature, can accelerate photoinhibition by altering the PSII repair mechanisms (Takahashi & Murata, 2008). Toxic active derivatives of oxygen (oxygen radicals), byproducts of photosynthesis, can also be over-produced under adverse environmental conditions (when exceeding the scavenging potential of cells) and can alter the biological integrity of cells (Ledford & Niyogi, 2005; Allakverdiev *et al.*, 2008).

To cope with excess light, energy is dissipated in the form of heat to rapidly regulate light harvesting; this mechanism is widespread in photoautotrophs (Raven & Geider, 2003). Increased thermal energy dissipation of excess light involves the xanthophyll cycle in brown algae, which plays a major role in the fast-dynamic acclimation to change in light, temperature and desiccation in macroscopic sporophytes (Fernandez-Marin *et al.*, 2011). However, the implication of xanthophyll cycle in the photoprotection process in kelp gametophytes has only been mentioned (Hanelt *et al.*, 1997; Altamirano *et al.*, 2004), without being clearly demonstrated.

Laminaria digitata Hudson Lamouroux (1813), a kelp species of high ecological and economical value, has been shown to be retreating from several sites along the French coasts (Arzel, 1998; Davoult *et al.*, 2011), sparking research to determine the reasons for this decline. Physical stress and environmental changes may contribute to a reduction in the fitness of the gametophytic developmental stages of *L. digitata*. In this study, we tested the sensitivity of *L. digitata* gametophytes to changing photon flux density and temperature. The investigations were carried out on two populations of *L.*

digitata along the French coast of the English Channel inhabiting contrasting environmental conditions (Delebecq *et al.*, 2013). The differences in local environmental conditions may result in different sensitivities among the populations that need to be taken into account in understanding the effect of environmental factors on organisms. In this study, we also measured the role of the xanthophyll cycle in the non-photochemical quenching in gametophytes of *L. digitata* in response to excess light.

Materials and Methods

Study site

A complete description of the two sites and environmental conditions during experiments is given in Delebecq *et al.*, (2013); consequently, we describe only the main characteristics of both sites here. We collected the seaweed material from two populations of *L. digitata*, growing in the upper subtidal zone (0-1 m) of a rocky shore in Roscoff (48°5'N, 3°6'W) and in Wissant (50°5'N, 1°4'E), located in the western and eastern part of the English Channel, respectively. The sites were selected for their large kelp stands and also for their contrasting environmental conditions. Light attenuation (m^{-1}) of photosynthetically active radiation (400–700 nm) ranged from 0.09 to 0.57 m^{-1} in Roscoff and from 0.19 to 0.96 m^{-1} in Wissant (Delebecq *et al.*, 2013) due to high turbidity in the eastern English Channel. Seawater surface temperature displays high seasonal variation at both sites, with a slightly broader annual temperature range in Wissant, from 5°C in winter to 20°C in summer, than in Roscoff, from 8°C in winter to 17°C in summer, due to the influence of the North Atlantic Ocean and depth of the

continental shelf. Environmental conditions in the two sites at the time of collection and 1 month prior to sampling are given in **Table 1**.

Sampling and culture conditions

Fertile sporophytes of *L. digitata* were collected at low tide in November 2008 in Roscoff, and in February 2009 in Wissant. *L. digitata* is reproductive most of the year in Roscoff, but the main spore/gamete release events generally occur in August-September and November-December in Northern Brittany (Arzel, 1998). In Wissant, *L. digitata* is reproductive at the end of winter and throughout spring. Mature sori were cut, cleaned, and dried at 10°C and in the dark for several hours. Sori were subsequently washed with distilled water and sterile seawater, and placed in 50 mL Falcon tubes (BD Biosciences, San Jose, CA, USA), filled with sterile Provasoli enriched seawater (PES) (Provasoli, 1968), and maintained overnight in the dark on a rotary shaking table to induce zoospore release. Zoospores in suspension were checked with inverted-light microscope to ensure that zoospores were viable, before being placed in Petri dishes (BD Falcon, Franklin Lakes, NJ, USA). Zoospores were allowed to settle and developing gametophytes were cultured in thermostatic chamber at 10°C under an irradiance of 35 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (photon flux density, PFD, 400-700 nm), produced by fluorescent tubes (L8W/840, cool white, Osram, Germany) in a light:dark cycle 12:12 h. PFD was measured with a cosine-corrected quantum sensor (Li-192SA, LiCor, Lincoln, Nebraska, USA), connected to a data logger (Li 1400, LiCor, Lincoln, Nebraska, USA). The medium was changed once a week. Experiments started approximately after 1 month of cultivation when the density was high enough for fluorescence measurements. The density of the gametophytes was $4711 \pm 1140 \text{ ind m}^{-2}$ for Roscoff ($43 \pm 3\%$ cover) and $3600 \pm 691 \text{ ind m}^{-2}$ for Wissant ($41 \pm 7\%$ cover). Gametophytes were composed of

few cells and arranged as homogenous thick layers at the bottom of the Petri dishes.

Gametophytes were isolated from three different parents at each site ($n = 3$) and were cultivated separately to ensure independent replicates.

Oxygen production

Dark respiration (R_d) and net oxygen production (NP) was measured polarographically at culture temperature (10°C), using a water-jacket thermostatic DW2/2 chamber combined with a “Clark-type” oxygen electrode and a computer-interfaced box CB1 (Hansatech Instruments, Kings Lynn, Norfolk, UK). The gametophytes were first detached from the substrate and resuspended several days prior to the experiment. Detaching gametophytes from their substrate does not affect their growth, their photosynthetic performance or their respiration (Fain & Murray, 1982). The gametophytes were held 12 h in the dark prior to the experiment, and then placed in the measurement chamber filled with 2 mL of PES and mixed with a magnetic stirrer. To prevent oxygen saturation, a quarter of the medium was renewed after each light step. To do so, a silk filtering mesh was used to retain the gametophytes in the media. Respiration was measured in the dark, and gametophytes then underwent light-increasing steps of 20 min each (13 light steps, ranging from 2.5 to $500\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$) using a halogen lamp (KL 2500 LCD, Schott, Germany) with a daylight cut-off filter (Schott, Germany). R_d , NP and gross oxygen production (GP) rates were calculated based on fresh weight (FW, $\mu\text{mol O}_2\ \text{g}_{\text{FW}}^{-1}\ \text{h}^{-1}$) and chlorophyll *a* content (chl *a*, $\mu\text{mol O}_2\ \text{nmoles chl } a^{-1}\ \text{h}^{-1}$). FW was measured after collecting gametophytes on a silk filtering mesh that had previously been weighed.

Fluorescence

189 *In vivo* chl *a* fluorescence of the photosystem II (PSII) of gametophytes was measured
190 using an underwater fluorometer (diving PAM; Heinz Walz, Effeltrich, Germany). The
191 optimal quantum yield (F_v/F_m) of PSII (Genty *et al.*, 1989), a measure of the maximum
192 efficiency of PSII, was measured using a 0.8 s saturating pulse (2500 $\mu\text{mol photons m}^{-2}$
193 s^{-1}) of white light. We calculated the relative F_v/F_m ratio (rel. F_v/F_m) by dividing all data
194 by the initial value measured at the beginning of the experiment in the dark.

195 The effective quantum yield of PSII (ϕ_{PSII}), the efficiency of PSII photochemistry, was
196 measured using a custom-made clip for Petri dishes to ensure a constant distance of 5
197 mm between the probe and the sample. The ϕ_{PSII} was calculated according to Genty *et*
198 *al.* (1989) and used to estimate the linear electron transport rate (relative electron
199 transport rate, $rETR$) (Gevaert *et al.*, 2003), an estimator of photosynthesis.

200 Non-photochemical quenching (NPQ) indicates thermal dissipation of excess light in
201 the PSII antennae, a photoprotective mechanism. We assumed that a stable NPQ level is
202 reached after a 10-min illumination period, as shown in several microalgal species
203 (Casper-Lindley & Björkman, 1998). F_m' values measured under very weak irradiance
204 were higher than F_m values measured after dark-adaptation; therefore NPQ values were
205 computed using the higher F_m' value instead of F_m (Serodio *et al.*, 2005).

206 $rETR$ and NPQ were measured on gametophytes at the end of each light-increasing step
207 of 10 min each (12 light steps, ranging from 2.5 to 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The NPQ
208 value measured under the maximal irradiance is referred to as NPQ_{250} .

209 *P-I* curves, $rETR-I$

210 The light-saturated maximum rate of GP (P_{max}), the light-saturated maximum rate of
211 relative electron transfer ($rETR_{max}$), the light-limited initial slope (α), and the saturation

onset irradiance level (I_k) were calculated by plotting computed oxygen production rates and $rETR$ against irradiance. P_{max} represents the maximal oxygen production, including all photosynthetic processes, while $rETR_{max}$ is an estimation of the linear electron transfer in PSII, an indication of the overall photosynthetic capacity. Data were fitted using the model of Eilers & Peeters (1988) to each replicate with a least-square regression, using the Simplex method in the Statistica computer package (Statsoft, Tulsa, OK, USA).

Response to high irradiance

To study high light stress, the settled gametophytes in a Petri dish filled with PES at cultivation temperature were exposed to 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 2 h, and then, to dim light (10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to allow recovery. F_v/F_m , ϕ_{PSII} , $rETR$ and NPQ were measured every 30 min. Samples for pigment analyses were simultaneously collected at the start of the experiment, at the end of the light stress, and 2 h after the return to dim light. Gametophytes were detached from the substrate and the gametophyte suspension was filtered on a 20 μm silk filtering mesh and subsequently deep-frozen in liquid nitrogen for further pigment analyses.

Response to temperature

First, $rETR-I$ curves were constructed for gametophytes acclimated 4 h at four different temperatures (5°C, 10°C, 15°C and 20°C) controlled by a thermo fluid circulator bath. During acclimation, F_v/F_m was measured hourly. Then, gametophytes underwent a progressive increase and decrease temperatures (2°C every 15 min) with an initial temperature set at 10°C. F_v/F_m was measured at the end of each temperature step.

Pigment analysis

Pigment contents of gametophytes were extracted by sonication and grinding in a cold mortar with methanol and methylene chloride. Extracts were centrifuged and supernatants were collected and dry-evaporated under nitrogen. Salt contents of the extracts were removed and the organic phase was evaporated and dissolved in methanol for injection. Pigment analysis was performed by high performance liquid chromatography (HPLC) (Beckman, system Gold, 126) with a reverse-phase column (C18 Allure, Restek). Separation was performed with a solvent delivery profile adapted from Arsalane *et al.*, (1994). Pigment contents were quantified using specific absorption coefficients and normalised to the total pigment content. The conversion of violaxanthin into antheraxanthin and zeaxanthin was estimated by calculating the de-epoxidation ratio (DR):

$$DR = (\text{antheraxanthin} + \text{zeaxanthin}) / (\text{violaxanthin} + \text{antheraxanthin} + \text{zeaxanthin})$$

Total chl *a* concentrations were normalised to the FW of samples. Fucoxanthin and chl *c* pigment concentrations normalised to chl *a* (moles per 100 mol of chl *a*) were pooled, and referred to as the antenna pigment pool. Violaxanthin, antheraxanthin and zeaxanthin concentrations normalised to chl *a* were pooled, referred to as the xanthophyll cycle pool.

Statistical analyses

Student's *t*-test (*t*) (with Welch correction) was used to test for the difference between means of the photosynthetic parameters (P_{max} , $rETR_{max}$, α et I_k), the F_v/F_m , NPQ values and pigment contents after the dataset was tested for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Response to light or temperature was fitted and response parameters were compared using Student's *t*-test. The DR response was compared

within sites using a t -test for paired samples ($t_{(p)}$) after checking the data for normality. Adjustment of P values for multiple testing was done using the Holm method. The effect of temperature on the response parameters of light-response curves was analysed using a PERMANOVA (Anderson, 2001) in the vegan package of R software. Post hoc power analysis ($1-\beta$) were performed on non significant results using G*Power software which automatically determine the effect size (Faul *et al.*, 2007). All experimental units had three replicates ($n = 3$).

Results

Comparison of the photosynthetic activity between the two sites

Gametophytes from the two sites showed differences in their photosynthetic parameters (**Fig. 1**). When normalized to chl a , $P_{max(chla)}$ values, the light-saturated maximal gross oxygen production values, were higher in Roscoff than in Wissant ($t = 8.0$, Holm-adjusted $P = 0.003$), with $0.27 \pm 0.01 \mu\text{mol O}_2 \cdot \text{nmol chl } a^{-1} \cdot \text{h}^{-1}$ in Wissant, and $0.95 \pm 0.15 \mu\text{mol O}_2 \cdot \text{nmol chl } a^{-1} \cdot \text{h}^{-1}$ in Roscoff (**Fig. 1 C**). $rETR_{max}$ values ($22.74 \pm 5.37 \mu\text{mol e}^{-} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in Wissant and $15.90 \pm 0.77 \mu\text{mol e}^{-} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in Roscoff), the light-saturated maximal electron transfer rates, and $P_{max(FW)}$ on a FW basis ($56.47 \pm 13.18 \mu\text{mol O}_2 \cdot \text{g}_{FW}^{-1} \cdot \text{h}^{-1}$ in Wissant and $38.33 \pm 1.12 \mu\text{mol O}_2 \cdot \text{g}_{FW}^{-1} \cdot \text{h}^{-1}$ in Roscoff), were not significantly different between two sites ($t = 2.9$, Holm-adjusted $P = 0.133$ for $rETR_{max}$; $t = 2.8$, Holm-adjusted $P = 0.142$ for $P_{max(FW)}$ values) (**Fig. 1 A-B**), likely due to low statistical power resulting from small sample sizes (0.39 for $rETR_{max}$ and 0.32 for $P_{max(FW)}$).

The same pattern appeared for the ascending slope under light limitation (α values). $\alpha_{(chl a)}$ values were significantly higher in Roscoff than in Wissant (respectively 0.005 ± 0.001 in Wissant and 0.023 ± 0.002 in Roscoff) ($t = 13.9$, Holm-adjusted $P = 0.001$) when expressed on a chl a basis, whereas no differences were observed when expressed in electron transport rates or when expressed on a FW basis (respectively 0.37 ± 0.06 in Wissant and 0.40 ± 0.03 in Roscoff; $t = 0.68$, Holm-adjusted $P = 0.534$ for $\alpha_{(rETR)}$ and 1.15 ± 0.17 in Wissant and 0.99 ± 0.09 in Roscoff $t = 1.39$, Holm-adjusted $P = 0.305$ for $\alpha_{(FW)}$) which can also result from very low statistical power (respectively 0.09 and 0.19).

Considering the light saturation parameter (I_k), no significant differences were found between the two sites but the low statistical powers suggest that there were not enough replication to resolve the differences ($t = 1.95$, Holm-adjusted $P = 0.245$ and $1-\beta = 0.30$ for $I_{k(rETR)}$, $t = 1.77$, Holm-adjusted $P = 0.152$ and $1-\beta = 0.28$ for $I_{k(FW)}$ and $t = 1.13$, Holm-adjusted $P = 0.322$ and $1-\beta = 0.14$ for $I_{k(chl a)}$). Despite this lack of significant differences, average I_k values in gametophytes from Wissant were always greater than I_k values in gametophytes from Roscoff ($62.67 \pm 20.20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in Wissant, and $39.71 \pm 1.73 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in Roscoff on an electron rate basis; $49.44 \pm 9.83 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in Wissant, and $38.83 \pm 3.42 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in Roscoff on a FW basis and 52.39 ± 13.49 in Wissant and 41.98 ± 8.56 in Roscoff on a chl a basis).

Despite high irradiance ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) in comparison with the culture conditions, there was no decrease in oxygen production observed.

Comparison of pigment contents

Pigment contents were similar at both sites (**Table 2**) except for chl *c* and violaxanthin contents, which were higher in Wissant than in Roscoff ($t = 4.69$, Holm-adjusted $P = 0.047$ for chl *c* and $t = 4.26$, Holm-adjusted $P = 0.047$ for violaxanthin). When expressed on a total pigment content basis, no differences were observed in chl *a* contents in both sites, but chl *a* contents were slightly lower in Wissant and statistical power was low (0.33). Therefore higher $\Sigma\text{XC}:\text{chl } a$ pigment ratio may have been expected in Wissant, but the lack of significant difference is believed to be the result of a low statistical power (0.53).

When chl *a* contents were expressed per unit fresh weight (respectively 47.74 ± 6.63 nmoles $\text{g}_{\text{FW}}^{-1}$ in Roscoff and 155.95 ± 38.22 nmoles $\text{g}_{\text{FW}}^{-1}$ in Wissant), they were significantly higher in Wissant ($t = 4.83$, Holm-adjusted $P = 0.036$).

Comparison of photoprotective capacities

NPQ was measured along with *rETR* (**Fig. 2**). *NPQ* values after prolonged darkness (12 h, corresponding to the end of the dark period of culture conditions) were higher than those under weak irradiances. Maximal F_m' values were reached under an average irradiance of $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in Roscoff, and $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in Wissant.

NPQ progressively developed with increasing irradiance. Maximal *NPQ* values (NPQ_{250}) were reached at the highest applied irradiance ($250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and these values were significantly higher in gametophytes from Roscoff than in gametophytes from Wissant ($t = 3.5$, Holm-adjusted $P = 0.047$), with NPQ_{250} values of 3.80 ± 0.42 in Roscoff and of 2.37 ± 0.56 in Wissant.

Intrinsic efficiency of PSII was altered in both populations, as indicated by the strong decline of rel. F_v/F_m values (**Fig. 3 A**) when gametophytes were exposed to 500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 2 h. It reached a constant level, decreasing by 70% of the initial F_v/F_m value after 2 h in both populations. In dim light, F_v/F_m recovered at a level within 5% of the initial F_v/F_m value after 1.5 h in Roscoff and after 4 h in Wissant.

During light stress, there was an increase in DR in gametophytes from Roscoff and from Wissant, corresponding to the progressive de-epoxidation of violaxanthin into antheraxanthin and zeaxanthin ($t_{(p)} = 7.9$, Holm-adjusted $P = 0.025$ for gametophytes from Wissant and $t_{(p)} = 8.9$, Holm-adjusted $P = 0.025$ for gametophytes from Roscoff) (**Fig. 3 B**). The increase in intrinsic efficiency of PSII under dim light was accompanied by a decrease in the DR values in gametophytes from Roscoff ($t_{(p)} = 11.6$, Holm-adjusted $P = 0.022$), indicating the reversion of zeaxanthin and antheraxanthin into violaxanthin. In spite of the difference in the absolute values of DR measured at both sites after 2 h of strong illumination, the percent increase in DR was similar at both sites with an increase of 160% of the initial DR values. There was no significant decrease in DR values in Wissant after 2 h under dim light.

Comparison of the response to temperature

Increasing the temperature resulted in a decrease in α values in Wissant (**Fig. 4 A**), and an increase in $rETR_{\text{max}}$ and I_k values for both sites (respectively **Fig. 4 B** and **4 C**) (**Table 3**).

NPQ_{250} was calculated at the end of each $rETR-E$ curves (**Fig. 4 D**). When exposed to 20°C, there was a slight decrease in the NPQ_{250} values, in comparison with the values reached at 10°C.

The sensitivity of F_v/F_m to temperature was tested on a broad range of temperature (**Fig. 5**). At 10°C, F_v/F_m values in the dark were 0.52 ± 0.09 in Roscoff and 0.53 ± 0.06 in Wissant. With the progressive increase in temperature (from 10°C to 32°C), the rel. F_v/F_m declined for temperatures greater than 20°C. There were no differences between sites in the response parameters from the regression analysis.

Discussion

Microscopic stages are thought to be the hardest life-cycle stages of kelp (tom Dieck 1993). They form a seed bank which persists through stressful environmental conditions and ensures the persistence of species when unfavourable conditions occur, as it may happen during unusual heat waves (Ladah & Zertuche-Gonzales, 2007, Bartsch et al., 2013). Therefore, a great tolerance of *L. digitata* gametophytes to stressful environmental conditions is essential. In this study, we tested their physiological tolerance to irradiation and temperature stress in two populations with different environmental conditions along the French coast. The main result of the present study is that gametophytes of *L. digitata* were highly resistant to the irradiation and temperature treatments that might be locally encountered in the field. It is reinforced by the fact that sori were sampled during months when irradiation and temperature stress are not prevalent: their temperature and irradiation resistance may be even higher during summer.

We set out to study the incidence of high light stress on the photosynthesis of *L. digitata* gametophytes. Our results demonstrate that gametophytes can cope with prolonged high

irradiance stress, confirming the great potential for high light tolerance in kelp gametophytes (Iizumi & Sakanishi, 1994; Hanelt *et al.*, 1997; Altamirano *et al.*, 2004). This tolerance arises from the efficient thermal dissipation of excess light (*NPQ*) and, mainly, through the formation of antheraxanthin and zeaxanthin, which induce the conformational change in the light harvesting complexes (LHC) II (Jahns & Holzwarth, 2009). This zeaxanthin-dependent quenching is a slow component of *NPQ*, and Garcia-Mendoza & Colombo-Pallota (2007) have shown that kelp may lack the fast energy or pH-dependent quenching. However, the large xanthophyll cycle pigment pool may accelerate *NPQ* development and thus compensate for this lack. Under dim light, epoxidation of zeaxanthin and antheraxanthin was virtually complete in less than 1 h. The slowly relaxing component of photoinhibition generally corresponds to the progressive re-activation of PSII or possibly to a conformational change of the LHC, and probably the aggregation of LHC due to zeaxanthin binding (Garcia-Mendoza & Colombo-Pallotta, 2007). Therefore, our study confirms that this efficient reversible conversion between violaxanthin, antheraxanthin and zeaxanthin in gametophytes (previously suggested by Hanelt *et al.*, 1997 and Altamirano *et al.*, 2004) is a widespread mechanism of fast-dynamic acclimation to abiotic stress in macroalgae (Fernandez-Marin *et al.*, 2011).

Another possible mechanism protecting *L. digitata* gametophytes from rapid light fluctuations is the maintenance of *NPQ* in the dark, a mechanism previously observed in microphytobenthos (Serôdio *et al.*, 2005) and in several phytoplankton species (Casper-Lindley & Björkman, 1998; Cruz *et al.*, 2011). In *Pelvetia canaliculata*, the *NPQ* of chl *a* fluorescence in the dark depends on the activation of violaxanthin de-epoxidase (Fernandez-Marin *et al.*, 2011), which is triggered by acidification of the lumen due to

metabolic activity in the dark (Cruz *et al.*, 2011). This activation may thus sustain ATP synthase activity (Casper-Lindley & Björkman, 1998; Serôdio *et al.*, 2005), and prevent the formation of oxygen radicals. *NPQ* in the dark may therefore represent a type of sustained photoprotection, maintaining a dissipative state through pre-formed zeaxanthin or antheraxanthin, as observed here.

Acclimation to higher irradiances than the culture irradiance used in this experiment and/or an intermittent light exposure may have also provided a higher tolerance to the two- hour light stress of 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Han and Kain, 1996; Lavaud *et al.*, 2002). Along the French coast of the English Channel, irradiance of 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and more can be recorded during spring and summer low spring tides (Gevaert *et al.*, 2003; Delebecq *et al.*, 2011; Delebecq *et al.*, 2013), but actual incident irradiances are generally deeply attenuated by the sporophyte canopy (Gerard, 1984) which may hypothesized that gametophytes display a great tolerance to high light.

This study also highlighted that the photosynthetic performance of *L. digitata* gametophytes were not negatively affected by short-term fluctuations in water temperature as it may be locally encountered in the field during periods of low tide. Gametophyte PSII — essential for the whole photochemistry process (Havaux & Tardy, 1996; Roleda, 2009) — shows high resistance to the temperatures currently encountered in the field. Increased temperature promotes electron transfer via the PSII through an increase in thylakoid membrane fluidity (Havaux & Tardy 1996) and results in an increase in maximum relative electron transfer rates ($rETR_{max}$) and in the onset of saturation parameters (I_k), as observed here. An increase in gross oxygen production with temperature can therefore be expected up to an optimum (Suklenik *et al.*, 1987),

417 since the relationship electron transport rate and gross oxygen production is robust,
418 provided that the temperature changes are not extremes (Morris & Kromkamp, 2003).

419 Above an optimal temperature (above 20°C in this study), a complex set of inactivation
420 and denaturation occur in thylakoïd, accompanied by the generation of elevated levels
421 of reactive oxygen species which reduces photosynthetic capacity (Sharkey, 2005;
422 Allakhverdiev *et al.*, 2008) and also the ability to cope with high light stress (Roleda,
423 2009). Superoptimal temperature also promotes electron-consuming processes (such as
424 cyclic electron transfer) and dark respiration (Fain & Murray, 1982; Sharkey, 2005;
425 Henkel & Hofmann, 2008) which can result in negative net photosynthesis.

426 However, short-term temperature responses do not provide insight into long-term
427 individual survival with regard to temperature: temperature optima for photosynthesis
428 can be several degrees higher than temperature optima for survival and growth
429 (Davison, 1991) as it is not possible to evaluate the extent of sublethal stress.

430 Concerning *L. digitata* gametophytes, tom Diek (1993) showed that they were able to
431 survive 8 weeks at 23°C, demonstrating that gametophytes are moderately resistant to
432 temperature extremes that are rarely encountered across its geographic range. Moreover,
433 there is seasonal acclimation of the temperature response of gametophytes (Lee &
434 Brinkhuis, 1988) and temperature resistance of PSII may be even higher in
435 gametophytes sampled in warmer months or acclimated to higher temperatures
436 (Mohring *et al.*, 2013).

437
438 The two sites from which gametophytes were obtained were known to display different
439 seasonal range of abiotic conditions (Berx & Hughes, 2009; Delebecq *et al* 2013), and a

strong photoacclimation to local environmental conditions of sporophytes of *L. digitata* from the two same sites was previously highlighted (Delebecq *et al.*, 2013). Here, we found some differences in the physiological characteristics of gametophytes between the two sites, however the small replication did not exhibit the full physiological diversity of the populations investigated, and replication was clearly not sufficient to resolve the differences as indicated by the very low statistical power of our analysis (never exceeding 53%). Moreover, results in the present study are confounded by the different environmental conditions at the time of sampling in the two sites (**Table 1**) that may influence the physiological response of gametophyte even after one month cultivation under lab conditions, depending on the time-scale of acclimation to new light and temperature treatments. More information on seasonal changes that can occur in the gametophytes from Roscoff and Wissant would be necessary for a more complete picture of the gametophyte response in the English Channel taking into account the variation of the whole set of past and present environmental conditions, and to test if the photosynthetic characteristics measured in sporophytes may be conserved in gametophytes.

The higher chl *a* concentrations and fucoxanthin contents in gametophytes from Wissant may suggest a greater light-harvesting efficiency and a higher density of reaction centres (Gerard, 1988), which generally result in higher photosynthetic rates. Although maximal photosynthetic rates ($rETR_{max}$ and P_{maxFW}) in Wissant were not significantly different from those in Roscoff, it is believed to result of low statistical power, as indicated by the differences observed in $P_{maxchl a}$. Hence, $P_{maxchl a}$ was higher in Roscoff than in Wissant, indicating less efficient light use per chl *a* molecule in the gametophytes from Wissant. This difference may be due to the self-shading of the LHC,

i.e. the fact that light absorption do not increase despite a higher pigment concentration (Falkowski & Raven, 1997). Daily underwater irradiances were shown to be similar in both sites at the time of sampling (Delebecq *et al.*, 2013). As thermal acclimation is known to induce change in photoacclimation status (Machalek *et al.*, 1996), those differences in photosynthetic characteristics may be partly due to the lower sea surface temperature in Wissant at the time of sampling (**Table 1**), if initial temperature encountered in the field still influence gametophyte's response to light, even after one month cultivation.

Then, the larger violaxanthin content in Wissant may compensate for the large antenna size (higher fucoxanthin contents) which is known to decrease the probability of encounters between violaxanthin and violaxanthin de-epoxidase and the speed of de-epoxidation under high light (Garcia-Mendoza & Colomba-Palotta, 2007). It may also be due to the faster changing light conditions imposed by higher light attenuation in Wissant. Violaxanthin is also involved in the response to other abiotic stressors (Havaux & Tardy, 1996; Fernandez-Marin *et al.*, 2011). Thus, a larger pool of violaxanthin may help gametophytes from Wissant to cope with other abiotic stressors not tested in this study.

We found no differences in the temperature response of the two sites with respect to the resistance of PSII quantum efficiency and increasing temperature. We expected that the wider temperature range encountered in Wissant may have induced a difference in thermostability of PSII. The small difference in temperature range may have not been sufficient to produce detectable differences. Moreover, at the time of sampling,

temperature was lower in Wissant (5°C) than in Roscoff (12°C) and this difference may have influenced the photosynthetic response of gametophytes in our study (Lee & Brinkhuis, 1988; Mohring *et al.*, 2013). Beside thermal conditions and sampling time, the low replication size of our experiments may have been not sufficient to draw a well supported conclusion on the lack of difference between the two sites.

Regarding the temperature response of gametophytes, Bolton & Lüning (1982) suggested that *Laminaria* species may show sufficient phenotypic plasticity to adjust to the temperature range along its distribution and is not composed of differentiated temperature-adapted ecotypes throughout its range. However, notwithstanding similar growth patterns, physiological differences in the response to temperature may nevertheless occur among populations of perennial brown seaweeds (Henkel & Hofmann, 2008; Staehr & Wernberg, 2009). In order to test for difference in temperature response of the *L. digitata*'s populations along the French coast, further experiments need to compare gametophytes from (1) the two sites at different times of the year during their respective sporulation periods (Mohring *et al.*, 2013) and (2) sites with more contrasting temperature ranges (Staehr & Wernberg, 2009). More generally, investigating the joint effect of light and temperature requires a multifactorial experiment (Fredersdorf *et al.*, 2009).

Gametophytes appeared to be resistant to the light and temperature conditions currently experienced in the field. Regarding vulnerability to environmental conditions, physiological studies on the interactive effects of multiple abiotic stressors are required to improve our understanding of the microscopic stages in kelp (Fredersdorf *et al.*, 2009). For instance, desiccation tolerance (Contreras-Porcia *et al.*, 2012), ultraviolet

radiation (Roleda *et al.*, 2006) and burial in sediment deposition (Roleda *et al.*, 2011) all affect microscopic stages. While the southern geographic range-limit of *L. digitata* is thought to be set by inhibition of reproduction, as it was observed by Bartsch *et al.* (2013) when temperature reaches 18°C, survival of microscopic stages remains essential to cope with unusual stressful conditions. There is also an important need to obtain more information on the persistence of these stages in the field. It could help in defining which environmental conditions are really encountered in the field by the different microscopic developmental stages. Despite the present study failed to report local variation in the physiological response of *L. digitata*, it remains essential to take into account local variation in predicting the impact of fast-changing conditions in coastal areas.

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Figures legends

Fig. 1 Photosynthesis-irradiance curves (*P-I* curves) of gametophytes in *L. digitata* from Wissant (□) and Roscoff (●) (*n* = 3), expressed as (A) relative electron transport rate (*rETR*, $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$), (B) gross oxygen production on a fresh weight basis (GP, $\mu\text{mol O}_2 \text{g}_{\text{FW}}^{-1} \text{h}^{-1}$) and (C) gross oxygen production per unit chl *a* (GP, $\mu\text{mol O}_2 \text{nmoles chl } a^{-1} \text{h}^{-1}$).

Fig. 2 *NPQ* of gametophytes in *L. digitata* from Wissant (□) and Roscoff (●) (*n* = 3) measured during the data collection for the *rETR-I* curves.

Fig. 3 (A) Relative optimal quantum yield (rel. F_v/F_m) and (B) de-epoxidation ratio of violaxanthin into antheraxanthin and zeaxanthin of gametophytes in *L. digitata* from Wissant (□) and Roscoff (●) (*n* = 3). Gametophytes were first exposed to high irradiance ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 2 h and then to dim white light ($10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

Fig. 4 Photosynthetic parameters (α (A), $rETR_{\text{max}}$ (B) and I_k (C)) and maximal *NPQ* (NPQ_{250}) (D) of gametophytes in *L. digitata* from Wissant (□) and Roscoff (■) (*n* = 3). Gametophytes acclimated at 5°C, 10°C, 15°C and 20°C exposed to increasing irradiance at the temperature of acclimation. Photosynthetic parameters (α , $rETR_{\text{max}}$ and I_k) were calculated using the model of Eilers & Peeters (1988).

Fig. 5 Relative optimal quantum yield (rel. F_v/F_m) of gametophytes in *L. digitata* from Wissant (□) and Roscoff (●) (*n* = 3). Gametophytes initially kept at 10°C were

780 separately exposed to increases and decreases in temperature (2°C steps at 15 min
781 intervals). Data were fitted using a non-linear regression analysis ($y = a * e^{(b * x / c)} + d$).

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784 **Table 1** Environmental conditions in the two sites at the time of collection and 1 month
785 prior to sampling. Environmental data were provided by T. Carriou from the Station
786 Biologique de Roscoff and the Somlit network.

	Roscoff		Wissant	
	Time of collection	1 month prior	Time of collection	1 month prior
Daylenght (hh:mm)	09:13	10:51	09:30	08:50
Daily surface irradiance (mol photons. m ⁻² . j ⁻¹)	4.13	11.12	5.45	8.04
Light attenuation of PAR (m ⁻¹)	0.21	0.20 ± 0.01	0.34	0.37 ± 0.06
Sea Surface Temperature (°C)	12.8	13.4 ± 0.8	5.1	5.3 ± 0.4
Salinity	35.2	35.2 ± 0.0	34.5	34.5 ± 0.1
Dissolved NO ₃ (μmol. L ⁻¹)	8.00	6.83 ± 1.07	13.62	13.42 ± 0.34
Dissolved PO ₄ (μmol. L ⁻¹)	0.49	0.45 ± 0.05	0.40	0.46 ± 0.07

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790 **Table 2** Pigment composition (moles per 100 moles of all pigments) and pigment ratios
791 (x 100) of gametophytes in *L. digitata* from Roscoff and Wissant (n = 3). Significant
792 results (Holm-adjusted $P < 0.05$) are highlighted in bold.

	Roscoff	Wissant	<i>t</i>	Holm- adjusted <i>P</i>	1- β
Pigment concentrations					
Chlorophyll <i>a</i> (chl <i>a</i>)	54.51 ± 2.64	50.05 ± 2.93	1.96	0.243	0.33
Chlorophyll <i>c</i> (chl <i>c</i>)	8.15 ± 0.65	3.10 ± 1.75	4.69	0.047	
Fucoxanthin	30.41 ± 3.00	37.85 ± 3.81	2.66	0.169	0.52
Violaxanthin (Vx)	2.11 ± 0.49	3.54 ± 0.31	4.26	0.047	
β carotene	0.61 ± 0.37	0.69 ± 0.33	0.27	0.802	0.06
Pigment ratios					
Antenna:chl <i>a</i> pigment ratio	77.13 ± 9.11	89.65 ±10.56	1.55	0.195	0.23
ΣXC :chl <i>a</i> pigment ratio	5.52 ± 1.53	9.28 ± 1.88	2.68	0.165	0.53
ΣXC:antenna pigment ratio	7.17 ± 2.01	10.28 ± 0.89	2.45	0.165	0.46

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Table 3 Results of multivariate PERMANOVA analysis to test for differences in temperature and sites. Data were normalised and dissimilarities calculated as Euclidian distances. *P*-values were calculated from 999 permutations of the residuals under the reduced model. Significant results ($P < 0.05$) are highlighted in bold.

		df	Mean squares	Pseudo-F	<i>P</i> (perm)
α	Site	1	0.0052	1.5444	0.237
	Temperature	3	0.0307	9.0127	0.009
	Site x Temperature Residual	1 20	0.0001 0.0034	0.0058	0.928
I_k	Site	1	745.0	2.742	0.100
	Temperature	3	20189.3	76.516	0.001
	Site x Temperature Residual	1 20	423.8 271.7	1.560	0.211
$rETR_{max}$	Site	1	15.35	0.717	0.417
	Temperature	3	1122.24	52.420	0.001
	Site x Temperature Residual	1 20	22.51 21.41	1.051	0.321
NPQ_{max}	Site	1	11.2083	11.21	0.001
	Temperature	3	12.0839	12.08	0.001
	Site x Temperature Residual	1 20	0.0335 0.5527	0.03 0.55	0.080

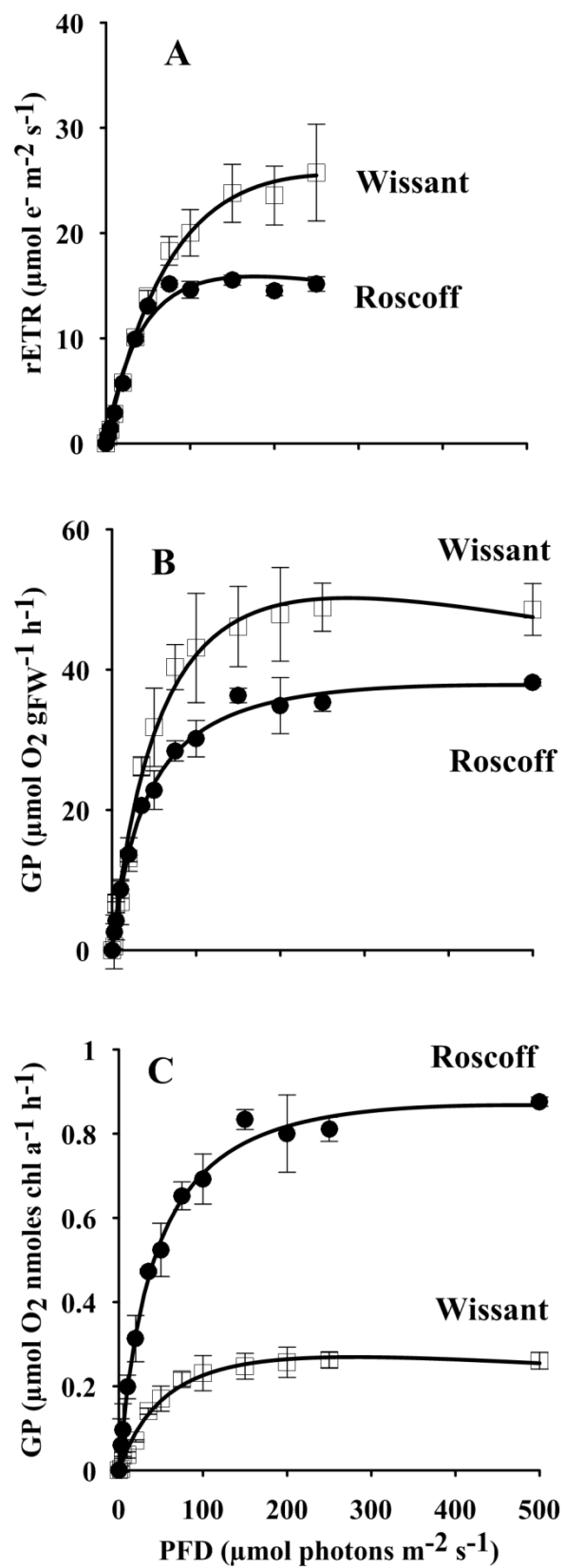


Fig. 1

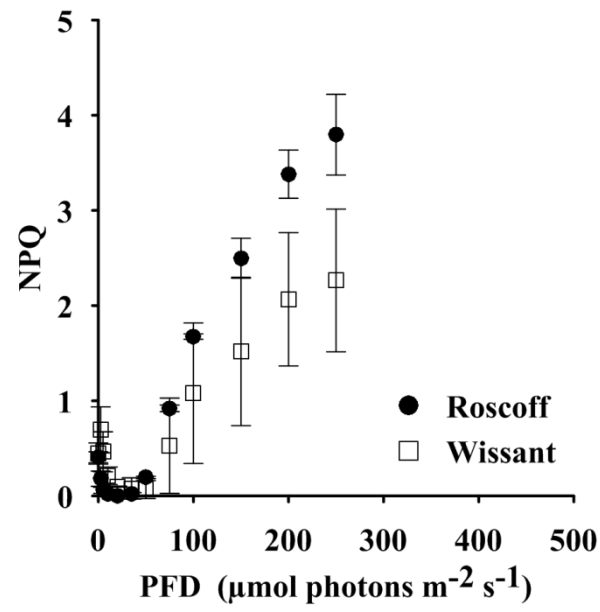


Fig. 2

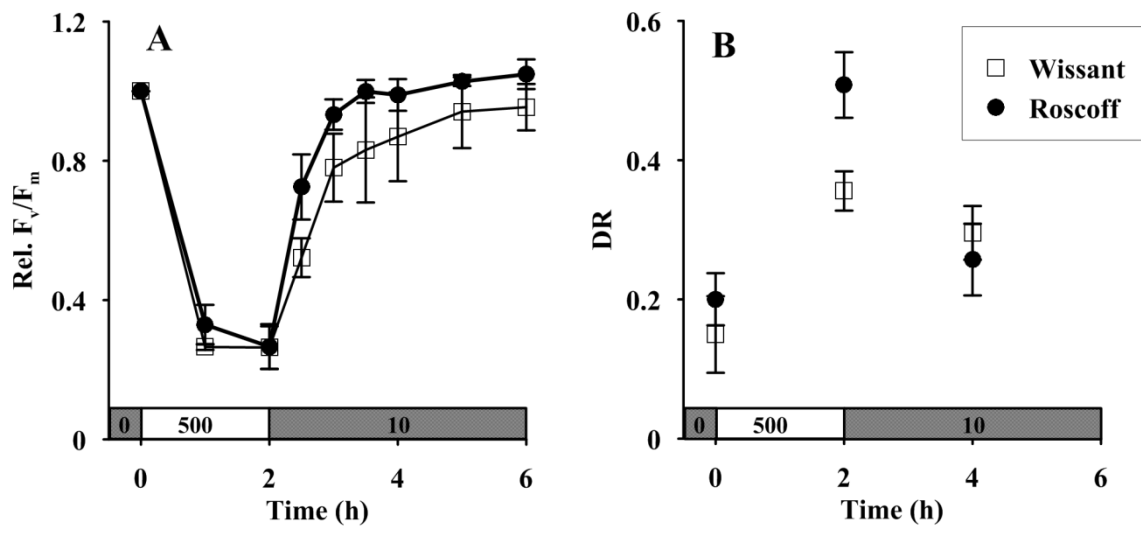


Fig. 3

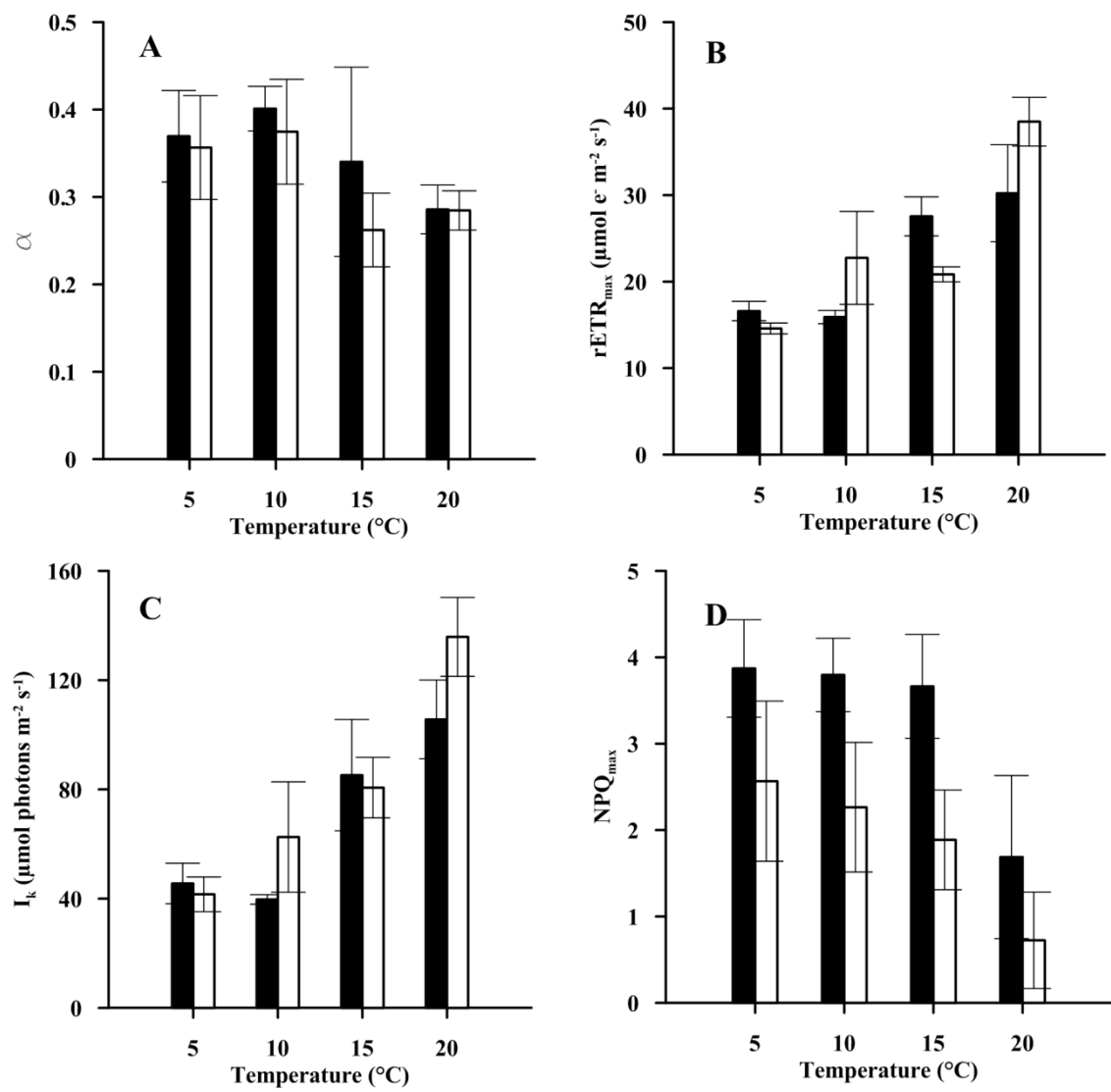


Fig. 4

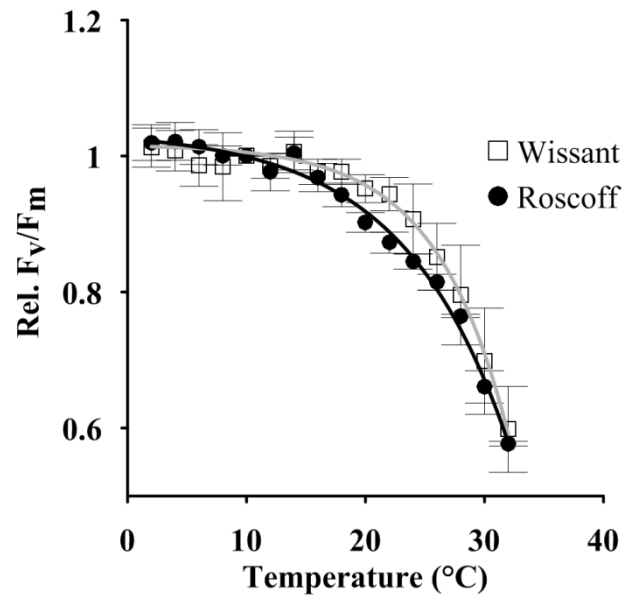


Fig. 5