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

3 Running head: Stress response in *Laminaria digitata* gametophytes

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25

26 **Summary**

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

43

44

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

47

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

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

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

77 Extremely high light has been shown to be adverse for the development and viability of
78 post-settlement stages (gametophytes and embryonic sporophytes) (Lüning & Neushul,
79 1978; Fetjek *et al.*, 2011). Likewise, extreme high temperature events (such as El Niño
80 events) have been shown to affect the reproductive success of gametophytes (Ladah &
81 Zertruche-Gonzales, 2007; Oppliger *et al.*, 2012).

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

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

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

95 macroscopic sporophyte stages (Gevaert *et al.*, 2003; Delebecq *et al.*, 2011), in the
96 laboratory on zoospores (Roleda, 2009) and in the gametophytic and embryonic
97 sporophyte stages of kelp (Hanelt *et al.*, 1997; Altamirano *et al.*, 2004). Factors other
98 than light, such as temperature, can accelerate photoinhibition by altering the PSII repair
99 mechanisms (Takahashi & Murata, 2008). Toxic active derivatives of oxygen (oxygen
100 radicals), byproducts of photosynthesis, can also be over-produced under adverse
101 environmental conditions (when exceeding the scavenging potential of cells) and can
102 alter the biological integrity of cells (Ledford & Niyogi, 2005; Allakverdiev *et al.*,
103 2008).

104 To cope with excess light, energy is dissipated in the form of heat to rapidly regulate
105 light harvesting; this mechanism is widespread in photoautotrophs (Raven & Geider,
106 2003). Increased thermal energy dissipation of excess light involves the xanthophyll
107 cycle in brown algae, which plays a major role in the fast-dynamic acclimation to
108 change in light, temperature and desiccation in macroscopic sporophytes (Fernandez-
109 Marin *et al.*, 2011). However, the implication of xanthophyll cycle in the
110 photoprotection process in kelp gametophytes has only been mentioned (Hanelt *et al.*,
111 1997; Altamirano *et al.*, 2004), without being clearly demonstrated.

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

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

126

127 **Materials and Methods**

128 *Study site*

129 A complete description of the two sites and environmental conditions during
130 experiments is given in Delebecq *et al.*, (2013); consequently, we describe only the
131 main characteristics of both sites here. We collected the seaweed material from two
132 populations of *L. digitata*, growing in the upper subtidal zone (0-1 m) of a rocky shore
133 in Roscoff (48°5'N, 3°6'W) and in Wissant (50°5'N, 1°4'E), located in the western and
134 eastern part of the English Channel, respectively. The sites were selected for their large
135 kelp stands and also for their contrasting environmental conditions. Light attenuation
136 (m^{-1}) of photosynthetically active radiation (400–700 nm) ranged from 0.09 to 0.57 m^{-1}
137 in Roscoff and from 0.19 to 0.96 m^{-1} in Wissant (Delebecq *et al.*, 2013) due to high
138 turbidity in the eastern English Channel. Seawater surface temperature displays high
139 seasonal variation at both sites, with a slightly broader annual temperature range in
140 Wissant, from 5°C in winter to 20°C in summer, than in Roscoff, from 8°C in winter to
141 17°C in summer, due to the influence of the North Atlantic Ocean and depth of the

142 continental shelf. Environmental conditions in the two sites at the time of collection and
143 1 month prior to sampling are given in **Table 1**.

144 *Sampling and culture conditions*

145 Fertile sporophytes of *L. digitata* were collected at low tide in November 2008 in
146 Roscoff, and in February 2009 in Wissant. *L. digitata* is reproductive most of the year in
147 Roscoff, but the main spore/gamete release events generally occur in August-September
148 and November-December in Northern Brittany (Arzel, 1998). In Wissant, *L. digitata* is
149 reproductive at the end of winter and throughout spring. Mature sori were cut, cleaned,
150 and dried at 10°C and in the dark for several hours. Sori were subsequently washed with
151 distilled water and sterile seawater, and placed in 50 mL Falcon tubes (BD Biosciences,
152 San Jose, CA, USA), filled with sterile Provasoli enriched seawater (PES) (Provasoli,
153 1968), and maintained overnight in the dark on a rotary shaking table to induce
154 zoospore release. Zoospores in suspension were checked with inverted-light microscope
155 to ensure that zoospores were viable, before being placed in Petri dishes (BD Falcon,
156 Franklin Lakes, NJ, USA). Zoospores were allowed to settle and developing
157 gametophytes were cultured in thermostatic chamber at 10°C under an irradiance of 35
158 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (photon flux density, PFD, 400-700 nm), produced by fluorescent
159 tubes (L8W/840, cool white, Osram, Germany) in a light:dark cycle 12:12 h. PFD was
160 measured with a cosine-corrected quantum sensor (Li-192SA, LiCor, Lincoln,
161 Nebraska, USA), connected to a data logger (Li 1400, LiCor, Lincoln, Nebraska, USA).
162 The medium was changed once a week. Experiments started approximately after 1
163 month of cultivation when the density was high enough for fluorescence measurements.
164 The density of the gametophytes was $4711 \pm 1140 \text{ ind m}^{-2}$ for Roscoff ($43 \pm 3\%$ cover)
165 and $3600 \pm 691 \text{ ind m}^{-2}$ for Wissant ($41 \pm 7\%$ cover). Gametophytes were composed of

166 few cells and arranged as homogenous thick layers at the bottom of the Petri dishes.
167 Gametophytes were isolated from three different parents at each site ($n = 3$) and were
168 cultivated separately to ensure independent replicates.

169 *Oxygen production*

170 Dark respiration (R_d) and net oxygen production (NP) was measured polarographically
171 at culture temperature (10°C), using a water-jacket thermostatic DW2/2 chamber
172 combined with a “Clark-type” oxygen electrode and a computer-interfaced box CB1
173 (Hansatech Instruments, Kings Lynn, Norfolk, UK). The gametophytes were first
174 detached from the substrate and resuspended several days prior to the experiment.
175 Detaching gametophytes from their substrate does not affect their growth, their
176 photosynthetic performance or their respiration (Fain & Murray, 1982). The
177 gametophytes were held 12 h in the dark prior to the experiment, and then placed in the
178 measurement chamber filled with 2 mL of PES and mixed with a magnetic stirrer. To
179 prevent oxygen saturation, a quarter of the medium was renewed after each light step.
180 To do so, a silk filtering mesh was used to retain the gametophytes in the media.
181 Respiration was measured in the dark, and gametophytes then underwent light-
182 increasing steps of 20 min each (13 light steps, ranging from 2.5 to 500 $\mu\text{mol photons}$
183 $\text{m}^{-2} \text{s}^{-1}$) using a halogen lamp (KL 2500 LCD, Schott, Germany) with a daylight cut-off
184 filter (Schott, Germany). R_d , NP and gross oxygen production (GP) rates were
185 calculated based on fresh weight (FW, $\mu\text{mol O}_2 \text{g}_{\text{FW}}^{-1} \text{h}^{-1}$) and chlorophyll *a* content (chl
186 *a*, $\mu\text{mol O}_2 \text{nmoles chl } a^{-1} \text{h}^{-1}$). FW was measured after collecting gametophytes on a
187 silk filtering mesh that had previously been weighed.

188 *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

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

219 *Response to high irradiance*

220 To study high light stress, the settled gametophytes in a Petri dish filled with PES at
221 cultivation temperature were exposed to $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 2 h, and then, to
222 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
223 measured every 30 min. Samples for pigment analyses were simultaneously collected at
224 the start of the experiment, at the end of the light stress, and 2 h after the return to dim
225 light. Gametophytes were detached from the substrate and the gametophyte suspension
226 was filtered on a $20 \mu\text{m}$ silk filtering mesh and subsequently deep-frozen in liquid
227 nitrogen for further pigment analyses.

228 *Response to temperature*

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

234 *Pigment analysis*

235 Pigment contents of gametophytes were extracted by sonication and grinding in a cold
236 mortar with methanol and methylene chloride. Extracts were centrifuged and
237 supernatants were collected and dry-evaporated under nitrogen. Salt contents of the
238 extracts were removed and the organic phase was evaporated and dissolved in methanol
239 for injection. Pigment analysis was performed by high performance liquid
240 chromatography (HPLC) (Beckman, system Gold, 126) with a reverse-phase column (C
241 18 Allure, Restek). Separation was performed with a solvent delivery profile adapted
242 from Arsalane *et al.*, (1994). Pigment contents were quantified using specific absorption
243 coefficients and normalised to the total pigment content. The conversion of violaxanthin
244 into antheraxanthin and zeaxanthin was estimated by calculating the de-epoxidation
245 ratio (DR):

$$246 \text{ DR} = (\text{antheraxanthin} + \text{zeaxanthin}) / (\text{violaxanthin} + \text{antheraxanthin} + \text{zeaxanthin})$$

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

252 *Statistical analyses*

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

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

265

266 **Results**

267 *Comparison of the photosynthetic activity between the two sites*

268 Gametophytes from the two sites showed differences in their photosynthetic parameters
269 (**Fig. 1**). When normalized to chl a , $P_{max(chla)}$ values, the light-saturated maximal gross
270 oxygen production values, were higher in Roscoff than in Wissant ($t = 8.0$, Holm-
271 adjusted $P = 0.003$), with $0.27 \pm 0.01 \mu\text{mol O}_2 \cdot \text{nmoles chl } a^{-1} \text{ h}^{-1}$ in Wissant, and $0.95 \pm$
272 $0.15 \mu\text{mol O}_2 \cdot \text{nmoles chl } a^{-1} \text{ h}^{-1}$ in Roscoff (**Fig. 1 C**). $rETR_{max}$ values (22.74 ± 5.37
273 $\mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$ in Wissant and $15.90 \pm 0.77 \mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$ in Roscoff), the light-
274 saturated maximal electron transfer rates, and $P_{max(FW)}$ on a FW basis (56.47 ± 13.18
275 $\mu\text{mol O}_2 \text{ g}_{FW}^{-1} \text{ h}^{-1}$ in Wissant and $38.33 \pm 1.12 \mu\text{mol O}_2 \text{ g}_{FW}^{-1} \text{ h}^{-1}$ in Roscoff), were not
276 significantly different between two sites ($t = 2.9$, Holm-adjusted $P = 0.133$ for $rETR_{max}$;
277 $t = 2.8$, Holm-adjusted $P = 0.142$ for $P_{max(FW)}$ values) (**Fig. 1 A-B**), likely due to low
278 statistical power resulting from small sample sizes (0.39 for $rETR_{max}$ and 0.32 for
279 $P_{max(FW)}$).

280 The same pattern appeared for the ascending slope under light limitation (α values).
281 $\alpha_{(chla)}$ values were significantly higher in Roscoff than in Wissant (respectively $0.005 \pm$
282 0.001 in Wissant and 0.023 ± 0.002 in Roscoff) ($t = 13.9$, Holm-adjusted $P = 0.001$)
283 when expressed on a chl a basis, whereas no differences were observed when expressed
284 in electron transport rates or when expressed on a FW basis (respectively 0.37 ± 0.06 in
285 Wissant and 0.40 ± 0.03 in Roscoff; $t = 0.68$, Holm-adjusted $P = 0.534$ for $\alpha_{(rETR)}$ and
286 1.15 ± 0.17 in Wissant and 0.99 ± 0.09 in Roscoff $t = 1.39$, Holm-adjusted $P = 0.305$ for
287 $\alpha_{(FW)}$) which can also result from very low statistical power (respectively 0.09 and 0.19).
288 Considering the light saturation parameter (I_k), no significant differences were found
289 between the two sites but the low statistical powers suggest that there were not enough
290 replication to resolve the differences ($t = 1.95$, Holm-adjusted $P = 0.245$ and $1-\beta = 0.30$
291 for $I_{k(rETR)}$, $t = 1.77$, Holm-adjusted $P = 0.152$ and $1-\beta = 0.28$ for $I_{k(FW)}$ and $t = 1.13$,
292 Holm-adjusted $P = 0.322$ and $1-\beta = 0.14$ for $I_{k(chla)}$). Despite this lack of significant
293 differences, average I_k values in gametophytes from Wissant were always greater than
294 I_k values in gametophytes from Roscoff ($62.67 \pm 20.20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in
295 Wissant, and $39.71 \pm 1.73 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in Roscoff on an electron rate basis;
296 $49.44 \pm 9.83 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in Wissant, and $38.83 \pm 3.42 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in
297 Roscoff on a FW basis and 52.39 ± 13.49 in Wissant and 41.98 ± 8.56 in Roscoff on a
298 chl a basis).
299 Despite high irradiance ($500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in comparison with the culture
300 conditions, there was no decrease in oxygen production observed.

301 *Comparison of pigment contents*

302 Pigment contents were similar at both sites (**Table 2**) except for chl *c* and violaxanthin
303 contents, which were higher in Wissant than in Roscoff ($t = 4.69$, Holm-adjusted $P =$
304 0.047 for chl *c* and $t = 4.26$, Holm-adjusted $P = 0.047$ for violaxanthin). When
305 expressed on a total pigment content basis, no differences were observed in chl *a*
306 contents in both sites, but chl *a* contents were slightly lower in Wissant and statistical
307 power was low (0.33). Therefore higher $\Sigma\text{XC}:\text{chl } a$ pigment ratio may have been
308 expected in Wissant, but the lack of significant difference is believed to be the result of
309 a low statistical power (0.53).

310 When chl *a* contents were expressed per unit fresh weight (respectively 47.74 ± 6.63
311 $\text{nmol g}_{\text{FW}}^{-1}$ in Roscoff and $155.95 \pm 38.22 \text{ nmol g}_{\text{FW}}^{-1}$ in Wissant), they were
312 significantly higher in Wissant ($t = 4.83$, Holm-adjusted $P = 0.036$).

313 *Comparison of photoprotective capacities*

314 *NPQ* was measured along with *rETR* (**Fig. 2**). *NPQ* values after prolonged darkness (12
315 h, corresponding to the end of the dark period of culture conditions) were higher than
316 those under weak irradiances. Maximal F_m' values were reached under an average
317 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
318 Wissant.

319 *NPQ* progressively developed with increasing irradiance. Maximal *NPQ* values
320 (NPQ_{250}) were reached at the highest applied irradiance ($250 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and
321 these values were significantly higher in gametophytes from Roscoff than in
322 gametophytes from Wissant ($t = 3.5$, Holm-adjusted $P = 0.047$), with NPQ_{250} values of
323 3.80 ± 0.42 in Roscoff and of 2.37 ± 0.56 in Wissant.

324 Intrinsic efficiency of PSII was altered in both populations, as indicated by the strong
325 decline of rel. F_v/F_m values (**Fig. 3 A**) when gametophytes were exposed to 500 μmol
326 photons $\text{m}^{-2} \text{s}^{-1}$ for 2 h. It reached a constant level, decreasing by 70% of the initial
327 F_v/F_m value after 2 h in both populations. In dim light, F_v/F_m recovered at a level within
328 5% of the initial F_v/F_m value after 1.5 h in Roscoff and after 4 h in Wissant.

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

340 *Comparison of the response to temperature*

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

344 NPQ_{250} was calculated at the end of each $rETR-E$ curves (**Fig. 4 D**). When exposed to
345 20°C, there was a slight decrease in the NPQ_{250} values, in comparison with the values
346 reached at 10°C.

347 The sensitivity of F_v/F_m to temperature was tested on a broad range of temperature (**Fig.**
348 **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
349 Wissant. With the progressive increase in temperature (from 10°C to 32°C), the rel.
350 F_v/F_m declined for temperatures greater than 20°C. There were no differences between
351 sites in the response parameters from the regression analysis.

352

353

354 **Discussion**

355 Microscopic stages are thought to be the hardest life-cycle stages of kelp (tom Dieck
356 1993). They form a seed bank which persists through stressful environmental conditions
357 and ensures the persistence of species when unfavourable conditions occur, as it may
358 happen during unusual heat waves (Ladah & Zertuche-Gonzales, 2007, Bartsch et al.,
359 2013). Therefore, a great tolerance of *L. digitata* gametophytes to stressful
360 environmental conditions is essential. In this study, we tested their physiological
361 tolerance to irradiation and temperature stress in two populations with different
362 environmental conditions along the French coast. The main result of the present study is
363 that gametophytes of *L. digitata* were highly resistant to the irradiation and temperature
364 treatments that might be locally encountered in the field. It is reinforced by the fact that
365 sori were sampled during months when irradiation and temperature stress are not
366 prevalent: their temperature and irradiation resistance may be even higher during
367 summer.

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

370 irradiance stress, confirming the great potential for high light tolerance in kelp
371 gametophytes (Iizumi & Sakanishi, 1994; Hanelt *et al.*, 1997; Altamirano *et al.*, 2004).
372 This tolerance arises from the efficient thermal dissipation of excess light (*NPQ*) and,
373 mainly, through the formation of antheraxanthin and zeaxanthin, which induce the
374 conformational change in the light harvesting complexes (LHC) II (Jahns & Holzwarth,
375 2009). This zeaxanthin-dependent quenching is a slow component of *NPQ*, and Garcia-
376 Mendoza & Colombo-Pallota (2007) have shown that kelp may lack the fast energy or
377 pH-dependent quenching. However, the large xanthophyll cycle pigment pool may
378 accelerate *NPQ* development and thus compensate for this lack. Under dim light,
379 epoxidation of zeaxanthin and antheraxanthin was virtually complete in less than 1 h.
380 The slowly relaxing component of photoinhibition generally corresponds to the
381 progressive re-activation of PSII or possibly to a conformational change of the LHC,
382 and probably the aggregation of LHC due to zeaxanthin binding (Garcia-Mendoza &
383 Colombo-Pallotta, 2007). Therefore, our study confirms that this efficient reversible
384 conversion between violaxanthin, antheraxanthin and zeaxanthin in gametophytes
385 (previously suggested by Hanelt *et al.*, 1997 and Altamirano *et al.*, 2004) is a
386 widespread mechanism of fast-dynamic acclimation to abiotic stress in macroalgae
387 (Fernandez-Marin *et al.*, 2011).

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

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

399 Acclimation to higher irradiances than the culture irradiance used in this experiment
400 and/or an intermittent light exposure may have also provided a higher tolerance to the
401 two- hour light stress of 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Han and Kain, 1996; Lavaud *et al.*,
402 2002). Along the French coast of the English Channel, irradiance of 500 $\mu\text{mol photons}$
403 $\text{m}^{-2} \text{s}^{-1}$ and more can be recorded during spring and summer low spring tides (Gevaert *et*
404 *al.*, 2003; Delebecq *et al.*, 2011; Delebecq *et al.*, 2013), but actual incident irradiances
405 are generally deeply attenuated by the sporophyte canopy (Gerard, 1984) which may
406 hypothesized that gametophytes display a great tolerance to high light.

407 This study also highlighted that the photosynthetic performance of *L. digitata*
408 gametophytes were not negatively affected by short-term fluctuations in water
409 temperature as it may be locally encountered in the field during periods of low tide.
410 Gametophyte PSII — essential for the whole photochemistry process (Havaux & Tardy,
411 1996; Roleda, 2009) — shows high resistance to the temperatures currently encountered
412 in the field. Increased temperature promotes electron transfer via the PSII through an
413 increase in thylakoid membrane fluidity (Havaux & Tardy 1996) and results in an
414 increase in maximum relative electron transfer rates ($rETR_{max}$) and in the onset of
415 saturation parameters (I_k), as observed here. An increase in gross oxygen production
416 with temperature can therefore be expected up to an optimum (Sukenic *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 thylakoid, 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

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

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

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

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

481

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

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

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

505 Gametophytes appeared to be resistant to the light and temperature conditions currently
506 experienced in the field. Regarding vulnerability to environmental conditions,
507 physiological studies on the interactive effects of multiple abiotic stressors are required
508 to improve our understanding of the microscopic stages in kelp (Fredersdorf *et al.*,
509 2009). For instance, desiccation tolerance (Contreras-Porcia *et al.*, 2012), ultraviolet

510 radiation (Roleda *et al.*, 2006) and burial in sediment deposition (Roleda *et al.*, 2011) all
511 affect microscopic stages. While the southern geographic range-limit of *L. digitata* is
512 thought to be set by inhibition of reproduction, as it was observed by Bartsch *et al.*
513 (2013) when temperature reaches 18°C, survival of microscopic stages remains essential
514 to cope with unusual stressful conditions. There is also an important need to obtain
515 more information on the persistence of these stages in the field. It could help in defining
516 which environmental conditions are really encountered in the field by the different
517 microscopic developmental stages. Despite the present study failed to report local
518 variation in the physiological response of *L. digitata*, it remains essential to take into
519 account local variation in predicting the impact of fast-changing conditions in coastal
520 areas.

521

522

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532

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760 **Figures legends**

761 **Fig. 1** Photosynthesis-irradiance curves (*P-I* curves) of gametophytes in *L. digitata* from
762 Wissant (□) and Roscoff (●) (n = 3), expressed as (A) relative electron transport rate
763 (*rETR*, $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$), (B) gross oxygen production on a fresh weight basis (GP, μmol
764 $\text{O}_2 \text{g}_{\text{FW}}^{-1} \text{h}^{-1}$) and (C) gross oxygen production per unit chl *a* (GP, $\mu\text{mol O}_2 \text{nmol chl } a^{-1}$
765 h^{-1}).

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

768 **Fig. 3** (A) Relative optimal quantum yield (rel. F_v/F_m) and (B) de-epoxidation ratio of
769 violaxanthin into antheraxanthin and zeaxanthin of gametophytes in *L. digitata* from
770 Wissant (□) and Roscoff (●) (n = 3). Gametophytes were first exposed to high
771 irradiance ($500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 2 h and then to dim white light ($10 \mu\text{mol}$
772 $\text{photons m}^{-2} \text{s}^{-1}$).

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

778 **Fig. 5** Relative optimal quantum yield (rel. F_v/F_m) of gametophytes in *L. digitata* from
779 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
Daylength (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 NO₃ (μmol. L⁻¹)	8.00	6.83 ± 1.07	13.62	13.42 ± 0.34
Dissolved PO₄ (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
Σ XC :chl <i>a</i> pigment ratio	5.52 ± 1.53	9.28 ± 1.88	2.68	0.165	0.53
Σ XC:antenna pigment ratio	7.17 ± 2.01	10.28 ± 0.89	2.45	0.165	0.46

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

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		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

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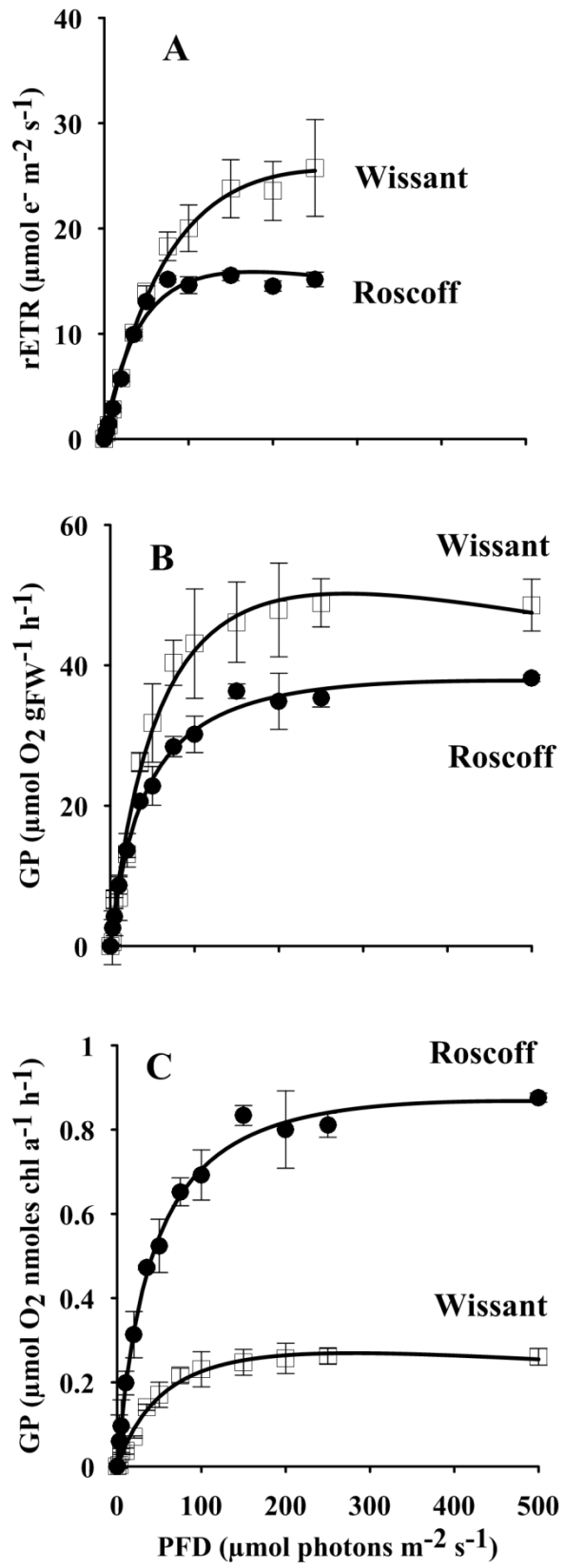


Fig. 1

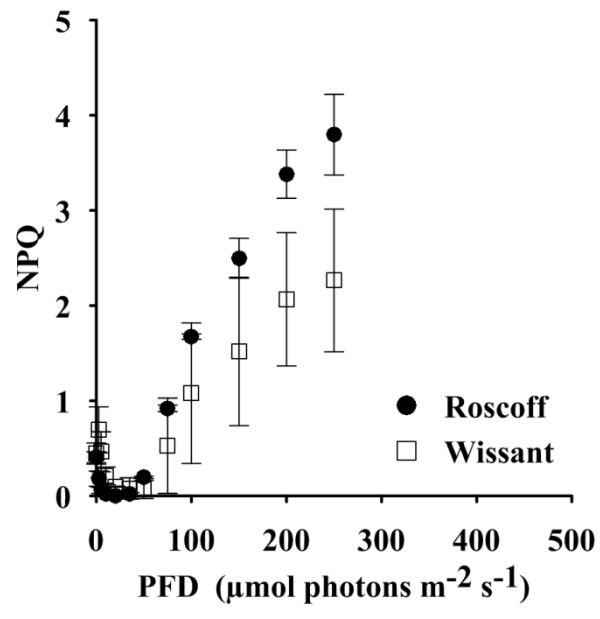


Fig. 2

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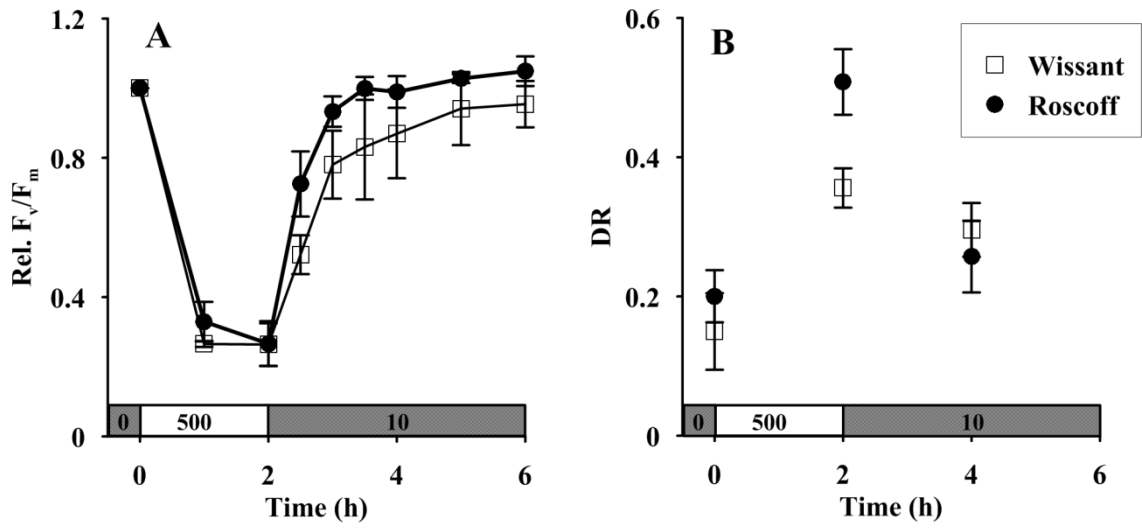


Fig. 3

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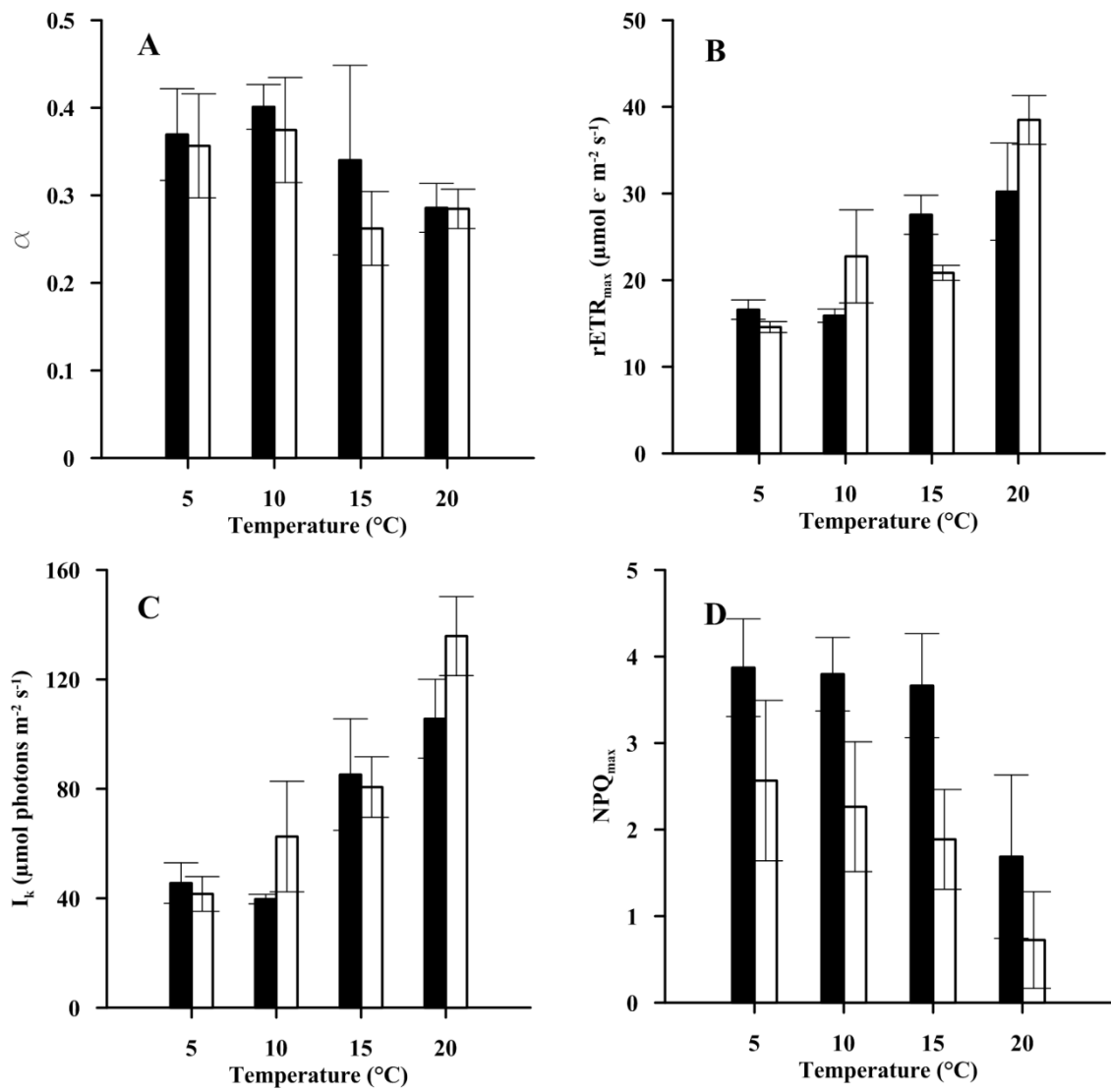


Fig. 4

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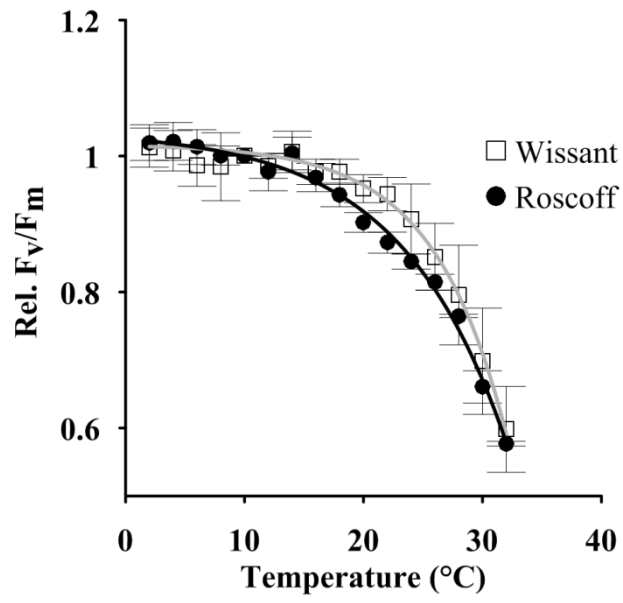


Fig. 5