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

Gaspard Delebecq, Dominique Davoult, Marie-Andrée Janquin, Luz Valeria Oppliger, Dominique Menu, Jean-Claude Dauvin, François Gevaert

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

4 Gaspard Delebecq • Dominique Davoult • Marie-Andrée Janquin • Luz Valeria

5 Oppliger •Dominique Menu • Jean-Claude Dauvin • François Gevaert

6 G. Delebecq* - M.-A. Janquin - F. Gevaert

7 Université Lille1, Univ Lille Nord de France, Station Marine, 62930 Wimereux, France

8 e-mail: gaspard.delebecq@gmail.com

9 G. Delebecq - D. Menu - M.-A. Janquin - F. Gevaert

10 CNRS, UMR 8187 LOG, 62930 Wimereux, France

11 D. Davoult

12 UPMC Université Paris 06, Station Biologique, 29680 Roscoff, France

13 D. Davoult

14 CNRS, UMR 7144 AD2 M, 29680 Roscoff, France

15 L. V. Oppliger

16 Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad

17 Católica de Chile, Post-code 6513677, Santiago, Chile.

18 J.-C. Dauvin

19 Université de Caen Basse Normandie, 14000 Caen, France

20 J.-C. Dauvin

21 CNRS, UMR 6143 M2C, 14000 Caen, France

22 * present address: Université de Bretagne Occidentale, Institut Universitaire Européen

23 de la Mer, 29280 Plouzané, France and CNRS, UMR 6539 LEMAR, 29280, Plouzané,

24 France

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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

Introduction

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

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

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

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

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 µmol photons m^{-2} 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 ± 1140 ind m^{-2} for Roscoff (43 ± 3% cover) and 3600 ± 691 ind m^{-2} for Wissant (41 ± 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 µmol photons m^{-2} 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, µmol O_2 g_{FW}^{-1} h^{-1}) and chlorophyll *a* content (chl *a*, µmol O_2 nmoles chl a^{-1} 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 µmol 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 µmol photons m^{-2} 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 µmol photons m^{-2} s^{-1} for 2 h, and then, to dim light (10 µmol photons m^{-2} 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 (C 18 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 = (antheraxanthin + zeaxanthin) / (violaxanthin + antheraxanthin + 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-β*) 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 ± 0.01 µmol O_2. nmoles chl a^{-1} h^{-1} in Wissant, and 0.95 ± 0.15 µmol O_2 nmoles chl a^{-1} h^{-1} in Roscoff (**Fig. 1 C**). $rETR_{max}$ values (22.74 ± 5.37 µmol e^- m^{-2} s^{-1} in Wissant and 15.90 ± 0.77 µmol e^- m^{-2} s^{-1} in Roscoff), the light-saturated maximal electron transfer rates, and $P_{max(FW)}$ on a FW basis (56.47 ± 13.18 µmol O_2 g_{FW}^{-1} h^{-1} in Wissant and 38.33 ± 1.12 µmol O_2 g_{FW}^{-1} 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). $\alpha_{(chla)}$ 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 $1\text{-}\beta$ = 0.30 for $Ik_{(rETR)}$, t = 1.77, Holm-adjusted P = 0.152 and $1\text{-}\beta$ = 0.28 for $Ik_{(FW)}$ and t = 1.13, Holm-adjusted P = 0.322 and $1\text{-}\beta$ = 0.14 for $Ik_{(chla)}$). Despite this lack of significant differences, average *Ik* values in gametophytes from Wissant were always greater than *Ik* values in gametophytes from Roscoff (62.67 ± 20.20 µmol photons m^{-2} s^{-1} in Wissant, and 39.71 ± 1.73 µmol photons m^{-2} s^{-1} in Roscoff on an electron rate basis; 49.44 ± 9.83 µmol photons m^{-2} s^{-1} in Wissant, and 38.83 ± 3.42 µmol photons m^{-2} 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 µmol photons m^{-2} 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 ΣXC: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 g_{FW}^{-1} in Roscoff and 155.95 ± 38.22 nmoles g_{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 µmol photons m^{-2} s^{-1} in Roscoff, and 50 µmol photons m^{-2} s^{-1} in Wissant.

NPQ progressively developed with increasing irradiance. Maximal *NPQ* values (NPQ_{250}) were reached at the highest applied irradiance (250 µmol photons m^{-2} 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 µmol photons m^{-2} 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 reconversion 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 $rETR_{max}$ 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 hardiest 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 if 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 µmol photons m^{-2} s^{-1} (Han and Kain, 1996; Lavaud *et al.*, 2002). Along the French coast of the English Channel, irradiance of 500 µmol photons m^{-2} $s^{-1}$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 (Sukenik *et al.*, 1987),

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

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

However, short-term temperature responses do not provide insight into long-term individual survival with regard to temperature: temperature optima for photosynthesis can be several degrees higher than temperature optima for survival and growth (Davison, 1991) as it is not possible to evaluate the extent of sublethal stress. Concerning *L. digitata* gametophytes, tom Diek (1993) showed that they were able to survive 8 weeks at 23°C, demonstrating that gametophytes are moderately resistant to temperature extremes that are rarely encountered across its geographic range. Moreover, there is seasonal acclimation of the temperature response of gametophytes (Lee & Brinkhuis, 1988) and temperature resistance of PSII may be even higher in gametophytes sampled in warmer months or acclimated to higher temperatures (Mohring *et al.*, 2013).

The two sites from which gametophytes were obtained were known to display different 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 $P_{maxchla}$. Hence, $P_{maxchla}$ 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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533 **References**

534 Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R.,
535 Mohanty, P. (2008). Heat stress: an overview of molecular responses in
536 photosynthesis. *Photosynthesis Research*, 98: 541-550.

537 Altamirano, M., Murakami, A., Kawai, H. (2004). High light stress in the Kelp *Ecklonia*
538 *cava*. *Aquatic Botany*, 79: 125-135.

539 Anderson (2001). A new method for non-parametric multivariate analysis of variance.
540 *Austral Ecology*, 26: 32-46.

541 Arsalane, W., Rousseau, B., Duval, J.C. (1994). Influence of the pool size of the
542 xanthophyll cycle on the effect of light stress in a Diatom: competition between
543 photoprotection and photoinhibition. Photochemistry and Photobiology, 60: 237-
544 243.

545 Arzel, P. (1998). *Les laminaires sur les côtes bretonnes. Evolution de l'exploitation et*
546 *de la flottille de pêche, état actuel et perspectives*, Plouzané, France.

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

552 Bartsch, I., Vogt, J., Pehlke, C., Hanlet, D. (2013). Prevailing surface temperature
553 inhibit summer reproduction of the kelp *Laminaria digitata* at Helgoland (North
554 Sea). *Journal of Phycology*, 49: 1061-1073.

555 Berx, B., Hughes, S.L. (2009). Climatology of surface and near-bed temperature and
556 salinity on the North-West European continental shelf for 1971-2000.
557 *Continental Shelf Research*, 29: 2286-2292.
558 Carney, L.T. (2011). A multispecies laboratory assessment of rapid sporophyte
559 recruitment from delayed kelp gametophytes. *Journal of Phycology*, 47: 244-
560 251.
561 Bolton, J.J., Lüning, K. (1982). Optimal growth and maximal survival temperatures of
562 Atlantic *Laminaria* species (Phaeophyta) in culture. *Marine Biology*, 66: 89-94.
563 Casper-Lindley, C., Björkman, O. (1998). Fluorescence quenching in four unicellular
564 algae with different light-harvesting and xanthophyll-cycle pigments.
565 *Photosynthesis Research*, 56: 277-289.
566 Christie, H., Jorgensen, N.M., Norderhaug, K.M., Waage-Nielsen, E., (2003). Species
567 distribution and habitat exploitation of fauna associated with kelp (*Laminaria*
568 *hyperborea*) along the Norwegian Coast. *Journal of the Marine Biological*
569 *Association of the United Kingdom* 83: 687-699.
570 Contreras-Porcia, L., Callejas, S., Thomas, D., Sordet, C., Pohnert, G., Contreras, A.,
571 Lafuente, A., Flores-Molina, M., Correa, J. (2012). Seaweeds early
572 development: detrimental effects of desiccation and attenuation by algal extracts.
573 *Planta*, 235: 337-348.
574 Cruz, S., Goss, R., Wilhelm, C., Leegood, R., Horton, P., Jakob, T. (2011). Impact of
575 chlororespiration on non-photochemical quenching of chlorophyll fluorescence
576 and on the regulation of the diadinoxanthin cycle in the diatom *Thalassiosira*
577 *pseudonana*. *Journal of Experimental Botany*, 62: 509-519.

578 Davison IR (1991) Environmental effects on algal photosynthesis: temperature. *Journal*
579 *of Phycology*, 27: 2-8.
580 Davison, I.R., Pearson, G.A. (1996). Stress tolerance in intertidal seaweeds. *Journal of*
581 *Phycology*, 32: 197-211.
582 Davoult, D., Engel, C.R., Arzel, P., Knoch, D., Laurans, M. (2011). Environmental
583 factors and commercial harvesting:exploring possible links behind the decline of
584 the kelp Laminaria digitata in Brittany, France. Cahiers de biologie marine, 52:
585 429-434.
586 Delebecq, G., Davoult, D., Menu, D., Janquin, M.-A., Migné, A., Dauvin, J.-C.,
587 Gevaert, F. (2011). *In situ* photosynthetic performance of *Laminaria digitata*
588 (Phaeophyceae) during spring tides in Northern Brittany. *Cahiers de Biologie*
589 *Marine*, 52: 405-414.
590 Delebecq, G., Davoult, D., Menu, D., Janquin, M.-A., Dauvin, J.C., Gevaert, F. (2013).
591 Influence of local environmental conditions on the seasonal acclimation process
592 and the daily integrated production rates of *Laminaria digitata* (Phaeophyta) in
593 the English Channel. *Marine Biology*, 160: 503-517.
594 tom Dieck (Bartsch), I. (1993). Temperature tolerance and survival in darkness of kelp
595 gametophytes (Laminariales, Phaeophyta): ecological and biogeographical
596 implications. *Marine Ecology Progress Series*, 100: 253-264.
597 Edwards, M.S., (2000). The role of alternate life-history stages of a marine macroalga: a
598 seed bank analogue? *Ecology* 81(9): 2404-2415.
599 Eilers, P.H.C., Peeters, J.C.H. (1988). A model for the relationship between light
600 intensity and the rate of photosynthesis in phytoplankton. Ecological Modelling,
601 42: 199-215.

602 Fain, S.R., Murray, S.N. (1982). Effects of light and temperature on net photosynthesis
603 and dark respiration of gametophytes and embryonic sporophytes of *Macrocystis*
604 *pyrifera*. Journal of Phycology, 18: 92-98.
605 Falkowski, P.G., Raven, J.A. (1997). *Aquatic photosynthesis*, Malden, Massachusetts.
606 Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A. (2007). G*Power 3: A flexible
607 statistical power analysis program for the social, behavorial and biomedical
608 sciences. *Behavior Research Methods*, 39: 175-191.
609 Fejtek, S.M., Edwards, M.S., Kim, K.Y. (2011). Elk Kelp, *Pelagophycus porra*,
610 distribution limited due to susceptibility of microscopic stages to high light.
611 *Journal of Experimental Marine Biology and Ecology*, 396: 194-201.
612 Fernández-Marín, B., Míguez, F., Becerril, J.M., García-Plazaola, J. (2011). Activation
613 of violaxanthin cycle in darkness is a common response to different abiotic
614 stresses: a case study in *Pelvetia canaliculata*. *BMC plant biology*, 11: 181.
615 Fredersdorf, J., Müller, R., Becker, S., Wiencke, C., Bischof, K. (2009). Interactive
616 effects of radiation, temperature and salinity on different life history stages of
617 the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia*, 160: 483-492.
618 Garcia-Mendoza, E., Colombo-Pallotta, M.F. (2007). The giant kelp *Macrocystis*
619 *pyrifera* presents a different nonphotochemical quenching control than higher
620 plants. *New Phytologist*, 173: 526-536.
621 Genty, B., Briantais, J.M., Baker, N.R. (1989). The relationship between the quantum
622 yield of photosynthetic electron transport and quenching of chlorophyll
623 fluorescence. *Biochimica et Biophysica Acta*, 990: 87-92.

624 Gerard, V.A. (1984). The light environment in a giant kelp forest: influence of
625 *Macrocystis pyrifera* on spatial and temporal variability. *Marine Biology*,
626 84:189-195.
627 Gerard, V.A. (1988). Ecotypic differentiation in light-related traits of the kelp
628 *Laminaria saccharina*. *Marine Biology*, 97: 25-36.
629 Gevaert, F., Créach, A., Davoult, D., Migné, A., Levavasseur, G., Arzel, P., Holl, A.-C.,
630 Lemoine, Y. (2003). *Laminaria saccharina* photosynthesis measured *in situ*:
631 photoinhibition and xanthophyll cycle during a tidal cycle. *Marine Ecology*
632 *Progress Series*, 247: 43-50.
633 Han, T., Kain, J.M. (1996). Effect of photon irradiance and photoperiod on young
634 sporophytes of four species of the Laminariales. *European Journal of*
635 *Phycology*, 31(3):233-240.
636 Hanelt, D., Wiencke, C., Karsten, U., Nultsch, W. (1997). Photoinhibition and recovery
637 after high light stress in different developmental and life-history stages of
638 *Laminaria saccharina* (Phaeophyceae). *Journal of Phycology*, 33: 387-395.
639 Harley, C.D.G., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L., Coyle, T.A.,
640 Graham, M.H. (2012). Effects of climate change on global seaweed
641 communities. *Journal of Phycology*, 48: 1064-1078.
642 Havaux, M., Tardy, F. (1996). Temperature-dependent adjustment of the thermal
643 stability of the photosystem II *in vivo*: possible involvement of xanthophyll-
644 cycle pigments. *Planta*, 198:324-333.
645 Henkel, S.K., Hofmann, G.E. (2008). Thermal ecophysiology of gametophytes cultured
646 from invasive *Undaria pinnatifida* (Harvey) Suringar in coastal California
647 harbors. *Journal of Experimental Marine Biology and Ecology*, 367: 164-173.

Iizumi, H., Sakanishi, Y. (1994). Temperature dependence of photosynthesis-irradiation (P-I) relationship of gametophytes of *Laminaria religiosa* Miyabe. *Bulletin of Hokkaido National Fisheries Research Institute*, 58: 45-51.

Ladah, L.B., Zertruche-Gonzalez, J.A. (2007). Survival of microscopic stages of a perennial kelp (*Macrocystis pyrifera*) from the centre and the southern extreme of its range in the Northern Hemsiphere after exposure to simulated El Nino stress. *Marine Biology*, 152: 677 – 686.

Jahns, P., Holzwarth, A.R. (2009). The role of xanthophylls cycle and of lutein in the photoprotection of photosystem II. *Biochimica et Biophysica Acta*, 1817:182-193.

Lavaud, J., Rousseau, B., van Gorkom, H.J., Etienne, A.-L. (2002) Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *Phaeodactylum tricornutum*. *Plant Physiology*, 129(3): 1398-1406.

Ledford, H.K., Niyogi, K.K. (2005). Singlet oxygen and photo-oxidative stress management in plants and algae. *Plant, Cell and Environment*, 28(8): 1037-1045.

Lee, J.A., Brinkhuis, B.H. (1988). Seasonal light and temperature interaction effects on development of *Laminaria saccharina* (Phaeophyta) gametophytes and juveniles sporophytes. *Journal of Phycology*, 24: 181-191.

Lüning, K. (1990). *Seaweeds: their environment, biogeography and ecophysiology*, Wiley, New York.

Lüning, K., Neushul, M. (1978). Light and temperature demands for growth and reproduction of laminarian gametophytes in Southern and Central California. *Marine Biology*, 45: 297-309.

672 Machalek, K.M., Davison, I.R., Falkowski, P.G. (1996). Thermal acclimation and
673 photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*.
674 *Plant, Cell and Environment*, 19: 1005-1016.
675 Mann, K.H. (1973). Seaweeds: their productivity and strategy for growth. The role of
676 large marine algae in coastal productivity is far more important than has been
677 suspected. *Science* 182, 4116: 975-981.
678 Martinez, E.A., Santelices, B. (1998). Selective mortality on haploid and diploid
679 microscopic stages of *Lessonia nigrescens* Bory (Phaeophyta, Laminariales).
680 *Journal of Experimental Marine Biology and Ecology*, 229: 219-239.
681 Matson, P.G., Edwards, M.S. (2007). Effects of ocean temperature on the southern
682 range limits of two understory kelps, Pterygophora californica and Eisenia
683 arborea, at multpiple life-stages. *Marine Biology* 151: 1941-1949.
684 Mohring, M.B., Kendrik, G.A., Wernberg, T., Rule, M.J., Vanderklift, M.A.(2013).
685 Environmental influences on kelp performance across the reproductive period:
686 an ecological trade-off between gametophyte survival and growth? *PLoS ONE*
687 8(6): e65310.
688 Morris, E.P., Kromkamp, J.C. (2003). Influence of temperature on the relationship
689 between oxygen- and fluorescence-based estimates of photosynthetic parameters
690 in a marine benthic diatom (*Cylindrotheca closterium*). *European Journal of*
691 *Phycology*, 38(2): 133-142.
692 Oppliger, L.V., Correa, J.A., Engelen, A.H., Tellier, F., Vieira, V., Faugeron, S.,
693 Valero, M., Gomez, G., Destombe, C. (2012). Temperature effects on
694 gametophyte life-history traits and geographic distribution of two cryptic kelp
695 species. *PLoSOne*, 7: e39289.

696 Provasoli, L. (1968). Media and prospects for the cultivation of marine algae Cultures
697 and Collections of Algae. *Proceedings of the US-Japan Conference*, Hakone,
698 September 1966, pp 63-75.
699 Raven, J.A., Geider, R.J. (2003). Adaptation, acclimation and regulation in algal
700 photosynthesis. In: Larkum, W.D., Douglas, S.E., Raven, J.A. (eds)
701 *Photosynthesis in Algae*. Kluwer Academic Publishers, Dordrecht, The
702 Netherlands, pp 385-412.
703 Raybaud, V., Beaugrand, G., Goberville, E., Delebecq, G., Destombe, C., Valero, M.,
704 Davoult, D., Morin, P., Gevaert, F., (2013). Decline in kelp in West Europe
705 and climate. *PLoSOne* 8:e66044.
706 Reed, D.C., Foster, M.S. (1984). The effects of canopy shading on algal recruitment and
707 growth in a giant kelp forest. *Ecology*, 65: 937-948.
708 Roleda, M.Y. (2009). Photosynthetic response of Arctic kelp zoospores exposed to
709 radiation and thermal stress. *Photochemical and Photobiological Sciences*, 8:
710 1302-1312.
711 Roleda, M.Y., Dethleff, D. (2011). Storm-generated sediment deposition on rocky
712 shores: Simulating burial effects on the physiology and morphology of
713 *Saccharina latissima* sporophytes. *Marine Biology Research*, 7: 213-223.
714 Roleda, M.Y., Hanelt, D., Wiencke, C. (2006). Exposure to ultraviolet radiation delays
715 photosynthetic recovery in Arctic kelp zoospres *Photosynthesis Research*, 88:
716 311 – 322.
717 Roleda, M.Y., Zacher, K., Wulff, A., Hanelt, D., Wiencke, C. (2008). Susceptibility of
718 spores of different ploidy levels from Antarctic *Gigartina skottsbergii*
719 (Gigartinales, Rhodophyta) to ultraviolet radiation. *Phycologia*, 47: 361-370.

720 Sauvageau, C. (1915). Sur la sexualite heterogamique d'une Laminaire (*Sacchoriza*
721 *bulbosa*). *Comptes rendus de l'académie des sciences de Paris*, 161: 796-799.
722 Serôdio, J., Cruz, S., Vieira, S., Brotas, V. (2005). Non-photochemical quenching of
723 chlorophyll fluorescence and operation of the xanthophyll cycle in estuarine
724 microphytobenthos. *Journal of Experimental Marine Biology and Ecology*, 326:
725 157-169.
726 Shapiro, S.S., Wilk, M.B. (1965). An analysis of variance test for normality (complete
727 samples). *Biometrika* 52, 591-611.
728 Sharkey, T.D. (2005). Effects of moderate heat stress on photosynthesis: importance of
729 thylakoid reactions, rubisco deactivation, reactive oxygen species, and
730 thermotolerance provided by isoprene. *Plant, Cell & Environment*, 28: 269-277.
731 Staehr, P.A., Wernberg, T. (2009). Physiological responses of *Ecklonia radiata*
732 (Laminariales) to a latitudinal gradient in ocean temperature. *Journal of*
733 *Phycology*, 45: 91-99.
734 Sukenik A, Bennett J, Falkowski PG (1987) Light-saturated photosynthesis - limitation
735 of electron transport or carbon fixation? *Biochimica et Biophysica Acta*, 891:
736 205-215.
737 Takahashi, S., Murata, N. (2008). How do environmental stresses accelerate
738 photoinhibition? *Trends in Plant Science*, 13: 178-182.
739 Walters, R.G. (2005). Towards an understanding of photosynthetic acclimation. *Jounal*
740 *of Experimental Botany*, 56: 435-447.
741 Wang, C., Fan, X., Wang, G., Niu, J., Zhou, B. (2011). Differential expression of
742 rubisco in sporophytes and gametophytes of some marine macroalgae.
743 *PLoSOne*, 6: e16351.

744 Wernberg, T., Russell, B.D., Moore, P.J., Ling, S.D., Smale, D.A., Campbell, A.,
745 Coleman, M.A., Steinberg, P.D., Kendrick, G.A., Connell, S.D. (2011a). Impact
746 of climate change in a global hotspot for temperate marine biodiversity and
747 ocean warming. *Journal of Experimental Marine Biology and Ecology* 400: 7-
748 16.
749 Wernberg, T., Russell, B.D., Thomsen, M.S., Gurgel, F.D., Bradshaw, C.J.A,
750 Poloczanska, E.S., Connell, S.D. (2011b). Seaweed communities in retreat from
751 ocean warming. *Current Biology* 21: 1828-1832.
752 Wernberg, T., Smale, D.A., Thomsen, M.S. (2012). A decade of climate change
753 experiments on marine organisms: procedures, patterns and problems. *Global*
754 *Change Biology*, 18: 1491-1498.
755 Wernberg, T., Smale, D.A., Tuya, F., Thomsen, M.S., Langlois, T.J., de Bettignies, T.,
756 Bennett, S., Rousseaux, C.S. (2013). An extreme climatic event alters marine
757 ecosystem structure in a global biodiversity hotspot. *Nature Climate Change*,
758 3:78-82.
759

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*, µmol e^- m^{-2} s^{-1}), (**B**) gross oxygen production on a fresh weight basis (GP, µmol
764 O_2 g_{FW}^{-1} h^{-1}) and (**C**) gross oxygen production per unit chl *a* (GP, µmol O_2 nmoles chl *a*$^-$
765 ^{1} 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 µmol photons m^{-2} s^{-1}) for 2 h and then to dim white light (10 µmol
772 photons m^{-2} s^{-1}).

773 **Fig. 4** Photosynthetic parameters (α (A), $rETR_{max}$ (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, $rETR_{max}$ 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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783

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	*t*	*Holm-adjusted P*	*1-β*
Pigment concentrations					
Chlorophyll *a* (chl *a*)	54.51 ± 2.64	50.05 ± 2.93	1.96	0.243	0.33
Chlorophyll *c* (chl *c*)	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 *a* pigment ratio	77.13 ± 9.11	89.65 ±10.56	1.55	0.195	0.23
ΣXC :chl *a* 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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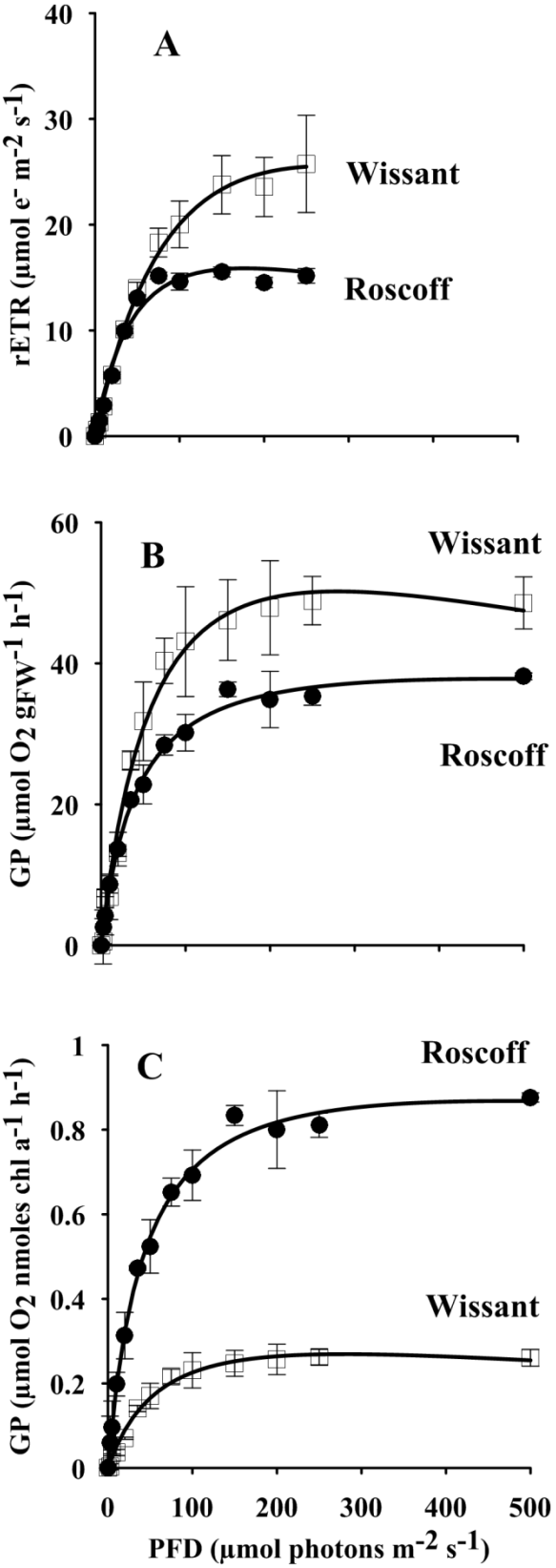
794

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.

799

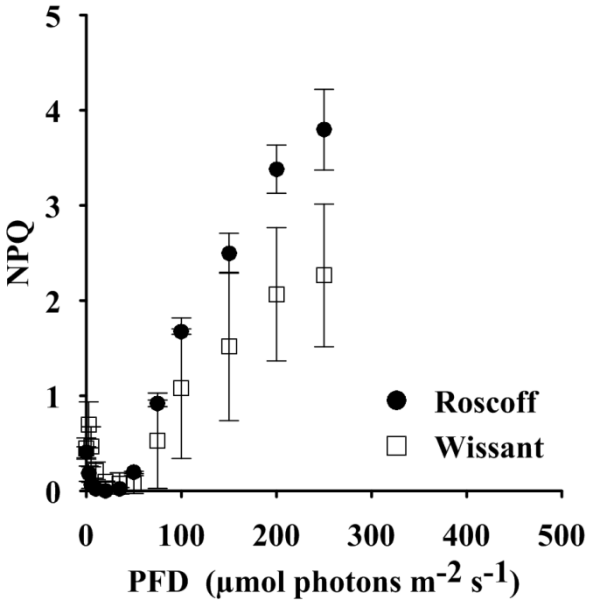
		df	Mean squares	Pseudo-F	*P* (perm)
α	**Site**	1	0.0052	1.5444	0.237
	Temperature	3	0.0307	9.0127	**0.009**
	Site x Temperature	1	0.0001	0.0058	0.928
	Residual	20	0.0034		
I_k	**Site**	1	745.0	2.742	0.100
	Temperature	3	20189.3	76.516	**0.001**
	Site x Temperature	1	423.8	1.560	0.211
	Residual	20	271.7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

800


A
rETR (µmol e- m-2 s-1)
40
30
20
10
0
Wissant
Roscoff
B
GP (µmol O2 gFW-1 h-1)
60
40
20
0
Wissant
Roscoff
C
GP (µmol O2 nmoles chl a-1 h-1)
1
0.8
0.6
0.4
0.2
0
Roscoff
Wissant
0
100
200
300
400
500
PFD (µmol photons m-2 s-1)

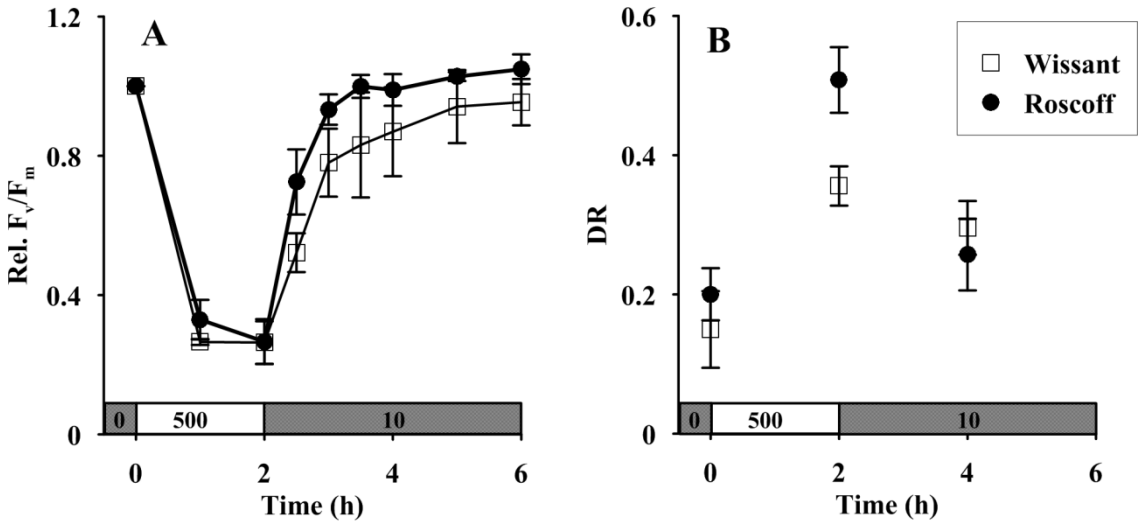
803

Fig. 1


NPQ
PFD (µmol photons m-2 s-1)
Roscoff
Wissant
0
1
2
3
4
5
0
100
200
300
400
500

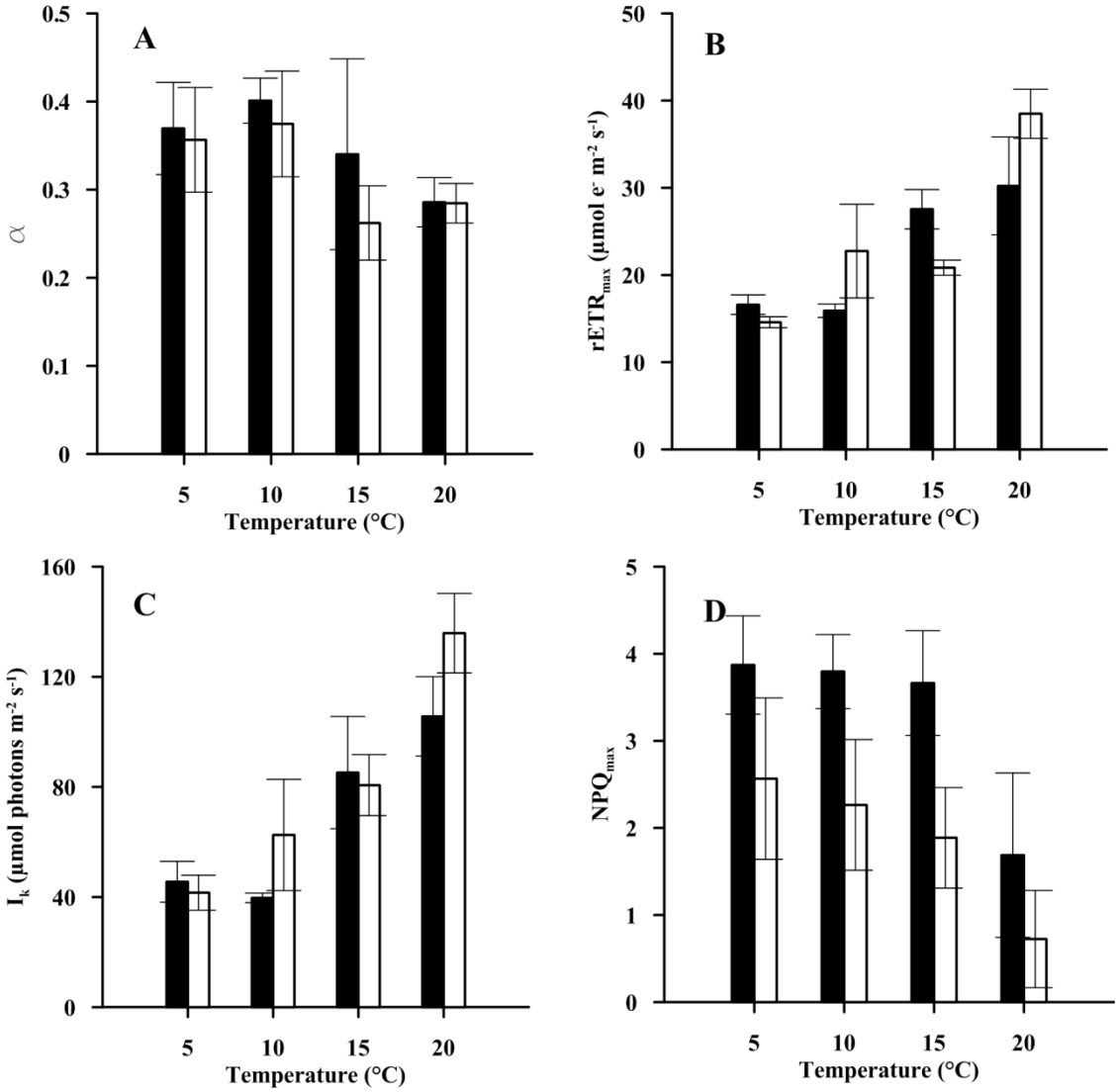
804 **Fig. 2**

805



806 **Fig. 3**

807


A
B
C
D
α
rETR$_{max}$ (µmol e$^-$ m^{-2} s^{-1})
I$_k$ (µmol photons m^{-2} s^{-1})
NPQ$_{max}$
Temperature (°C)
5
10
15
20

808 **Fig. 4**

809

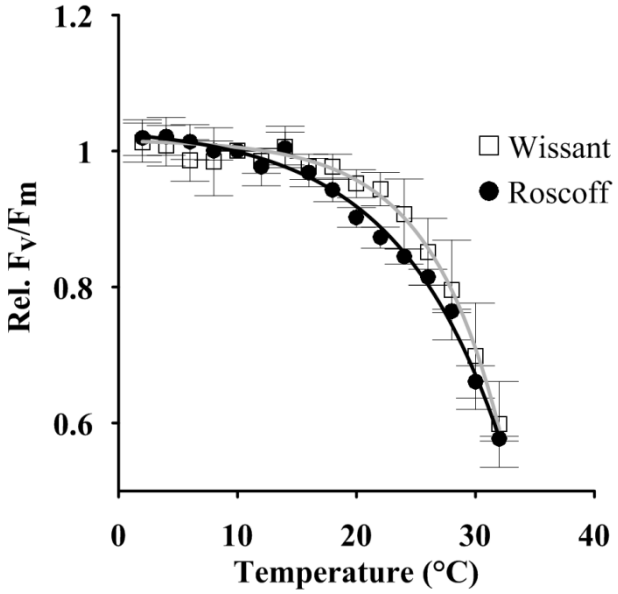


Fig. 5