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► To cite this version:

Martin Laviale, Alexandre Barnett, João Ezequiel, Bernard Lepetit, Silja Frankenbach, et al.. Response of intertidal benthic microalgal biofilms to a coupled light-temperature stress: evidence for latitudinal adaptation along the Atlantic coast of Southern Europe.. *Environmental Microbiology*, 2015, pp.3662-3677. 10.1111/1462-2920.12728 . hal-01097838

HAL Id: hal-01097838

<https://hal.science/hal-01097838v1>

Submitted on 9 Jan 2015

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1 **Response of intertidal benthic microalgal biofilms to a coupled light-temperature stress:**
2 **evidence for latitudinal adaptation along the Atlantic coast of Southern Europe.**

3

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21

22 Running title: microphytobenthos response to light-temperature stress

23

24 **Summary:**

25 Although estuarine microphytobenthos (MPB) is frequently exposed to excessive light and
26 temperature conditions, little is known on their interactive effects on MPB primary
27 productivity. Laboratory and *in situ* experiments were combined to investigate the short-term
28 joint effects of high light (HL) and high temperature (37°C vs. 27°C) on the operating
29 efficiency of photoprotective processes (vertical migration vs. non-photochemical quenching:
30 NPQ) exhibited by natural benthic diatom communities from two intertidal flats in France
31 (FR) and Portugal (PT). A clear latitudinal pattern was observed, with PT biofilms being more
32 resistant to HL stress, regardless the effect of temperature, and displaying a lower relative
33 contribution of vertical migration to photoprotection and a stronger NPQ *in situ*. However,
34 higher temperature leads to comparable effects, with photoinhibition increasing to about 3-
35 times (i.e. from 3 to 10 % and from 8 to 22 % in PT and FR sites, respectively). By using a
36 number of methodological novelties in MPB research (lipid peroxidation quantification, Lhcx
37 proteins immunodetection), this study brings a physiological basis to the previously reported
38 depression of MPB photosynthetic productivity in summer. They emphasize the joint role of
39 temperature and light in limiting, at least transiently (i.e. during emersion), MPB
40 photosynthetic activity *in situ*.

41

42 **Introduction**

43 Estuarine tidal flats are one of the most productive ecosystems on Earth, largely owing to the
44 photosynthetic productivity of benthic microalgae communities, or microphytobenthos (MPB)
45 (Underwood and Kromkamp, 1999). MPB communities are often dominated by motile
46 pennate diatoms (Haubois *et al.*, 2005; Méléder *et al.*, 2007; Ribeiro *et al.*, 2013). In fine
47 sediment habitats, they undergo rhythmic vertical migration patterns in the upper layers of the
48 sediment following tidal/dial cycles (Saburova and Polikarpov, 2003; Consalvey *et al.*, 2004;
49 Coelho *et al.*, 2011). During daylight emersion, the upward migration of cells results in the
50 formation of transient photosynthetic biofilms which are thus periodically exposed to variable
51 and extreme environmental conditions, due to the complex interplay of timing of tidal
52 exposure and weather conditions (Admiraal 1984; Paterson and Hagerthey, 2001). Excessive
53 light exposure generates intra-cellular oxidative stress (Roncarati *et al.*, 2008; Waring *et al.*,
54 2010) which can lower photosynthetic efficiency (i.e. photoinhibition; Wu *et al.*, 2012;
55 Cartaxana *et al.*, 2013) and ultimately community-level primary productivity (Blanchard *et al.*
56 *et al.*, 1996; Guarini *et al.*, 2006). Furthermore, benthic diatom's photosynthesis often operates
57 under the combined action of high light and other potentially stressful environmental factors
58 such as extreme temperature (Blanchard *et al.*, 1997; Serôdio and Catarino, 1999). Although
59 photosynthesis is known to be highly sensitive to temperature (Mathur *et al.*, 2014), effects of
60 high temperature on MPB productivity, either alone or in combination with excess light have
61 received little attention (Grant, 1986; Vieira *et al.*, 2013 and references therein). However,
62 recent results showed implications on both dial and seasonal scales (Hancke *et al.*, 2014). On
63 a longer term perspective, the cumulative effects of light and temperature could be relevant as
64 well, as climate change is expected to influence not only average values but also the
65 frequency and the intensity of extreme events such as heat waves (Schär *et al.*, 2004; Stott *et al.*,
66 *et al.*, 2004; Solomon *et al.*, 2007).

67 The actual photoinhibitory effects depend on the photoadaptive strategy of the diatoms of
68 MPB, i.e. the operating efficiency of a range of mechanisms which include ‘behavioural’ and
69 ‘physiological’ photoprotective processes (Barnett *et al.*, 2014). The ‘behavioural
70 photoprotection’ consists of a strong negative phototaxis resulting in downward migration
71 under supersaturating light intensities (Kromkamp *et al.*, 1998; Perkins *et al.*, 2010; Cartaxana
72 *et al.*, 2011; Serôdio *et al.*, 2012). The vertical migration of cells within the steep light
73 gradient of the sediment photic zone (Paterson and Hagerthey, 2001; Cartaxana *et al.*, 2011)
74 has been hypothesized to allow for the optimization of light harvesting and the avoidance of
75 excess light (Admiraal, 1984). Strong evidence for a photoprotective role of vertical migration
76 was provided by the use of the diatom motility inhibitor Latrunculin A (Lat A) on undisturbed
77 biofilms (Perkins *et al.*, 2010; Cartaxana *et al.*, 2011; Serôdio *et al.*, 2012). ‘Physiological
78 photoprotection’ mainly includes the excess energy-dissipative non-photochemical quenching
79 of chlorophyll (Chl) *a* fluorescence (NPQ). NPQ is mainly controlled by the presence of the
80 xanthophyll pigment diatoxanthin (DT) and of Lhcx proteins in the light-harvesting
81 complexes of photosystem II (PSII) (Depauw *et al.*, 2012; Lepetit *et al.*, 2012, 2013; Lavaud
82 and Goss, 2014). In diatoms, DT is produced by the (dark/low light) reversible light-
83 dependent conversion of the xanthophyll diadinoxanthin (DD) in the so called xanthophyll
84 cycle (XC) (Goss and Jakob, 2010). Besides its involvement in NPQ, it seems to have another
85 function as antioxidant (Lepetit *et al.*, 2010).

86 Despite the crucial role of NPQ and XC in the photoprotective response of diatoms to
87 excessive fluctuating light regimes, especially in field situations (Brunet *et al.*, 2010; Lavaud
88 and Lepetit, 2013; Lavaud and Goss, 2014), they have only scarcely been studied in MPB.
89 They show a relation to diurnal and tidal cycles, season, latitude (Serôdio *et al.*, 2005; van
90 Leeuwe *et al.*, 2008; Chevalier *et al.*, 2010), to vertical position of diatoms within the
91 sediment (Jesus *et al.*, 2009; Cartaxana *et al.*, 2011) or along the intertidal elevation gradient

92 (Jesus *et al.*, 2009), with significant differences between the main growth forms (i.e. epipellic
93 and epipsammic: Cartaxana *et al.*, 2011; Barnett *et al.*, 2014). As for the implication of Lhc
94 proteins in diatom NPQ, its characterization has been limited to few model species (Bailleul *et*
95 *al.* 2010; Zhu *et al.*, 2010). Lhc1 is involved probably at least by binding DT (Bailleul *et al.*,
96 2010; Büchel, 2014). It is present at low light and its transcript level/synthesis is slightly
97 enhanced under high light (HL) (Bailleul *et al.*, 2010; Wu *et al.*, 2012; Lepetit *et al.*, 2013).
98 While the transcript levels/synthesis of other examined isoforms (i.e. Lhc2, Lhc3, Lhc6)
99 strongly increase during HL (Zhu *et al.*, 2010; Lepetit *et al.*, 2013) and temperature stress (for
100 Lhc6; Wu *et al.*, 2012), their exact physiological role remains unknown, although Lhc3 and
101 Lhc6 are suspected to be involved in the binding of *de novo* synthesized DT and/or the
102 sustain part of NPQ (i.e. qI) during a prolonged HL stress (Zhu *et al.*, 2010; Lepetit *et al.*,
103 2013).

104 This work intended to study the combined effects of high light (HL) and high temperature on
105 the photophysiology of intertidal diatom-dominated MPB communities. Light stress-recovery
106 experiments (LSRE) were performed to distinguish physiological photoprotection efficiency
107 from photoinhibitory effects, and to quantify behavioural photoprotection (Serôdio *et al.*,
108 2012). Until now, these processes have been characterized only to the extent of chlorophyll
109 fluorescence measurements and XC pigments analysis. The present study combines
110 chlorophyll fluorescence imaging, pigment analysis and for the first time on intact biofilms,
111 lipid peroxidation quantification and immunodetection of Lhc proteins. LSRE were
112 performed on MPB from two locations in France and Portugal with different solar exposure
113 and temperature regimes, likely to support the establishment of diatom communities with
114 contrasting photo- and thermo-adaptive strategies. Moreover, the potential role of temperature
115 in modulating the HL-induced stress was investigated by carrying out LSRE at two
116 experimental temperature conditions ('optimal' vs. high temperature). In parallel, the

117 estimated photosynthetic and protective indices were further compared with field situation at
118 the two sites during a typical summer diurnal emersion.

119

120 **Results**

121 *Environmental conditions, in situ photosynthetic activity and taxonomic composition of*
122 *microphytobenthos in PT and FR sites*

123 Environmental conditions and MPB photosynthetic activity were assessed during typical
124 summer diurnal emersion at both Portuguese (PT) and French (FR) intertidal flats. The
125 Photosynthetic Active Radiation (PAR) reached its daily maximum ($2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$)
126 at both sites (Fig. 1A-B) with a comparable light dose over the emersion (~ 19 to 23 mol
127 photons m^{-2} , Table S1). Temperature was higher at PT site (Fig. 1A-B). Average temperatures
128 at the sediment surface were 37.5°C and 28.8°C for PT and FR sites, respectively.
129 Temperature decreased with sediment depth with a stronger effect in PT site (-7°C and -6°C
130 in the first 0.5 cm for PT and FR sites, respectively: Table S1, Fig. 1A-B).

131 At the beginning of emersion, the effective PSII quantum yield (Φ_{PSII}) was close to its
132 optimum at both sites (~ 0.70 : Fig. 1C-D, open squares). It decreased/increased opposite to
133 the PAR evolution over the emersion period, reaching values as low as ~ 0.40 . The maximal
134 non-photochemical quenching of Chl *a* fluorescence (NPQ_{max}) was about 4-5 at PT site and
135 lower than 1 at FR site at the beginning of emersion (Fig. 1C-D, closed circles). It decreased
136 continuously at PT site to reach values around 3 at the end of the emersion, while it covaried
137 with PAR at FR site, reaching a maximum value of about 6 (matching the sudden increase in
138 PAR level) and values of 3-4 at the end of the emersion.

139 At both sites, MBP communities were dominated by epipellic (i.e. motile) benthic diatoms of
140 the genus *Navicula* (Table S2). *Navicula phyllepta* sensu lato (Vanellander *et al.*, 2009) was
141 the dominant species at PT site (relative abundance: 44 %) while FR site was co-dominated
142 by *N. spartinetensis* (33 %) and *N. phyllepta* s.l. (28 %).

143

144 *Comparison between in situ and experimental conditions*

145 The two temperature treatments used for the laboratory experiments during the LSREs were
146 $27.4 \pm 1.2^{\circ}\text{C}$ and $37.3 \pm 0.7^{\circ}\text{C}$ (thereafter named 27°C and 37°C temperature treatments,
147 respectively), which was close to the average temperatures monitored at the sediment surface
148 for both sites (see above and Table S1). The light exposure of 3 h of $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$
149 represented a light dose of $12.96 \text{ mol photons m}^{-2}$ (Serôdio *et al.*, 2012). This light dose was
150 reached after 2 h 53 min and 3 h 34 min in PT and FR sites, respectively (Table S1).

151

152 *LSRE-induced photoinhibition and NPQ in sediment from FR site*

153 ΦPSII remained constant in samples maintained under LL throughout the experiment ($0.66 \pm$
154 0.02 , Fig. 2). In comparison, samples exposed to HL exhibited dramatic changes over time
155 (time: $P < 0.001$, see Table S3a for detailed statistical outputs). After 3h HL, ΦPSII decreased
156 down to values between 0.13 ± 0.03 (37°C +Lat A) and 0.34 ± 0.02 (27°C Ctrl). It increased
157 during LL recovery but did not reach back its initial level (0.46 ± 0.05 (37°C +Lat A) and
158 0.61 ± 0.01 (27°C Ctrl). The differences observed between temperature and chemical
159 treatments were significant (temperature: $P < 0.001$; chemical: $P < 0.01$; Table S3a), and they
160 both increased with time (time \times temperature: $P < 0.001$; time \times chemical: $P < 0.05$; Table
161 S3a). Similarly, NPQ of samples maintained under LL remained constant (0.38 ± 0.04 , Fig. 3)
162 and changed over time according to light (time: $P < 0.001$, Table S3b): it increased under HL
163 (between 4.4 ± 0.5 and 3.4 ± 0.4 from 37°C +Lat A and 27°C Ctrl treatments, respectively)
164 but only partially relaxed during the subsequent 15 min LL incubation (between 1.9 ± 0.1 and
165 1.1 ± 0.1 ; Fig. 3). The effect of temperature was highly significant (temperature: $P < 0.001$,
166 Table S3b) and the observed differences increased with time (time \times temperature: $P < 0.001$;
167 Table S3b). In comparison, the effect of lat A was less significant (chemical: $P < 0.05$ and
168 time \times temperature: $P = 0.11$; Table S3b).

169

170 *LSRE-induced allomerization of Chl a and xanthophyll cycle in sediment from FR site*

171 All samples exhibited typical diatom pigment signatures, including Chl *a*, Chls *c*,
172 fucoxanthin, the carotenoid β , β carotene (data not shown) and the xanthophyll cycle pigments
173 DD and DT. The allomer form of Chl *a* (Chl *a*-allo) accumulated during the LSRE as
174 illustrated by the Chl *a* allomerization molar ratio ($AR = \text{Chl } a\text{-allo} / \text{total Chl } a \times 100$, Fig.
175 4A). AR increased ~ 2-fold under HL and remained stable thereafter (time: $P < 0.001$; Table
176 S3c). No significant changes were observed with temperature (temperature: $P = 0.48$; Table
177 S3c), whereas AR was significantly higher in +Lat A samples than in Ctrl (chemical: $P <$
178 0.01 ; Table S3c), with mean maximum values of $11.9 \pm 5.0\%$ and $8.4 \pm 2.1\%$, respectively
179 (Fig. S1). AR did not significantly change in samples maintained in LL during 3 h ($5.9 \pm$
180 2.7% on average).

181 The pool of DD+DT (in mol. 100 mol Chl *a*⁻¹) increased ~ 3-fold under HL and remained
182 stable during the LL recovery (Fig. 4B, time: $P < 0.001$; Table S3d). Overall, it reached
183 higher values at 37°C than at 27°C (temperature: $P < 0.01$; Table S3d) but it was comparable
184 in Ctrl and +Lat A samples ($P = 0.58$; Table S3d). At 37°C, DD+DT increased even in the
185 samples maintained under LL for 3 h (from 15.0 ± 4.0 to 23.8 ± 6.6 mol. 100 mol Chl *a*⁻¹,
186 Tukey HSD: $P < 0.05$, Fig. S2). DD de-epoxidation ($DES = \text{DT} / \text{DD+DT} \times 100$) correlated
187 well with light whatever the temperature in both Ctrl and Lat A-treated samples ($P < 0.001$)
188 (Fig. 4C-D). DES increased under HL and decreased during LL recovery while it remained
189 stable in samples maintained in LL for 3 h ($14.0 \pm 3.2\%$ on average) (Fig. S3). DES was
190 significantly higher in +Lat A samples than in Ctrl ones (chemical: $P < 0.001$; Table S3d), and
191 at 37°C than at 27°C (temperature: $P < 0.001$; Table S3d) with maxima of $67.2 \pm 7.5\%$ and
192 $33.8 \pm 7.5\%$ at 37 °C and 27 °C, respectively, in +Lat A samples (Fig. S3). DES recovered
193 much faster at 27°C than at 37°C, especially for Ctrl samples. In contrast to AR and the
194 DD+DT pool, interaction between time and temperature was significant for DES (time \times

195 temperature: $P < 0.001$; Table S3d). Moreover, NPQ and DES were significantly positively
196 correlated according to the chemical treatment (Ctrl/+Lat A), *i.e.* the slope of the NPQ *vs.*
197 DES linear regression was 2.5 lower in +Lat A samples (Fig. S4) while for both treatments
198 the origin of the regression was similar and non-nul (Ctrl: 17.6 ± 0.4 ; +Lat A: 16.4 ± 0.4), *i.e.*
199 some DT was synthesized without NPQ development.

200

201 *LSRE-induced Lhcx synthesis in sediment from FR site*

202 The anti-FCP6 (*i.e.* Lhcx1) revealed a clear Lhcx isoform in FR samples (Fig. 5A). Its size of
203 about 24 kDa was slightly higher than the one of Lhcx 3 in *Phaeodactylum tricornutum* and
204 its amount increased after 3 h HL exposure especially in +Lat A samples. Interestingly, *N.*
205 *phyllepta* s.l., the dominant species of the MPB assemblage, showed an Lhcx pattern similar
206 to *P. tricornutum* so that three isoforms (Lhcx1, Lhcx2 and Lhcx3) could be identified. The
207 anti-Lhcx6 from *Thalassiosira pseudonana* revealed two Lhcx isoforms in FR samples, the
208 size of which was different from Lhcx6 and was neither present in HL exposed *P. tricornutum*
209 cells (Fig. 5B). *N. phyllepta* showed two isoforms, one which size was slightly higher than
210 Lhcx6 and the other one with a similar size to the 33-50 kDa isoform from FR samples. With
211 such size, this isoform could be a hypothetical Lhcx dimer (given that the available sizes of
212 Lhcx monomers deduced from the genomes of *P. tricornutum*, *T. pseudonana* and
213 *Fragilariopsis cylindrus* range between 21 and 29 kDa) but different than in *P. tricornutum*.
214 The 17-23 kDa isoform showed a size similar to Lhcx 1 in *P. tricornutum* and *N. phyllepta*
215 (see Fig. 5A). Interestingly, the two isoforms reacted to light, temperature and Lat A in
216 different ways: while the 17-23 kDa isoform appeared not to react to any of the treatments,
217 the 33-50 kDa isoform positively reacted to HL and 37°C with an effect enhanced by Lat A at
218 37°C (Fig. 5C).

219

220 *LSRE-induced lipid peroxidation in sediment from FR site*

221 Common markers of membrane lipid peroxidation (TBARS-thiobarbituric acid reactive
222 substances) were quantified. In Lat A treated samples, TBARS content normalized to the
223 surface microphytobenthos biomass (I_{diat} biomass index) did not change significantly over
224 time and was not significantly affected by chemical or temperature treatments ($P = 0.71$,
225 0.99 and 0.19 , respectively; Table S3f), exhibiting a mean value of $1.19 \pm 0.29 \cdot 10^{-6}$ nmol eq
226 MDA mL^{-1} (Fig. 6).

227

228 *Comparison between FR and PT sites: NPQ, photoinhibition, lipid peroxidation and Lhcx.*

229 ΦPSII and NPQ measured on PT samples at T0 (0.67 ± 0.02 and 0.40 ± 0.05 , respectively)
230 were comparable to the one measured on FR ones (Fig.2 and 3: 0.66 ± 0.01 and 0.39 ± 0.01 ,
231 respectively), and they exhibited the same general trend under HL stress. However, PT
232 samples were less affected by the HL treatment, as ΦPSII recovered faster under LL (site: $P <$
233 0.001 , Table S4a), regardless of temperature or Lat A application (Fig. 7A). Moreover, NPQ
234 did not relax entirely, but the level of this sustained NPQ was lower than in FR samples (site:
235 $P < 0.01$, Table S4b), although post-hoc multiple comparison tests did not show any statistical
236 differences between sites (Tukey HSD: $P > 0.05$; Fig. 7B).

237 For each site and temperature, the extent of photoinhibition and the contribution of vertical
238 migration to the overall photoprotection capacity of MPB were estimated as in Serôdio *et al.*
239 (2012) (Table 1). Photoinhibition was higher in FR than PT site but it increased similarly (~3
240 fold) with temperature. The contribution of vertical migration to photoprotection remained
241 below 15 % but was always lower in PT biofilms (< 5%).

242 In Lat A treated samples, TBARS content normalized to surface biomass did not change
243 significantly at PT site whatever the temperature (Fig. S5). Mean value was $3.26 \pm 0.66 \cdot 10^{-6}$
244 nmol eq MDA mL^{-1} , which was significantly higher than for FR samples (t-test: $P < 0.001$).

245 While the anti-FCP6 (i.e. Lhcx1) revealed two clear Lhcx isoforms in PT samples (Fig. 8), the
246 anti-Lhcx6 did not bring any signal probably due to its unspecificity as regards to the species
247 composing the microphytobenthic community of PT site. Their respective size were close
248 with one at about 23 kDa, the size of Lhcx 3 in *P. tricornutum* and *N. phyllepta*, and the other
249 one slightly higher (about 24 kDa) which was also present in FR samples (Fig. 5A). Both
250 isoforms (although the 24 kDa reacted in a stronger way) were enhanced after 3 h of HL
251 exposure as well as by the higher temperature; this effect was increased by the addition of Lat
252 A. Interestingly, at 27°C, the 24 kDa isoform was not present under LL and HL exposure, it
253 appeared with the addition of Lat A solely (LL 27°C +) and its content was increased by HL
254 exposure (HL 27°C +). Similarly, the 23 kDa isoform positively reacted to Lat A to a level
255 even similar to HL exposure without Lat A (compare 27°C LL+ and 27°C HL-).

256

257 **Discussion**

258 In this study, higher temperature significantly increased photoinhibition susceptibility of
259 epipelagic MPB biofilm. Communities from the Portuguese (PT) site were clearly more resistant
260 to a coupled high light-temperature stress, in comparison to biofilms from the French (FR)
261 site. At both site, photoprotection was based on the interplay between physiological (NPQ,
262 DT and Lhcx proteins) and behavioural (vertical motility) response, nevertheless PT biofilms
263 displayed a lower relative contribution of vertical migration and a stronger NPQ. The
264 apparent latitudinal pattern observed at the community level ('MPB biofilm') might be driven
265 by the individual response of the respective dominant diatom species/ecotypes in each site.
266 These different aspects are discussed below.

267

268 *Evidence for a latitudinal adaptation in the response of epipelagic MPB to a coupled light-*
269 *temperature stress*

270 Our results suggest that PT MPB communities were adapted to a higher light and/or
271 temperature environment than FR ones. Overall, PT samples were more resistant than FR
272 ones to HL stress, regardless the effect of temperature. At 27°C, PT control samples
273 recovered almost entirely from HL exposure (97 %), and photoinhibition increased only from
274 3 to 10 % at 37°C. This trend was also obvious *in situ*: the time course and level of Φ PSII
275 were comparable between sites despite the potentially more stressful conditions at PT site
276 (similar light conditions but 8-9°C higher average surface temperature). One of the reasons
277 for this differential latitudinal adaptation might lie in the photoprotective strategies the two
278 MPB communities exhibited. PT MPB communities showed higher NPQ_{max} average values,
279 an indication of a strong(er) physiological photoprotective response (Serôdio *et al.*, 2005). PT
280 NPQ_{max} was already high right from the beginning of the emersion although light intensity
281 was still moderate and it remained relatively stable along the emersion. Consequently, vertical

282 migration contributed less to the overall photoprotection capacity (< 5 %) in comparison to
283 FR biofilms. FR NPQ_{max} tended to follow the fluctuations of light intensity along the
284 emersion which would be in agreement with a more dynamics MPB response, regarding both
285 motility and physiology. Only few studies have examined the link between seasonal and/or
286 latitudinal photoacclimation and the coupling between behavioural and physiological
287 photoprotection in MPB communities. A similar increase of migration contribution was
288 reported for communities acclimated to lower irradiances (i.e. seasonal acclimation: Serôdio
289 *et al.*, 2012). Another study provided evidence for the differential role of migration *vs.* XC
290 along a latitudinal gradient (Ireland, UK and Portugal), although the role of sediment type (i.e.
291 mud *vs.* sand) could not be ruled out (van Leeuwe *et al.*, 2008).

292 In contrast, the contribution of thermal adaptation is difficult to ascertain. Although PT
293 samples were more resistant and resilient, higher temperature leads to comparable effects:
294 photoinhibition increased to about 3-times (i.e. from 3 to 10 % and from 8 to 22 %, in PT and
295 FR sites, respectively) and lipid peroxidation was comparable. The pattern of the detected
296 Lhcx isoforms was also similar, although no quantification was possible. Average surface and
297 -0.5 cm temperature conditions experienced by the FR and PT communities were clearly
298 different. The fact that this difference was lower than the observed amplitude over an
299 emersion could explain the apparent lack of temperature latitudinal pattern. This is in
300 agreement with other comparative studies on short-term (i.e. over few hours) temperature
301 effects on MPB photosynthesis (Grant, 1986; Blanchard *et al.*, 1997; Hancke and Glud,
302 2004). Instead, the different average temperature conditions at PT and FR sites recorded in
303 this study, which are representative of typical summer conditions in these areas (Guarini *et*
304 *al.*, 1997; Lillebo *et al.*, 2010), might have played a role on a longer time scale. As
305 temperature and light adaptations rely on similar strategies (Davison, 1991; this study, see
306 below), it is likely that the difference in average temperatures influenced the susceptibility of

307 MPB to photoinhibition, directly and/or via its photoacclimation status, together with e.g.
308 daily light dose, day length and/or UV exposure.

309 The latitudinal pattern in ecophysiological characteristics observed at the community level
310 coincided with a shift in the diatom species composition. Although it is difficult to separate
311 ‘community’ response from ‘individual species’ response within the biofilm, it is possible that
312 the observed responses at each site were driven by the few dominant species (representing c.a.
313 50% of the total abundance). The two dominant species, *N. phyllepta* s.l. and *N.*
314 *spartinetensis*, are known to be widespread along the European Atlantic and Northwestern
315 coasts (Vanellander *et al.*, 2009; Witkowski *et al.*, 2012). Epipellic diatoms generally exhibit
316 a low NPQ, in comparison to non-motile ones (Barnett *et al.*, 2014). This does not preclude
317 the existence of interspecific variability between organisms sharing the same life style,
318 considering both behavioural (Underwood *et al.*, 2005; Du *et al.*, 2010, 2012) and
319 physiological photoprotective mechanisms (Barnett *et al.*, 2014). Furthermore, the occurrence
320 of *N. phyllepta* s.l. at both sites can be explained by the pseudocryptic feature of this
321 taxonomic group. It comprises different closely related species morphologically
322 indistinguishable but exhibiting important ecophysiological differences and environmental
323 preferences (Vanellander *et al.*, 2009). To which extent the differences observed between PT
324 and FR sites (i.e. stronger NPQ_{max} in PT assemblage regardless of the stress treatment;
325 different Lhcx patterns) can be explained by the differential relative abundances of *N.*
326 *phyllepta* s.l. vs. *N. spartinetensis* and/or pseudocryptic species or ecotypes of *N. phyllepta*
327 s.l. is a question that remains to be answered.

328

329 *Higher temperature increases photoinhibition susceptibility in epipellic MPB*

330 To our knowledge, this is the first study which shows that on intact migratory MPB biofilms
331 high temperature and HL acts synergistically in decreasing photosynthesis, a feature so far

332 reported only for individual species (Salleh and McMinn, 2011). Other works have shown that
333 temperature alone impacts photosynthesis in benthic diatoms, in monospecific cultures
334 (Morris and Kromkamp, 2003; Yun *et al.*, 2010), MPB suspensions (Blanchard *et al.*, 1997),
335 and intact MPB biofilms (Vieira *et al.*, 2013). In conditions where photosynthesis is light-
336 saturated, benthic diatom photosynthesis can respond to a transient temperature increase if it
337 remains within a 25-35°C range. The high temperature used here ($37.3 \pm 0.7^\circ\text{C}$) falls slightly
338 outside this range, a situation likely to occur regularly *in situ* in summer, as confirmed by our
339 field data.

340 *In situ* measurements showed that ΦPSII correlated well with irradiance during the emersion
341 period. During LSRE, a strong decrease was also observed after 3 h of HL exposure.
342 Although the applied light dose was lower than *in situ*, our results show that it was strong
343 enough to induce photoinhibition at the community level. The incomplete recovery of ΦPSII
344 after 15 min LL coincided with a sustained phase of NPQ which was induced during the
345 prolonged HL exposure. This can be partly attributed to photoinhibition and illustrates the
346 physiological response to harsh stress (Zhu *et al.*, 2010; Wu *et al.*, 2012; Lavaud and Lepetit,
347 2013). At optimal temperature (27°C; see Blanchard *et al.*, 1997 and Experimental procedures
348 section), photoinhibition remained below 20 %, which is consistent with a previous study
349 using a similar experimental approach (Serôdio *et al.*, 2012). Photoinhibitory effects may be
350 attributed to the progressive accumulation of reactive oxygen species (ROS, Roncarati *et al.*,
351 2008; Waring *et al.*, 2010), as evidenced by the increase of AR at the end of LSRE. Indeed,
352 ROS have been shown to be directly involved in Chl *a* allomerization (Hynninen *et al.*, 2010
353 and references therein). This is also consistent with the accumulation of Chl *a* allomers in
354 response to oxidative stress in the diatom *P. tricornutum* (Cid *et al.*, 1995). When
355 superimposed to HL stress, significantly increased photoinhibition (c.a. 3-times increase in
356 the controls from 27 to 37°C) which is not surprising as PSII is the most thermosensitive

357 component of the photosynthetic apparatus (Mathur *et al.*, 2014). Nevertheless, the amount of
358 MDA and other aldehydes (TBARS) were not affected, suggesting that antioxidant activities
359 were sufficient to prevent from substantial ROS-mediated membrane damage during the 3 h
360 stress exposure, even when migration was inhibited. In such short-term experiments (i.e. < 24
361 h), it seems that only stronger oxidative stress such as UV- or herbicides-induced leads to
362 noticeable lipoperoxidation (Rijstenbil, 2005; Wang *et al.*, 2011).

363

364 *New evidences on the interplay between behavioural and physiological response to light-*
365 *temperature stress in epipelagic microphytobenthos*

366 In response to HL alone, diatoms which were free to move (i.e. in the controls) could reach
367 deeper layers of sediment with lower light conditions, potentially closer to their
368 photosynthetic optimum (Kromkamp *et al.*, 1998). This downward migration contributed to a
369 reduction of the deleterious effects of HL observed in Lat A treated samples (i.e. immobilized
370 cells at the surface of sediment) such as an enhanced accumulation of Chl *a* allomers and a
371 slower Φ PSII recovery. Our results are in agreement with previous works using Lat A on
372 natural biofilms (Perkins *et al.*, 2010; Serôdio *et al.*, 2012). Similarly, the motile diatoms
373 could reach a depth with lower and more stable temperature, as shown by the *in situ*
374 temperature depth profiles monitored here. Nevertheless, our measurements were not refined
375 enough to conclude about the steepness of the vertical temperature gradient in muddy
376 sediment, as for light (Paterson and Hagerthey, 2001; Cartaxana *et al.*, 2011). Additionally,
377 high temperature alone is known to directly affect the motility of MPB diatoms, i.e. over 35°C
378 cell motility significantly drops-down (Cohn *et al.*, 2003; Du *et al.*, 2012). Such feature might
379 partly explain the stronger photoinhibition observed at 37°C in Lat A free samples, together
380 with a direct impact of temperature on physiological mechanisms.

381 NPQ developed in the same range of mean values (i.e. 3-4) *in situ* than during LSRE with HL
382 stress alone. Concomitantly, the DD+DT pool size as well as DES increased. DT synthesis
383 was based not only on DD de-epoxidation (as illustrated by DES) but also by additional DT
384 *de novo* synthesis (Lavaud and Lepetit, 2013; Lepetit et al., 2013) which enlightened the high
385 level of stress the cells were exposed to. Whatever the source, additional DT provides higher
386 physiological photoprotection via higher NPQ and/or via higher prevention of oxidative lipid
387 peroxidation (Lepetit et al., 2010; Lavaud and Lepetit, 2013). DT synthesis (DD+DT and
388 DES) is enhanced by the high temperature stress alone (Salleh and McMinn, 2011), and this
389 effect was even stronger in conditions of a coupled HL and high temperature stress.
390 Specifically, DD+DT and DES showed a slower recovery which could represent a feed-back
391 reaction (i.e. related to HL/high temperature impairment of the Calvin cycle activity, Mathur
392 *et al.*, 2014) for the maintenance of a photoprotection capacity to cope with prolonged
393 stressful temperature conditions, even under LL (Lavaud and Lepetit, 2013; Lepetit *et al.*,
394 2013). When the diatom motility was inhibited by Lat A, DES significantly increased
395 independently of the temperature, and its recovery was slower. This was apparently not due to
396 a higher DT *de novo* synthesis as illustrated by the similar DD+DT pool size with and without
397 Lat A. Higher DT synthesis in presence of Lat A had no significant effect on NPQ, except for
398 its increased sustainability when in conjunction with high temperature. Higher sustained NPQ
399 (i.e. qI) is known to be related with both sustained DT and photoinhibition in conditions of a
400 strong stress (Lavaud and Lepetit, 2013; Lepetit et al., 2013). As a consequence, the DT
401 quenching efficiency (i.e. the slope of the NPQ-DT relationship) was lower in presence of Lat
402 A, likely due to the differential involvement of DT molecules in NPQ (Lavaud and Lepetit,
403 2013): in presence of Lat A, the additional DT molecules instead most probably acted as ROS
404 scavengers in prevention of lipid peroxidation (Lepetit *et al.*, 2010) arguing for a pronounced
405 stress (i.e. HL + high temperature + inhibition of motility) level.

406 Although, as expected, the Lhcx pattern in sediment samples was not as clear as the one of
407 monocultures of *N. phyllepta*, one of the dominant species common to the FR and PT MPB
408 communities, the confirmation of the presence of some of the known isoforms was possible.
409 While an isoform with a similar size than Lhcx1 apparently did not react to any of the
410 treatments, we found that the synthesis of two isoforms were enhanced by HL, high
411 temperature and Lat A: one isoform with a size similar to Lhcx3, and one isoform with the
412 size of a dimer which especially positively reacted to high temperature and Lat A treatment.
413 Interestingly, these two isoforms were also present in *N. phyllepta*. A third isoform (of about
414 24 kDa), which was not detected in *N. phyllepta*, was synthesized in all treatments. Based on
415 our knowledge of the synthesis and roles of Lhcx proteins during prolonged stress, it is
416 reasonable to argue that these isoforms participated, at least in part, to the above described
417 NPQ and XC patterns during the coupled HL and high temperature stress. Interestingly, the
418 Lhcx3 and 24 kDa isoforms strongly reacted to Lat A out of any stress and to a level
419 comparable to HL stress. It suggests that the inhibition of motility might generate a strong
420 preventive physiological answer in diatom cells blocked at the surface of sediment (which can
421 occur *in situ* during sediment desiccation events for instance), an additional hint that
422 physiological and behavioural photoprotection are closely related in epipelagic diatoms. This
423 compensation, together with a stronger NPQ induction and XC activity, was nevertheless not
424 strong enough to prevent immobilized cells from additional photoinhibition, as Φ PSII
425 recovery was always lower in Lat A treated samples than in migratory biofilms.

426

427

428 *Conclusions and perspectives*

429 By using a number of methodological novelties in MPB research (a new cryo-sampling
430 method, lipid peroxidation markers for photooxidative stress-TBARS, photosynthetic protein

431 markers for physiological protection-Lhcx), the present study clearly strengthens the previous
432 statement of a coupled and complementary action of physiological and behavioural processes
433 in protecting the photosynthetic activity of MPB diatoms in stressful environmental
434 conditions (Perkins et al., 2010, Cartaxana et al., 2011; Serôdio et al., 2012). Moreover, our
435 results bring a physiological basis to the previously reported depression of MPB
436 photosynthetic productivity in summer (Guarini et al., 1997). They emphasize the joint role of
437 temperature and light in limiting, at least transiently (i.e. during emersion), the photosynthetic
438 activity of MPB biofilm *in situ*. They confirm that high temperature, together with high light,
439 is an important environmental driver which supports seasonal, spatial and potentially
440 latitudinal MPB biodiversity and biomass distribution at the scale of intertidal mudflats along
441 the Atlantic coast of Southern Europe (Guarini et al., 1997; Brito et al., 2013).

442 Moreover, temperature being expected to increase due to global change (Solomon *et al.*,
443 2007), further longer-term experiments are required in order to question its importance in
444 modulating MPB photosynthetic activity. Our results support previous works suggesting that
445 MPB gross photosynthesis will most probably not be affected by a small increase (even if
446 significant, i.e. +1°C to +4°C for the most extreme previsions; Solomon *et al.*, 2007) of
447 average temperature (Vieira *et al.*, 2013; Hancke *et al.*, 2014). However, it does not preclude
448 a potential change in the community structure, as observed for associated invertebrate
449 assemblages (Hicks *et al.*, 2011), which might be of importance in driving the whole
450 community response as reported here. Further experiments should rather focus on the
451 cumulative effects of successive extreme events such as heat waves experienced during
452 spring/summer (Schär *et al.*, 2004; Stott *et al.*, 2004), with an emphasis on the
453 ‘species/individual’ vs. ‘community/biofilm’ response.

454

455 **Experimental procedures**

456 *Study areas and experimental design*

457 Two intertidal mudflats were sampled along the Atlantic Coast of Southern Europe, in France
458 and Portugal (FR and PT sites, respectively): a macrotidal one in the Baie de l'Aiguillon near
459 Esnandes (FR, 46°15.36'N, 1°8,55'W) and a mesotidal estuary in the Ria de Aveiro near Vista
460 Alegre (PT, 40°35' N, 8°41' W). Both sites are composed of fine muddy sediments (dominant
461 particle size < 63 µm) and are known to be colonized by epipellic diatom-dominated MPB
462 throughout the year (Haubois *et al.*, 2005; Serôdio *et al.*, 2012, respectively).

463 All experiments were performed consecutively in early summer (weeks 26-27, June-July
464 2012). For each site, natural migratory MPB was studied *in situ* over one diurnal emersion
465 period. In parallel, light stress-recovery experiments (LSRE) were carried out in controlled
466 conditions in the laboratory at two temperatures using freshly collected samples (see section
467 below). During LSRE, samples were sequentially exposed to (i) low light (LL) level to
468 determine pre-stress reference state; (ii) high light (HL) intensity to induce photoinhibitory
469 effects; (iii) LL to monitor recovery from HL stress (Serôdio *et al.*, 2012).

470

471 *In situ measurements*

472 For each site, the day for carrying out the experiment was selected so that the emersion period
473 matched with maximum light and temperature exposure, i.e. noon/early afternoon low tides.
474 Photosynthetic Active Radiation (PAR) and temperature at the surface of the sediment were
475 assessed every 30 s with a universal light-meter and data logger (ULM-500, Walz Effeltrich
476 Germany) equipped with a plane light/temperature sensor (accessory of the ULM-500) and a
477 plane cosine quantum sensor (Li-COR). Depth temperature profiles were measured with Hobo
478 sensors (Hobo Pro V2, Massachussets, USA) fixed on a home-made stick that was vertically
479 inserted into the sediment to position the sensors at four (-0.5 cm, -2 cm, -5 cm, -10 cm). The

480 effective photosystem II quantum yield (Φ PSII) and the non-photochemical quenching of
481 chlorophyll *a* (Chl *a*) fluorescence (NPQ) of MPB was assessed with a Water-PAM (micro-
482 fiber version, Walz GmbH, Effeltrich, Germany) as described in Lefebvre *et al.* (2011). Φ PSII
483 was calculated as $\Delta F / F_m' = (F_m - F) / F_m'$. Rapid light curves (RLCs) with 30 s light steps
484 were used to measure NPQ vs irradiance (NPQ-E) curves (as recommended in Lefebvre *et al.*,
485 2011). NPQ-E curves were fitted with the model by Serôdio and Lavaud (2011) in order to
486 estimate NPQ_{max} where $NPQ = (F_m - F_m') / F_m$. F_m and F_m' are the maximum Chl *a*
487 fluorescence levels measured in dark-adapted and illuminated (at the end of each RLCs light
488 steps) MPB, respectively, while F is the steady-state Chl *a* fluorescence level measured at the
489 end of each RLC light step, just before F_m' was assessed. F was measured with the application
490 of a non-actinic modulated beam of 455 nm, and F_m and F_m' with the application of over-
491 saturating (about 4000 μ mol photons $m^{-2} s^{-1}$) light pulses (800 ms). Because in *in situ* as well
492 as in laboratory (see below) conditions (i.e. light-responsive migratory biofilm) it is virtually
493 impossible to measure a true F_m level, for the calculation of NPQ, F_m was in reality the
494 maximum F_m' of the respective RLC (i.e. $F_m'_{max} \sim F_m$, see Lefebvre *et al.*, 2011).

495

496 *Light stress-recovery experiments (LSRE)*

497 LSRE were carried out following a protocol adapted from Serôdio *et al.* (2012). The top 1 cm
498 of sediment was collected at the beginning of low tide and immediately transported to the
499 laboratory where it was sieved (0.5 mm mesh) to remove debris and macrofauna. Sediment
500 was thoroughly mixed and spread in trays, forming a 2-4 cm thick slurry, and it was covered
501 with seawater from the sampling site and left undisturbed overnight in the dark at room
502 temperature (~ 20 - $25^\circ C$). The next morning, the slurry was homogenized and transferred to
503 24-well plates using a 100 mL syringe, filling the wells completely (ca. 3 mL). The well
504 plates were then exposed to constant LL of 50 μ mol photons $m^{-2} s^{-1}$, provided by three slide

505 projectors (Reflecta Diamator AF, Reflecta GmbH, Rottenburg, Germany) containing halogen
506 lamps (Quartzline DDL 150W, General Electric, USA) to induce the upward migration of
507 MPB. Once the MPB biofilm was formed (typically after 3 h), the plates were transferred to a
508 water bath of constant temperature of 25°C or 40°C and exposed to constant LL for 30 min.
509 These two experimental temperatures were defined based on a previous study (Blanchard *et*
510 *al.*, 1997) which defined 25°C as an optimum and 35-40°C as an extreme maximum for the
511 photosynthetic productivity of MPB. A fixed volume (200 µL) of Lat A solution (10 µM final
512 concentration) or of filtered sea water (Control samples: Ctrl) was added to each well. Lat A
513 solution was freshly prepared daily from a 1 mM stock solution of purified Lat A (Sigma-
514 Aldrich) dissolved in dimethylsulfoxide and stored at -20°C (Cartaxana and Serôdio, 2008).
515 After 15 min waiting for the inhibitor to diffuse, the plates were exposed to HL (1200 µmol
516 photons m⁻² s⁻¹, 3 h). This light dose (12.96 mol photons m⁻²) was previously shown to be
517 excessive for MPB photosynthesis (Serôdio *et al.*, 2012). After 3 h, samples were placed
518 under LL for 15 min. In parallel, additional samples (for both Ctrl and +Lat A treatments)
519 were maintained during 3 h under continuous LL. Temperature of the sediment in the top 5
520 mm was regularly checked using the same sensor previously described. The duration of each
521 step described above was always the same, but the timing was adjusted so that HL period
522 always started at the low tide maximum expected *in situ*.

523 For each chemical and light treatment (Ctrl and +Lat A; HL or continuous LL), several
524 endpoints were assessed on 3-6 independent samples at three times: just before the HL stress
525 (T₀), at the end of the HL stress and after 15 min of LL recovery (Recov). For the experiments
526 with FR samples, Chl *a* fluorescence was measured using an Imaging-PAM fluorometer
527 (Maxi-PAM M-series, Walz GmbH, Effeltrich, Germany) and samples were collected for
528 further quantification of photosynthetic pigments, lipid peroxidation, and Lhcx proteins. For
529 PT samples, Chl *a* fluorescence was measured with a FluorCAM 800MF, open version (PSI,

530 Brno, Czech Republic, see Serôdio *et al.*, 2013). Samples were collected for the same
531 analyses than for FR site, except that pigments were not quantified. Samples were collected
532 for the same analyses than for FR site, except that pigments were not quantified. Φ PSII and
533 NPQ were calculated as previously described (Serôdio *et al.*, 2012).

534 For the sediment sampling in wells, a contact-corer specially designed was used to collect
535 rapidly (< 30 s) the uppermost layer of sediment (0.5 mm thick, 1.5 cm² area) in each well. It
536 is based on the cryo-sampling of the biofilm, through the contact of the sediment surface with
537 a metal cylinder of known length previously cooled in liquid nitrogen (Laviale *et al.*,
538 submitted). Each core was immediately frozen in liquid nitrogen and stored at -80°C until
539 further analysis.

540

541 *Pigment analysis*

542 Pigment extraction and HPLC analysis were carried out following Lepetit *et al.* (2013), with
543 the exception of the first extraction step which was performed after 24 h lyophilisation of the
544 sediment cores (3 mL of 4°C mixture extraction buffer: 90% methanol/0.2 M ammonium
545 acetate (90/10 vol/vol) + 10% ethyl acetate). Extracted samples were analyzed with a Hitachi
546 LaChrom Elite HPLC system equipped with a 10°C-cooled autosampler and a Nucleosil 120-
547 5 C18 column (Macherey-Nagel). The allomerization of Chl *a* was calculated as the ratio AR
548 = $Chl\ a\text{-}allo / tot\ Chl\ a \times 100$ where $tot\ Chl\ a = (Chl\ a\text{-}allo + Chl\ a' + Chl\ a)$ and Chl *a*-allo
549 and Chl *a'* are the allomer and epimer forms of Chl *a*, respectively. The pigment molar
550 concentrations (expressed as mol. 100 mol Chl *a*⁻¹) for the xanthophyll cycle (XC) pigments
551 diadinoxanthin (DD) and diatoxanthin (DT) were used to calculate the DD de-epoxidation
552 state (DES) as follows: $DES = [DT / (DD + DT)] \times 100$.

553

554 *Lipid peroxidation*

555 Lipid peroxidation generated by oxidative stress was estimated by quantifying thiobarbituric
556 acide reactive substances (TBARS) according to Kwon and Watts (1964). Each sample was
557 resuspended in 0.8 mL of 10% trichloroacetic acid (TCA, Sigma-Aldrich) and 500 mg L⁻¹
558 butylated hydroxytoluene (BHT, Sigma-Aldrich) and sonicated (U200S control; IKA
559 Labortechnik, Ika-Werke GmbH, Staufen, Germany) on ice 5 times during 30 s (cycle: 1,
560 amplitude: 30%) with intervals of 30 s to prevent overheating. After extraction, 0.8 mL of
561 0.5% thiobarbituric acid (TBA, Sigma-Aldrich) in 10% TCA was added. The mixture was
562 heated for 30 min at 90 °C and immediately put on ice to stop the reaction. Samples were then
563 centrifuged (15 min, 10000 g) and 250 µL of supernatant were transferred in a 96 well-plate
564 (Sarsted). TBARS were measured by subtracting the nonspecific turbidity (at 600 nm) from
565 the TBARS absorption maximum (532 nm) using a Synergy HT absorbance microplate reader
566 (BioTek Instruments Inc., Vermont, USA). Blanks consisted of the same mixture without
567 sediment sample. TBARS concentration was expressed in malondialdehyde (MDA)
568 equivalent (nmol mL⁻¹) using an extinction coefficient of 155 mM⁻¹ cm⁻¹. As it was not
569 possible to obtain paired measurements of Chl *a* and MDA, values were normalized to the
570 microphytobenthos biomass present at the sediment surface (measured by spectral reflectance:
571 see below). As migration was susceptible to occur in Ctrl samples, only Lat A-treated samples
572 could be compared.

573

574 *MPB surface biomass*

575 MPB biomass present in the surface layers of the sediment was estimated by spectral
576 reflectance analysis with a fiber optic spectrometer (USB2000-VIS-NIR, grating #3, Ocean
577 Optics, Duiven, The Netherlands) according to Serôdio *et al.* (2009). In particular,
578 measurements were carried out under LL (50 µmol photons m⁻² s⁻¹) supplied with an halogen
579 lamp (120W, Plusline ES, Philips Lighting) positioned with a 45° angle to the sediment

580 surface while the sensor was at a fixed distance (~2 cm) perpendicularly to it. Reflectance
581 measurements at 550, 600 and 675 nm (ρ_{550} , ρ_{600} and ρ_{675} , respectively) were used to
582 calculate the normalized index I_{diat} as follows: $I_{\text{diat}} = 2 \times \rho_{600} / (\rho_{550} + \rho_{675})$ (modified from
583 Kazemipour et al 2012). It is based on the spectral peak centered at 600 nm between the
584 absorbance bands due to the fucoxanthin at 550 nm and the Chl *a* at 675 nm. In comparison to
585 other MPB spectral indices available (Méléder *et al.*, 2010), it was shown to be less affected
586 by water or detrital organic matter contents, thus allowing a better comparison between sites
587 (data not shown).

588

589 *Lhcx* protein analysis

590 Protein extraction from sediment cores, SDS-PAGE, Western-blot and ECL immunodetection
591 were carried out following the protocol by Coesel et al. (2009) modified by Lepetit *et al.*
592 (2013): In order to improve the proteins extraction, sediment samples were exposed to eight
593 freeze thaw cycles in the extraction buffer (500 μL lysis buffer + 500 μL protease inhibitor,
594 Sigma-Aldrich, USA) and then incubated at RT (total extraction was 90 min). Chl *a*
595 concentration of extracted samples was determined according to Jeffrey and Humphrey
596 (1975) and samples corresponding to an amount of 0.5 μg Chl *a* were loaded on the gel.
597 Correct blotting was verified by correct transfer of pre-stained protein markers on the PVDF
598 membrane (Amersham Hybond-P, GE Healthcare life Sciences, USA) and by staining gels
599 with Coomassie R-250 (Pierce Imperial Protein stain, Thermo scientific, Rockford, USA).
600 Anti-FCP6 (Lhcx1) from *Cyclotella cryptica* (Westermann and Rhiel, 2005) and anti-Lhcx6
601 from *T. pseudonana* (Zhu and Green, 2010) were applied in a 1:1000 dilution for LR samples
602 overnight (respective incubations with the secondary antibody were 2 h and 1 h). For PT
603 samples, the anti-Lhcx6 antibody did not yield a good signal; anti-FCP6 was used at a dilution
604 of 1:1000 (incubation with the secondary antibody, 1 h). Note that because of the species-

605 specific immunodetection pattern no quantification *per se* was possible. However, the
606 comparison with the planktonic models *P. tricornutum* (Bailleul *et al.*, 2010; Lepetit *et al.*,
607 2013) and *T. pseudonana* (Zhu *et al.*, 2010) enabled to possibly distinguish different isoforms
608 including some for which behaviour under HL and temperature stress and their role is known
609 or suspected (see Introduction; Bailleul *et al.*, 2010; Zhu *et al.*, 2010; Lepetit *et al.*, 2013;
610 Büchel, 2014). For *P. tricornutum*, *T. pseudonana* and *N. phyllepta* samples, the procedure
611 was as in Lepetit *et al.* (2013): exponentially growing cells were exposed to HL stress (2 h at
612 2000 $\mu\text{mol photons. m}^{-2}. \text{s}^{-1}$) to induce protein expression of several Lhcx isoforms which
613 were separated on the LDS-gel.

614

615 *Taxonomic composition*

616 For each site, the first cm of sediment was collected and stored at -20 °C until analysis.
617 Extraction from sediment of the organic fraction, including microalgae cells, was done
618 following Méléder *et al.* (2007). Preliminary observations of the organic fraction using optical
619 microscopy (Olympus AX70 using 200-fold magnification) were performed to confirm the
620 absence of other classes of microalgae than diatoms. Permanent slides of cleaned diatom
621 frustules (cremation: 2 h at 450 °C) were then prepared in a high refractive index medium
622 (Naphrax; Northern Biological Supplies Ltd, Ipswich, UK). The taxonomic composition was
623 determined to the species level by examining 200-300 frustule valves at 500 fold
624 magnification on the basis of morphological criteria using reference works (Ribeiro *et al.*,
625 2013, and references therein). Scanning electron microscopy was used to confirm the analysis
626 (JEOL JSM 7600F reaching 50 000-fold magnification).

627

628 *Statistics*

629 Data presented are mean \pm one standard deviation (SD). Each LSRE experiment was
630 performed twice. Analyses of variance (ANOVA), Tukey's honestly significant difference
631 (HSD) tests, Student's t-tests and linear regressions were performed using the R statistical
632 computing environment (v 2.15.1, Ihaka and Gentleman, 1996). In particular, potential
633 interactions between factors were tested considering data set from each site individually, with
634 time (T_0 , after 3 h of HL, after 15 min recovery under LL), temperature (27°C and 37°C) and
635 chemical (Ctrl or +Lat A) as fixed factors. The fact that Chl *a* fluorescence measurements
636 were made on the same samples over the experiment was also taken into account using the
637 sample name as random effect in a linear mixed effect model (lme function of the nlme
638 package: Pinheiro and Bates, 2000). Comparisons between sites (FR, PT) were performed on
639 data gathered at the end of the LSRE (after recovery), expressed as % of T_0 . Site, temperature
640 and chemical were considered as fixed factors. Data normality (Shapiro-Wilk test) and
641 homoscedasticity (Bartlett test) were checked using the residuals.

642

643 **Acknowledgements**

644 The present study was supported by the Egide/Campus France-PHC Pessoa exchange
645 program (n°27377TB, JL and JS), the Centre National de la Recherche Scientifique-CNRS
646 (JL), the University of La Rochelle-ULR and the Contrat Plan Etat Région-CPER ‘Littoral’
647 (JL, BL post-doc grant), the Region Poitou-Charentes (AB Ph.D. grant), the Deutscher
648 Akademischer Austausch Dienst-DAAD (BL post-doc grant), European Funds through
649 COMPETE, the Portuguese Science Foundation (FCT) through projects PEST (PEst-
650 C/MAR/LA0017/2013) and MigROS (PTDC/MAR/112473/2009: JS, ML post-doc grant and
651 SF research assistant grant), and through grants SFRH/BSAB/962/2009 (JS), SFRH/BD/
652 44860/2008 (JE Ph.D. grant). The authors thank Dr. E. Rhiel (Univ. Oldenburg, Germany)
653 and Prof. B. Green (Univ. British Columbia, Canada) for the kind gifts of the anti-FCP6 and
654 of the anti-Lhcx6, respectively, Prof. B. Green for her help analyzing the Lhcx6 Western-blot,
655 C. Pais for her technical assistance with the TBARS quantification, Dr L. Ribeiro (Univ.
656 Lisboa, Portugal) for his help in confirming the identification of *N. phyllepta* s.l., and Sr
657 Armindo do Evaristo for his cheerful support. We thank the two anonymous reviewers for
658 their constructive comments on the manuscript.

659

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888

889 **Figure legends**

890 **Figure 1**

891 **In situ environmental conditions and photosynthetic activity of microphytobenthos**
892 **during a summer diurnal emersion in Portuguese (Ria de Aveiro, Vista Alegre, A- and**
893 **C-) and French (Baie de l'Aiguillon, Esnandes, B- and D-) intertidal flats. A- and B-**
894 **Photosynthetic Active Radiation (PAR, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, black line) at the surface of the**
895 **sediment and temperature ($^{\circ}\text{C}$) at five depths (surface, -0.5 cm, -2 cm, -5 cm, -10 cm, grey**
896 **lines); C- and D- effective PSII quantum yield (ΦPSII : open squares) and maximum non-**
897 **photochemical quenching of Chl *a* fluorescence (NPQ_{max} : closed circles). Local time (FR:**
898 **UTC+1, PT: UTC).**

899

900 **Figure 2**

901 **Index of photoinhibition (decrease in ΦPSII) of microphytobenthos harvested at the**
902 **French site (FR) during light stress recovery experiments performed on sediment**
903 **exposed at two temperatures (27°C , A- or 37°C , B-) and with an inhibitor of cell motility**
904 **(+ Lat A) or without (Ctrl). T_0 , beginning of the experiment under low light ($50 \mu\text{mol}$**
905 **photons $\text{m}^{-2} \text{s}^{-1}$); HL, after 3 h high light ($1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$); Recov, after 15 min**
906 **recovery under low light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) following the 3 h high light; LL, after 3 h**
907 **of low light ($50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Values are mean \pm SD (n=6).**

908

909 **Figure 3**

910 **Index of photoprotection (NPQ) of microphytobenthos harvested at the French site (FR)**
911 **during light stress recovery experiments performed on sediment exposed at two**
912 **temperatures (27°C, A- or 37°C, B-) and with an inhibitor of cell motility (+Lat A) or**
913 **without (Ctrl). Legend as in Fig. 2. Values are mean ± SD (n=6).**

914

915 **Figure 4**

916 **Pigment content makers of photooxidative stress (Chl *a* allomerization ratio: AR) and of**
917 **photoprotection (DD+DT pool; DD de-epoxidation state: DES) of microphytobenthos**
918 **harvested at the French site (FR) during light stress recovery experiments performed on**
919 **sediment exposed at two temperatures (27°C or 37°C) and with an inhibitor of cell**
920 **motility (+Lat A) or without (Ctrl). A- AR, data from 27 and 37°C experiments were**
921 **pooled, * significant difference compared to Ctrl at the same time (P < 0.05, Tukey HSD); B-**
922 **DD+DT pool (in mol.100 mol Chl *a*⁻¹), data from Ctrl and Lat A-treated samples were pooled,**
923 **** significant difference compared to 27 °C at the same time (P < 0.01, Tukey HSD); C- and**
924 **D- DES, *** significant difference compared to Ctrl at the same time (P < 0.001, Tukey**
925 **HSD). Labels as in Fig. 2. Values are mean ± SD (n=12: A,B or n=6: C,D) expressed as %T₀.**

926

927 **Figure 5**

928 **Western blot of the LhcX proteins of microphytobenthos harvested at the French site**
929 **during light stress recovery experiments performed on sediment exposed at two**
930 **temperatures (27°C, A- or 37°C, B-) and with an inhibitor of cell motility (+) or without**
931 **(-). HL, after 3 h HL (1200 μmol photons m⁻² s⁻¹); LL, after 3 h of LL (50 μmol photons**
932 **m⁻² s⁻¹). All samples were normalized to the same Chl *a* concentration (15 μg Chl *a* mL⁻¹). A-**

933 Lhcx proteins were detected using a monoclonal anti-FCP6 (Lhcx1). This antibody detects,
934 similarly as the anti-LHCSR of *C. reinhardtii* (Lepetit *et al.*, 2013), also Lhcx2 and Lhcx3 in
935 *P. tricornutum* (strain 'P.t.1') used here as a control. *N. phyllepta* s.l. (N.p.), the dominant
936 species of the MPB community, was also tested. B- and C- Lhcx proteins were detected using
937 a monoclonal anti-Lhcx6 from *T. pseudonana* (T.p.) (Zhu and Green, 2010) which also
938 potentially detect Lhcx3 and an Lhcx dimer in *P. tricornutum* ; C- is a special focus on the
939 band between 33-50 kDa in 37°C exposed samples. Revelation times were: A- 30 s; B- 15 s
940 for the bands between 17-23 kDa, 45 s for the band between 33-50 kDa; C- 30 s;. Note that
941 for A- all samples exposed at 37°C did not yield satisfying signal; and for B-, the sample HL+
942 27°C was lost.

943

944 **Figure 6**

945 **Amount of lipid peroxidation marker (TBARS content in 10^{-6} nmol MDA equivalent**
946 **mL⁻¹) in microphytobenthos harvested at the French site (FR) during light stress**
947 **recovery experiments performed on sediment exposed at two temperatures (27°C, A- or**
948 **37°C, B-) with an inhibitor of cell motility (+Lat A). Labels as in Fig. 2. Values are mean \pm**
949 **SD (n=6) normalized to surface biomass (I_{diat} biomass index).**

950

951 **Figure 7**

952 **Comparison of photoinhibition level (decrease in ΦPSII) and photoprotection (NPQ) in**
953 **microphytobenthos harvested at the Portuguese (PT) and the French (FR) sites during**
954 **light stress recovery experiments performed on sediment exposed at two temperatures**
955 **(27°C or 37°C) and with an inhibitor of cell motility (+Lat A) or without (Ctrl). ΦPSII**

956 recovery (A) and sustained NPQ (B) after 15 min incubation under LL (50 $\mu\text{mol photons m}^{-2}$
957 s^{-1}) following 3 h of HL exposure (1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), *** significant difference
958 between sites ($P < 0.001$, Tukey HSD). Values are mean \pm SD (n=6).

959

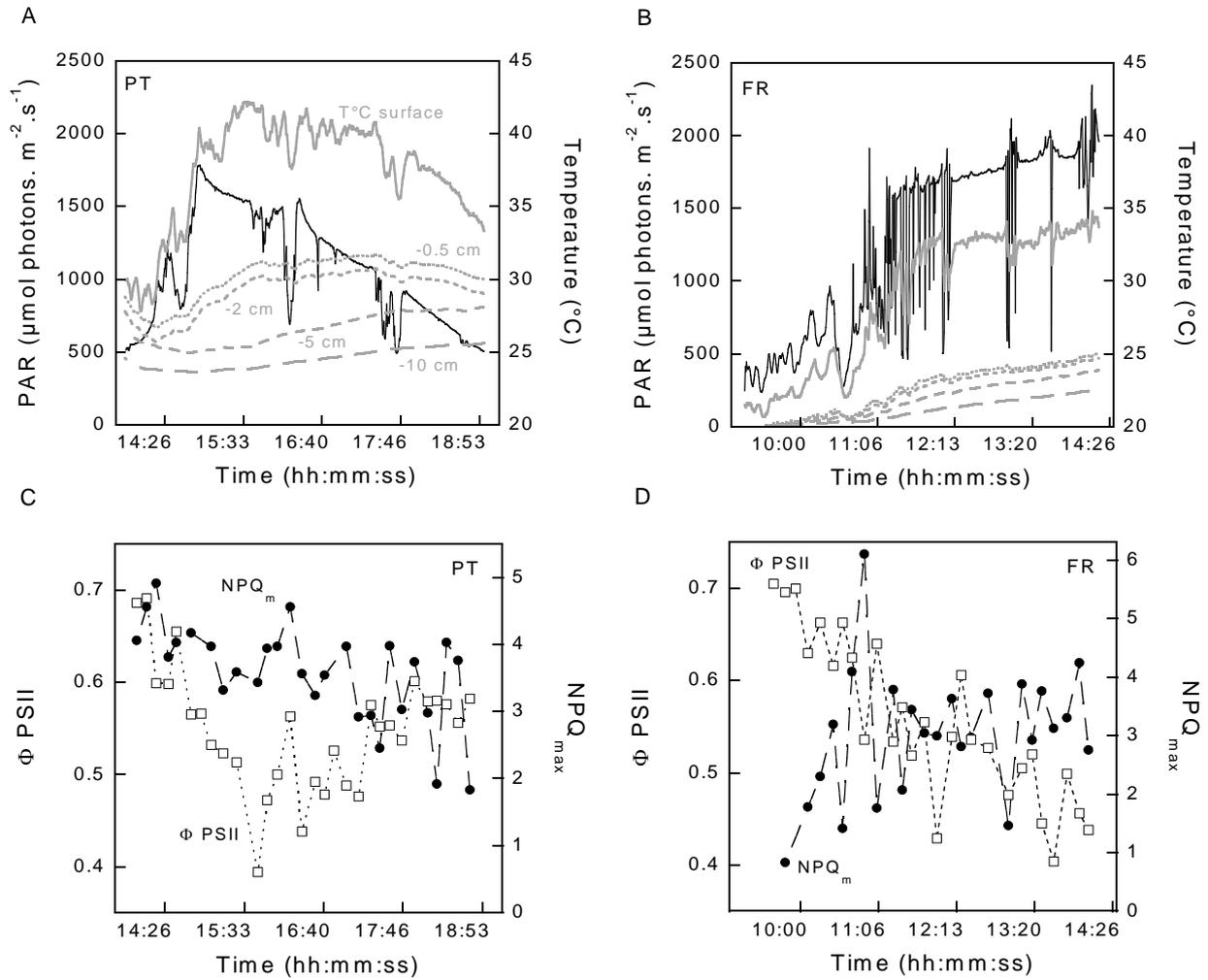
960 **Figure 8**

961 **Western-blot of the Lhc_x proteins of microphytobenthos harvested at the Portuguese**
962 **site during light stress recovery experiments performed on sediment exposed at two**
963 **temperatures (27°C or 37°C) and with an inhibitor of cell motility (+) or without (-). HL,**
964 **after 3 h HL (1200 $\mu\text{mol photons. m}^{-2} \text{s}^{-1}$) treatment; LL, after 3 h of LL exposure. All**
965 **samples were normalized to the same Chl *a* concentration (15 $\mu\text{g Chl } a \text{ mL}^{-1}$). Lhc_x proteins**
966 **were detected using a monoclonal anti-FCP6 (Lhc_{x1}). This antibody detects, similarly as the**
967 **anti-LHCSR of *C. reinhardtii* (Lepetit *et al.*, 2013), also Lhc_{x2} and Lhc_{x3} in *P. tricorutum***
968 **(strain ‘P.t.1’) used here as a control. Note that i) the 27°C and 37°C samples were not loaded**
969 **on the same gel but the revelation time was the same (30 s), ii) the use of the monoclonal anti-**
970 **Lhc_{x6} from *T. pseudonana* did not yield any signal with these samples.**

971

972

973 **Figure 1_Laviale et al.**

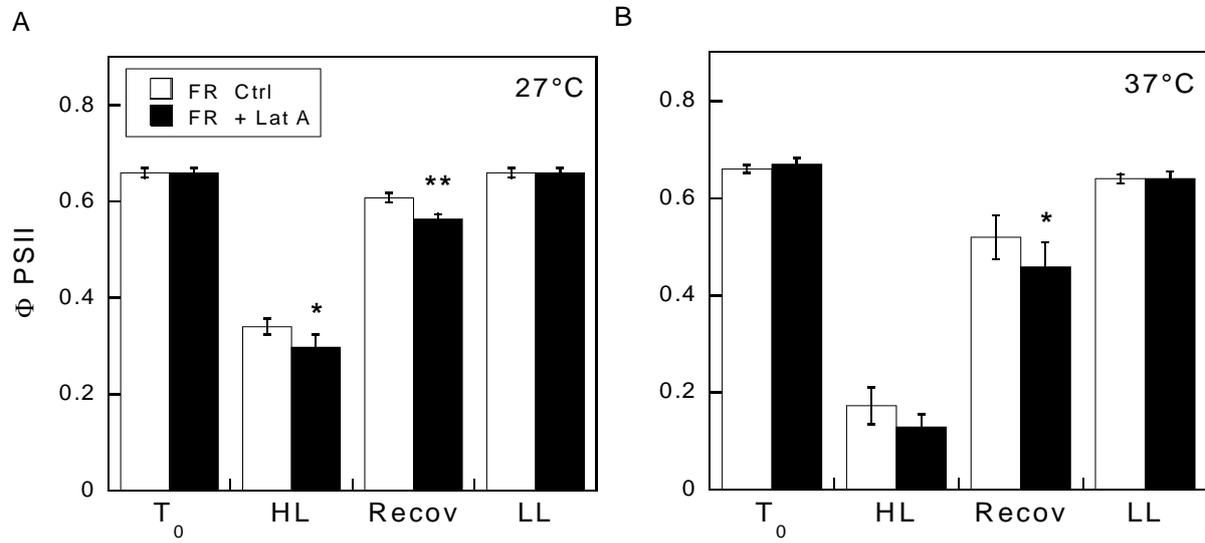


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977 **Figure 2_Laviale et al.**

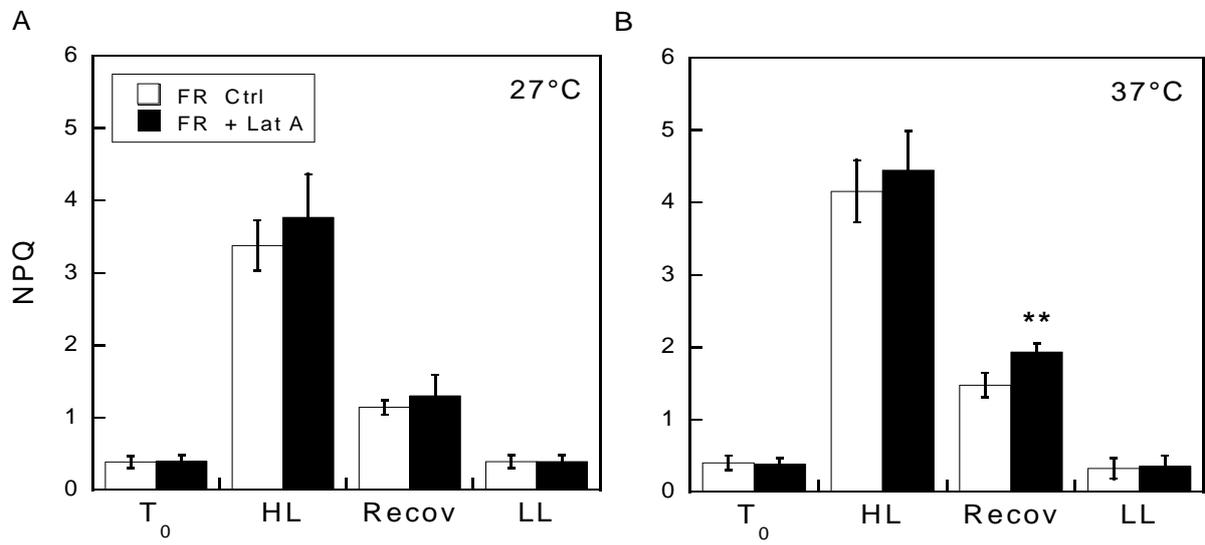


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981 **Figure 3_Laviale et al.**

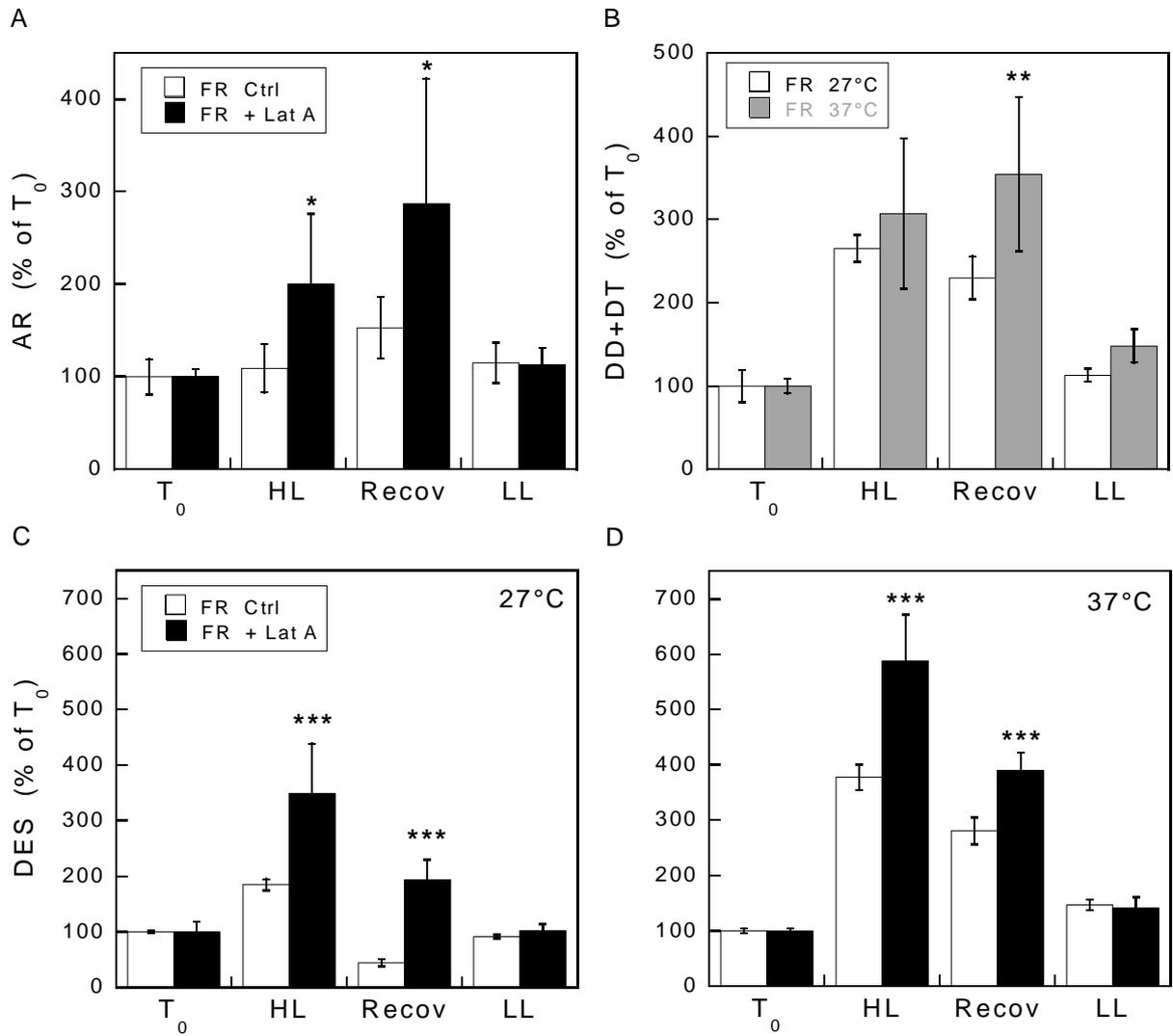


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985 **Figure 4_Laviale et al.**



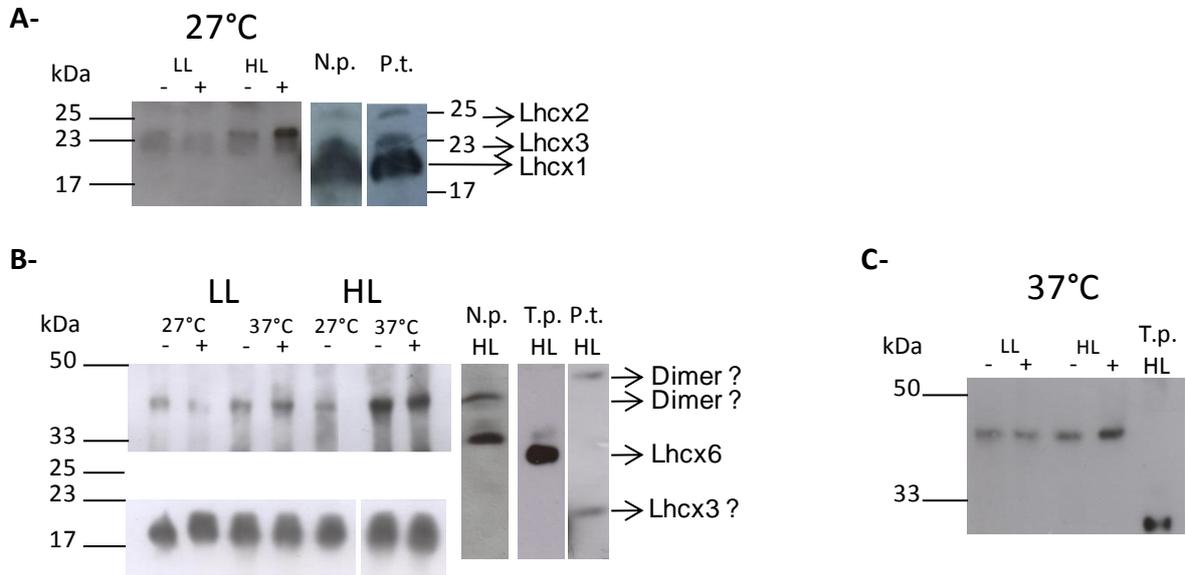
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989 **Figure 5_Laviale et al.**

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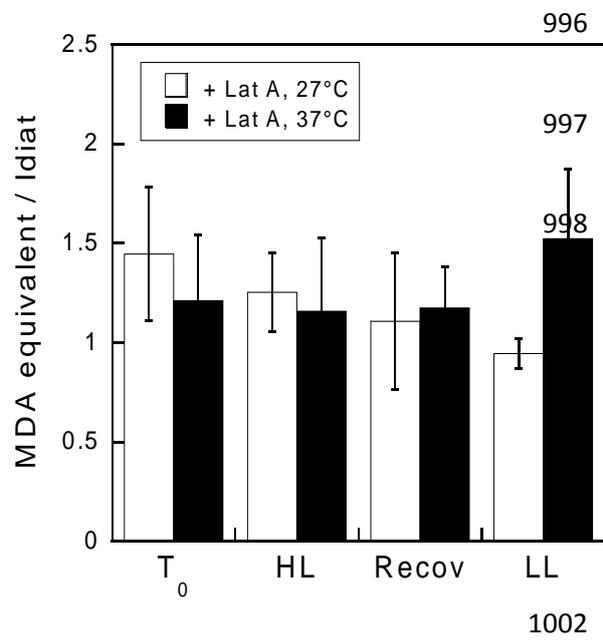
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995 **Figure 6_Laviale et al.**

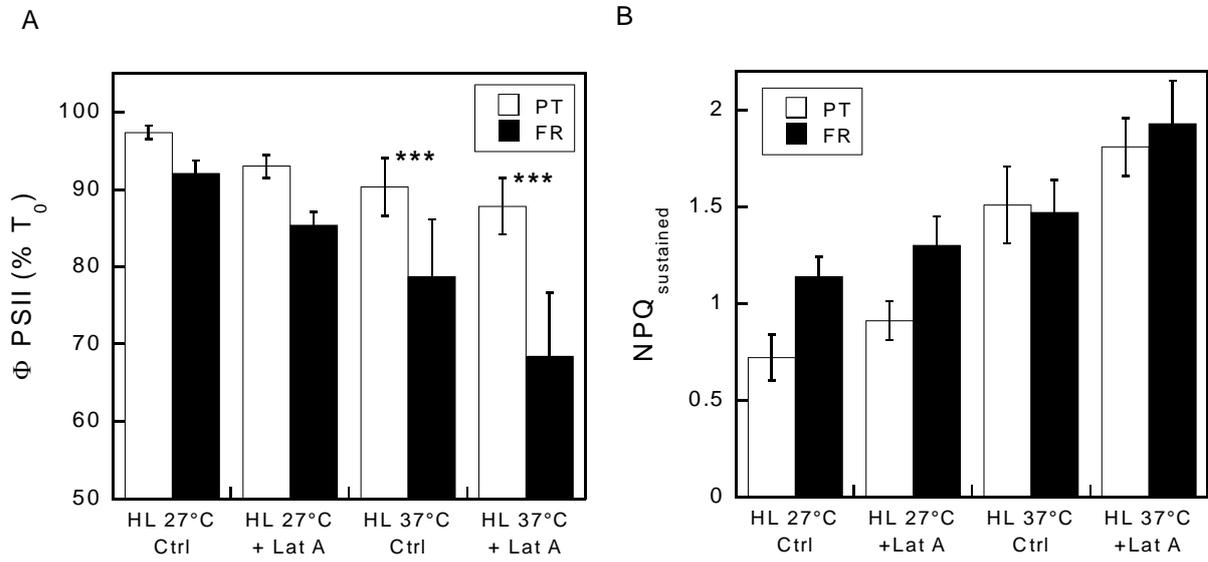


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1006 **Figure 7_Laviale et al.**



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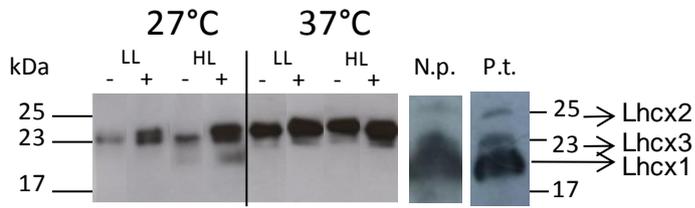
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1011 **Figure 8_Laviale *et al.***

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