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1 Growth form defines physiological photoprotective capacity in intertidal benthic diatoms

2

3 Alexandre Barnett<sup>1</sup>, Vona Méléder<sup>1,2</sup>, Lander Blommaert<sup>1,3</sup>, Bernard Lepetit<sup>1#</sup>, Pierre

4 Gaudin<sup>1,2§</sup>, Wim Vyverman<sup>3</sup>, Koen Sabbe<sup>3</sup>, Christine Dupuy<sup>1</sup> & Johann Lavaud<sup>1\*</sup>

5

6 <sup>1</sup> UMR7266 LIENSs ‘Littoral, Environnement et Sociétés’, CNRS/Université de La Rochelle,

7 Institut du Littoral et de l’Environnement, 2 rue Olympe de Gouges, 17000 La Rochelle,

8 France.

9 <sup>2</sup> UPRES EA 2160 MMS ‘Mer, Molécules, Santé’, Université de Nantes, Faculté des Sciences

10 et Techniques, 2 rue de la Houssinière, BP 92208, 44322 Nantes cedex 3, France.

11 <sup>3</sup> Laboratory of Protistology & Aquatic Ecology, Department of Biology, Ghent University,

12 Krijgslaan 281-S8, B-9000 Ghent, Belgium.

13 \* Corresponding author:

14 UMR 7266 ‘LIENSs’, CNRS/Université de La Rochelle, Institut du Littoral et de

15 l’Environnement (ILE), 2 rue Olympe de Gouges, 17000 La Rochelle, France

16 Phone: +33-(0)5-46-50-76-45, Fax: +33-(0)5-46-45-82-64, E-mail: [johann.lavaud@univ-lr.fr](mailto:johann.lavaud@univ-lr.fr)

17 # Current address: Group of Plant Ecophysiology, Department of Biology, University of

18 Konstanz, Universitätsstraße 10, 78457 Konstanz, Germany

19 § Current address: UMR6112 ‘LPGN’, CNRS / Université de Nantes, Faculté des Sciences et

20 Techniques, 2 rue de la Houssinière, BP 92208, 44322 Nantes cedex 3, France.

21

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26 **Abstract**

27 In intertidal marine sediments, characterized by rapidly fluctuating and often extreme light  
28 conditions, primary production is frequently dominated by diatoms. We performed a  
29 comparative analysis of photophysiological traits in 15 marine benthic diatom species  
30 belonging to the four major morphological growth forms (epipelon, motile and non-motile  
31 epipsammon and tychoplankton) found in these sediments. Our analyses revealed a clear  
32 relationship between growth form and photoprotective capacity, and identified fast regulatory  
33 physiological photoprotective traits (i.e. non-photochemical quenching and the xanthophyll  
34 cycle) as key traits defining the functional light response of these diatoms. Non-motile  
35 epipsammon and motile epipelon showed the highest and lowest non-photochemical  
36 quenching respectively, with motile epipsammon showing intermediate values. Like epipelon,  
37 tychoplankton had low non-photochemical quenching, irrespective of whether they were  
38 grown in benthic or planktonic conditions, reflecting an adaptation to a low light  
39 environment. Our results thus provide the first experimental evidence for the existence of a  
40 trade-off between behavioural (motility) and physiological photoprotective mechanisms (non-  
41 photochemical quenching and the xanthophyll cycle) in the four major intertidal benthic  
42 diatoms growth forms using unialgal cultures. Remarkably, while motility is restricted to the  
43 raphid pennate diatom clade, raphid pennate species which have adopted a non-motile  
44 epipsammic or a tychoplanktonic life style display the physiological photoprotective response  
45 typical of these growth forms. This observation underscores the importance of growth form  
46 and not phylogenetic relatedness as the prime determinant shaping the physiological  
47 photoprotective capacity of benthic diatoms.

48

## 49 **Introduction**

50 Functional trait-based approaches are increasingly adopted to explain and understand the  
51 distribution and diversity of phytoplankton communities (Litchman and Klausmeier, 2008;  
52 Barton *et al.*, 2013; Edwards *et al.*, 2013). Various morphological and physiological traits  
53 have been shown to define the ecological niches of phytoplankton species, including size,  
54 temperature response and resource acquisition and utilization traits. For example, in  
55 planktonic diatoms, which play a key role in marine primary production and biogeochemical  
56 cycling (Armbrust, 2009), pronounced species-specific differences in photosynthetic  
57 architecture and photophysiological strategies have been documented (e.g. Dimier *et al.*,  
58 2007; Key *et al.*, 2010; Schwaderer *et al.*, 2011; Wu *et al.*, 2012) and related to their *in situ*  
59 light environment (Strzepek and Harrison, 2004; Lavaud *et al.*, 2007; Dimier *et al.*, 2009;  
60 Petrou *et al.*, 2011). A high capacity for physiological photoprotection is generally observed  
61 in highly fluctuating light climates and/or under on average high irradiances. This suggests  
62 that photoprotective capacity is an adaptive trait that shapes the distribution of planktonic  
63 diatoms in the environment (Lavaud *et al.*, 2007; Dimier *et al.*, 2009; Bailleul *et al.*, 2010;  
64 Petrou *et al.*, 2011; Lavaud and Lepetit, 2013).

65 Benthic marine environments, and especially intertidal environments, are characterized by  
66 even more changeable and extreme light climates resulting from the interplay of weather  
67 conditions, tides, water column turbidity and sediment composition (and hence light  
68 penetration) (Admiraal, 1984; Underwood and Kromkamp, 1999; Paterson and Hagerthey,  
69 2001). Nevertheless, intertidal sediments rank amongst the most productive ecosystems on  
70 Earth, largely owing to the primary production of highly diverse assemblages of benthic  
71 diatoms (Underwood and Kromkamp, 1999). To date however, little is known about the role  
72 of functional traits, and especially photophysiological traits, in shaping the structure,

73 dynamics and function of benthic diatom assemblages. In most studies, diatom functional  
74 groups are defined on the basis of morphological growth form (e.g. Gottschalk and Kahlert,  
75 2012; Larson and Passy, 2012) and not physiological traits. In addition, photoprotective  
76 ability (limited to the measurement of the ‘xanthophyll cycle’, XC) and its relationship with  
77 ecology has only been studied in natural communities with mixed assemblages of functional  
78 groups (e.g. Jesus *et al.*, 2009; van Leeuwe *et al.*, 2009; Cartaxana *et al.*, 2011).

79 In temperate seas, intertidal benthic communities are largely dominated by diatoms (Méléder  
80 *et al.*, 2007; Ribeiro *et al.*, 2013), which display a high degree of taxonomic, phylogenetic and  
81 functional diversity (Kooistra *et al.*, 2007). Several growth forms can be distinguished, which  
82 mainly differ in their attachment mode and degree of motility (see Ribeiro *et al.* (2013) for a  
83 detailed description): (1) the epipelon (EPL) comprises larger (usually > 10 µm) motile  
84 diatoms which can move freely in between sediment particles and typically form biofilms (cf.  
85 (Herlory *et al.*, 2004); (2) the epipsammon (EPM) groups smaller (usually < 10 µm) diatoms  
86 which live in close association with individual sand grains; and (3) the tychoplankton  
87 (TYCHO), which is an ill-defined and rather enigmatic group of largely non-motile diatoms  
88 which presumably have an amphibious life style (both sediment and water column) (e.g.  
89 Sabbe *et al.* (2010)). Within the epipsammic group, non-motile (EPM-NM) species are firmly  
90 attached (either stalked or adnate) to sand particles, while motile forms (EPM-M) can move  
91 within the sphere of individual sand grains. From a phylogenetic perspective, motile forms  
92 (i.e. all epipelon and motile epipsammon) exclusively belong to the pennate raphid clade  
93 (Kooistra *et al.*, 2007), possessing a raphe allowing motility. Most non-motile epipsammon  
94 belongs to the pennate araphid lineage, but also includes some raphid pennates, such as  
95 *Biremis lucens*, which firmly attaches to sand grains (Sabbe *et al.*, 1995). Tychoplankton  
96 includes both centric and pennate raphid forms. Intertidal benthic diatom species, but also

97 growth forms, show distinct distribution patterns in time and space, suggesting pronounced  
98 (micro)niche differentiation (Sabbe, 1993; Méléder *et al.*, 2007, Ribeiro *et al.*, 2013). For  
99 example, epipsammon dominates non-cohesive sandy sediments (Méléder *et al.*, 2007), while  
100 epipelon dominates cohesive muddy sediments (Haubois *et al.*, 2005). Epipelon typically  
101 display vertical ‘micromigration’ in the sediment following endogenous tidal/dial rhythms  
102 and environmental stimuli (Saburova and Polikarpov, 2003; Consalvey *et al.*, 2004; Coelho *et*  
103 *al.*, 2011): during daylight emersion, they migrate to the sediment surface, while during  
104 immersion they migrate to deeper sediment layers.

105 To prevent photoinhibition (Serôdio *et al.*, 2008), benthic diatoms utilize behavioural and  
106 physiological responses (Mouget *et al.*, 2008; van Leeuwe *et al.*, 2009; Perkins *et al.*, 2010b;  
107 Cartaxana *et al.*, 2011; Serôdio *et al.*, 2012). Behavioural photoprotection involves motility,  
108 allowing cells to position themselves in light gradients and escape from prolonged exposure  
109 to excess light (Admiraal, 1984; Kromkamp *et al.*, 1998; Consalvey *et al.*, 2004; Serôdio *et*  
110 *al.*, 2006). In addition, both motile and non-motile species employ fast regulatory  
111 physiological processes for photoprotection (i.e. ‘physiological photoprotection’; Lavaud,  
112 2007; Goss and Jakob, 2010; Depauw *et al.*, 2012; Lepetit *et al.*, 2012). In diatoms, two  
113 processes are important in field situations (Lavaud, 2007): photosystem II cyclic electron  
114 transfer (PSII CET) and non-photochemical quenching of chlorophyll (Chl) fluorescence  
115 (NPQ) (Depauw *et al.*, 2012; Lepetit *et al.*, 2012; Lavaud and Lepetit, 2013). NPQ is  
116 controlled by several regulatory partners including the light-dependent conversion of  
117 diadinoxanthin (DD) to diatoxanthin (DT) by the DD de-epoxidase (i.e. the XC) (Brunet and  
118 Lavaud, 2010; Goss and Jakob, 2010). In benthic diatoms however, XC-NPQ has only rarely  
119 been studied, and mostly *in situ*: it has been shown to vary with diurnal and tidal cycles,  
120 season, latitude (Serôdio *et al.*, 2005; van Leeuwe *et al.*, 2009; Chevalier *et al.*, 2010), the

121 organisms' position within the sediments and along the intertidal elevation gradient (Jesus *et*  
122 *al.*, 2009; Cartaxana *et al.*, 2011). On the basis of their *in situ* measurements, the latter authors  
123 hypothesized the existence of a trade-off between behavioural and physiological  
124 photoprotection mechanisms in benthic diatoms as a stronger XC was shown to occur in  
125 sandy *vs.* muddy sediments. However, at least the sandy sediments contained a mix of both  
126 epipsammic and epipellic forms (Jesus *et al.*, 2009; Cartaxana *et al.*, 2011), and even when the  
127 latter are not numerically dominant, they can still make a substantial contribution to biomass  
128 due to their much larger biovolumes (see e.g. Hamels *et al.* 1998).

129 Our study represents a comprehensive characterization of fast regulatory physiological  
130 photoprotection capacity in typical representatives of the major diatom growth forms  
131 occurring in intertidal marine sediments. Given the highly dynamic and often extreme  
132 intertidal light climate, we hypothesize that photoprotective features are key traits shaping  
133 niche differentiation between benthic growth forms, as has been proposed before for  
134 phytoplankton (Huisman *et al.*, 2001; Litchman and Klausmeier, 2008; Dimier *et al.*, 2009;  
135 Petrou *et al.*, 2011; Lavaud and Lepetit, 2013). In this respect, we predict that the largely  
136 immotile epipsammic life forms are better able to cope with pronounced and rapid changes in  
137 light intensity at the physiological level than the motile epipellic forms which can actively  
138 position themselves in the sediment light gradient.

139

140 **Materials and methods**

141 *Diatom culturing and harvesting (Table 1)*

142 Fifteen benthic diatom strains were used (Table 1). All species were assigned to their  
143 respective growth form on the basis of microscopical observations on natural assemblages.  
144 They were grown in batch cultures at 20°C in sterile artificial F/2 seawater medium enriched  
145 with NaHCO<sub>3</sub> (80 mg L<sup>-1</sup> final concentration). Tychoplankton species were also grown in  
146 continuously flushed airlift (i.e. with air bubbling) to mimic ‘planktonic’ growth conditions.  
147 Two light intensities (E, 20 and 75 μmol photons m<sup>-2</sup> s<sup>-1</sup>) were used with a 16 h light:8 h dark  
148 photoperiod white fluorescent tubes, L58W/840, OSRAM, Germany. Cultures were  
149 photoacclimated to the above conditions at least 2 weeks before measurements and  
150 experiments (see below). Diatom suspensions for the experiments were prepared to a final  
151 concentration of 10 μg chlorophyll *a* (Chl *a*) mL<sup>-1</sup>. For this purpose, Chl *a* concentration was  
152 determined according to the (Jeffrey and Humphrey, 1975) spectrophotometric method.  
153 Diatoms suspensions were continuously stirred at 20°C under the growth E (i.e. 20 or 75  
154 μmol photons m<sup>-2</sup> s<sup>-1</sup>) at least 1 h before the start of the experiments and all along the course  
155 of the experiments (Lavaud *et al.*, 2007). This kept the photosynthetic machinery in an  
156 oxidized state and prevented NPQ.

157

158 *Growth rates and biovolumes*

159 Specific growth rates, μ (d<sup>-1</sup>), were calculated from regression of the natural logarithm of the  
160 number of diatom cells during their exponential growth phase as microscopically determined  
161 in a Malassez’s counting chamber. Biovolumes (μm<sup>3</sup>) were calculated using the formula of  
162 (Hillebrand *et al.*, 1999) based on measurements performed on fifteen specimens per species.

163

164



165 *HPLC pigment analyses*

166 Chl *a*, Chlorophyll *c* (Chl *c*), fucoxanthin (Fx), DD, DT and  $\beta$ -carotene ( $\beta$ -car) content, all  
167 normalized to Chl *a* (i.e. expressed as mol. 100 mol Chl *a*<sup>-1</sup>), were measured using HPLC as  
168 described in Jakob *et al.* (1999). 1 mL of diatom suspension was rapidly filtered (Isopore 1.2  
169  $\mu$ m RTTP filters, Merck Millipore, Ireland) and immediately frozen in liquid nitrogen before  
170 extraction in a cold (4°C) mixture of 90% methanol/0.2 M ammonium acetate (90/10 vol/vol)  
171 and 10% ethyl acetate. The pigment extraction was improved by the use of glass beads  
172 (diameter 0.25-0.5 mm, Roth, Germany) and included several short (20 s) vortexing steps.  
173 Supernatants were collected after centrifugation (5 min, 10 000 g, 4°C) and immediately  
174 injected into an HPLC system (Hitachi Lachrom Elite, Japan) equipped with a cooled auto-  
175 sampler and a photodiode array detector (L-2455). Chromatographic separation was carried  
176 out using a Nucleosil 120-5 C18 column (125 mm long, 4 mm internal diameter, 5  $\mu$ m  
177 particles, Macherey-Nagel, Germany) equipped with a pre-column (CC 8/4 Nucleosil,  
178 Macherey-Nagel, Germany) for reverse phase chromatography during a 25 min elution  
179 program. The solvent gradient followed Jakob *et al.* (1999) with an injection volume of 50  $\mu$ L  
180 and a flow rate of 1.5 mL min<sup>-1</sup>. Pigments were identified from absorbance spectra (400-800  
181 nm) and retention times (Roy *et al.*, 2011), and their concentrations were obtained from the  
182 signals in the photodiode array detector at 440 nm. The de-epoxidation state (DES in %) was  
183 calculated as  $[(DT / DD + DT) \times 100]$ , where DD is the epoxidized form and DT is the de-  
184 epoxidized form. Chl *a* concentration per cell was determined during exponential growth  
185 based on cell counts (see above) and the Chl *a* measurements.

186

187 *Chl fluorescence yield and light curves (Table 2)*

188 For a complete overview of the definition and measurement of the photophysiological  
189 parameters, see Table 2. Chl fluorescence yield was monitored with a Diving-PAM

190 fluorometer (Walz, Germany) on a 2.5 mL stirred and 20°C controlled diatom suspension  
191 (Lavaud et al 2004). Before measurement, the cells were dark-adapted for 15 min, and a  
192 saturating pulse ( $3600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , duration 0.4 ms) was fired to measure  $F_0$ ,  $F_m$  and  
193  $F_v/F_m$ . Two types of light curves were performed: Non Sequential and Rapid Light Curves  
194 (NSLCs and RLCs) (Perkins *et al.*, 2010a). For NSLCs, continuous light (KL-2500 lamp,  
195 Schott, Germany) was applied for 5 min at different  $E_s$  ( $48\text{-}1950 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ ); a new  
196 diatom suspension was used for each  $E$ . At the end of each exposure,  $F_m'$  and NPQ were  
197 measured. For RLCs, one diatom suspension was exposed to 8 successive, incrementally  
198 increasing  $E_s$  ( $29\text{-}1042 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ ) of 30 s each (Perkins *et al.*, 2006) (Table S1).  
199 RLCs allow constructing rETR *vs.*  $E$  and NPQ *vs.*  $E$  curves. The NPQ *vs.*  $E$  curve is based on  
200 a 3-parameter Hill equation model and it is described by the equation  $\text{NPQ}(E) = \text{NPQ}_m \times$   
201  $[E^{n_{\text{NPQ}}}/(E_{50_{\text{NPQ}}}^{n_{\text{NPQ}}} + E^{n_{\text{NPQ}}})]$  (Serôdio and Lavaud, 2011). From the fitted rETR- $E$  curves  
202 (Eilers and Peeters, 1988) and NPQ- $E$  curves (Serôdio and Lavaud, 2011),  $r\text{ETR}_m$ ,  $\alpha$ ,  $E_k$ , and  
203  $\text{NPQ}_m$ ,  $E_{50_{\text{NPQ}}}$ ,  $n_{\text{NPQ}}$  can be derived, respectively. All parameters are described in the Table 2.  
204  $n_{\text{NPQ}}$  is the Hill coefficient or the sigmoidicity coefficient of the NPQ- $E$  curve (Serôdio and  
205 Lavaud, 2011). It informs on the onset of NPQ at moderate  $E_s$ , i.e. when the DT molecules  
206 are being 'activated' with increasing  $E_s$  to effectively participate to NPQ: DT 'activation'  
207 depends on its enzymatic conversion and its binding to the PSII light-harvesting antenna  
208 complex in order to promote the antenna switch to a dissipative state of excess energy which  
209 is measurable by NPQ (see Lavaud and Lepetit, 2013). When  $n_{\text{NPQ}}$  is  $< 1$ , the NPQ- $E$  curve  
210 shows an asymptotic saturation-like increase towards  $\text{NPQ}_m$ , while when  $n_{\text{NPQ}}$  is  $> 1$ , the  
211 NPQ- $E$  curve shows a sigmoidal shape. In the later case, the Hill reaction (i.e. NPQ onset) is  
212 allosteric (as proposed for the NPQ mechanism, see Lavaud and Lepetit, 2013),  $n_{\text{NPQ}}$  thus  
213 informing on the degree of allostery of the NPQ- $E$  curve. The higher  $n_{\text{NPQ}}$ , the more  
214 positively cooperative the Hill reaction is;  $n_{\text{NPQ}}$  around 2 being the highest values reported so

215 far (Serôdio and Lavaud, 2011). The same fitting procedure can obviously be used for the  
216 DT-E and the DES-E curves, thereby extracting analogous parameters as from the fitted NPQ-  
217 E curves.

218

### 219 *O<sub>2</sub> yield and the PSII CET*

220 The relative O<sub>2</sub> yield produced during a sequence of single-turnover saturating flashes at a  
221 frequency of 2 Hz was measured with a home-made rate electrode (Lavaud *et al.*, 2002). The  
222 steady-state O<sub>2</sub> yield per flash (Y<sub>SS</sub>) was attained for the last 4 flashes of a sequence of 20  
223 when the S-state cycle oscillations were fully damped (Lavaud *et al.*, 2002). Y<sub>SS</sub> of 15 min  
224 dark-adapted (**D**) and illuminated (**L**, samples taken at the end of each NSLC) cells was used to  
225 calculate the PSII CET (Lavaud *et al.*, 2002; Lavaud *et al.*, 2007) as follows:  $[\{(20 \times Y_{SS} \text{ L}) - (\sum (Y_{1...20}) \text{ L})\} - \{(20 \times Y_{SS} \text{ D}) - (\sum (Y_{1...20}) \text{ D})\}] / Y_{SS} \text{ D}$ .

227

### 228 *Statistics*

229 Statistical analyses were conducted using the statistical software package SAS 9.3. Species  
230 were compared using the general linear model PROC GLM. Growth forms (groups) were  
231 compared using the mixed linear model PROC MIXED. Groups were regarded as fixed  
232 effects. Data were log- or square root-transformed when needed to allow the best possible fit.  
233 Where necessary, estimated least squares means (lsmeans) and standard errors (SE) were  
234 back-transformed as in Jørgensen and Pedersen (1998).

235

## 236 **Results**

### 237 *Growth rate and photosynthetic properties (Fig. S1, Tables 3, S2, S3)*

238 The Chl *a* concentration per cell showed an exponential relationship with biovolume with  
239 relatively small changes at the smaller cell volumes (Fig. S1) The average diatom  
240 biovolumes were independent of growth form (Table 3, Fig. S1). Growth rate did not differ  
241 significantly between the growth forms at growth  $E = 20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Relative  
242 concentrations of the light-harvesting pigments Chl *c* and Fx were comparable among growth  
243 forms.  $\beta$ -car, which is mainly associated with the photosystem cores, was only slightly but  
244 significantly higher in epipelon than in non-motile epipsammon. DD+DT content was  
245 significantly lower in epipelon than in the other growth forms. Because the cells were grown  
246 at low  $E$ , DES was generally low, with no significant differences between the growth forms.  
247 The highest DD+DT ( $16.95 \pm 2.56 \text{ mol } 100 \text{ mol Chl } a^{-1}$ ) and DES ( $16.4 \pm 6.2 \%$ ) values were  
248 observed in *Plagiogramma staurophorum* (non-motile epipsammon). There were no  
249 significant differences in  $F_v/F_m$ ,  $\alpha$ ,  $rETR_m$ ,  $E_k$  and PSII  $CET_{\text{max}}$  between the growth forms.  $E_k$   
250 was on average 3 to 4 times the growth  $E$  in all growth forms. PSII  $CET_m$  was close to 3 (its  
251 maximum, Lavaud *et al.*, 2002) for the two epipsammon growth forms, and about 2 in  
252 epipelon and tycho plankton.

253

### 254 *NPQ properties (Figs 1, S2, Tables 4, S4-S6)*

255 At  $E$  values  $\geq 230 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , NPQ was significantly higher in non-motile  
256 epipsammon than in both epipelon and tycho plankton; the same holds true for motile  
257 epipsammon vs. epipelon and tycho plankton at  $E$  values  $\geq 1050 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . NPQ  
258 was also significantly higher in non-motile epipsammon than in motile epipsammon except at  
259 the lowest and highest  $E$  values. Likewise, NPQ<sub>m</sub> was significantly higher (x 3.5 and x 2.4,  
260 respectively) in non-motile epipsammon and motile epipsammon than in epipelon and

261 tychoplankton. In epipelon and tychoplankton, the NPQ-E curves showed a lower variability  
262 than in the two epipsammon growth forms. Non-motile epipsammon had the lowest  $E50_{NPQ}$ ,  
263 significantly lower than all other groups. In contrast, tychoplankton  $E50_{NPQ}$  was significantly  
264 higher than in the other groups. Epipellic and motile epipsammonic  $E50_{NPQ}$  did not differ  
265 significantly from each other. In contrast,  $n_{NPQ}$  was not significantly different and varied  
266 around its optimum (i.e. 2, Serôdio and Lavaud, 2011) in most species except the  
267 tychoplanktonic ones (which is significantly lower than in epipsammon non-motile).

268

269 *XC properties (Figs 1-2, Tables 4, S4, S6, S7)*

270 DES was only significantly different between epipelon and both tychoplankton and motile  
271 epipsammon at  $105 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and between epipelon and both epipsammonic forms at  
272  $230 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .  $DES_m$  varied between  $21.2 \pm 3.4$  for epipelon,  $22.7 \pm 4.4$  for  
273 tychoplankton,  $28.7 \pm 4.4$  for motile epipsammon-M and  $29.4 \pm 3.8$  for non-motile  
274 epipsammon (lsmmeans  $\pm$  SE). The slight difference between epipelon and the epipsammon  
275 growth forms, although not significant, in combination with the significantly higher DD+DT  
276 in the latter, generated a significantly lower  $DT_m$  in epipelon than in the epipsammon growth  
277 forms.  $E50_{DT}$  was close to the  $E50_{NPQ}$  in all growth forms except in tychoplankton where it  
278 was lower; no significant differences between the epipsammon and epipelon were observed,  
279 only non-motile epipsammon and tychoplankton  $E50_{DT}$  differed significantly.  $n_{DT}$  was  
280 significantly lower in motile epipsammon and tychoplankton than in epipelon and non-motile  
281 epipsammon.  $NPQ/DT$  was about half its optimum (= 1 under these experimental conditions)  
282 in all groups except non-motile epipsammon. It roughly followed the same order as observed  
283 for  $NPQ_m$ , i.e. non-motile epipsammon > motile epipsammon > epipelon  $\cong$  tychoplankton,  
284 with a 2x higher value in non-motile epipsammon. The difference between non-motile  
285 epipsammon and the other growth forms, however, was not significant due to the low

286 NPQ/DT value in *Plagiogramma staurophorum*. Fig 2 shows that in all growth forms except  
287 motile epipsammon there were species (*Seminavis robusta*, *Fragilaria. cf. subsalina*, *P.*  
288 *staurophorum*, *Brockmanniella brockmannii*) for which a low NPQ developed without DT  
289 synthesis, while two motile epipsammon species (*Amphora* sp. and *Planothidium*  
290 *delicatulum*) showed DT synthesis ( $0.17 \pm 0.03$  mol 100 mol Chl  $a^{-1}$ ) without NPQ. All other  
291 species showed a NPQ/DT relationship with an origin close to 0, as expected.

292

293 *Effect of high light acclimation on the NPQ and XC properties (Figs 3-4, Tables S8-S9)*

294 All species were grown under an E ( $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) roughly corresponding to the  
295 mean  $E_k$  for the low E acclimated cells ( $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , Table 3). Only epipelon had  
296 significantly higher growth rates at  $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . DD+DT significantly increased  
297 with a factor 1.6-1.7 in epipelon and epipsammon, and 2.3 in tychoplankton. There was a  
298 significant increase in DES at  $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  in all growth forms except in motile  
299 epipsammon. The increase in DD+DT and DES at the higher light intensity was most  
300 pronounced in tychoplankton and resulted in a pronounced, significant difference in both  
301 parameters between tychoplankton and epipelon at this light intensity. The comparison of Chl  
302 fluorescence yield and light curve parameters could only be performed for a selection of six  
303 species (covering all growth forms) and is summarised in Fig. 4. As expected, the Chl *a*  
304 content per cell decreased, roughly with a factor of 2 in all species (except *Navicula*  
305 *phyllepta*). There was only a slight (up to about 10 %) decrease in  $F_v/F_m$  in all species,  
306 illustrating the unstressed state of the cells (note that in *Seminavis robusta* and *Planothidium*  
307 *delicatulum* this decrease was slightly significant).  $DES_m$  significantly increased in *S. robusta*  
308 only. Together with the overall increase in DD+DT, this resulted in a significant increase in  
309  $DT_m$  (by a factor of 4) in this species, but also in *P. delicatulum* and *Plagiogrammopsis*  
310 *vanheurckii*. The corresponding  $NPQ_m$  did not follow the same trend: it significantly

311 increased in all species (except for *P. delicatulum* and *Opephora* sp.) but only by a factor of  
312 maximally 2. NPQ/DT remained low (0.2 to 0.5) in all species (and significantly decreased in  
313 *Opephora* sp.).  $E50_{NPQ}$  was significantly higher only in the non-motile epipsammic species  
314 *Plagiogramma staurophorum*.

315

316 *Effect of 'planktonic' growth on the NPQ and XC properties of tychoplankton (Fig. 5, Table*  
317 *S10)*

318 The three tychoplanktonic species were grown under 'planktonic' conditions (at 20  $\mu\text{mol}$   
319  $\text{photons.m}^{-2}.\text{s}^{-1}$ ) for a comparison with growth under 'benthic' conditions. *Brockmaniella*  
320 *brockmannii* responded most strongly to a switch from 'benthic' to 'planktonic' growth: it  
321 showed a significantly lower growth rate and a higher DES and  $\text{DES}_m$  but a lower  $\text{NPQ}_m$ ,  
322 suggesting photosynthetic stress and investment of additional DT in other processes than  
323 NPQ. *Plagiogrammopsis vanheurckii* and *Cylindrotheca closterium* showed very little  
324 change, apart from a significantly higher growth rate during planktonic growth in *P.*  
325 *vanheurckii*, a slight decrease in NPQ/DT in *C. closterium*, and an increase in DES in both  
326 species. The most pronounced and consistent change in tychoplankton thus concerned an  
327 increase in DES when grown in suspension. Note that there is also an overall decrease in  
328  $\text{rETR}_m$ , but this decrease was just not significant ( $p=0.08$ ).

329 **Discussion**

330 The present work constitutes the first comparative experimental study, using unialgal cultures  
331 in standardized conditions, of fast regulatory photoprotective mechanisms in the four main  
332 benthic diatom growth forms present in intertidal marine sediments (epipelon, motile and  
333 non-motile epipsammon and tychoplankton). Because no sediment was added in our  
334 experiments, motile diatoms were not able to position themselves in a light gradient, hence  
335 effectively incapacitating their behavioural response. As the growth rate and photosynthetic  
336 characteristics (main pigments,  $F_v/F_m$ ,  $\alpha$ ,  $E_k$ ,  $rETR_m$ ) of the studied species were comparable  
337 between the growth forms at  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , we were able to compare their purely  
338 physiological light response.

339 Our study revealed a highly significant and pronounced difference in NPQ between the four  
340 growth forms. NPQ was significantly lower in epipellic and tychoplanktonic than in  
341 epipsammic species; differences in DES were only observed between epipellic and other forms  
342 at lower light intensities. Within the epipsammon, NPQ capacity was significantly higher in  
343 the non-motile than in the motile forms. As all growth forms included both small and large  
344 species, the functional light response (NPQ capacity) apparently did not depend on biovolume  
345 or the Chl *a* concentration per cell, as has also been observed *in situ* (Jesus *et al.*, 2009). The  
346 absence of significant differences in PSII CET between growth forms underscores the  
347 importance of NPQ as the main fast photoprotective process in intertidal benthic diatoms,  
348 confirming earlier results for these organisms (Lavaud *et al.*, 2002) but in contrast with  
349 planktonic diatoms (Lavaud *et al.*, 2002; Lavaud *et al.*, 2007). By analogy with previous  
350 studies on planktonic diatoms (Dimier *et al.*, 2009; Lavaud *et al.*, 2007; Lavaud and Lepetit,  
351 2013; Petrou *et al.*, 2011; Strzepek and Harrison, 2004), our data suggest that epipellic and  
352 tychoplanktonic diatoms are adapted to a less fluctuating light climate and/or to a lower



353 average irradiance, and vice versa for epipsammic diatoms. This result fits well with the  
354 ecology of these growth forms. Epipelon is not only more abundant in muddy cohesive  
355 sediments where light penetration is more restricted than in sandy sediments (Paterson and  
356 Hagerthey 2001; Cartaxana *et al.*, 2011), but, more importantly, their (micro-)migratory  
357 behaviour allows positioning at the optimal irradiance in the vertical light gradient and rapid  
358 escape from periodic excess light (Kromkamp *et al.*, 1998; Conn *et al.*, 2004; Consalvey *et*  
359 *al.*, 2004; Serôdio *et al.*, 2006). This alleviates the need to invest in a strong physiological  
360 capacity to respond to light stress as previously proposed (Jesus *et al.*, 2009; Cartaxana *et al.*,  
361 2011), although the right balance between motility and physiology still remains essential (van  
362 Leeuwe *et al.*, 2009; Perkins *et al.*, 2010b; Cartaxana *et al.*, 2011; Serôdio *et al.*, 2012).

363 Such balance is more crucial in the motile epipsammic species, which can move but have only  
364 limited control over their immediate light environment as movement is restricted, usually  
365 within the sphere of individual sand grains. As expected, they showed a significantly lower  
366 NPQ and a higher  $E_{50_{NPQ}}$  than non-motile epipsammon, which have no behavioural control  
367 over their light environment. An alternative, but not exclusive, explanation could be related to  
368 the difference in exopolysaccharide (EPS) secretion between motile and non-motile growth  
369 forms. EPS secretion could work as an alternative electron sink under stressful conditions (i.e.  
370 high light, nutrient limitation, etc.) in order to limit the over-reduction of the photosynthetic  
371 machinery ('overflow' hypothesis; Staats *et al.*, 2000), alleviating the need for a strong NPQ.  
372 However, EPS secretion is not as fast as NPQ (minutes/hours *vs.* seconds/minutes) and may  
373 not be useful to the cells for responding to rapid light changes but only to cope with  
374 prolonged high light exposure. Additionally, while the 'overflow' hypothesis is often  
375 proposed (Underwood and Paterson, 2003; Stal, 2009), it was never clearly proven. A few  
376 studies have shown a positive relationship between light intensity and EPS production

377 (Underwood, 2002; Wolfstein and Stal, 2002) but other studies have reported a negative  
378 relation with light intensity and no relationship with nutrient limitation (Hanlon *et al.*, 2001;  
379 Perkins *et al.*, 2006). To date there is no information on EPS production in different benthic  
380 diatom growth forms, and only epipelagic species have been compared (Underwood and  
381 Paterson, 2003), showing no clear relationship between light response and EPS secretion. To  
382 our knowledge, there are no reports on a relationship between NPQ-XC capacity and EPS  
383 production. Finally, tychoplankton typically alternates between resuspension in a highly  
384 turbid shallow water column at high tide and deposition and burial in the upper sediment  
385 layers of muddy sediments at low tide (deposition in sandy sediments does not occur due to  
386 the intense hydrodynamic disturbance in these sediments). As such, the tychoplankton  
387 resembles planktonic diatoms adapted to subtle light fluctuations and/or on average low  
388 irradiance (Bailleul *et al.*, 2010; Lavaud and Lepetit, 2013).

389 The reason for the NPQ differences between epipelagic and epipsammonic can be explained by  
390 its main control: the XC dynamics. Previous *in situ* studies reported a consistently stronger  
391 DES under light stress in epipsammonic than in epipelagic diatom communities (i.e. in sandy vs.  
392 muddy sediments) and related growth form with differential (behavioural vs. physiological)  
393 photoregulatory strategies (Jesus *et al.*, 2009; Cartaxana *et al.*, 2011). As recently shown, a  
394 high NPQ is supported by the strong effective involvement of DT which first depends both on  
395 a high DD+DT content and a high DES (Lavaud and Lepetit, 2013). The slope of the  
396 NPQ/DT relationship has been proposed as a good indicator of light climate adaptation: the  
397 higher the NPQ/DT slope, the better the adaptation to a highly fluctuating and/or on average  
398 high light climate (Dimier *et al.*, 2009; Lavaud and Lepetit, 2013). All epipsammonic species,  
399 and especially the non-motile ones, showed XC parameter values which are characteristic for  
400 a high NPQ capacity, viz. a higher DD+DT content and  $DT_m$  which was 2x higher than in

401 epipelon. Non-motile epipsammon also tended to show a higher efficiency in promoting NPQ  
402 (NPQ/DT), but this difference was not significant due to high intra-group variability.

403 Within the epipsammon, NPQ is clearly more efficient in non-motile than motile epipsammic  
404 species. In motile epipsammon, the discrepancy between  $E_{50_{NPQ}}$  and  $E_k$  is more pronounced  
405 than in non-motile forms: while there is no significant difference in  $E_k$  between both growth  
406 forms,  $E_{50_{NPQ}}$  is significantly higher in the motile growth forms. This suggests a weaker  
407 relationship between NPQ development and photochemistry in the latter group, with slower  
408 NPQ development with increasing E. Remarkably,  $E_{50_{DT}}$  does not significantly differ  
409 between both growth forms, and the significantly higher initial induction of DT synthesis  
410 ( $n_{DT}$ ) but not NPQ ( $n_{NPQ}$ ) in the motile group, together with the fact that some representatives  
411 of this group show DT synthesis without NPQ, suggests that either DT is less or not involved  
412 in NPQ development, or that the light-dependent built-up of the transthylakoidal proton  
413 gradient (which is involved in both the activation of the DD de-epoxidase and the molecular  
414 control of NPQ) and the onset of NPQ are uncoupled (Lavaud *et al.*, 2012; Lavaud and  
415 Lepetit, 2013). Our observations thus suggest that in contrast to the non-motile group, motile  
416 epipsammic species rely more on a behavioural response (motility) and/or involve DT in  
417 other photoprotective processes such as the prevention of lipid peroxidation by reactive  
418 oxygen species (ROS) (Lepetit *et al.*, 2010). The increase in  $E_{50_{NPQ}}$  in the non-motile  
419 epipsammic species *Plagiogramma staurophorum* during a shift to higher light illustrates the  
420 ability to physiologically modulate the NPQ *vs.* E development kinetics to its light  
421 environment in contrast to motile epipsammon, epipelon and tychoplankton.

422 The influence of DT on the inter-group/species NPQ differences was further investigated by  
423 the acclimation to higher light ( $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , close to the mean  $E_k$  for cells  
424 acclimated to  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). High light exposure is known to induce constitutive

425 DT synthesis (Schumann *et al.*, 2007) and in field conditions, DT is usually even present in  
426 significant amounts in cells adapted to low/moderate light (Jesus *et al.*, 2009; van Leeuwe *et*  
427 *al.*, 2009; Chevalier *et al.*, 2010; Cartaxana *et al.*, 2011). Acclimation to higher light resulted  
428 in a significant increase in XC pigments (DD+DT) and DES in most growth forms,  
429 suggesting that although epipelon uses behavioural photoprotection, the XC is still important  
430 (cf. above). NPQ<sub>m</sub> increased in most of the species examined, mainly due to a higher DT<sub>m</sub>  
431 resulting from a higher DD+DT rather than a higher DES<sub>m</sub>. The discrepancy between DES<sub>m</sub>  
432 and NPQ<sub>m</sub> as well as the low NPQ/DT may be due to the fact that the additional DT primarily  
433 served in the prevention of lipid peroxidation rather than in NPQ as previously reported in  
434 high light acclimated diatoms (see also above).

435 While under low light conditions, the growth, photosynthetic and steady-state light-response  
436 features of tycho plankton were similar to those of epipellic diatoms (i.e. low NPQ, NPQ<sub>m</sub> and  
437 DT<sub>m</sub>), their dynamic light response was significantly different, i.e. higher E50<sub>NPQ</sub>.  
438 Surprisingly, E50<sub>NPQ</sub> was beyond the natural light maximum (2000-2500 μmol photons m<sup>-2</sup> s<sup>-1</sup>)  
439 illustrating the inability of tycho plankton to strongly and/or continuously develop NPQ in  
440 the environmental high light range (a situation also encountered in one epipellic species:  
441 *Navicula phyllepta*). In contrast, its low n<sub>NPQ</sub> supported a relatively strong onset of NPQ at  
442 low Es. Both E50<sub>DT</sub> and n<sub>DT</sub> were correspondingly high and low, respectively (and  
443 significantly different from epipelon for n<sub>DT</sub>), although E50<sub>DT</sub> was much lower than E50<sub>NPQ</sub>  
444 suggesting a discrepancy between DT synthesis and NPQ development (cf. above). The  
445 response of tycho plankton to higher light was much more pronounced, with the strongest  
446 increase in XC pigments and DES of all growth forms. However, the NPQ<sub>m</sub> and DT<sub>m</sub> data  
447 (only available however for one representative species, *Plagiogrammopsis vanheurckii*) did  
448 not show a similar response, with DT<sub>m</sub> showing a more pronounced increase than NPQ<sub>m</sub>,

449 suggesting that NPQ development was low and that DT may have mainly been involved in  
450 other processes than NPQ. For most parameters, the response of the tychoplankton species to  
451 growth in suspension ('planktonic' growth) was limited and largely species-specific, except  
452 for a general increase in DES and a decrease (albeit just non-significant) in  $rETR_m$ . These data  
453 suggest that representatives of the tychoplanktonic growth form are well-adapted to their  
454 amphibious life style, which is characterized by an on average low irradiance (MacIntyre *et*  
455 *al.*, 1996). In contrast, epipellic species do not grow well in suspended, turbulent conditions (J.  
456 Lavaud, pers. observation).

457 Our study for the first time shows that intertidal benthic diatoms display growth form specific  
458 variation in fast regulatory physiological mechanisms for photoprotective capacity (NPQ and  
459 the XC), which mirrors their behavioural light response. In epipellic motile diatoms,  
460 exclusively belonging to the raphid pennate clade, the physiological response is not well  
461 developed, as these diatoms appear to largely rely on motility to control their immediate light  
462 environment. In the motile epipsammon however the physiological response remains essential  
463 because their movement is restricted to the sphere of individual sand grains. The evolution of  
464 the raphe system, the hallmark synapomorphy of the raphid pennate diatom clade which  
465 enables locomotion, has therefore been essential for the colonization of intertidal sediments  
466 by not only migratory epipellic biofilms but also motile epipsammon. In contrast, NPQ and  
467 XC capacity is high in non-motile araphid pennate diatoms which passively have to abide  
468 often pronounced variations in the intertidal light climate. Tychoplanktonic diatoms, which  
469 alternate between high tide resuspension in a turbulent and turbid water column, and low tide  
470 deposition in muddy sediments, appear to be adapted to an on average low light environment,  
471 with low NPQ and XC capacity.

472 While we made no formal analysis of the relationship between functional and phylogenetic  
473 diversity, it is obvious that despite the fact that a behavioural photoprotective response  
474 (motility) is restricted to the raphid pennate diatom clade, differences in the studied  
475 physiological traits are more strongly driven by growth form than phylogenetic relatedness.  
476 For example, the epipsammic species *Biremis lucens*, despite being a raphid pennate species,  
477 has a non-motile growth form, and shows a NPQ capacity which is more similar to non-motile  
478 epipsammon than to the (phylogenetically more closely related) motile epipsammon and  
479 epipelon. Likewise, photophysiological features of pennate raphid (*Cylindrotheca closterium*)  
480 and centric (*Plagiogrammopsis vanheurckii* and *Brockmanniella brockmannii*) tychoplankton  
481 species were similar as reported before in planktonic centric/pennate species (Lavaud *et al.*,  
482 2004). Raphid pennate diatoms which have colonized an epipsammic or tychoplanktonic  
483 niche thus display a reverse evolutionary trade-off switch towards a much more performant  
484 physiological response. Our observations thus suggest that photoprotective capacity in  
485 diatoms is a highly adaptive trait which is to a certain degree constrained by clade-specific  
486 evolutionary innovations (the evolution of the raphe system and hence a behavioural  
487 response) but also, and more importantly, by growth form, which ultimately defines the  
488 balance between the physiological and behavioural photoprotective response in these  
489 organisms. Such differential adaptation is of primary importance for the regulation of the  
490 photosynthetic productivity *vs.* light, as has been demonstrated before in planktonic diatoms,  
491 where the photochemical *vs.* the photoprotective energy allocation as a function of light is  
492 drastically different in species adapted to a fluctuating *vs.* a more stable light environment  
493 (Wagner *et al.*, 2006; Lavaud *et al.*, 2007; Petrou *et al.*, 2011; Lavaud and Lepetit, 2013).  
494 However, unlike in planktonic environments, the trade-off between a physiological and  
495 behavioural response in benthic diatoms allows local co-existence of different growth forms  
496 under the same overall light environment.

497

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505

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507

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509

510

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702

703 **Titles and legends to figures and tables.**

704

705 **Figure 1.** Non-photochemical quenching of Chl fluorescence (NPQ) (A), de-epoxidation state  
706 of the diadinoxanthin (DD) to diatoxanthin (DT) [DES = DT / (DD + DT) x 100] (B), and DT  
707 content (C) as a function of light intensity (E from darkness to 1950  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$   
708 which is equivalent to full sunlight in the field) measured during Non-Sequential Light  
709 Curves (NSLCs) in the four benthic diatom growth forms (EPM-NM, epipsammon non-  
710 motile, EPM-M, epipsammon motile; EPL, epipelon; TYCHO, thychoplankton). Cells were  
711 grown at 20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The NPQ-E curves for the individual species can be found  
712 in Fig. S2. Values are estimated least squares means  $\pm$  estimated standard errors (PROC  
713 MIXED procedure).

714

715 **Figure 2.** Non-photochemical quenching of Chl fluorescence (NPQ) as a function of the  
716 amount of diatoxanthin (DT) measured during Non-Sequential Light Curves (NSLCs) in the  
717 five species of epipelon (EPL) (A), the four species of motile epipsammon (EPM-M) (B), the  
718 three species of thychoplankton (TYCHO) (C), and the three species of non-motile  
719 epipsammon (EPM-NM) (D). Cells were grown at 20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The full names  
720 and classification of all species is listed in Table 1.

721

722 **Figure 3.** Growth rate ( $\mu$ ) (A), diadinoxanthin (DD) + diatoxanthin (DT) content (B) and de-  
723 epoxidation state of DD to DT [DES = (DT / DD+DT x 100)] (C) in the four benthic diatom  
724 growth forms (EPM-NM, epipsammon non-motile, EPM-M, epipsammon motile; EPL,  
725 epipelon motile; TYCHO, thychoplankton) for cells grown at light intensities of 20 and 75  
726  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  respectively. All parameters were measured on cells in exponential  
727 growth and sampled 2 h after the onset of light; growth conditions were 16 h light:8 h dark,



728 20°C. The values for all species in 20 and 75  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  conditions are found in  
729 Tables S2 and S8, respectively. Values are estimated least squares means  $\pm$  estimated standard  
730 errors (PROC MIXED procedure).

731

732 **Figure 4.** Comparison of photosynthetic, non-photochemical quenching of Chl fluorescence  
733 (NPQ) and xanthophyll cycle (XC) parameters measured in diatom species representative of  
734 the four benthic diatom growth forms grown at light intensities of 20 and 75  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$   
735 respectively. For each parameter the ratio of the values obtained at 75 and 20  $\mu\text{mol}$   
736  $\text{photons m}^{-2} \text{ s}^{-1} - 1$  was calculated (i.e. the 0 line is equal to a 75/20 ratio = 1 which is  
737 equivalent to no change of values between light intensities). Significant changes between both  
738 light intensities are indicated with an asterisk. The values used for the 20 and the 75  $\mu\text{mol}$   
739  $\text{photons m}^{-2} \text{ s}^{-1}$  conditions can be found in Tables S2/S4 and S8/S9 respectively.

740

741 **Figure 5.** Comparison of growth, photosynthetic, pigment, non-photochemical quenching of  
742 Chl fluorescence (NPQ) and xanthophyll cycle (XC) parameters measured in the three  
743 tychoplankton diatom species in ‘benthic’ and ‘planktonic’ conditions. For each parameter the  
744 ratio of the values obtained under benthic and planktonic conditions – 1 was calculated (i.e.  
745 the 0 line is equal to a planktonic/benthic ratio = 1 which is equivalent to no change of values  
746 between ‘benthic’ and ‘planktonic’ conditions). Chl *a* per cell (in pg cell<sup>-1</sup>) and growth rates  
747 (in day<sup>-1</sup>) were measured on cells in exponential growth phase sampled 2 h after the onset of  
748 light; growth conditions were 20  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , 16 h light:8 h dark, 20°C. Significant  
749 changes between both light intensities are indicated with an asterisk. The values used for the  
750 ‘benthic’ and ‘planktonic’ growth conditions can be found in Tables S2/S4 and S10  
751 respectively.

752

753

754 **Table 1: List of the fifteen diatom species used in this study with their growth form**  
755 **classification, collection number, origin and average biovolume.**

756 Abbreviations: NCC, Nantes Culture Collection-France ; UTCC, University of Toronto  
757 Culture Collection of Algae and Cyanobacteria-Canada (now the Canadian Phycological  
758 Culture Collection-CPCC); CCY, Culture Collection Yerseke-The Netherlands; DCG: BCCM  
759 (Belgian Coordinated Collections of Microorganisms) Diatom Culture Collection hosted by  
760 Laboratory for Protistology & Aquatic Ecology, Ghent University, Belgium ; n.d. not  
761 determined.

762

763 **Table 2: Photophysiological parameters used in this study, their photophysiological**  
764 **meaning and measurement method and conditions.** Abbreviations: Chl, chlorophyll; DD,  
765 diadinoxanthin; DT, diatoxanthin; E, light intensity; NSLCs, Non-Sequential Light Curves;  
766 PSII, photosystem II; RLCs, Rapid Light Curves. See the Materials and Methods section for  
767 further details.

768

769 **Table 3: Growth rate, pigment content and photosynthetic properties of the four growth**  
770 **forms of benthic diatoms.** All parameters were measured on cells in exponential growth  
771 phase sampled 2 h after the onset of light. Growth conditions were  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ,  
772 16 h light:8 h dark,  $20^\circ\text{C}$ . Abbreviations: EPL, epipelon; EPM-M, motile epipsammon; EPM-  
773 NM, non-motile epipsammon; TYCHO, tychoplankton.  $\mu$ , growth rate ( $\text{day}^{-1}$ ); Chl  $a \text{ cell}^{-1}$ ,  
774 content of chlorophyll  $a$  (in pg) per diatom cell; other pigments are expressed in mol 100 mol.  
775 Chl  $a^{-1}$ : Chl, chlorophyll; Fx, fucoxanthin;  $\beta$ -car,  $\beta$ -carotene; DD, diadinoxanthin; DT,  
776 diatoxanthin. Definitions and conditions of measurement of all parameters are listed in Table

777 2. The values for the individual species can be found in Table S2. Values are least squares  
778 means estimates and estimated standard errors (PROC MIXED procedure).

779

780 **Table 4: Non-photochemical quenching (NPQ) and xanthophyll cycle (XC) properties of**  
781 **the four growth forms of benthic diatoms.** Abbreviations: EPL, epipelon; EPM-M, motile  
782 epipsammon; EPM-NM, non-motile epipsammon; TYCHO, tychoplankton. Definitions and  
783 conditions of measurement of all parameters are listed in Table 2. The values for the  
784 individual species can be found in Tables S4. Values are least squares means estimates and  
785 estimated standard errors (PROC MIXED procedure).

786

787

Figure 1

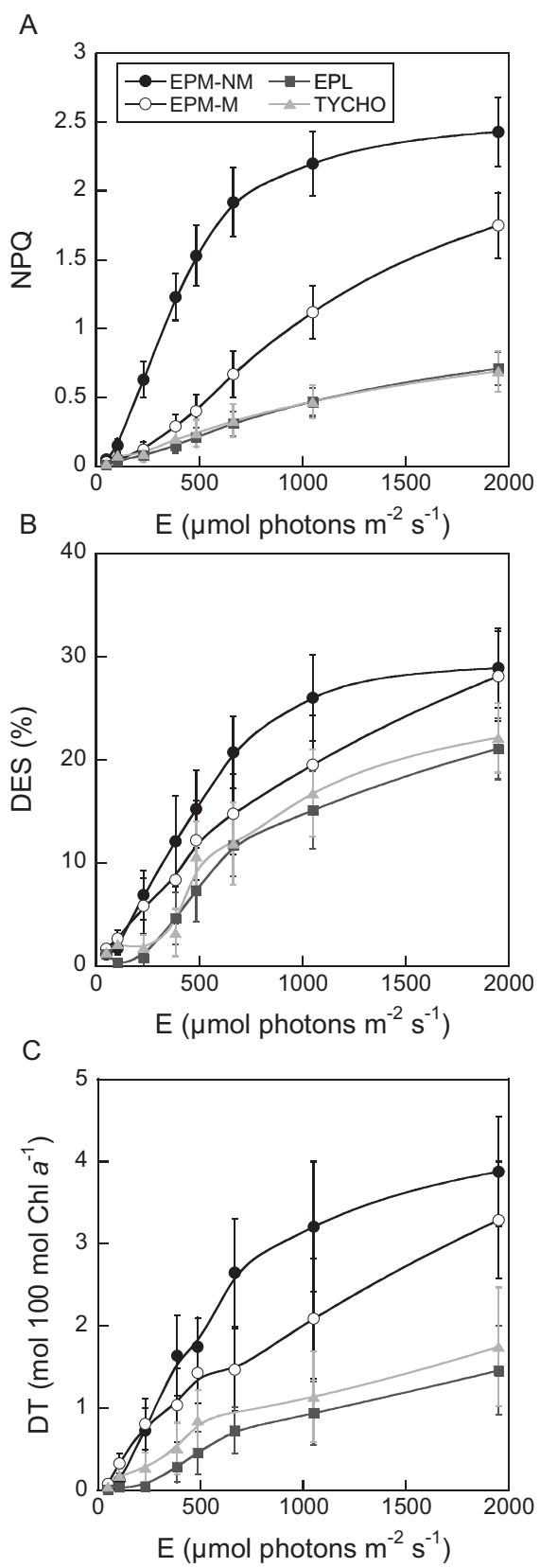
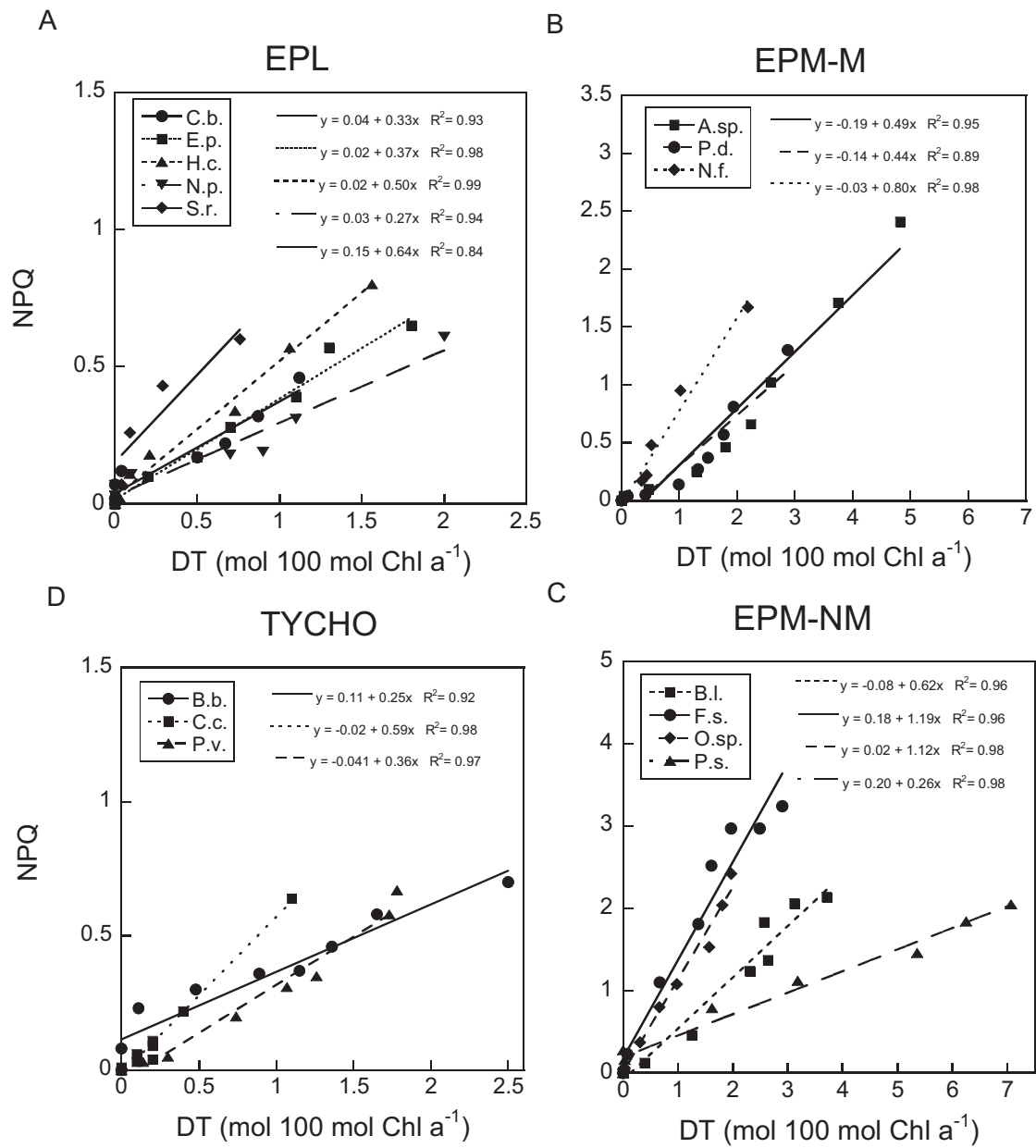


Figure 2



**Figure 3**

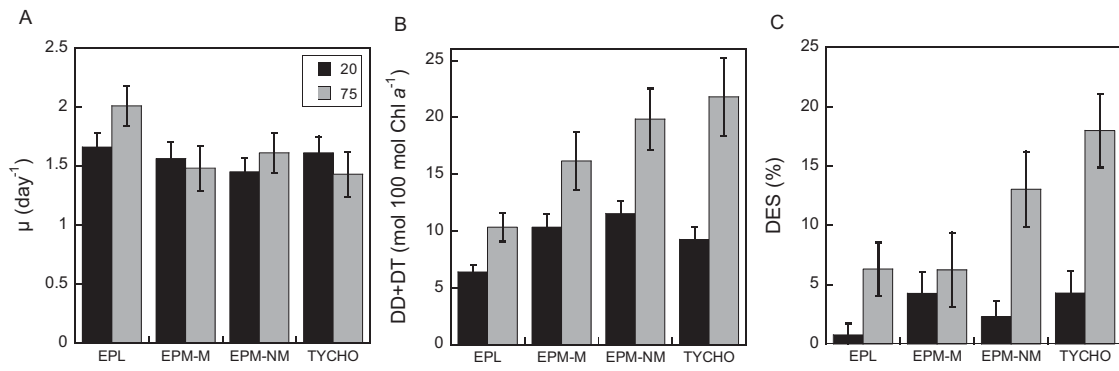


Figure 4

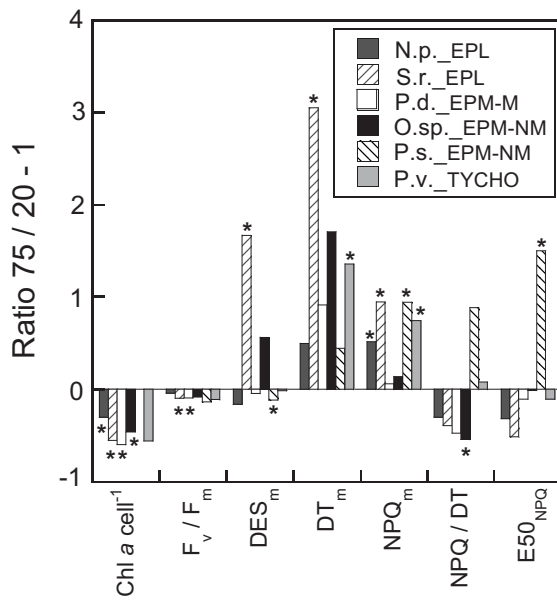
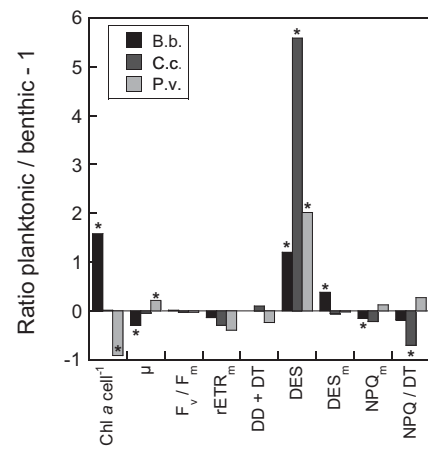


Figure 5





Species	Growth form		Collection n°	Sampling place	Average biovolume (µm <sup>3</sup> )
<i>Craspedostauros britannicus</i> C.b.	Epipelon (EPL)		NCC195-06-2	Pouliguen, Atlantic, France	1740
<i>Entomoneis paludosa</i> E.p.			NCC18-1	Bay of Bourgneuf, Atlantic, France	1081
<i>Halamphora coffeaeformis</i> H.c.			UTCC58	Victoria, British Columbia, Pacific, Canada	126
<i>Navicula phyllepta</i> N.p.			CCY9804	Westerschelde estuary, North sea, The Netherlands	218
<i>Seminavis robusta</i> S.r.			DCG 0105	Progeny of strains from Veerse Meer, The Netherlands	1790
<i>Amphora</i> sp. A. sp.	Epipsammon (EPM)	motile (EPM-M)	DCG 0493	Rammekenshoek, North sea, The Netherlands	39
<i>Nitzschia</i> cf. <i>frustulum</i> N.f.			DCG 0494	Rammekenshoek, North Sea, The Netherlands	29
<i>Planothidium delicatulum</i> P.d.			NCC363	Bay of Bourgneuf, Atlantic, France	242
<i>Biremis lucens</i> B.l.		non-motile (EPM-NM)	NCC360.2	Bay of Bourgneuf, Atlantic, France	242
<i>Fragilaria</i> cf. <i>subsalina</i> F.s.			DCG 0492	Rammekenshoek, North sea, The Netherlands	165
<i>Opephora</i> sp. O. sp.			DCG 0448	Rammekenshoek, North Sea, The Netherlands	86
<i>Plagiogramma staurophorum</i> P. s.			DCG 0495	Rammekenshoek, North sea, The Netherlands	n.d.
<i>Brockmanniella brockmannii</i> B.b.	Tychoplankton (TYCHO)		NCC161	Bay of Bourgneuf, Atlantic, France	105
<i>Cylindrotheca closterium</i> C.c.			Collection Univ. Aveiro	Ria de Aveiro, Atlantic, Portugal	247
<i>Plagiogrammopsis vanheurckii</i> P.v.			NCC186-2	Bay of Bourgneuf, Atlantic, France	737



Table 2

Parameter	Unit	Definition	Photophysiological meaning	Measurement conditions
$F_0$	No units	Minimum PSII Chl fluorescence yield	Used to calculate $F_v/F_m$ (see below)	Measured with NSLCs after 15 min of dark acclimation
$F_m$	No units	Maximum PSII Chl fluorescence yield	Used to calculate $F_v/F_m$ and NPQ (see below)	Measured with NSLCs during a saturating pulse after 15 min of dark acclimation
$F_v/F_m$	No units	Maximum photosynthetic efficiency of PSII; $F_v = F_m - F_0$	Maximum quantum efficiency of PSII photochemistry	See the above measurement conditions for $F_0$ and $F_m$
$F_m'$	No units	$F_m$ for illuminated cells	Used to measure NPQ and rETR	Measured with NSLCs during a saturating pulse after 5 min of illumination at specific E
NPQ	No units	Non-photochemical quenching of Chl fluorescence; $NPQ = F_m'/F_m' - 1$	Estimates the photoprotective dissipation of excess energy	Measured with NSLCs
rETR	$\mu\text{mol electrons m}^{-2} \text{s}^{-1}$	Relative electron transport rate of PSII; $rETR = \Phi_{PSII} \times E$ where $\Phi_{PSII} = F_m' - F/F_m'$	Effective quantum yield of photochemistry vs. E	Measured with RLCs; F is the steady-state of Chl fluorescence measured after 30 s illumination at a given E
$\alpha$	Relative units	rETR-E curve initial slope	Maximum light efficiency use	Derived from fitted rETR-E curves measured with RLCs (Eilers and Peeters, 1988)
rETR <sub>m</sub>	$\mu\text{mol electrons m}^{-2} \text{s}^{-1}$	rETR-E curve asymptote	Maximum relative photosynthetic electron transport rate	Derived from fitted rETR-E curves measured with RLCs (Eilers and Peeters, 1988)
$E_k$	$\mu\text{mol photons. m}^{-2} \text{s}^{-1}$	$E_k = rETR_m / \alpha$	Light saturation coefficient	Derived from fitted rETR-E curves measured with RLCs (Eilers and Peeters, 1988)
NPQ <sub>m</sub>	No units	NPQ-E curve asymptote	Maximum NPQ	Measured with NSLCs
$E_{50_{NPQ}}$	$\mu\text{mol photons. m}^{-2} \text{s}^{-1}$	E for reaching 50% of NPQ <sub>m</sub>	Pattern of NPQ induction vs. E	Derived from fitted NPQ-E curves (Seródio and Lavaud, 2011) measured with NSLCs
$n_{NPQ}$	No units	NPQ-E curve sigmoidicity coefficient	Onset of NPQ induction for moderate Es (< $E_{50_{NPQ}}$ )	Derived from fitted NPQ-E curves (Seródio and Lavaud, 2011) measured with NSLCs
DT <sub>m</sub>	mol. 100 mol Chl $\alpha^{-1}$	DT-E curve asymptote	Maximum DT concentration	Measured with NSLCs
$E_{50_{DT}}$	$\mu\text{mol photons. m}^{-2} \text{s}^{-1}$	E for reaching 50% of DT <sub>max</sub>	Pattern of DT synthesis vs. E	Derived from fitted DT-E curves (Seródio and Lavaud, 2011) measured with NSLCs
$n_{DT}$	No units	DT-E curve sigmoidicity coefficient	Onset of DT synthesis for moderate Es (< $E_{50_{NPQ}}$ )	Derived from fitted DT-E curves (Seródio and Lavaud, 2011) measured with NSLCs
DES <sub>m</sub>	%	DES-E curve asymptote; $DES = [DT / (DD+DT) \times 100]$	Maximum de-epoxidation state	Measured with NSLCs
NPQ / DT	No units	NPQ-DT curve slope	Effective involvement of DT in NPQ for all Es (Lavaud et Lepetit, 2013)	Measured with NSLCs

**Table 3**

Growth Form	Pigments							Photosynthetic parameters				
	$\mu$	Chl <i>a</i> cell <sup>-1</sup>	Chl <i>c</i>	Fx	$\beta$ -car	DD+DT	DES	F <sub>v</sub> /F <sub>m</sub>	$\alpha$	rETR <sub>m</sub>	E <sub>k</sub>	PSII CET <sub>m</sub>
EPL	1.66 ± 0.12	12.55 ± 12.91	18.91 ± 3.05	65.99 ± 7.90	3.91 ± 0.39	6.39 ± 0.61	0.75 ± 0.93	0.72 ± 0.01	0.68 ± 0.03	52.41 ± 5.90	78.93 ± 9.79	2.09 ± 0.23
EPM-M	1.56 ± 0.14	1.45 ± 0.78	16.05 ± 3.34	64.29 ± 10.21	2.76 ± 0.43	10.34 ± 1.17	4.25 ± 1.79	0.68 ± 0.02	0.65 ± 0.04	51.50 ± 7.36	80.41 ± 12.89	2.86 ± 0.33
EPM-NM	1.45 ± 0.12	2.13 ± 1.63	20.12 ± 3.63	70.52 ± 8.83	2.11 ± 0.43	11.52 ± 1.13	2.30 ± 1.33	0.67 ± 0.02	0.63 ± 0.04	39.20 ± 4.88	61.01 ± 8.52	2.82 ± 0.23
TYCHO	1.61 ± 0.14	1.72 ± 2.45	24.81 ± 5.17	79.36 ± 10.12	3.04 ± 0.51	9.25 ± 1.09	4.29 ± 1.83	0.73 ± 0.02	0.71 ± 0.04	58.32 ± 8.44	82.79 ± 13.40	2.03 ± 0.26

**Table 4**

Growth form	NPQ <sub>m</sub>	E50 <sub>NPQ</sub>	n <sub>NPQ</sub>	DES <sub>m</sub>	DT <sub>m</sub>	E50 <sub>DT</sub>	n <sub>DT</sub>	NPQ/DT
EPL	0.69 ± 0.09	866.45 ± 200.24	1.88 ± 0.26	21.20 ± 3.38	1.34 ± 0.52	714.73 ± 128.29	2.39 ± 0.20	0.46 ± 0.10
EPM-M	1.71 ± 0.28	1061.25 ± 310.20	2.04 ± 0.34	28.68 ± 4.37	3.08 ± 1.36	809.41 ± 164.71	1.38 ± 0.20	0.52 ± 0.14
EPM-NM	2.41 ± 0.34	360.61 ± 91.42	2.27 ± 0.29	29.43 ± 3.79	3.45 ± 2.21	465.91 ± 80.04	2.30 ± 0.21	0.67 ± 0.16
TYCHO	0.66 ± 0.11	3887.42 1105.58	1.12 ± 0.34	22.73 ± 4.39	1.78 ± 0.61	1099.82 ± 341.05	1.42 ± 0.19	0.36 ± 0.10