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Behavioural versus physiological photoprotection in epipellic and epipsammic benthic diatoms

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Running head: Photoprotection strategies in intertidal diatoms

22 **Abstract**

23

24 Benthic diatoms are dominant primary producers in intertidal marine sediments, which
25 are characterized by widely fluctuating and often extreme light conditions. To cope with
26 sudden increases in light intensity, benthic diatoms display both behavioural and
27 physiological photoprotection mechanisms. Behavioural photoprotection is restricted to
28 raphid pennate diatoms, which possess a raphe system that enables motility and hence
29 positioning in sediment light gradients (e.g. via vertical migration into the sediment).
30 The main physiological photoprotection mechanism is to dissipate excess light energy
31 as heat, measured as Non-Photochemical Quenching (NPQ) of chlorophyll
32 fluorescence. A trade-off between vertical migration and physiological photoprotection
33 (NPQ) in benthic diatoms has been hypothesized before, but this has never been
34 formally tested. We exposed five epipelagic diatom species (which move in between
35 sediment particles) and four epipsammic diatom species (which live in close association
36 with individual sand grains) to high light conditions, and characterized both NPQ and
37 the relative magnitude of the migratory response to high light. Our results reveal the
38 absence of a significant downward migratory response in an araphid diatom, but also in
39 several raphid epipsammic diatoms, while all epipelagic species showed a significant
40 migratory response upon high light exposure. In all epipsammic species the
41 upregulation of NPQ was rapid and pronounced; NPQ relaxation in low light
42 conditions, however, occurred faster in the araphid diatom, compared to the raphid
43 epipsammic species. In contrast, all epipelagic species lacked a strong and flexible NPQ
44 response and showed higher susceptibility to photodamage when not able to migrate.
45 While overall our results support the vertical migration-NPQ trade-off, the lack of
46 strong relationships between the capacity for vertical migration and NPQ within the

47 epipsammic and epipelagic groups suggests that other factors as well, such as cell size,
48 substrate type and photoacclimation, may influence photoprotective strategies.

49

50 **Keywords:** diatom, microphytobenthos, non-photochemical quenching,
51 photoprotection, trade-off, vertical migration.

52

53

54 **Introduction**

55

56 Light is an indispensable but often highly variable resource for microalgae. While traits
57 associated with light utilization are plastic, they can also differ between taxa, often in
58 relation to the specific light climate in their respective habitats (Litchman & Klausmeier
59 2008). For example, rapid physiological photoprotection mechanisms, such as excess
60 energy dissipation as heat through Non-Photochemical Quenching of chlorophyll
61 fluorescence [NPQ, related to de-epoxidation of xanthophyll pigments in the so-called
62 xanthophyll cycle (XC), (Lavaud & Goss 2014)] are more strongly developed in diatom
63 species which inhabit strongly mixed and turbid coastal environments than in those
64 inhabiting open ocean environments with a more stable light climate (Lavaud *et al.*,
65 2007; Dimier *et al.*, 2009; Bailleul *et al.*, 2010).

66 At low tide, benthic diatoms living in intertidal sediments experience a light
67 climate similar to the terrestrial environment, with often fast and unpredictable
68 fluctuations in light (Lavaud & Goss 2014), and display a high NPQ capacity (Perkins
69 *et al.*, 2010a). Large differences in NPQ capacity have been observed between benthic
70 growth forms (Jesus *et al.*, 2009, Cartaxana *et al.*, 2011, Barnett *et al.*, 2015, Pniewski
71 *et al.*, 2015, Cartaxana *et al.*, 2016b). Dense biofilms composed of epipellic diatoms
72 form on fine-grained sediments (Sabbe 1993; Ribeiro *et al.*, 2013). Epipellic diatoms
73 live freely on and in sediments and possess a raphe structure through which mucilage is
74 secreted allowing movement (Round *et al.*, 1990; Aumeier & Menzel 2012). In addition
75 to endogenous vertical migration rhythms in response to diurnal and tidal cycles
76 (Consalvey *et al.*, 2004), epipellic diatoms can also actively position themselves within
77 sediment light gradients in order to maximize photosynthesis and/or avoid overexposure

78 (Admiraal 1984; Serôdio *et al.*, 2006; Cartaxana *et al.*, 2016a). While in situ epipellic
79 diatom communities also activate the XC as a response to high light (Chevalier *et al.*,
80 2010), downward vertical migration (VM) into the sediment is considered to be their
81 prime response to high light stress (Perkins *et al.*, 2010b). This behavioural response
82 could as such minimize the need for physiological photoprotection (Serôdio *et al.*, 2001;
83 Raven, 2011).

84 In more sandy sediments, epipellic communities are largely replaced by
85 communities of epipsammic diatoms. These diatoms are either araphid (and hence non-
86 motile) and firmly attached to sand grains (either adnate or via a mucilage stalk), or
87 raphid. In the latter case, it is hypothesized that their movement is largely restricted to
88 the sphere of individual sand grains (Sabbe 1997; Ribeiro *et al.*, 2013). As in situ
89 communities living on sandy sediments showed no migratory behaviour and exhibited
90 higher diatoxanthin/diadinoxanthin (Dtx/Ddx) ratios than communities living on silt, a
91 trade-off between behavioural (VM) and physiological photoprotection (NPQ) was
92 proposed (van Leeuwe *et al.*, 2008; Jesus *et al.*, 2009). Exposing both silt- and sand-
93 inhabiting communities to high light in controlled laboratory conditions supported this
94 hypothesis as the sand-inhabiting communities showed higher Dtx/Ddx ratios while not
95 migrating downward in response to high light (Cartaxana *et al.*, 2011). These
96 observations were confirmed by Barnett *et al.* (2015) who used unialgal cultures to
97 show that epipsammic diatoms indeed have a higher capacity for NPQ and XC than
98 epipellic species. In addition, non-motile epipsammic species show a stronger coupling
99 between NPQ development and the light saturation point (E_k) than motile epipsammic
100 species. Finally, Laviale *et al.* (2016) showed that in epipellic communities light

101 induction of VM occurs at a similar rate as NPQ induction, an essential condition for a
102 migration-physiology trade-off.

103 While all above studies support a trade-off between NPQ and VM, combined
104 measurements of both traits are limited to natural communities (Perkins *et al.*, 2010b;
105 Serôdio *et al.*, 2012; Laviale *et al.*, 2015, 2016). Natural communities however usually
106 contain a mix of growth forms and species (Hamels *et al.*, 1998), which can hamper the
107 interpretation of NPQ and XC measurements as both growth form and species responses
108 can be quite specific (Underwood *et al.*, 2005; Barnett *et al.*, 2015; Cohn *et al.*, 2015).
109 The photoprotection capacity of raphid and araphid epipsammic growth forms has
110 rarely been investigated in unialgal cultures (Barnett *et al.*, 2015; Blommaert *et al.*,
111 2017), while the capacity for vertical migration in epipsammic species has to our
112 knowledge never been investigated in cultures.

113 Here we investigated the relationship between NPQ activation and relaxation
114 and vertical migration capacity for a set of common epipellic (5) and epipsammic (4)
115 diatom species. As it is important that trade-offs are studied with all else being equal
116 (Litchman & Klausmeier 2008), we quantified both traits under identical conditions,
117 with all strains acclimated to the same light climate.

118

119

120

121 **Materials and Methods**

122

123 Epipellic and epipsammic diatom strains were obtained from the diatom culture
124 collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms
125 (<http://BCCM.belspo.be>) and the Nantes Culture Collection-France (NCC),
126 (<http://ncc.univ-nantes.fr/>). Accession numbers are given in Table 1. Photographs of all
127 species were taken with an Axiophot2 microscope (Carl Zeiss AG, Oberkochen,
128 Germany), equipped with a monochrome digital camera, AxioCam MRm (Carl Zeiss
129 AG, Oberkochen, Germany) (Fig. 1). Species were grown at 20°C in batch cultures in a
130 day/night regime of 16/8 h with a light intensity of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ using two
131 L58W/840 Lumilux cool white tubes and one L58W/865 cool daylight fluorescent tube
132 (Osram, Munich, Germany). Cells were cultured in Provasoli's enriched f/2 seawater
133 medium using Tropic Marin artificial sea salt (Dr. Beiner GmbH, Wartenberg,
134 Germany) (34.5 g l⁻¹) enriched with NaHCO₃ (80 mg l⁻¹ final concentration). Cultures
135 were acclimated to these culturing conditions for at least 2 weeks prior to the
136 experiments.

137 **Preparation of monospecific biofilms**

138 Light brown kaolin (Carl Roth GmbH, Karlsruhe, Germany) was used as a standard test
139 substrate for diatom motility as it has similar properties as mudflat sediment (Hay *et al.*,
140 1993) and is commercially available. Differences in sediment light climate between
141 natural sediments and kaolin, however, could not be excluded. A schematic overview of
142 artificial biofilm preparation is shown in Fig. 2. 24-well plates were filled with 0.75 g
143 kaolin in each well and mixed with one ml of medium to obtain a homogenous
144 suspension. The sediment was pelleted by centrifugation at 1000 RCF for 5 min. For

145 epipellic diatoms, monospecific suspensions (0.5 ml, 6-10 $\mu\text{g Chl } a/\text{ml}$) were mixed with
146 0.1 g kaolin, pipetted on top of the kaolin within the wells and centrifuged at 50 RCF
147 for 1 min. The diatom suspensions were mixed with kaolin because centrifugation
148 without kaolin resulted in an uneven distribution of the diatom layer on the sediment.
149 For epipellic species 10 $\mu\text{g Chl } a/\text{ml}$ suspensions were used, except for both *Navicula*
150 species (6 $\mu\text{g Chl } a/\text{ml}$). After a second centrifugation step (to conform with the
151 epipsammic treatment, see below), the supernatant medium was removed and the
152 diatoms were allowed to migrate to the surface for 6 h in 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at
153 20°C. Higher light intensities were not used for upward migration to avoid a
154 photophobic response or a change in photophysiology. For epipsammic diatoms, 0.1 g
155 kaolin, mixed with 0.5 ml artificial seawater but without diatoms, was first added to the
156 wells already containing 0.75 g centrifuged kaolin, as described above, and then
157 centrifuged at 50 RCF for 1 min. Afterwards, 1 ml suspension of epipsammic diatoms
158 (2 $\mu\text{g Chl } a/\text{ml}$) was added and centrifuged at 50 RCF for 1 min after which the
159 supernatant medium was removed. The epipsammic treatment was slightly different
160 from the epipellic one because the cells were either non-motile or did not migrate to the
161 sediment surface within the same time frame used for the epipellic diatoms. By analogy
162 with the epipellic treatment, the epipsammic species were then placed in 20 μmol
163 $\text{photons m}^{-2} \text{s}^{-1}$ at 20°C for 6 h before high light exposure. Surface biomass (expressed
164 as Normalized Difference Vegetation Index (NDVI), see below), measured before the
165 start of the experiments, ranged between 0.1 and 0.25 for all species. To quantify the
166 extent of vertical migration, relative values were calculated, thus standardizing for
167 differences in initial surface biomass.

168 **NDVI and chlorophyll fluorescence measurements**

169 Both pulse amplitude modulated (PAM) fluorescence imaging and NDVI were
170 measured with a standard MAXI Imaging PAM *M-series* (Heinz Walz GmbH,
171 Effeltrich, Germany), equipped with an IMAG-K4 camera and mounted with an IMAG-
172 MAX/F filter. The illumination unit of the imaging system contains red (660 nm) and
173 near-infrared (NIR, 780 nm) LEDs, providing monochromatic pulse modulated light.
174 The reflectance images of the monochromatically illuminated samples were captured by
175 the same CCD-chip that captures chlorophyll fluorescence.

176 In two separate experiments, NPQ and VM (see below) were measured
177 immediately before (0 min) and after 2.5, 5, 10, 15, 20, 25 and 30 min HL illumination
178 ($1900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, photosynthetically available radiation), provided by the
179 MAXI Imaging PAM Blue LED-panel. The outer wells of the 24-well plates were not
180 included to avoid inhomogeneity in light intensity.

181 The intensities of both red and NIR illumination sources were calibrated with a
182 18% grey standard (Neutral Grey Card 4963, FOTOWAND-Technic Dietmar Meisel,
183 Sudwalde, Germany), placed in the middle of the camera field of view as during HL
184 exposure the NIR reflectance decreased while red reflectance increased. Red and NIR
185 reflectance images were captured automatically using the script function in the
186 ImagingWin software. Areas of interest (AOI) were placed in the middle of each well to
187 avoid edge-effects using the ImagingWin (v2.41a) software (Heinz Walz GmbH,
188 Effeltrich, Germany). Sediment temperature was maintained at 20°C by working in an
189 air-conditioned room, removing the Perspex eye-protection hood and providing
190 additional cooling by a fan, and keeping the 24-well plate, which was perforated in
191 between the wells, in a water bath on a stirring plate.

192 For NDVI measurements, a saturating pulse (0.8 s, INT 8) was fired at the end
193 of each HL interval to create a new file in which only one red and NIR image could be
194 saved. Red and NIR were captured after 10 s of darkness after this saturating pulse to
195 avoid interference. NDVI was calculated as $(R780 - R660)/(R780 + R660)$ using the
196 AOI averages (Rouse *et al.*, 1974). An NDVI vs. chlorophyll *a* (Chl *a*) calibration curve
197 was constructed by creating artificial biofilms by centrifuging (cf. epipsammic
198 treatment above) suspensions of known Chl *a* content (determined
199 spectrophotometrically, Jeffrey & Humphrey, 1975) of the diatom *Phaeodactylum*
200 *tricornutum* K. Bohlin (Fig. 3). The extent of vertical migration was calculated as the
201 relative (percentage) decline in initial NDVI values (Laviale *et al.*, 2016) after
202 subtraction of the NDVI value of kaolin without diatoms, which was recorded
203 simultaneously. Even though we controlled for changes in red and NIR illumination, we
204 observed a decline in recorded NDVI of a sheet of green paper (absorbing in the red
205 spectrum) under the same light conditions, possibly due to a shift in LED-spectrum
206 resulting from the heating up of the LED panel during HL illumination. We corrected
207 for this artifact (see Supplemental Fig. S1) by adding the average decline of 15
208 observations (3 independent measurements of 5 AIOs in the green sheet) to the recorded
209 data for diatoms.

210 NPQ was measured on diatom suspensions in 24-well plates (1 ml containing 1
211 μg Chl *a*, measured as above) using the same light conditions as the vertical migration
212 assay. As no sediment was present, a behavioural photoprotection response was not
213 possible. NPQ reversal was measured during an additional 30 min low light (LL)
214 recovery period ($15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Saturating pulses (0.8 s, INT 8) were fired
215 automatically each 5 min. A fluorescence standard (Heinz Walz GmbH, Effeltrich,

Germany) was measured simultaneously to correct for deviations in measuring light intensity during HL. NPQ was calculated as $(F_m - F_m')/F_m'$, where F_m is the Maximum PSII chlorophyll fluorescence yield and F_m' is maximum PSII Chl fluorescence yield (F_m) during illumination. As F_m' values recorded during HL were lower than the minimum PSII Chl fluorescence yield F_0 (as observed in diatoms by Lavaud *et al.*, 2002), qN (as used by Laviale *et al.*, 2016) was not determined. Photosynthetic efficiency of PSII ($\Delta F/F_m'$) was calculated as $(F_m' - F')/F_m'$ and expressed as a percentage, taking the maximal photosynthetic efficiency (F_v/F_m), measured immediately before HL onset as 100%.

The NPQ induction (1) and recovery (2) rates (k) were calculated by fitting an exponential decay function (nonlinear regression), derived from Olaizola & Yamamoto (1994):

$$(1) \text{ NPQ}(t) = \text{NPQ}_{\max} + [\text{NPQ}_0 - \text{NPQ}_{\max}]e^{-kt}$$

where t represents time during HL and $\text{NPQ}_{\max}$ and NPQ_0 represent NPQ after 30 min HL and before HL onset, respectively.

$$(2) \text{ NPQ}(t) = \text{NPQ}_r + [\text{NPQ}_{\max} - \text{NPQ}_r]e^{-kt}$$

where t represents time during recovery and $\text{NPQ}_{\max}$ and NPQ_r represent NPQ at the start of the recovery period and after 30 min of recovery in LL, respectively. Statistical analyses were conducted using the statistical software package SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Exponential decay functions were fitted using the nonlinear regression procedure (PROC NLIN). Measured and fitted parameters (3 biological replicates per species) were compared between species using ANOVA, followed by a Tukey's test, using the General Linear Model procedure (PROC GLM). *P*-values of

240 0.05 or less were considered statistically significant. A decrease in surface biomass
241 was evaluated as a one-sided t-test (only a decrease was considered).

242 **Results**

243

244 **NDVI measured with the MAXI Imaging PAM *M-series***

245 A dilution series of *Phaeodactylum tricornutum* suspensions of known Chl *a* content
246 was centrifuged on kaolin sediment to create artificial biofilms. NDVI of these biofilms
247 correlated very well ($R^2=0.98$, $p < 0.0001$) with Chl *a* content (Fig 3). As the y-intercept
248 was larger than zero ($p = 0.0002$), a blank with bare sediment was included and its
249 NDVI measurement subtracted from all samples in all VM experiments.

250 **Behavioural photoprotection - vertical migration (VM)**

251 All epipelagic species showed a significant (one-sided t-test, $p < 0.05$) decrease in surface
252 biomass (20-40%) by the end of the HL illumination period (VM₃₀)(Fig. 4a, b;
253 significant p -values listed in Table S1). As only two replicates were included for
254 *Entomoneis paludosa*, this species was excluded from statistical analysis. None of the
255 epipsammic species, including the motile ones, showed a significant surface biomass
256 decline during the HL period (Fig. 4a, b; p -values listed in Table S1). Of the epipelagic
257 species, only *Craspedostauros britannicus* showed a small but significant decrease in
258 surface biomass after 2.5 min ($p = 0.001$). After 15 min of HL, *C. britannicus*, *Navicula*
259 *phyllepta* and *N. arenaria* had significantly lower surface biomass than at the start of the
260 experiment (one-sided t-test). The smallest epipelagic species *N. phyllepta* exhibited the
261 most pronounced vertical migration (Fig. 4a); decreasing its surface biomass
262 significantly more than any other tested epipelagic species within the 30 min HL exposure
263 period (ANOVA and Tukey's post hoc pairwise comparison, p -values reported in Table
264 S2). No significant differences in surface biomass decrease were found between the

265 other epipelagic diatoms after 30 min of HL (Table S2). A control experiment, using only
266 the largest (*N. arenaria*) and smallest (*N. phyllepta*) epipelagic diatoms (2 technical
267 replicates each), showed no VM in growth light conditions (20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$,
268 Supplemental Fig. S2).

269 **Physiological photoprotection - NPQ**

270 All investigated epipsammic species displayed a strong NPQ induction, resulting in
271 significantly higher NPQ values than epipelagic species after 5 min HL (Fig. 4c, d)
272 (ANOVA and Tukey's post hoc pairwise comparison, *p*-values reported in Table S3).
273 No significant differences in NPQ were observed between diatoms of the same growth
274 form at this time point (Table S3). The NPQ induction rate was lowest in *C. britannicus*
275 (rate constant $k = 0.08 \text{ min}^{-1}$; SD = 0.02), which was significantly lower than in *B.*
276 *lucens*, *E. paludosa*, *O. guenter-grassii* and *P. delicatulum* (ANOVA and Tukey's post
277 hoc pairwise comparison, *p*-values reported in Table S4). The epipsammic species
278 showed a comparable but lower further increase in NPQ during the rest of the HL
279 period. In the epipelagic species, NPQ diverged by the end of the HL exposure period.
280 Both *C. britannicus* and *E. paludosa* showed a strong NPQ increase during the HL
281 period, resulting in significantly higher NPQ values than the other tested epipelagic
282 species (except for the difference between *E. paludosa* and *N. phyllepta* which was not
283 significant, ANOVA and Tukey's post hoc pairwise comparison, *p*-values reported in
284 Table S5).

285 During the 30 min low light recovery period NPQ relaxed rapidly in all
286 epipsammic species, whereas only one epipelagic species, *N. arenaria*, showed clear NPQ
287 relaxation. Sustained quenching (NPQs) after 30 min recovery was highest for *C.*

288 *britannicus*, followed by *E. paludosa*. Fitting NPQ relaxation with exponential decay
289 functions revealed significantly faster NPQ relaxation in the araphid epipsammic diatom
290 *Opephora guenter-grassii* (rate constant $k = 0.52 \text{ min}^{-1}$; SD = 0.03), whereas no
291 significant differences were observed between the raphid epipsammic diatoms and *N.*
292 *arenaria* (rate constant $k = 0.13 \text{ min}^{-1}$; SD = 0.06) (ANOVA and Tukey's post hoc
293 pairwise comparison, p -values reported in Table S4). The recovery of the quantum yield
294 of PSII ($\Delta F/F_m'$) after 30 min of LL (Fig. 5) was higher in all epipsammic species
295 compared to the investigated epipellic species, with the notable exception of the epipellic
296 species *N. arenaria* which showed an equally high recovery (ANOVA and Tukey's post
297 hoc pairwise comparison, p -values reported in Table S6).

298 Plotting the NPQ values after 5 min HL versus VM₃₀ (% NDVI decrease after 30
299 min) (Fig. 6) revealed a clear VM-NPQ trade-off between the epipellic and epipsammic
300 diatom groups, with epipellic diatoms displaying high VM₃₀ and low NPQ, and
301 epipsammic diatoms showing no clear VM and high NPQ. No VM₃₀/NPQ trade-offs,
302 however, were observed within both functional groups.

303

304 **Discussion**

305

306 As the ability to vertically migrate (VM) away from high light to avoid photoinhibition
307 might minimize the costs associated with high and flexible NPQ (Raven, 2011), we
308 compared NPQ and VM capacity as photoprotection mechanisms in a set of five
309 epipellic and four epipsammic species and confirm a general NPQ-VM trade-off
310 between both functional groups.

311 The fact that no vertical movement was observed in the epipsammic species
312 shows that the migration observed in the epipellic species was not caused by a passive
313 process such as water percolation. To further test whether the response was indeed
314 caused by high light, we performed a control experiment with the largest (*N. arenaria*)
315 and smallest (*N. phyllepta*) epipellic diatoms in which we exposed them to growth light
316 conditions (i.e. low light) instead of high light conditions. No migratory response was
317 observed (see supplemental Fig. S2), clearly demonstrating that the migratory response
318 was caused by high light.

319 While all epipellic species showed significant VM, they migrated less fast
320 compared to natural epipellic communities, where surface biomass decreased up to 30%
321 within the first 2.5 min of high light (Laviale *et al.*, 2016). In our experiments, where
322 VM was prevented under HL conditions, NPQ was initially low for all epipellic species,
323 but for some species, it increased considerably during the course of the HL exposure.
324 Most of this NPQ, however, coincided with high sustained NPQ (NPQs, see below).
325 Epipsammic species compensated for the absence of significant VM with a strong NPQ
326 response during HL onset. Consistent differences in NPQ induction rate between
327 epipellic and epipsammic diatoms were not observed, possibly due to the fact that we

328 have no data for the first five minutes of high light, during which most of the NPQ
329 induction, due to Ddx de-epoxidation, takes place (Serôdio *et al.*, 2005).

330 Besides a strong NPQ after HL onset, epipsammic diatoms were also able to
331 relax NPQ rapidly during low light conditions. Within both growth forms, trade-offs
332 between both photoprotective strategies were not observed. However, the only araphid
333 (and hence by definition non-motile) epipsammic diatom included here showed
334 considerably faster relaxation of NPQ after high light exposure than the raphid
335 epipsammic diatoms and is therefore able to more efficiently track rapid changes in
336 irradiance intensity (Lavaud *et al.*, 2007; Lavaud & Lepetit 2013). Taken together, our
337 results confirm that epipelagic and epipsammic growth forms can be seen as different
338 functional groups exhibiting contrasting primary photoprotection strategies.

339 NPQ differences between epipelagic and epipsammic growth forms were studied
340 by Barnett *et al.* (2015), and agree with our observations after 5 min HL. Higher NPQ
341 values in epipsammic diatoms were attributed to higher Dtx production, originating
342 from a larger Ddx + Dtx pool rather than a higher Ddx de-epoxidation state. The
343 measured NPQ values in two epipelagic species (*C. britannicus* and *E. paludosa*),
344 however, increased to similar levels as in the epipsammic diatoms after 30 min HL, a
345 feature not observed by Barnett *et al.* (2015) as only 5 min illumination periods were
346 used. During the low light recovery period, however, all epipelagic species except *N.*
347 *arenaria* showed high NPQs and a low recovery of PSII quantum yield. High NPQs has
348 been observed after exposure to high light conditions or a combination of high light and
349 elevated temperatures (Zhu *et al.*, 2010; Lavaud & Lepetit 2013; Laviale *et al.*, 2015),
350 and has been attributed to a slow epoxidation of Dtx back to Ddx (Lavaud & Lepetit
351 2013; Lavaud & Goss 2014; Blommaert *et al.*, 2017) and/or photoinhibition (qI). In

352 contrast with our observations, epipellic communities freshly obtained from the field are
353 able to withstand high light doses (up to 1200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) for up to three
354 hours, even when VM is inhibited (Serôdio *et al.*, 2012; Laviale *et al.*, 2015). Moreover,
355 they show higher NPQ values, while relaxing their NPQ more in low light conditions
356 (Serôdio *et al.*, 2005, 2008, 2012), suggesting that these field communities are less
357 sensitive to photoinhibition than monospecific epipellic cultures. This could be due to
358 the fact that the diatom cultures used in our study were acclimated to rather low light
359 intensities (20 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and exposed to relatively high light intensities.
360 Acclimation to higher irradiances increased the NPQ capacity of epipellic diatoms (Cruz
361 & Serôdio 2008; Ezequiel *et al.*, 2015; Barnett *et al.*, 2015) and caused them to
362 accumulate at higher light intensities in a light gradient (Ezequiel *et al.*, 2015). As a
363 result, the VM/NPQ trade-off between epipsammic and epipellic diatoms observed in the
364 field may not be as pronounced as observed in our experiments.

365 Alternatively, the high NPQs/qI in epipellic species, as observed in this study,
366 could be related by the origin of the strains. Diatom communities originating from
367 Portuguese mudflats, as used in the above studies, tend to have overall higher NPQ and
368 less NPQs and/or photoinhibition after high light exposure than communities sampled at
369 higher latitudes along the Atlantic Coast (Laviale *et al.*, 2015). Pniewski *et al.* (2015)
370 also observed photoinhibition (measured as a decline in oxygen evolution-irradiance
371 curves) in epipellic communities from Aiguillon Bay (Atlantic coast, France). The
372 observed absence or low amount of NPQs/qI and in general higher recovery of PSII
373 quantum yield in epipsammic diatoms in this study confirms that the energy dissipating
374 mechanisms of these diatoms are capable of tracking light fluctuations (cf. Blommaert
375 *et al.*, 2017) and optimizing photosynthesis in rapidly fluctuating and high light

376 conditions, as photosynthesis can be forgone if energy dissipating mechanisms fail to
377 relax in light-limiting conditions (Raven, 2011). Finally, sustained NPQ in MPB
378 diatoms may be advantageous to keep the antenna system in a basal dissipative state,
379 allowing the cells to cope with a sudden increase in light intensity after a long dark
380 period as may happen during immersion and night emersion (Lavaud & Goss 2014).

381 Differences in motility of epipellic diatoms (here mainly between *N. phyllepta*
382 and larger species) were not reflected in NPQ capacity and thus do not point to a VM-
383 NPQ trade-off within the epipellic group. *N. phyllepta* is much smaller (~13 µm long)
384 than the other studied epipellic species (>30 µm long) but exhibited the strongest
385 migratory response, whereas no differences in VM were observed between the larger
386 species. The difference in VM between *N. phyllepta* and the larger species may be due
387 to the fact that because of its smaller size (and assuming comparable pigment
388 concentration per unit biovolume) pigment self-shading may be lower, rendering the
389 cells more vulnerable to photoinactivation (Key *et al.*, 2010) and therefore requiring
390 higher photoprotection (i.e. VM). In this respect it is interesting to note that in a field
391 study small naviculoid diatoms were mainly observed at the sediment surface in early
392 morning when light intensity was still relatively low whereas larger species dominated
393 the intertidal surface biofilm at noon (Underwood *et al.*, 2005). It should also be noted
394 that while VM was observed in all epipellic species, most diatom biomass stayed at the
395 sediment surface as observed by Laviale *et al.* (2015, 2016). Therefore, epipellic diatoms
396 might have used alternative photoprotection strategies or displayed within-population
397 cyclical micromigration at the sediment surface (Kromkamp *et al.*, 1998), as such
398 obscuring differences in VM as a photoprotection strategy.

399 An NPQ-VM capacity trade-off was also not observed within the epipsammic group: no
400 significant NPQ differences were detected during the high light period and no
401 significant VM was observed. However, all epipsammic species we tested, with the
402 exception of *O. guenter-grassii*, were raphid and therefore in principle capable of
403 movement. However, despite the fact that some of them were in the same size range
404 (see Fig. 1) as *N. phyllepta* (which displayed the strongest migratory response), they did
405 not migrate down in response to high light. The absence of vertical migration is in
406 accordance with the lack of endogenous migratory rhythms in epipsammic communities
407 (Jesus *et al.*, 2009) and the observation that epipsammic diatoms in the field do not
408 seem to migrate down in response to high light (Cartaxana *et al.*, 2011). Barnett *et al.*
409 (2015) did observe differences in NPQ capacity between motile and non-motile
410 epipsammic species but not at the highest light intensity (2000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$).
411 The most notable difference between the epipsammic species in our study was the faster
412 NPQ relaxation in the araphid species *O. guenter-grassii*, which is due to fast Dtx
413 epoxidation in low light conditions (Blommaert *et al.*, 2017). The slower relaxation in
414 raphid epipsammic species may suggest that they do use motility, not to perform VM
415 but to move to slightly more shaded areas on the sand grain surface, such as
416 depressions, where epipsammic diatoms are often seen to aggregate (Miller *et al.*, 1987;
417 Jewson *et al.*, 2006; Sabbe, unpubl. obs.). While it has been hypothesized that cell
418 accumulations in depressions can represent a strategy to protect against abrasion (Miller
419 *et al.*, 1987), it could also be a way to reduce high light stress through increased cell
420 shading caused by cell accumulation and the microtopography of the sand grains. This
421 may slightly reduce the need for the very rapid NPQ relaxation observed in the araphid
422 species. An alternative explanation for the absence of significant VM in raphid

423 epipsammic species in sandy sediments is that because light is scattered and penetrates
424 deeper than in silty sediments (Kühl *et al.*, 1994; Cartaxana *et al.*, 2016b), downward
425 VM would probably not drastically change the experienced light climate (Cartaxana *et*
426 *al.*, 2016b). A third potential explanation for the observed difference in NPQ relaxation
427 between the araphid and raphid epipsammic species could be that slower NPQ
428 relaxation represents a phylogenetic signal typical for raphid, motile diatoms which was
429 retained in raphid taxa which adopted an epipsammic growth form.

430 Finally, it needs to be pointed out that the distinction between araphid and raphid
431 epipsammon does not necessarily coincide with a difference in motility. Some raphid
432 species, such as *Biremis lucens* are usually observed as small colonies which are
433 attached to the sand grain surface via their girdle side (Sabbe *et al.*, 1995). For this
434 reason, this species was classified as non-motile in Barnett *et al.*, (2015). In the present
435 study, we focused on the distinction between araphid and raphid, as raphid diatoms are
436 at least potentially motile as they possess a raphe. Motility in most of these forms,
437 however, has not yet been properly characterized.

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439
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605

606 **Figures legends**

607

608 **Fig. 1**

609 Fig. 1: Light microscopy photographs of the species used in this study. a: *Navicula*
610 *arenaria* var. *rostellata*, b: *Craspedostauros britannicus*, c: *Seminavis robusta*, d:
611 *Entomoneis paludosa*, e: *Navicula phyllepta*, f: *Planothidium delicatulum*, g: *Biremis*
612 *lucens*, h: *Nitzschia* cf. *frustulum*, i: *Opephora guenter-grassii*. Scale bar = 10 µm.

613 **Fig. 2**

614 Fig. 2: A schematic overview of artificial biofilm preparation.

615 **Fig. 3**

616 Fig. 3: The linear regression of the Normalized Difference Vegetation Index (NDVI) with
617 chlorophyll *a* content, determined spectrophotometrically, on a *Phaeodactylum*
618 *tricornutum* dilution series centrifuged on kaolin.

619 **Fig. 4a, b, c, d**

620 Fig. 4a, b, c, d: The decrease in surface biomass, measured as NDVI, of epipellic
621 diatoms (a) and epipsammic diatoms (b) on kaolin during 30 min of HL. Note that the
622 y-axis in these plots starts at 50%. Non-photochemical quenching (NPQ) for epipellic

623 diatoms (d) and epipsammic diatoms (c), measured during 30 min HL and 30 min LL
624 recovery. Values represent averages of three independent measurements $\pm$ standard
625 deviations.

626 **Fig. 5**

627 Fig. 5: The quantum yield of PSII ($\Delta F/F_m'$), after 30 min of HL and 30 min of LL
628 recovery, expressed in percentage of the maximal photosynthetic efficiency of PSII
629 (F_v/F_m) before HL exposure for epipelagic (black bars) and epipsammic diatoms (white
630 bars). Values represent averages of three independent measurements $\pm$ standard
631 deviations.

632

633 **Fig. 6**

634 Fig. 6: The extent of vertical migration (measured as the decrease in surface biomass in
635 percentage, cf. Fig. 4a, b), measured after 30 min of HL in function of the NPQ capacity,
636 measured after 5 min of HL to avoid the effect of photoinhibition for epipelagic (black
637 symbols) and epipsammic diatoms (white symbols). Three replicates per species are
638 plotted (with exception of *E. paludosa*, $n = 2$).

Epipelon

Epipsammon