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1 **Fitness of teratological morphotypes and heritability of deformities in the diatom**
2 ***Gomphonema gracile***

3

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13 **Abstract**

14 Diatom teratologies have intrigued scientists since the XIXth century, with respect to their
15 causes and origins. These deformities, mainly observed in long-term cultures or under high
16 levels of pollution, were poorly considered, until they were recently found to be potential
17 indicators of toxic impairment of freshwaters. However, very little is known about their
18 ecology.

19 In this study, the growth and fitness of morphologically distinct descendants of the same cell
20 line of *Gomphonema gracile* (teratological vs. non teratological forms) were compared over a
21 typical growth cycle. Contrary to expectations, teratological populations grew slightly faster,
22 at a rate of 0.47 ± 0.03 divisions.day⁻¹, versus 0.41 ± 0.04 for the normal morphotype. They
23 had similar physiological performances as non-teratological forms. They did not differ in their
24 movement velocities, but the trajectory of teratological forms was more linear, likely as a
25 consequence of their elongated outline. Under the same culture conditions, no competitive
26 exclusion of one phenotype over the other was demonstrated on the time scale of an
27 exponential growth cycle (9 days). Moreover, the deformities were faithfully reproduced over
28 time, and no evidence of decreased viability in teratological forms was provided.

29 These new insights call into question the common hypothesis that deformed diatoms are
30 altered individuals produced by unfavorable conditions and thus highlight ecosystem
31 dysfunction. They call for further investigations of their ecology.

32

33 **Keywords**

34 *Gomphonema gracile*, morphology-based assessment, teratology, transmissibility of
35 morphological characters, phenotypic variability, fitness

36

37 **Highlights**

- 38 • The growth, physiology and behavior of normal and deformed *Gomphonema gracile* were
39 analyzed.

- 40 • Teratological diatoms were able to survive and reproduce.
- 41 • Teratology was faithfully passed on with cell division over 9 days.
- 42 • Deformed individuals performed similarly to normal ones.

43

44

45 1. Introduction

46 Diatoms are unicellular brown microalgae with an important ecological role in the functioning
47 of freshwaters (Morin et al., 2016). Diatom species identification is based on the
48 morphological features of their siliceous cell wall, the frustule: valve shape and symmetry,
49 cell dimensions, length:width ratio, specific ornamentations (presence and number of raphes,
50 orientation and density of striae, etc.). Practically, diatom identification assumes that the
51 features of the frustule are constant for a given species. A range of length and width
52 variability exists, as the result of the natural morphological variation in diatoms occurring over
53 their life cycle, consequence of their peculiar reproduction cycle (Mann, 2011).

Code de champ modifié

54 Besides morphological variations associated with development, polymorphism can also
55 result from environmental stress (phenotypic variation) or natural genetic variability
56 (Håkansson and Chepurinov, 1999). Notably, Rose & Cox (2014) documented morphological
57 changes over the life cycle in clones of *Gomphonema parvulum*, complicating the definition
58 of clear species boundaries. Continued culturing resulted in cell size reduction and changes
59 in morphology, potentially leading to the identification of 3 different species. Polymorphism
60 can be an adaptive response of diatom species to a shift in environmental conditions and can
61 result from changing selective pressures (Kocielek and Stoermer, 2010). Trobajo (2007) also
62 detected changes in the morphology of *Nitzschia frustulum* stemming from environmental
63 variations; diatoms were significantly more elongate under increasing salinities and nitrogen
64 concentrations. Frustule deformities or teratologies (abnormal outline or ornamentations) are
65 beyond normal phenotypic variations. They can be induced during valve formation, either by
66 inimical conditions or under long-term artificial culture conditions (see review in Falasco et
67 al., 2009a). Numerous observations of deformed diatoms in laboratory cultures were
68 attributed to cell crowding (Barber and Carter, 1981) or culture senescence (Falasco et al.,
69 2009a). Recently, Windler et al. (2014) reported the major influence of bacteria associated
70 with diatoms in the long-term maintenance of laboratory cultures. They found that cultivation
71 under axenic conditions reduced population growth and promoted morphological changes
72 (cell size reduction, frustule aberrations) in pennate diatoms, compared to xenic cultures.

73 The teratological character is, thus, commonly accepted to result from unhealthy conditions,
74 as discussed in Lavoie et al. (2017), either in laboratory cultures (lack of essential
75 accompanying bacteria, Windler et al., 2014) or in the field (induction by toxic contamination,
76 Falasco et al., 2009a). For this reason, lower fitness is expected in teratological diatoms, and
77 some types of deformities are even suspected to be lethal (Arini et al., 2013). The
78 assumption that abnormal diatoms may be outcompeted by normal phenotypes under
79 optimal conditions, and ultimately eliminated from the populations, is based on the following
80 empirical observations. First, their rare occurrences: teratologies are infrequent and generally
81 recorded at relative abundances not exceeding 1% (Morin et al., 2012a), in particular under
82 field conditions. Second, they generally appear under altered environments, and were shown
83 to progressively disappear with a return to normal conditions. For instance, Arini et al. (2013)
84 observed that the abundances of cadmium-induced teratologies in *Planothidium*
85 *frequentissimum* decreased with decontamination. Consistently, the recovery of normal
86 morphology after sexual reproduction in deformed diatoms from long-term cultures suggests

87 that teratologies do not result from genetic drift (Granetti, 1968). Still, the fact that
88 teratological forms are rarely dominant in samples has until now limited the possibility to
89 experimentally validate the hypothesis of competitive exclusion of deformed phenotypes over
90 time.

91 Given this background, an experiment was carried out to assess the heritability of the
92 teratological character and the viability of abnormal cells. To this end, cell lines descending
93 from laboratory-cultured *Gomphonema gracile* which had diverged into two morphological
94 variants were used: a normal phenotype and a markedly deformed one. The objectives of
95 this study were: 1) to compare the fitness of both phenotypes under the same culture
96 conditions, and 2) to assess the heritability of the teratological character in diatoms, taking
97 into account the potential influence of closely-associated bacteria. To reach these objectives,
98 both cultures were assessed over 9 days for population dynamics (viability, growth kinetics),
99 cell morphology (cell dimensions, altered frustule), physiology (photosynthesis) and behavior
100 (mobility features). The first hypothesis was that the deformities would be reproduced with
101 cell duplications, while normal phenotypes would be restored with sexual reproduction. The
102 second hypothesis was that the teratological character, even if heritable, would alter the
103 global metabolism of the species, which would be observable through reduced performances
104 in the endpoints studied.

105 **2. Materials & Methods**

106 *2.1. Biological material.*

107 A pennate diatom was isolated from a field sample (Rebec, upstream section of the Leyre
108 river, Southwest France) in December 2013 and established in culture (Coquillé et al., 2015).
109 During the first month that they were cultured (March 2014), cells exhibited typical features of
110 *Gomphonema gracile* Ehrenberg (Reichardt, 2015). Approximately one year later, the
111 parental line of *G. gracile* (further named GGRA) gave rise to two morphologically distinct
112 (but homogenous within one culture) descendant cell lines continuing to reproduce
113 vegetatively. The first phenotype, called GNF (*Gomphonema* normal form), was smaller than
114 the parent culture, less lanceolate and had more rounded apices. The second one, called
115 GTF (*Gomphonema* teratological form), was 2-fold larger than GNF, and all cells exhibited a
116 typical deformity in valve outline (“boomerang shaped”, Type 1 deformity in Falasco et al.,
117 2009b).

118 Before the experiment, GNF and GTF populations were almost pure. They were maintained
119 in separate cultures in sterile Dauta medium (Dauta, 1982) at 17°C in a thermostatic
120 chamber 610 XAP (LMS LTD[®], UK) at $67 \pm 0 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ with a dark:light cycle of 8:16 h.
121 Non-axenic cultures were grown in 100-mL round borosilicate sterile glass flasks previously
122 heated to 450°C for 6 h and autoclaved 20 min at 121°C.

123 *2.2. Experimental design.*

124 The experiment, carried out in March 2015, was run during nine days under the conditions
125 affording *Gomphonema*'s growth without nutrient depletion over time, as used in Coquillé et
126 al. (2015). GNF and GTF cultures were inoculated at 30,000 cells.mL⁻¹ in sterile Dauta
127 medium (40 mL final volume). As it was likely that a small percentage of the other phenotype
128 would be found in those cultures, the experimental cultures were labelled Ctrl (control) and
129 Trtg (teratological), respectively, in order to avoid confusion between phenotypes and
130 treatments. Triplicate cultures were used for each treatment (Ctrl and Trtg) and incubated
131 simultaneously in a thermostatic chamber 610 XAP (LMS LTD[®], UK), under the same
132 environmental conditions as the mother cultures.

133 On days 1, 2, 3, 6, 7, 8 and 9, population dynamics (viability, growth kinetics), cell
134 morphology (cell dimensions, deformities), physiology (photosynthesis) and behavior
135 (mobility features) were analyzed. On each sampling day, parameters derived from
136 chlorophyll-a fluorescence were first measured, directly on the cultures. Then, the flasks
137 were gently shaken to homogenize the suspensions before sampling 1 mL of culture under
138 the flame. The sample was then aliquoted as follows: 125 μL for the quantitative analysis of
139 live and dead diatoms, 100 μL for mobility measurements, and 200 μL to prepare permanent
140 diatom slides. On the last day of the experiment (day 9), 3 mL of homogenized suspension
141 was used to perform Rapid Light Curves and the remaining volume of culture was softly
142 filtered on previously ashed Whatman GF/C[®] filters that were immediately frozen at -20°C
143 before molecular analyses of the associated bacteria.

144 2.3. Chlorophyll-a fluorescence-related endpoints.

145 Parameters derived from chlorophyll-a fluorescence were measured on the intact biofilms, by
146 means of a PHYTO-PAM (Heinz Walz, GmbH, Germany) equipped with an emitter-detector
147 unit (PHYTO-EDF). Benthic settlement potentially leads to the formation of cell aggregates;
148 therefore ten randomly selected measurements of the effective quantum yield
149 (photosynthetic efficiency) and chlorophyll-a content estimated by chlorophyll-a fluorescence
150 were performed. To do this, a home-made system was used for reproducible direct
151 measurements on the bottom of the flasks. The median of 10 values per sample was then
152 used for statistical analyses.

153 On day 9, 3 mL of suspension were used to perform Rapid Light Curves (RLC, White and
154 Critchley, 1999) generated with the PHYTO-PAM in ED mode. Briefly, samples adapted to
155 low light irradiance ($50 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) for 20 minutes were sequentially exposed to increasing
156 actinic irradiances (9 light levels ranging from 64 to $1,964 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) for 10 seconds,
157 separated by a saturating flash. The relative electron transport rate of photosystem II in
158 response to the light pulse was then plotted against irradiance. The following parameters
159 were extracted from the curves fitted on the model of Eilers & Peeters (1988): photosynthetic
160 efficiency at low light intensity (initial slope of the curve), maximal electron transport rate and
161 onset of light saturation.

162 2.4. Determination of population growth kinetics.

163 Diatom counts were carried-out on fresh material in a Nageotte counting chamber, using light
164 microscopy at 400x magnification (Olympus BX51, Olympus Optical Co. GmbH, Germany).
165 Diatom cell density and mortality were estimated for each replicate sample as described in
166 Morin et al. (2010). Solitary cells and those forming associations in the counts were
167 distinguished. Density data were recorded as cells.mL^{-1} and the logarithmic increase in
168 population was plotted as a function of time unit, to calculate growth rates (GR, expressed in
169 $\text{divisions.day}^{-1}$ during exponential growth) following the formula provided in Morin et al.
170 (2008), and the carrying capacity (i.e. maximum size reached during the stationary phase) of
171 the cultures.

172 2.5. Mobility features.

173 The percentage of motile cells, as well as their velocity and trajectory, were determined using
174 CASA (computer-assisted sperm analysis) plug-in (Wilson-Leedy and Ingermann, 2007) of
175 ImageJ software under the specific measurement conditions adapted to *G. gracile* by
176 Coquillé et al. (2015). A 20- μL drop of sample was deposited onto a microscope slide and
177 video acquisition (Archimed[™], Microvision Instruments) was performed after 2–3 min.
178 Velocity average path (VAP), velocity curvilinear (VCL), velocity straight line (VSL) and

179 linearity (LIN = VSL/VAP, describing the curvature of the trajectory) (Rurangwa et al., 2004)
180 were then calculated from 260 frames corresponding to 10 seconds of film in total.

181 2.6. *Diatom cell morphology.*

182 Organic matter in the samples was removed by adding hydrogen peroxide to the diatom
183 suspensions and kept overnight at room temperature. They were then rinsed with distilled
184 water and centrifuged, before preparing permanent slides according to EN 14407:2014, by
185 mounting the clean samples in Naphrax (provided by Brunel Microscopes Ltd, UK). Then,
186 they were observed at 1,000x magnification (Leica DMRB, Germany), for the determination
187 of the percentage of normal (symmetrical) and deformed (asymmetrical) *Gomphonema*
188 individuals, based on >100 individuals per replicate sample. Additionally, at least 10
189 measurements of cell linear dimensions (length, width, thickness) and of striae density were
190 performed in each replicate sample. Average cell biovolume was then calculated using the
191 mathematical equation of Hillebrand et al. (1999) for weakly heteropolar forms (elliptic
192 cylinder).

193 A return to the origin material (i.e. samples from Coquillé et al., 2015) was made in order to
194 measure, in the same way, the morphological characters of GGRA cells, thus providing
195 elements for comparison and understanding of the morphological range of variation of this
196 culture after 1 year.

197 2.7. *Bacterial fingerprinting.*

198 Each replicate filter collected on day 9 was used to analyze the bacterial communities closely
199 associated with the diatoms, i.e. embedded in their extracellular polysaccharides (EPS).
200 Nucleic acids were extracted with the FastDNA SPIN kit for soils (MP Biomedicals, France)
201 and the 16S–23S intergenic spacer region from the bacterial rRNA operon was amplified as
202 described in Pesce et al. (2016), using the universal primers S-D-Bact-1522b-S-20 and L-D-
203 Bact-132-a-A-18. Automated ribosomal intergenic spacer analyses (ARISA) were performed
204 on an Agilent 2100 Bioanalyzer (Agilent Technologies, France) and the resulting profiles
205 were exported from the Agilent 2100 Expert software.

206 2.8. *Data analysis.*

207 All statistical analyses were performed with R 3.2.2 (Ihaka and Gentleman, 1996).

208 After having checked for normality and homogeneity of variances, linear models for fixed
209 effects (lm) were implemented (nlme package). For each endpoint, the effect of culture (Ctrl
210 vs. Trtg), date, and their interaction, were tested sequentially by the model. The results are
211 illustrated as figures in the manuscript. Detailed values and statistics output can be found in
212 Appendix 1.

213 A t-test was used to compare the parameters extracted from the curves fitted for growth
214 kinetics and RLC between cultures. Additionally, for growth, the kinetics of the two
215 phenotypes in each culture were compared.

216 For all analyses, a *p*-value below 0.05 was considered statistically significant.

217 The endpoints raising significant culture and/or date effects were plotted in a Principal
218 Component Analysis (PCA), followed by Hierarchical Clustering to identify the main groups of
219 data, using the FactoMineR package (Lê et al., 2008).

220

221 3. Results

222 3.1. Diatom morphology: cell dimensions and teratology

223 During the first months of culture (2014), cells from the original material (GGRA) were
224 identified as small specimens of *Gomphonema gracile*. Valves were quite isopolar in outline
225 and were $28.91 \pm 0.12 \mu\text{m}$ long and $5.94 \pm 0.04 \mu\text{m}$ wide (Figure 1). Striking differences were
226 observed between the parental line (GGRA) and the cultures used in this study (Figure 1).
227 Indeed, GNF individuals were slightly more heteropolar with more rounded apices and valves
228 were smaller ($21.92 \pm 0.09 \mu\text{m}$ long and $5.92 \pm 0.03 \mu\text{m}$ wide). Moreover, GTF cells were
229 more lanceolate ($33.88 \pm 0.10 \mu\text{m}$ long and $6.17 \pm 0.03 \mu\text{m}$ wide) but showed a deformed
230 valve outline (boomerang shape, Figure 2). In all cases, the invagination of the frustule was
231 located opposite to the stigma present in the central area. No difference in the density of
232 striae was found, with average values of 17.00 ± 0.09 striae in $10 \mu\text{m}$ ($n=200$).

233 As a consequence of these differences in linear dimensions, the cell biovolumes calculated
234 over the duration of the experiment (7 days for GGRA and 9 days for GTF and GNF) showed
235 significant differences ($p<0.001$) between GGRA ($457 \pm 14 \mu\text{m}^3$, $n=150$), GNF ($334 \pm 11 \mu\text{m}^3$,
236 $n=210$) and GTF ($543 \pm 16 \mu\text{m}^3$, $n=210$). Cell biovolume of both descendant cell lines tended
237 to slightly decrease with time, and significantly smaller cell sizes were found on days 6 and 9
238 ($p<0.05$) (Figure 3A).

239 Very few deformed individuals were observed in the Ctrl cultures ($0.34 \pm 0.12\%$, $n=2617$)
240 (Figure 4). Cells were in the size range of GNF individuals, but the deformity was similar in
241 location as in GTF (Figure 4). In Trtg cultures, only $7.7 \pm 0.7\%$ of the cells ($n=2693$) were not
242 deformed, and were morphologically very close to GNF individuals ($21.07 \pm 0.38 \mu\text{m}$ long,
243 $6.00 \pm 0.07 \mu\text{m}$ wide; $n=65$).

244 3.2. Growth kinetics

245 Significant differences in cell densities were observed as a function of time in both cultures,
246 featuring typical growth kinetics with the stationary phase reached from day 6 (Figure 3B).
247 The percentage of dead diatoms decreased continuously on the first dates, simultaneously to
248 the exponential cell increase. Then, mortality increased when the stationary phase was
249 reached (culture x date interaction, $p<0.05$, Figure 3C).

250 Despite the absence of any statistical difference in overall growth rates between Ctrl
251 ($\text{GR}=0.46 \pm 0.01 \text{ divisions}\cdot\text{day}^{-1}$) and Trtg cultures ($\text{GR}=0.49 \pm 0.04 \text{ divisions}\cdot\text{day}^{-1}$) in the
252 exponential growth phase ($p=0.63$), t-tests revealed statistical differences in the specific
253 growth rates of GNF and GTF phenotypes in Ctrl and Trtg cultures (Figure 5, $p<0.001$ in both
254 cases). Indeed, GNF populations grew faster in the Ctrl units ($0.46 \pm 0.01 \text{ divisions}\cdot\text{day}^{-1}$)
255 than in the Trtg ones ($0.37 \pm 0.08 \text{ divisions}\cdot\text{day}^{-1}$), where they reached their stationary phase
256 one day later (day 8 instead of day 7). GTF had the same growth characteristics as GNF in
257 the Ctrl ($\text{GR}=0.45 \pm 0.06 \text{ divisions}\cdot\text{day}^{-1}$, stationary phase starting on day 7). Conversely, in
258 the Trtg units, they showed higher division rates ($0.49 \pm 0.04 \text{ divisions}\cdot\text{day}^{-1}$ vs.
259 $0.37 \pm 0.08 \text{ divisions}\cdot\text{day}^{-1}$ for GNF) and consequently attained their maximal cell density
260 earlier (on day 6, Figure 3B). Therefore, significant interaction between culture and date was
261 highlighted for cell densities, as a result of the delay in reaching maximal cell numbers in
262 GNF (on day 7, Figure 3B).

263 The carrying capacity (maximum population size) in cell density was about one third lower for
264 Trtg cultures ($397,800 \pm 31,700 \text{ cell}\cdot\text{mL}^{-1}$, average values for days 7 to 9) compared to Ctrl
265 cultures ($616,200 \pm 44,400 \text{ cell}\cdot\text{mL}^{-1}$, average values for days 7 to 9) (Figure 3B). However,

266 the total biovolume occupied (i.e. cell densities x individual biovolume) did not differ between
267 experimental units from day 7, with an average of $197 \pm 12 \cdot 10^6 \mu\text{m}^3 \cdot \text{mL}^{-1}$ (Figure 3D).

268 3.3. Physiology

269 No significant difference was observed in chlorophyll-a derived parameters between Ctrl and
270 Trtg ($p=0.42$). Chlorophyll-a fluorescence followed a bell-shaped curve and peaked on day 6
271 (Figure 3E). Photosynthetic activity (Figure 3F) increased on the first days of exponential
272 growth, and then slightly decreased over time (from day 6).

273 The RLC did not highlight any differences between treatments in the use of light by the
274 cultures (Appendix 2). Indeed, similar values were obtained for Ctrl and Trtg photosynthetic
275 efficiency at low light intensity (0.23 ± 0.00 in average, $p=0.09$), as well as the maximum
276 electron transport rate ($95.1 \pm 7.6 \mu\text{mol electrons} \cdot \text{m}^2 \cdot \text{s}^{-1}$, $p=0.83$), reached from the
277 saturating irradiance of $415.9 \pm 30.1 \mu\text{mol} \cdot \text{m}^2 \cdot \text{s}^{-1}$ ($p=0.90$).

278 3.4. Behavior

279 In both cultures, cells grew benthically, and were mostly solitary. Up to $40 \pm 2\%$ cells (Ctrl,
280 day 2) had the potential to form aggregates, principally forming star-shaped associations
281 (Figure 3G). The abundance of cell clumps was always higher in Ctrl cultures ($p<0.001$) and
282 decreased over time whatever the culture from day 2 ($p<0.01$). For this reason, on the first
283 days, the percentage of mobile diatoms was so low that mobility parameters could not be
284 determined appropriately. When the cultures reached their stationary phase, the percentage
285 of cells forming aggregates drastically decreased and the percentage of mobile cells
286 increased significantly (Figure 3H), simultaneously to a decrease of benthic forms (as
287 assessed through the chlorophyll-a settled on the bottom of the flasks, Figure 3E). Striking
288 differences in motility were observed between cultures ($p<0.001$): in the Ctrl, the percentage
289 of mobile cells gradually increased from $<5\%$ (day 6) to $18.9 \pm 3.4\%$ (day 9). Oppositely,
290 $36.8 \pm 2.7\%$ cells were mobile in the Trtg cultures from day 6 to 8, then decreased to
291 $21.5 \pm 3.4\%$ on the last day of the experiment. On the last days, the percentage of
292 associated cells was low ($5.6 \pm 2.6\%$ in Ctrl and $1.3 \pm 0.5\%$ in Trtg cultures), and the cells
293 grew mostly in suspended, loose agglomerates, potentially indicating biofilm senescence. No
294 significant difference was found between cultures with respect to movement velocity;
295 however, the trajectory was more linear in Trtg ($p<0.05$) (Appendix 1).

296 3.5. Global patterns in sample variation

297 The two first dimensions of the PCA explained the same amount of data variability, reaching
298 68.43% of combined variance (Figure 6A). The optimal clustering reached after applying the
299 PCA discriminated four groups (Figure 6B).

300 The first component of the PCA expressed a temporal gradient. It discriminated between
301 samples collected on the first days of sampling (exponential growth phase), located on the
302 left half-panel, and cultures corresponding to the stationary phase (last sampling days),
303 located on the right half-panel. Positive values along this dimension were correlated with
304 increasing carrying capacity (total cell biovolume, cell density) as well as other endpoints
305 related to termination of growth: higher mortality, increasing percentages of mobile cells
306 associated with decreasing cell aggregation.

307 The second PCA component separated treatments, with Ctrl clustered on the lower half-
308 panel and Trtg on the upper half-panel. Sample discrimination was mostly related to the
309 morphological descriptors (cell dimensions, biovolume, percentage of teratologies), and with
310 more linear trajectories in Trtg on the last days of sampling.

311 3.6. ARISA profiles

312 Amplification of the 16S rDNA genes on the filters did not give rise to banding patterns in one
313 replicate sample of Ctrl (likely due to an experimental error), which was consequently
314 discarded. Fingerprinting of bacterial communities (Appendix 3) highlighted that Ctrl and Trtg
315 cultures shared 3 major peaks (above a threshold of 100 relative fluorescence units in height
316 from the baseline), and that no major differences were found between Ctrl and Trtg.
317 However, a minor peak (below 50 fluorescence units) was detected around 623bp, but only
318 in the Trtg samples.

319 **4. Discussion**

320 4.1. Implications of deformities for fitness and survival

321 4.1.1. Teratologies did not impact performance in GTF populations

322 Results of this study are in complete disagreement with common assumptions that deformed
323 individuals are impaired. Indeed, the deformed culture of *Gomphonema gracile* (GTF) did not
324 exhibit a longer lag phase compared to the controls and growth rates of the GTF populations
325 were similar or higher than those of GNF (Figure 5). Growth rates of the GTF population
326 were higher than that of GNF in Trtg cultures, and similar in Ctrl cultures, possibly resulting in
327 higher competitive abilities of GTF individuals, compared to GNF ones, under specific
328 conditions. This is in contradiction with the observations made by Windler et al. (2014),
329 where teratological cultures had grown more slowly than normal cultures. However, it is
330 worth noting that, in their study, teratologies were suspected to result from axenicity, which
331 was not the case here.

332 The carrying capacity (maximum cell density in the stationary phase) was certainly lower in
333 Trtg than in Ctrl (~400,000 cells.mL⁻¹ vs. ~600,000 cells.mL⁻¹). However, these differences
334 were likely caused by space availability more than growth abilities: total biovolume occupied
335 in the stationary phase was similar in Ctrl and Trtg cultures. In the same way, Windler et al.
336 (2014) observed that their normal and teratological cultures of *Achnantheidium minutissimum*
337 reached similar chlorophyll concentrations in the stationary phase. Thus, competition for
338 space (and subsequent access to resources such as nutrients and/or light) was probably the
339 main driving factor in kinetics for both cultures in this experiment.

340 Besides growth kinetics, the physiological and behavioral endpoints assessed did not show
341 lower performances of GTF populations, contrarily to what was expected. Photosynthetic
342 efficiency was similar in Trtg compared to Ctrl cultures and fell into the range of other
343 measurements carried-out on *G. gracile* (Coquillé et al., 2015; Table 1). In this experiment,
344 the mobility features (percentage of mobile cells and velocities) of GNF and GTF individuals
345 were below the values reported by Coquillé et al. (2015). Nevertheless, differences were
346 found between Ctrl and Trtg: more mobile individuals were found within GTF populations and
347 they moved more linearly than GNF cells (Figure 6). This may be a consequence of their
348 more elongated valve outline. No differences were found, however, in movement velocity, in
349 agreement with Bertrand's work on the motility of several diatom species (1990, 1999).
350 Based on analyses of five distinct populations, he also demonstrated that velocity was
351 independent of cell size within a species (Bertrand, 1990).

352 To sum up, the morphological changes that occurred in *Gomphonema gracile* did not impair
353 its performance, based on the endpoints assessed, when cultured alone. The fact that GNF
354 and GTF displayed similar responses in cultures where they were dominant does not
355 prejudice their behavior when found together. Superseding of GTF by GNF would be

356 expected based on Arini et al. (2013)'s work, arguing for a relative decrease in the
357 percentage of deformed diatoms under optimal growth conditions.

358 4.1.2. Coexistence of GNF and GTF morphotypes

359 The teratological forms had higher growth rates and higher cell dimensions, thus higher cell
360 surface in contact with their environment for nutrient uptake. Moreover, the cells tended to
361 form large floating bulks of solitary individuals, whereas they formed benthic mats in Coquillé
362 et al. (2015). This may stem from ageing with long-term culturing, as well as from the
363 morphological changes over time in *G. gracile*. These associations were more striking in the
364 GTF cultures; deformed diatoms may thus be less adhesive than the normal forms. This
365 slightly distinct lifestyle can be an adaptation of this phenotype, larger than GNF and more
366 silicified, to modify its sinking rates and/or maximize its access to resources (nutrients, light).
367 Such adaptive mechanisms have been observed in small lacustrine *Stephanodiscus* species
368 (Kociolek and Stoermer, 2010). Therefore, these adaptations may confer a competitive
369 advantage to GTF individuals over the normal morphotype under certain circumstances. The
370 low percentage of non-teratological forms in GTF, stable over time, tends to confirm this
371 hypothesis.

372 The percentage of deformed individuals in Ctrl fell into the range of the abundances
373 occurring "naturally" in the field, i.e. below 0.35% (Morin et al., 2012a). The GTF phenotype
374 did not significantly increase over time in Ctrl cultures, indicating that GNF was not
375 outcompeted (in agreement with the GR values of GNF and GTF in Ctrl units, Figure 3).
376 However, the differential growth of GNF and GTF in Trtg units suggests that a massive
377 outbreak of teratological individuals, owing to an extreme event and/or repeated toxic
378 exposure, could lead to competitive exclusion of their normal congeners. Neury-Ormanni et
379 al. (2017) performed a competition experiment with a balanced GNF:GTF inoculum under the
380 same physicochemical conditions as in the present study. In their experiment, no significant
381 differences in growth were found, compared to the individual kinetics of GNF and GTF, and
382 both phenotypes co-occurred at a 1:1 ratio until the stationary phase was reached.
383 Therefore, dedicated competition experiments using several GNF:GTF ratios would
384 constitute a potential way to confirm this theory and determine a threshold above which
385 teratological diatoms would irreversibly dominate over normal ones. However, there is
386 evidence from translocation experiments (Tolcach and Gómez, 2002) and laboratory
387 investigations (Morin et al., 2012b) that diatom immigration may contribute to re-colonization
388 when toxic contamination is removed, and mask a small population of deformed individuals
389 kept alive. Indeed, in the field, continuous immigration of normal morphotypes and
390 competition with several other species likely impedes the sustainable presence of
391 teratological diatoms under uncontaminated conditions.

392 4.2. Transmission of the teratological character over time

393 4.2.1. Consistency in the teratological feature

394 In this study, the culture of deformed *Gomphonema gracile* (GTF) was maintained with
395 classical growth kinetics at abundances exceeding 90%, suggesting that the deformity was
396 non-lethal. In long-term cultures, frustule aberrations were demonstrated to increase as
397 valves decrease in length in several diatom species (e.g. Hostetter and Rutherford, 1976;
398 Torgan et al., 2006). In this study, on the contrary, GTF cells were larger than the other
399 population, although at the same development stage as the non-teratological populations
400 (GNF). All deformed diatoms from Trtg and Ctrl exhibited a typical deformed valve outline
401 with symmetry loss (Figure 1 and Figure 2), although the few deformed diatoms in Ctrl were
402 smaller than the GTF populations (Figure 2).

403 Valve asymmetry is the most frequent type of deformity in *Gomphonema* species (Falasco et
404 al., 2009b). Similarly incised valves have been described as abnormal *G. parvulum* (Victoria
405 and Gómez, 2010) and *G. gracile* (Morin and Coste, 2006) in natural communities under
406 toxic contamination, where they were associated with normal specimens. Indeed, the
407 consistency of this feature reproduced over time in the Trtg units suggested either that a new
408 species emerged (with another strain of associated bacteria as suggested by ARISA profiles;
409 Appendix 3), or that boomerang-shaped outline deformation preferentially occurs in
410 *Gomphonema*.

411 4.2.2. Heritability of the teratology throughout the experiment

412 Heritability of teratologies was empirically demonstrated through the reproduction of
413 deformed *Gomphonema gracile* in the Trtg cultures over the 9-day experiment. Indeed, the
414 percentage of GTF cells was stable through time, although cultures grew in cell numbers.
415 This may have resulted from i) mechanical transmission through generations by vegetative
416 division (Round et al., 1990; Von Dassow et al., 2006) of the deformed clones and/or ii)
417 higher growth rates in GTF cultures, potentially masking the recovery of normal forms (with
418 lower growth rates, as inferred by GNF growth kinetics in Trtg cultures; also see section
419 4.1.1). Restoration of maximal size in GTF on day 7 (Table 1), coinciding with stabilization of
420 cell numbers (plateau phase) and the decrease of benthic cells suggested that they
421 underwent sexual reproduction at the timescale of the 9-day experiment. Even though a
422 slight decrease in the percentages of deformities was observed in GTF after sexual
423 reproduction ($89.4 \pm 4.0\%$ on day 8, Figure 4), it seems that the genetic and/or epigenetic
424 cause of teratologies is most likely.

425 Reversibility of teratologies is generally assumed; for instance Granetti (1968) observed in
426 long-term cultures that deformed *Navicula* species restored their normal morphology after
427 sexual reproduction, leading him to discard the genetic cause of teratologies. However,
428 McLaughlin (1988) stated that, if the variation of morphology is well defined, consistent and
429 reproducible, teratologies should be considered as varieties of the species. In line with this,
430 Jüttner et al. (2013) described a *Gomphonema* with a deflection in the head and foot poles
431 as a new species, based on its frequent occurrence and large geographical distribution, thus
432 assuming genetic distinctness. As generally observed in long-term cultures, continuous
433 culturing resulted in a decrease in cell size over time for both phenotypes. Size differences
434 between GNF and GTF populations were perpetuated and maintained for 6 extra months
435 (Ezzedine and Vedrenne, 2015): in August 2015, GNF cells averaged $17.35 \pm 0.15 \mu\text{m}$
436 ($n=160$) in length. In the same way, continuous decrease also occurred in GTF populations
437 that were $29.59 \pm 0.14 \mu\text{m}$ ($n=320$) long. The deformities were not reversed after the
438 predictable rounds of sexual reproduction over this extra 6-month period. The continuity of
439 the teratological feature, repeated throughout the GTF population in varying sizes of
440 individuals, also argues in favor of a new variety (McLaughlin, 1988). Further investigations
441 would thus be required (i) to characterize both GNF and GTF genomes in depth in order to
442 identify the potential mutations, to discard or not the genetic causes of diatom teratologies
443 and (ii) to characterize the two epigenomes via the study of DNA methylation and epigenetic
444 causes of diatom teratologies.

445 4.2.3. Long term persistence of the teratological feature suggests genetic and/or 446 epigenetic control

447 In this study, both Ctrl and Trtg cultures were issued from a unique parental line which had
448 diverged morphologically. Initially (i.e. when GTF appeared), deformation of this cell line may
449 have been induced by mechanical causes such as crowding in the cultures or by changes in

450 the accompanying bacteria (rather than axenicity, as bacteria were found in both cultures at
451 the end of the experiment). The ARISA profiles differed slightly between cultures by the
452 presence of one small peak in Trtg, suggesting that another strain of bacteria was present in
453 these cultures. Hypotheses regarding a previous exposure to chemical insults or
454 inappropriate culture conditions can be discarded. Indeed, the diatom was isolated from a
455 pristine site in Rebec tributary, and then cultivated using a culture medium under laboratory
456 conditions that were previously shown to be adapted to *G. gracile*'s growth (Coquillé et al.,
457 2015). Besides, both GNF and GTF morphotypes were cultivated under identical conditions
458 that proved to be appropriate given their growth kinetics, thus endorsing the deformity as
459 teratological.

460 Although the body of work dealing with diatom teratologies is vast (Falasco et al., 2009a),
461 Lavoie et al. 2017), the processes involved in the formation of abnormal cells are still unclear
462 and it has never been proved whether they resulted or not from epigenetic mechanisms or
463 genetic alteration. Better knowledge at the molecular level and more specifically at the
464 epigenetic level is necessary because of the conservation of the epigenetic machinery in
465 diatoms, its potentially role in phenotypic modification (Tirichine et al., 2017) and, contrary to
466 genetic factors, ease in evaluating some parameters such as DNA methylation. Indeed, the
467 identification of genetic alteration is hardly assessable directly since few genomes have been
468 sequenced and annotated, making it difficult to target the genetic loci responsible for frustule
469 morphology and to identify potential alterations.

470 **5. Conclusions**

471 The phenotypic plasticity in diatoms makes heritability difficult to assess, but thanks to
472 unexpected changes in cultures from the same parental line, cultures of morphological
473 variants of *Gomphonema gracile* (normal and abnormal phenotypes) made it possible to
474 comparatively study them. The results of this study showed that teratologies were
475 reproduced over time in both Ctrl and Trtg cultures, to variable extent depending on the
476 experimental units considered. Moreover, teratology did not significantly affect physiology nor
477 behavior, disputing the previous assumptions that abnormal diatoms are not competitive and
478 are only found as a transient state, useful to point out extremely adverse environmental
479 conditions. Thus, the similarity in the performance of GTF individuals, compared to normal
480 forms (GNF), has implications for the improvement of knowledge about the ecology of
481 teratological diatoms, and for the environmental interpretations made in hydrosystems where
482 they are found. Indeed, this study provides evidence that abnormal diatoms, likely to appear
483 by chance, are viable individuals and may hold out against normal forms under "favorable"
484 conditions. Consequently, much care must be paid to them in biomonitoring studies, before
485 stating that the presence of teratologies reflects the degradation of water quality.

486 Apart from the ability to transmit teratological characters as seen in the microscopical
487 observations of diatom morphology and discussed in this paper, the growth and physiology
488 data strongly advocate for a genetic and/or epigenetic origin in GTF. In future, special
489 attention should be given to diatom teratologies from the genetic and epigenetic standpoint to
490 understand if they result from alterations in genome and/or epigenetic modifications, to their
491 ecological preferences.

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624

625 *Figure captions:*

626 Figure 1. Diatom cell dimensions (length and width, in μm) in the Ctrl (white diamonds, $n=210$) and
627 Trtg (black diamonds, $n=210$) cultures, compared to the parental culture (GGRA, grey diamonds,
628 $n=150$). Significant differences in length were found between Ctrl and Trtg ($p<0.001$).

629 Figure 2. External (left) and internal (right) views of teratological valves from GTF cultures (SEM
630 microphotographs).

631 Figure 3. Temporal evolution in Ctrl (white diamonds) and Trtg (black diamonds) cultures of A-
632 Individual cell biovolumes (μm^3), B- Diatom cell densities (cells.mL^{-1}), C- Mortality (%), D- Total cell
633 biovolume (μm^3 per mL), E- Photosynthetic efficiency (relative units r.u.), F- Chlorophyll-*a* measured
634 on the bottom of the flasks ($\mu\text{g.cm}^{-2}$), G- Percentage of cells forming associations (%) and H-
635 Percentage of mobile cells (%; n.d. = not determined because the number of mobile individuals was
636 too low). Linear mixed-effects models highlight a significant culture effect (*) for individual biovolumes
637 (A, $p<0.001$), significant date effects (†) for individual biovolumes (A, $p<0.05$), photosynthetic
638 efficiency (E, $p<0.05$) and chlorophyll-*a* content (F, $p<0.001$), while interactive effects between culture
639 and date (‡) are found for growth kinetics (cell densities B, $p<0.001$; total cell biovolume C, $p<0.001$
640 and mortality D, $p<0.05$) and the percentage of cells in associations or motile (G and H, $p<0.01$).

641 Figure 4. Teratological forms. Left panel: Percentage of deformed individuals (%) in Ctrl (white
642 diamonds, $n=2617$) and Trtg (black diamonds, $n=2693$) cultures. * denotes a significant culture effect
643 ($p<0.001$) identified by the linear mixed-effects model. Right panel: Light microscopy
644 photomicrographs of the teratological *Gomphonema* found in Ctrl (down) and in Trtg cultures (top).
645 Scale bar: 10 μm .

646 Figure 5. Population dynamics of GNF and GTF phenotypes during the experiment, in the Ctrl (A) and
647 Trtg (B) cultures. Experimental results are shown by: open symbols, GNF; solid symbols, GTF. The
648 dashed and solid lines indicate the best-fitting growth curves for normal (GNF) and teratological (GTF)
649 forms, respectively; growth rates (GR, in $\text{divisions.day}^{-1}$) and duration of exponential phase considered
650 are specified for each data series. Note the logarithmic scale on the Y-Axis.

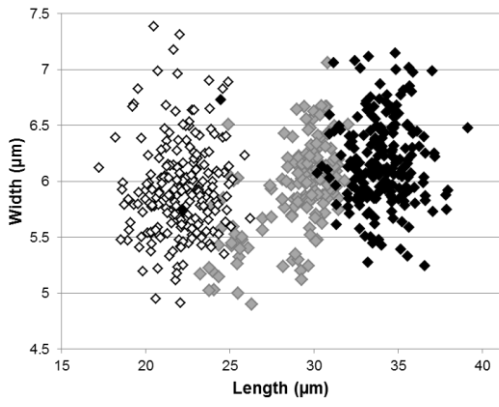
651 Figure 6. Principal component analysis (PCA) based on the endpoints significantly affected by
652 treatment (culture) and/or date. A- Variables factor map. B- Projection of the individuals, labelled
653 according to a *posteriori* clustering outputs. Grey arrows show temporal changes for both cultures.
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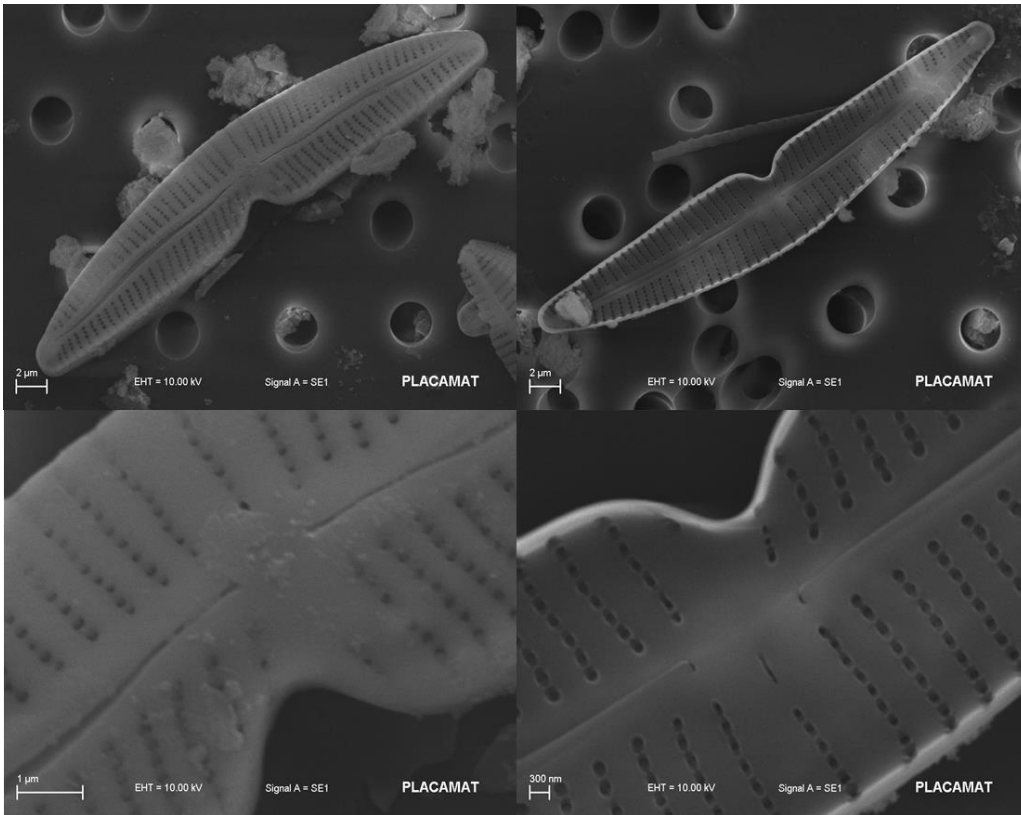
658 Fig. 1



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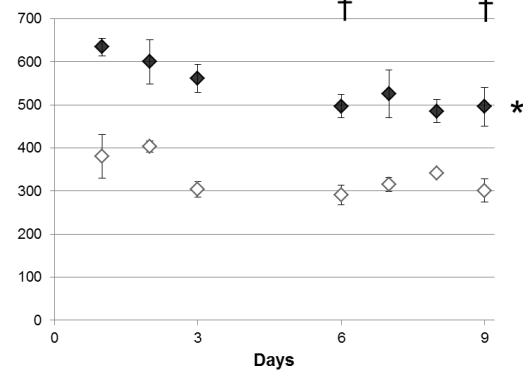
661 Fig. 2



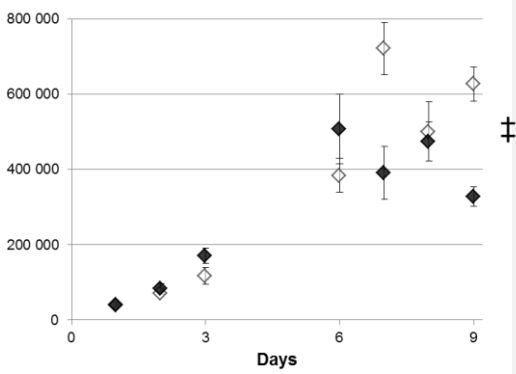
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665 Fig. 3

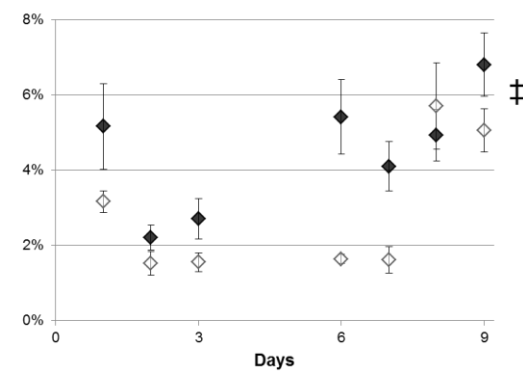
A- Measured cell biovolume (μm^3)



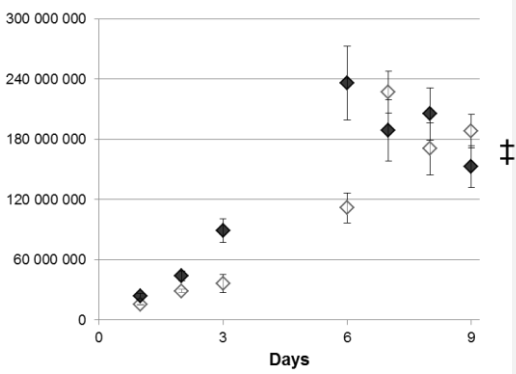
B- Cell density (cell.mL^{-1})



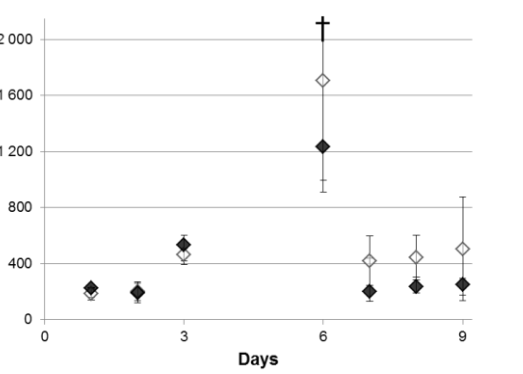
666 C- Mortality



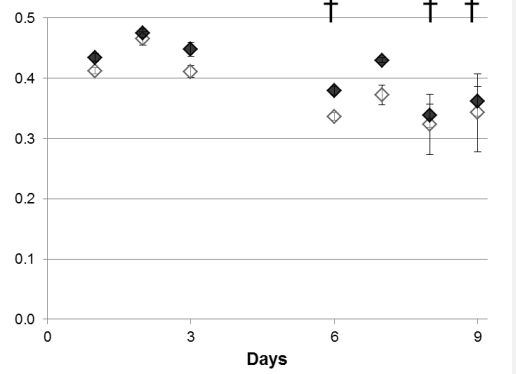
D- Total biovolume ($\mu\text{m}^3.\text{mL}^{-1}$)



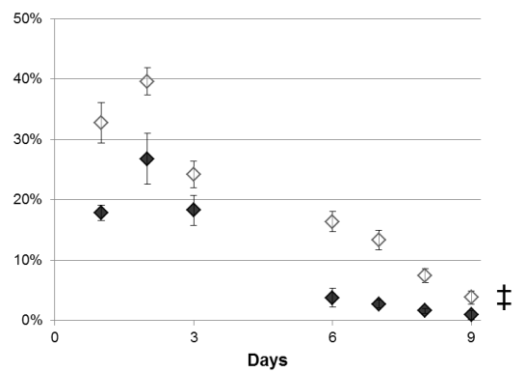
667 E- Chlorophyll a concentration ($\mu\text{g.cm}^2$)



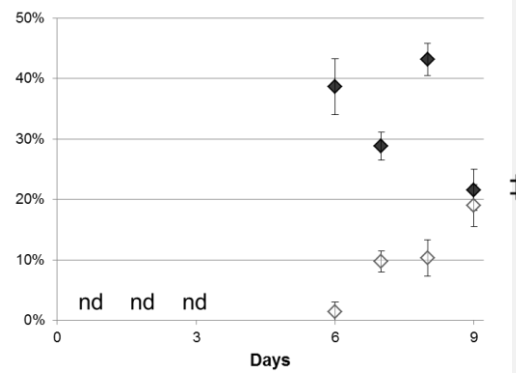
F- Photosynthetic efficiency (r.u.)



668 G- Cells in association



H- Mobile cells

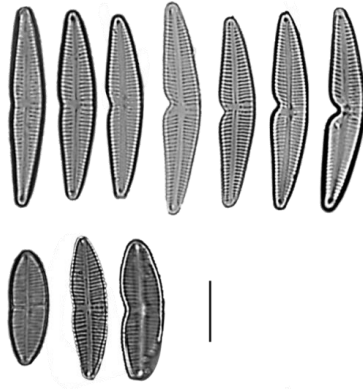
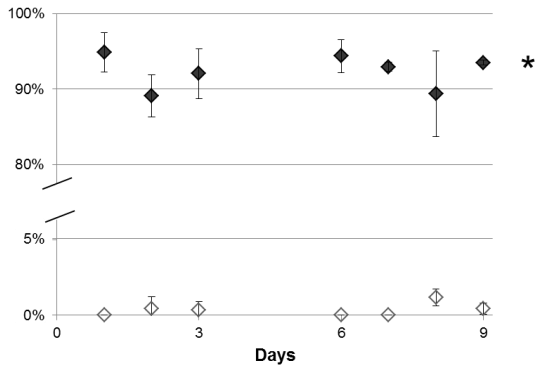


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671 Fig. 4

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Teratological forms

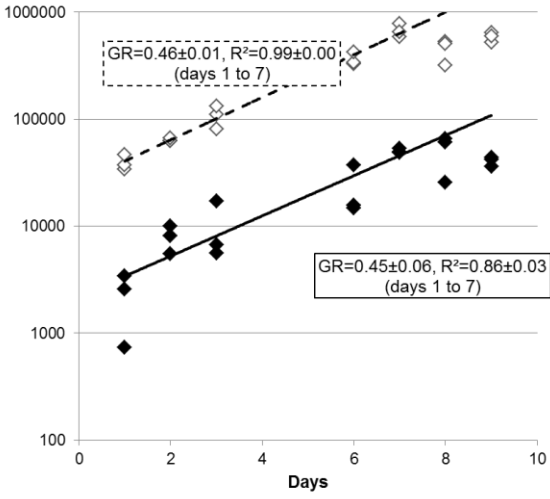


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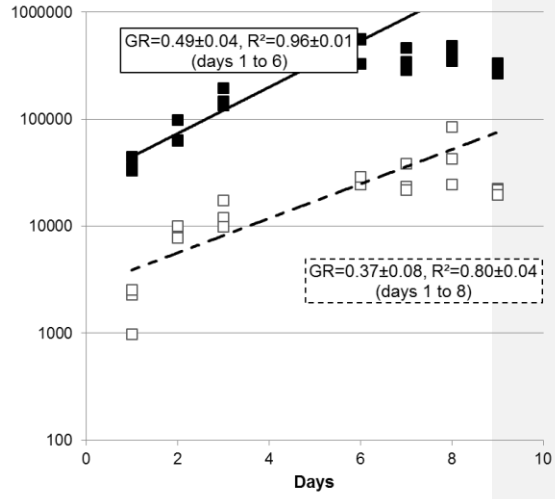
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676 Fig. 5
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A- Cell densities (cell.mL⁻¹) in Ctrl cultures

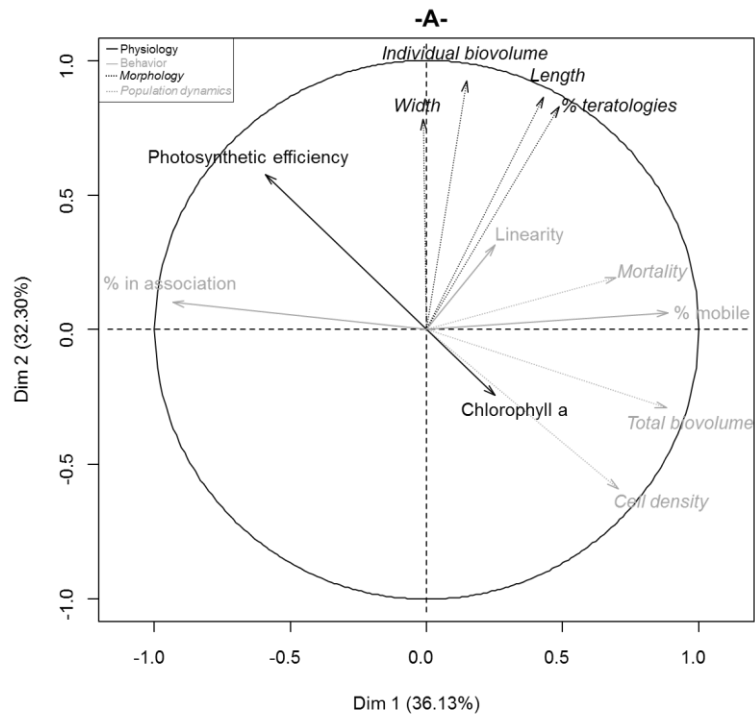


B- Cell densities (cell.mL⁻¹) in Trtg cultures



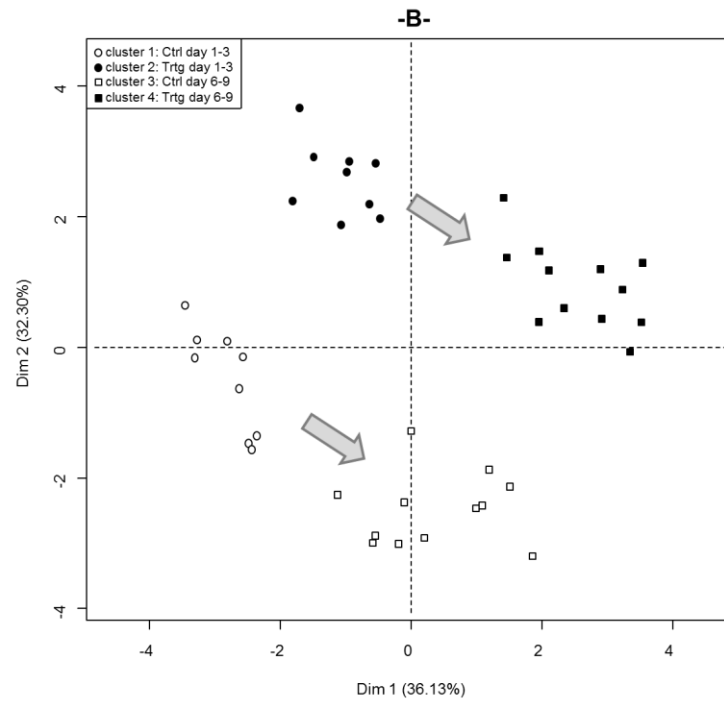
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680 Fig. 6



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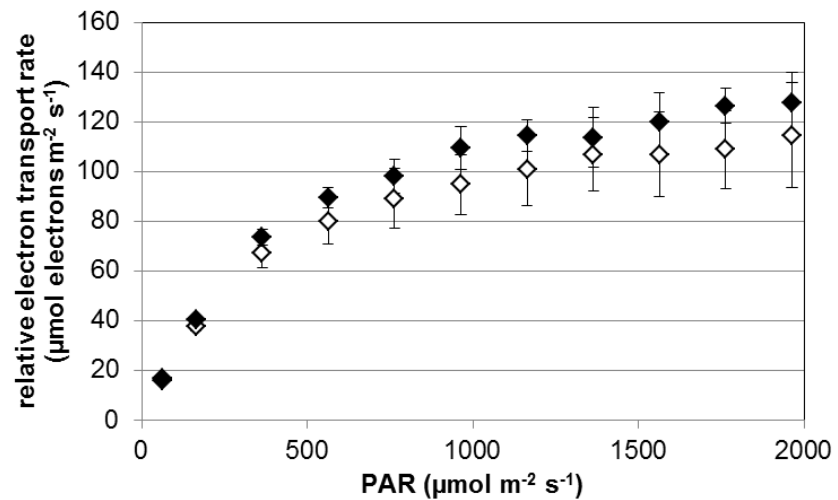
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Appendix 1. Characteristics of the diatom cultures analyzed in this study (Ctrl and Trtg) compared to the control cultures (GGRA) used in Coquillé et al. (2015). All values are mean \pm standard error over the experiment, except * corresponding to the stationary phase and † that were only measurable on days 6 to 9; n=number of individuals measured/counted, N= number of samples. [£]: GGRA values are from Coquillé et al. (2015; control cultures). Effects of Culture, Date and Interaction between culture and date result from modelling; n.s.: not significant.

	Ctrl	Trtg	Culture effect	Date effect	Interaction	GGRA
Morphology						
Length (μm)	21.92 \pm 0.09 (n=420)	33.88 \pm 0.10 (n=420)	$p < 0.001$	n.s.	n.s.	28.91 \pm 0.12 (n=300)
Width (μm)	5.92 \pm 0.03 (n=210)	6.17 \pm 0.03 (n=210)	n.s.	$p < 0.05$	n.s.	5.94 \pm 0.04 (n=150)
Cell biovolume (μm^3)	334 \pm 11 (n=210)	543 \pm 16 (n=210)	$p < 0.001$	$p < 0.05$	n.s.	457 \pm 14 (n=150)
Cells with abnormal outline (%)	0.34 \pm 0.12 (n=2617)	92.32 \pm 0.74 (n=2693)	$p < 0.001$	n.s.	n.s.	0.25 \pm 0.12 (n=1571)
Population dynamics						
Cell density ($10^3 \text{ cells} \cdot \text{mL}^{-1}$)	616.2 \pm 44.4* (N=9)	397.8 \pm 31.7* (N=9)	n.s.	$p < 0.001$	$p < 0.001$	421.0 \pm 42.7* (N=3)
Total biovolume ($10^6 \mu\text{m}^3 \cdot \text{mL}^{-1}$)	195.3 \pm 20.3* (N=9)	198.6 \pm 23.4* (N=9)	n.s.	$p < 0.001$	$p < 0.01$	210.1 \pm 31.1* (N=3)
Mortality (%)	2.9 \pm 0.4 (N=21)	4.5 \pm 0.4 (N=21)	n.s.	$p < 0.05$	$p < 0.05$	1.1 \pm 0.7 (N=24) [£]
Physiology						
Photosynthetic efficiency (r.u.)	0.38 \pm 0.00 (N=21)	0.41 \pm 0.00 (N=21)	n.s.	$p < 0.05$	n.s.	0.37 \pm 0.00 [£] (N=24)
Chlorophyll-a concentration ($\mu\text{g} \cdot \text{cm}^{-2}$)	561 \pm 143 (N=21)	410 \pm 87 (N=21)	n.s.	$p < 0.001$	n.s.	732 \pm 155 [£] (N=24)
Behaviour						
Cells in association (%)	19.6 \pm 5.4 (N=21)	10.3 \pm 4.3 (N=21)	$p < 0.001$	$p < 0.01$	$p < 0.01$	9.76 \pm 1.47 [£] (N=24)
Mobile cells (%)	10.1 \pm 2.2 [†] (N=12)	33.0 \pm 2.9 [†] (N=12)	$p < 0.001$	$p < 0.05$	$p < 0.01$	36.2 \pm 4.6 [£] (N=24)
Linearity	0.85 \pm 0.02 [†] (N=10)	0.90 \pm 0.02 [†] (N=12)	$p < 0.05$	n.s.	n.s.	0.91 \pm 0.01 [£] (N=24)
VAP ($\mu\text{m} \cdot \text{s}^{-1}$)	2.7 \pm 0.2 [†] (N=10)	3.5 \pm 0.2 [†] (N=12)	n.s.	n.s.	n.s.	7.9 \pm 0.2 [£] (N=24)
VSL ($\mu\text{m} \cdot \text{s}^{-1}$)	2.3 \pm 0.3 [†] (N=10)	3.1 \pm 0.3 [†] (N=12)	n.s.	n.s.	n.s.	6.8 \pm 0.2 [£] (N=24)
VCL ($\mu\text{m} \cdot \text{s}^{-1}$)	2.9 \pm 0.2 [†] (N=10)	3.7 \pm 0.2 [†] (N=12)	n.s.	n.s.	n.s.	8.5 \pm 0.2 [£] (N=24)

Appendix 2. Rapid Light Curves for Ctrl (white diamonds) and Trtg (black diamonds) cultures on day 9. Values are mean±standard errors of three replicates.



Appendix 3. PCR-ARISA patterns of amplified bacterial fragments in the replicate samples (Ctrl cultures: Ctrl_1 to 3, Trtg cultures: Trtg_1 to 3). Ref stands for the internal standards. Band intensity and position represent the ARISA peak intensity and corresponding migration duration [s] or fragment size [bp].

