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**Diatom teratologies as biomarkers of contamination: Are all deformities ecologically meaningful?**

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(Article begins on next page)

1 **Diatom teratologies as biomarkers of contamination: are all deformities ecologically**  
2 **meaningful?**

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46

47 ABSTRACT

48

49 Contaminant-related stress on aquatic biota is difficult to assess when lethal impacts are not observed.  
50 Diatoms, by displaying deformities (teratologies) in their valves, have the potential to reflect sub-lethal  
51 responses to environmental stressors such as metals and organic compounds. For this reason, there is  
52 great interest in using diatom morphological aberrations in biomonitoring. However, the detection and  
53 mostly the quantification of teratologies is still a challenge; not all studies have succeeded in showing a  
54 relationship between the proportion of abnormal valves and contamination level along a gradient of  
55 exposure. This limitation in part reflects the loss of ecological information from diatom teratologies  
56 during analyses when all deformities are considered. The type of deformity, the severity of aberration,  
57 species proneness to deformity formation, and propagation of deformities throughout the population are  
58 key components and constraints in quantifying teratologies. Before a metric based on diatom deformities  
59 can be used as an indicator of contamination, it is important to better understand the “ecological signal”  
60 provided by this biomarker. Using the overall abundance of teratologies has proved to be an excellent  
61 tool for identifying contaminated and non-contaminated environments (presence/absence), but refining  
62 this biomonitoring approach may bring additional insights allowing for a better assessment of  
63 contamination level along a gradient. The dilemma: are all teratologies significant, equal and/or  
64 meaningful in assessing changing levels of contamination? This viewpoint article examines numerous  
65 interrogations relative to the use of diatom teratologies in water quality monitoring, provides selected  
66 examples of differential responses to contamination, and proposes solutions that may refine our  
67 understanding and quantification of the stress. Hopefully, this paper highlights the logistical problems  
68 associated with accurately evaluating and interpreting teratologies and stimulates more discussion and  
69 research on the subject to enhance the sensitivity of this metric in bioassessments.

70

71 **Key words:** Bioassessment, biomarker, contaminants, deformities, diatoms, teratologies

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73 **Highlights:**

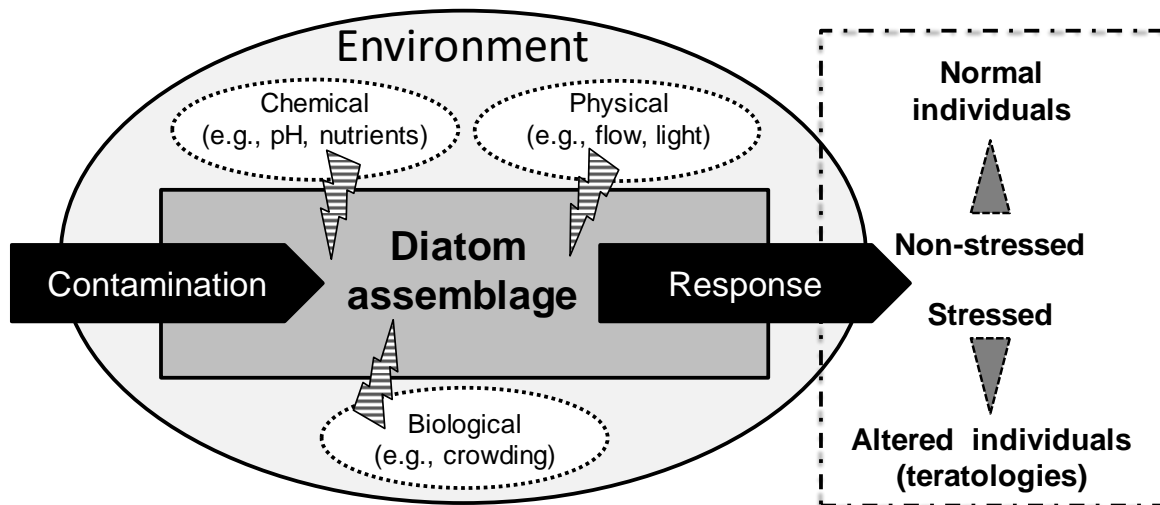
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- 75 • Diatom teratologies are valuable metrics to assess toxic contamination.
- 76 • Bioassessment could be improved by weighing deformities by their type and severity.
- 77 • Species proneness to deformities could be an interesting metric to consider.
- 78 • Abnormal valve shapes are multiplied during cell division; can this be ignored?

79

80 1. INTRODUCTION

81  
82 Diatoms are useful tools in the bioassessment of freshwater ecosystem integrity and are presently  
83 included in numerous water quality monitoring programs worldwide. A variety of diatom-based indices  
84 have been developed using different approaches (e.g., Lavoie et al., 2006; 2014 and references therein;  
85 Stoermer and Smol, 1999 and references therein). Most indices were created to assess ecosystem health  
86 reflecting general water quality and regional climate. There are also countless studies reporting the  
87 response of diatom assemblages to metal contamination (see review in Morin et al., 2012) and to organic  
88 contaminants (Debenest et al., 2010). However, diatom-based indices have not been developed to  
89 directly assess toxic contaminants (e.g., metals, pesticides, hydrocarbons). Contaminant-related stress on  
90 biota is difficult to assess when lethal impacts are not observed. Diatoms, by displaying aberrations in  
91 their valves (deviation from normal shape or ornamentation), have the potential to reflect sub-lethal  
92 responses to environmental stressors including contaminants. Observed deformities can affect the  
93 general shape of the valve, the sternum/raphe, the striation pattern, and other structures, or can be a  
94 combination of various alterations (Falasco et al., 2009a). Other stressors such as excess light, nutrient  
95 depletion, and low pH also have the potential to induce frustule deformities (see review in Falasco et al.,  
96 2009a; Fig. 1). However, the presence of abnormal frustules (also called teratologies or deformities) in  
97 highly contaminated environments is generally a response to toxic chemicals. For this reason, there is  
98 great interest in using morphological aberrations in biomonitoring. Teratologies may be a valuable tool  
99 to assess ecosystem health and it can be assumed that their frequency and severity are related to  
100 magnitude of the stress. Other descriptors such as species diversity and diatom valve densities are used  
101 to evaluate the response of diatom assemblages to contaminations as they are known to decrease,  
102 however we focussed our main discussion on teratologies as biomarkers.  
103



104 Fig. 1. Conceptual model representing the response of a diatom assemblage to contamination. Additional  
105 environmental stressors (other than contaminants such as metals and organic compounds) have the  
106 potential to induce a response from the diatom assemblage.  
107  
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109 Based on the current literature, the presence of deformities in contaminated environments is considered  
110 an indication of stress; however detection and quantification of teratologies is still a challenge. In other  
111 words, not all studies have succeeded in showing a relationship between the proportion of abnormal  
112 valves and contamination level along a gradient of exposure (see sections 3.2 and 5.1 for examples).  
113 Before a metric based on diatom teratologies can be used as an indicator of contamination, we believe it

114 is imperative to better understand the “ecological information” provided by the different types of  
115 deformities and their severity. Furthermore, how are teratologies passed through generations of cell  
116 division? These aspects may influence our assessment and interpretation of water quality.

117  
118 This paper will not provide a detailed review of the abundant literature on the subject of diatom valve  
119 morphogenesis or the different types of teratologies and their causes, but will examine numerous  
120 interrogatives relative to the use of diatom teratologies for the assessment of various types of  
121 contamination. This work is an extension of the discussion issued from the collaborative poster entitled  
122 “*Diatom teratologies in bioassessment and the need for understanding their significance: are all*  
123 *deformities equal?*” presented at the 24th International Diatom Symposium held in Quebec City (August  
124 2016). The participants were invited to take part in the project by adding comments, questions and  
125 information directly on the poster board, and by collaborating on the writing of the present paper.  
126 Numerous questions were presented (Table 1) related to the indicator potential of different types of  
127 deformities and their severity, the transmission of teratologies as cells divide, and species proneness to  
128 deformities. These questions, we believe, are of interest when using diatom teratologies as biomarkers of  
129 stress. This topic is especially of concern because diatom teratologies are increasingly used in  
130 biomonitoring as shown by the rising number of publications on diatom malformations (Fig. 2). With  
131 this paper we hope to initiate a discussion on the subject. Hopefully, this discussion will create new  
132 avenues for using teratologies as biomarkers of stress and contamination. The ultimate goal would be the  
133 creation of an index including additional biological descriptors to complement the teratology-based  
134 metric.

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Table 1. List of questions that initiated this communication as well as questions raised by participants during the 24<sup>th</sup> International Diatom Symposium (IDS 2016, Quebec City).

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#### TERATOLOGY FORMATION AND TRANSMISSION

- A) How are deformities transmitted to the subsequent generations?
- B) The newly-formed valve is an exact copy (or smaller) of the mother cell; in this case, how does the first deformity of the valve outline appear?
- C) Are abnormal ornamentation patterns observed on both valves?
- D) Are deformed cells able to survive and reproduce?

#### ECOLOGICAL MEANING

- E) Are deformities equal between different species? Are all type of deformities equal within the same species?
- F) Are all toxicants likely to induce similar deformities? (or are deformities toxicant-specific?)
- G) Should a deformity observed on a “tolerant” species (versus a “sensitive” species) have more weight as an indicator of stress?

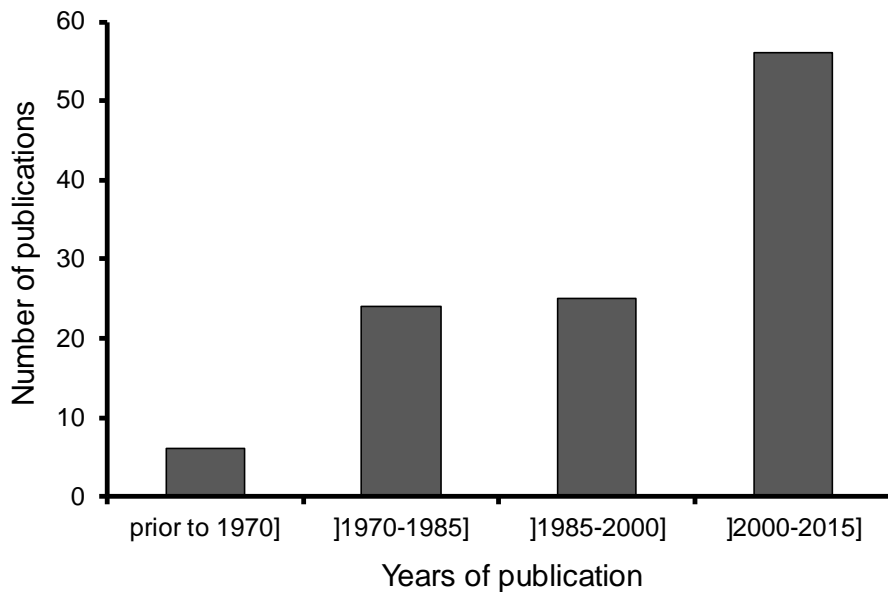
#### ISSUES WITH TERATOLOGY ASSESSMENT

- H) Certain types of deformities are difficult or impossible to see under a light microscope, particularly for small species. Should problematic taxa be included in bioassessments based on teratologies?
- I) How to assess deformities on specimen that are in girdle view?
- J) How should the “severity” of a teratology be assessed?

## IMPLICATIONS FOR BIOMONITORING

- K) The sternum is the initial structure to be formed; should an abnormal sternum (including the raphe) be considered to be more important/significant than other types of aberrations?
  - L) Proneness to produce abnormal valves and sensitivity to specific contaminants are key factors for the inclusion of teratological forms in diatom indices. How to quantify them?
  - M) What is the significance of deformities in a single species versus multiple species in an assemblage?
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145 Fig. 2. Number of papers on the topic of diatom teratologies in freshwater environments (natural and  
146 laboratory conditions) published from 1890 to 2015. Database provided in Supplementary Material.

147

## 148 2. TERATOLOGY FORMATION AND TRANSMISSION

149

### 150 2.1. Valve formation

151

152 Current routine identifications of diatom species are based on morphological characters such as  
153 symmetry, shape, stria density, and ornamentation. The characteristic shape of each diatom species  
154 results from a combination of genetic and cellular based processes that are regulated by environmental  
155 factors. There is a wealth of literature on valve morphogenesis, based both on ultrastructure observations  
156 and cellular (molecular and biochemical) processes. Descriptions of the processes involved in valve  
157 formation are provided, among others, by the following authors: Cox (2012); Cox et al. (2012); Falasco  
158 et al. (2009a); Gordon et al. (2009); Knight et al. (2016); Pickett-Heaps et al. (1979); Round et al.  
159 (1990); Sato et al. (2011); Schmid and Schulz (1979), and Kröger et al. (1994, 1996, 1997). Although a  
160 detailed description of cellular processes involved in valve formation is far beyond the scope of this  
161 discussion, the following section briefly summarizes the information given in the above-mentioned  
162 publications.

163  
164 Diatoms have external cell walls (frustule) composed of two valves made of amorphous polymerized  
165 silica. They mainly reproduce asexually during the life cycle with short periods of sexual activity.  
166 During cell division (mitosis), a new hypotheca (internal valve) is formed after cytokinesis. Silica  
167 polymerization occurs in a membrane-bound vesicle (silica deposition vesicle; SDV) within the  
168 protoplast (Knight et al., 2016). In pennate species, a microtubule center is associated with initiation of  
169 the SDV (Pickett-Heaps et al., 1979; 1990). The sternum (with or without a raphe) is the first structure to  
170 be formed followed by a perpendicular development of virgae (striae). In raphid diatoms the primary  
171 side of the sternum develops, then curves and fuses with the later-formed secondary side; the point of  
172 fusion generally appears as an irregular striae called the Voigt discontinuity or Voigt fault (Mann, 1981).  
173 Sketches and pictures of valve morphogenesis are presented in Cox (2012), Cox et al. (2012) and in Sato  
174 et al. (2011). The size of the new hypotheca formed by each daughter cell is constrained by the size of  
175 the parent valves, resulting in a gradual size reduction over time. Sexual reproduction initiates the  
176 formation of auxospores which can ultimately regenerate into large initial frustules (see Sato et al. 2008  
177 for information on auxosporulation). Asexual spore formation (Drebes 1966; Gallagher 1983) may also  
178 lead to large initial frustules and a larger population. Auxospore initial cells may differ greatly in  
179 morphology compared to cells from later in the cell line and these differences in cell shape should not be  
180 confused with deformity. These initial cells are however rather rare.

## 181 182 *2.2. Overview of teratogenesis*

183  
184 Deformities are commonly observed in natural diatom assemblages, but their frequency of occurrence is  
185 generally low (<0.5% according to Arini et al. 2012 and Morin et al. 2008a). The presence of multiple  
186 stressors, however, can significantly increase the proportions of deformed individuals. Falasco et al.  
187 (2009a) reviewed different types of deformities observed on diatom valves and the various potential  
188 mechanisms involved, as well as numerous environmental factors known to be responsible for such  
189 aberrations. We are aware that various stresses may induce teratologies, but here we focus our  
190 observations and discussion on the effects of toxic contaminants such as metals and organic compounds.

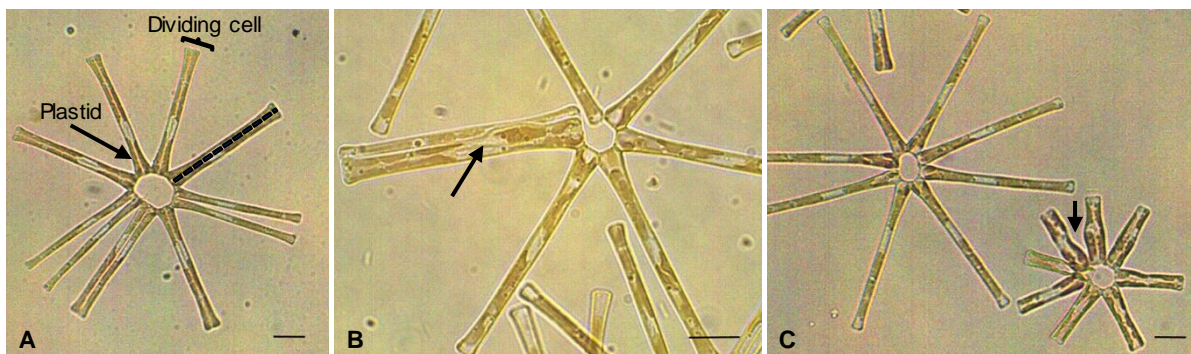
191  
192 Based on the current literature, mechanisms inducing teratologies are not fully understood. Due to  
193 physical (e.g., crowding, grazing) or chemical stresses (e.g., metals, pesticides, nutrient depletion),  
194 cellular processes involved in cell division and valve formation may be altered (Barber and Carter, 1981;  
195 Cox, 1890). One reliable explanation for teratology formation involves the microtubular system, an  
196 active part in the movement of silica towards the SDV. Exposure to anti-microtubule drugs (Schmid  
197 1980) or a pesticide (Debenest et al. 2008), can affect the diatom microtubular system (including  
198 microfilaments), leading to abnormal nucleus formation during cell division and to the deformation of  
199 the new valve. Despite this, Licursi and Gómez (2013) observed a significant increase in the production  
200 of abnormal nuclei (dislocation and membrane breakage) in mature biofilms exposed to hexavalent  
201 chromium. No teratological forms were observed, but the biofilm was exposed to the contaminant only  
202 for a short duration (96 h).

203  
204 Malformations can also be induced by other independent factors. For instance, malfunctions of proteins  
205 involved in silica transport and deposition (Knight et al., 2016; Kröger et al., 1994, 1996, 1997; Kröger  
206 and Poulsen, 2008), or proteins responsible for maintenance, structural and mechanical integrity of the  
207 valve (Kröger and Poulsen, 2008; Santos et al., 2013) would have significant impacts on teratologies.  
208 Metals could also inhibit silica uptake due to metal ion binding on the cell membrane (Falasco et al.,  
209 2009a). Likewise the initial formation of the valve can be affected by a lack of transverse perizonial

210 bands on the initial cell (Chepurnov et al., 2004; Mann, 1982, 1986; Sabbe et al., 2004; Sato et al., 2008;  
211 Toyoda et al., 2005; von Stosch, 1982; Williams 2001). Finally, biologically-induced damage related to  
212 bottom-up and top-down processes (e.g., parasitism, grazing, crowding) represent natural stresses that  
213 may result in abnormal valves (Barber and Carter, 1981; Huber-Pestalozzi, 1946; Stoermer and  
214 Andresen, 2006).

215  
216 Deformities can also be the consequence of plastid abnormalities or mis-positioning during cell division,  
217 as observed in standard laboratory cultures of *Asterionella formosa* Hassall (Kojadinovic-Sirinelli,  
218 Bioénergétique et Ingénierie des Protéines Laboratory UMR7281 AMU-CNRS, France; unpublished  
219 results) and under metal exposure in *Tabellaria flocculosa* (Roth) Kütz. (Kahlert, Swedish University of  
220 Agricultural Sciences; unpublished results). When considering normal cellular morphotypes of *A.*  
221 *formosa*, plastids are symmetrically positioned within dividing cell (Fig. 3A). In some cases, the plastids  
222 are significantly larger than normal, which may be the consequence of a microtubular system defect.  
223 This seems to induce formation of curved epivalve walls (Fig. 3B). As a consequence, daughter cells  
224 appear deformed (Fig. 2C). Extreme curvatures of the valve results in the formation of much smaller  
225 daughter cells (15–20  $\mu\text{m}$ ; Fig. 3C) compared to the mother cells (about 40–50  $\mu\text{m}$ ). The “small-cell”  
226 characteristic is then transmitted to subsequent daughter cells, resulting in colonies of small individuals.  
227 In this case, the deformity and reduction in size **does not seem to decrease cell fitness**, because the  
228 small-sized cells reproduce as efficiently as the normally-sized cells, or even faster. In this case, the  
229 abrupt size reduction is certainly a response to the environment. Interestingly, abnormally small cells  
230 seem to appear at the end of the exponential growth phase and to increase in frequency as cultures age  
231 (Falasco et al., 2009b). This may suggest that the “small-size aberration” was a consequence of nutrient  
232 depletion or the production of secondary metabolites that could stress *A. formosa*. Sato et al. (2008) also  
233 reported a sharp decrease in cell size accompanied by deformed individuals bearing two valves of  
234 unequal size in old cultures.

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239 Fig 3: Light micrographs of *Asterionella formosa* grown in laboratory conditions. Micrographs were  
240 made on a culture in late exponential growth phase. A: Normal cellular morphotype of an *A. formosa*  
241 colony. The dashed line represents septum position in a dividing cell. B: Abnormal morphotype. The  
242 arrow points to a curved epivalve wall. C: Colony of normally-sized cells (about 50  $\mu\text{m}$  long) cohabiting  
243 with a colony of small and deformed cells (about 15–20  $\mu\text{m}$  long). Scale bars represent 10  $\mu\text{m}$ .

244  
245  
246 According to Hustedt (1956) and Granetti (1968), certain morphological alterations are not induced by  
247 genetic changes, because the diatoms return to their typical form during the subsequent sexual cycle. In



248 contrast other authors have elevated altered forms to the variety or species level (e.g., Jüttner et al.,  
249 2013), thus assuming taxonomic distinctness. Biochemical and molecular investigations of clones with  
250 distinct morphotypes would thus be required to assess whether deformities are short term phenotypic  
251 responses, problems with gene expression (i.e., assembly line malfunction) or true alterations in the  
252 genes. The evolution of a species, at least in part, is a temporal process of physiological (teratological)  
253 changes resulting in “deviations from the normal type of organism/species”. The gain or loss of any  
254 structure, like for example rimoportulae, potentially represents a new species. Even a change in the  
255 position of a structure can constitute a new species. Teratologies under temporal changes can influence  
256 populations or species. For the purpose of this discussion paper, longer temporal events of teratology  
257 (reproduction of selected deformity over generations) can lead to speciation events, while short term  
258 teratologies (not reproducible in the next generation after sexual reproduction) are considered dead end  
259 and non-taxonomically significant.

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### 262 *2.3 Abnormal overall shape*

263

264 The initial question here would be: “when does an atypical valve outline fall into the abnormal  
265 category”? For the purpose of this discussion, an abnormal outline is when aberrations affect valve  
266 symmetry, or when defects alter the “normal” shape of the diatom. This working definition excludes  
267 deviations from expected shape changes as cells get smaller (natural variability). Variability in shape  
268 related to post auxosporulation is difficult to differentiate from an abnormal form, but these forms are  
269 considered as rare. The second question is when does the deviation from the “common shape” become  
270 significant enough to be deformed? This question is particularly relevant when aberrations are subtle and  
271 subjectively identified with variability between analysts. On the other hand, marked deviations from the  
272 normal shape are easy to notice and classify as aberrant. **Deformities** affecting the general valve outline  
273 **are assumed to be passed along from generation to generation through asexual cell division.**  
274 Replication of the deformity happens because the newly formed valves must “fit into” the older valves;  
275 thus the aberration is copied and the number of abnormal valves increases even though “new errors” do  
276 not occur. This scenario is clearly stated in numerous publications, as for instance:

277

278 *“A morphological variation in the frustules outline is easily transmitted through generations, others, like*  
279 *the pattern and distribution of the striae, are not: this is the reason for the lower frequency of the latter*  
280 *alterations.” Falasco et al. (2009a)*

281

282 *“If the damaged cells survive, they will be able to reproduce: in this case, the daughter clones will build*  
283 *their hypotheca on the basis of the damaged epitheca, spreading the abnormal shape through the*  
284 *generations” Stoermer and Andresen (2006)*

285

286 This propagation of abnormal valves during cell division may explain why valve outline deformities are  
287 the most frequently reported in the literature and with the highest abundances. For example, Leguay et  
288 al. (2016) observed high abundances of individuals presenting abnormal valve outlines in two small  
289 effluents draining abandoned mine tailings (50% and 16%, all of the same *Eunotia* species). Valve  
290 outline deformities reaching 20 to 25% (on *Fragilaria pectinalis* (O.F.Müll.) Lyngb.) were observed at a  
291 site located downstream of textile industries introducing glyphosate in the Cleurie River, Vosges, France  
292 (Heudre, DREAL Grand Est, Strasbourg, France; unpublished results). Kahlert (2012) found deformities  
293 of up to 22% on *Eunotia* species in a Pb contaminated site. This proportion of abnormal valves is  
294 markedly elevated and to our knowledge no other field study has observed such high numbers. The

295 effect of carry-over from cell division could explain the high frequency of abnormal individuals  
296 (reaching up to >90% with a marked indentation) in a culture of *Gomphonema gracile* Ehrenb. from the  
297 IRSTEA-Bordeaux collection in France (Morin, IRSTEA-Bordeaux, France; unpublished results).

298  
299 If cell division is the key agent for the transmission of valves with abnormal outlines due to the “copying  
300 effect”, then this raises the question of **how does the first frustule get deformed?** An initial abnormal  
301 valve must start the cascade of teratologies: logically, we could argue that the initial deformity appears  
302 during sexual reproduction when the frustule of the new cells is formed without the presence of an  
303 epivalve as a template. Hustedt (1956) discussed this scenario where he suggested that particular  
304 environmental conditions during auxospore formation may induce morphological changes that are  
305 perpetuated during vegetative reproduction, giving rise to a population with a morphology different from  
306 the parental line. This new abnormal cell would then divide by mitosis and legate the abnormal shape to  
307 all subsequent daughter cells, as also suggested by Stoermer (1967). This is in-line with the observation  
308 that the above-mentioned *G. gracile* bearing the marked incision on the margin is ca. 50% larger than its  
309 “normal” congeners of the same age. On the other hand, there is also the possibility or hypothesis in the  
310 gradual appearance of an abnormal outline that is accentuated from generation to generation. First, a  
311 very subtle deviation from the normal pattern appears on the forming hypovalve and a deformity is not  
312 noticed. This subtle deviation from the normal shape is progressively accentuated by the newly forming  
313 hypovalve leading to a very mild abnormality of the overall shape, and so on through multiple  
314 successive divisions resulting in a population of slightly abnormal to markedly deformed individuals. If  
315 this scenario is possible, then the opposite situation could also be plausible: the subtle deviation from the  
316 normal overall shape is “fixed” or “repaired” during subsequent cell divisions instead of being  
317 accentuated. In another scenario, the epivalve could be normal and the hypovalve markedly deformed,  
318 potentially resulting in an individual that would not be viable. Sato et al. (2008) reported something  
319 similar in old cultures of *Grammatophora marina* (Lyngb.) Kütz. where drastic differences in valve  
320 length between epivalve and hypovalve (up to 50% relative to epitheca) were observed, suggesting that a  
321 “perfect fit” is not always necessary. These authors also observed cells that had larger hypothecae than  
322 epithecae, implying expansion before or during cell division. In this case, are these growth forms viable  
323 and sustainable?

324

#### 325 *2.4 Other deformities*

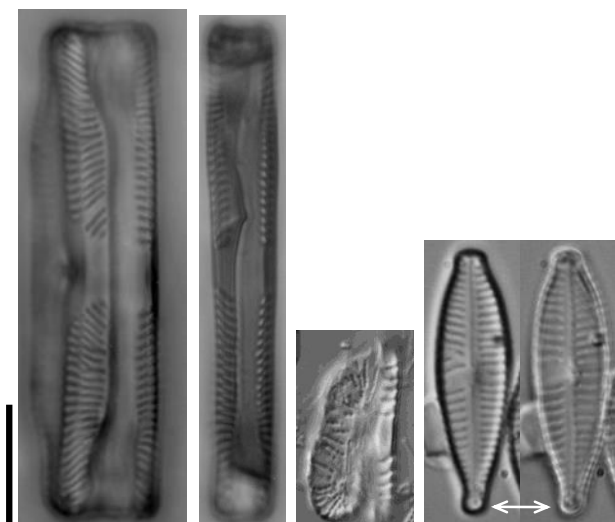
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327 Although irregular valve outlines appear to be a common and frequent type of teratology, it is not  
328 always the dominant type of deformity observed within a given population. For instance, Arini et al.  
329 (2013) found abnormal striation patterns and mixed deformities to be the most frequently observed  
330 aberration in a Cd exposure experiment using a culture of *Planothidium frequentissimum*. Deformities  
331 on the same species were observed more frequently on the rapheless-valve and the structure affected was  
332 generally the sinus/cavum and less frequently the striae (Falasco, Aquatic Ecosystem Lab., DBIOS,  
333 Italy; unpublished results from field samples).

334

335 The sternum is the first structure to be produced by the SDV; if an aberration occurs in this region,  
336 other/additional aberrations may subsequently appear in striation patterns occurring later during valve  
337 formation. This could therefore be considered as “collateral damage” because of an abnormal sternum  
338 (including the raphe), leading to mixed deformities. For example, Estes and Dute (1994) have shown  
339 that raphe aberrations can lead to subsequent valve and virgae (striae) distortions. However, abnormal  
340 striation patterns have also been observed on valves showing a normal raphe or sternum system.  
341 Because the appearance of striae aberrations is believed to happen later during valve formation, should

342 these teratologies be considered as a signal reflecting a mild deleterious effect? The same reasoning  
343 applies to the general valve outline; should it be considered as a minor response to stress or as collateral  
344 damage? Another interesting deformity is the presence of multiple rimoportulae on *Diatoma vulgaris*  
345 valves. Rimoportulae are formed later in the morphogenesis process; should this type of alteration be  
346 considered equal to raphe or striae abnormalities? Our observations on raphid diatoms suggest that  
347 individuals generally exhibit **abnormal striation or sternum/raphe anomalies only in one valve**,  
348 while the other valve is normal (Fig. 4). The possibility of an abnormal structure on the two valves of a  
349 cell is not excluded, and would therefore suggest two independent responses to stress. A mother cell  
350 with one abnormal valve (e.g., raphe aberration) will produce one normal daughter cell and one  
351 abnormal daughter cell, resulting in a decreasing proportion of teratologies if no additional “errors”  
352 occur. This makes deformities in diatom valve structure, other than the abnormal outline category, good  
353 biomarkers of stress because the deformity is not directly transmitted and multiplied through cell  
354 division. In other words, aberrations occurring at different stages of valve formation may not all have the  
355 same significance/severity or ecological signal, and this may represent important information to include  
356 in bioassessments. The problem, however, is that these abnormalities are often rare.  
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360 Fig. 4. Examples of diatom frustules showing deformities on one valve, while the other valve is normal.  
361 The first example represents a type (iii) deformity (striae aberration), the second a type (i) deformity  
362 (shape aberration), the third shows a type (iv) deformity (mixed) and the last two pictures are valves of  
363 one individual where only the left picture shows striae deformities while the other valve is normal (see  
364 section 3.1. Types of deformities). Scale bar = 10 microns.

#### 365 366 2.5. Are deformed diatoms viable, fit and able to reproduce? 367

368 Based on numerous laboratory observations made by authors of this publication, it seems clear that  
369 deformed diatoms in cultures are able to reproduce, even sometimes better than the normal forms (e.g.,  
370 deformed *Asterionella formosa*, section 2.2 and deformed *Gomphonema gracile*, section 2.3). However,  
371 the ability of abnormal cells to survive and compete in natural environments is potentially affected.  
372 Teratologies have different impacts on physiological and ecological sustainability depending on the  
373 valve structure altered.. Valve outline deformation, for instance, could prevent the correct linking spine  
374 connections during colony formation. Alterations in the raphe system could limit the locomotion of  
375 motile diatoms, although this has not been observed based on preliminary experiments conducted on *G.*

376 *gracile* (Morin, IRSTEA-Bordeaux, France; unpublished results). Motility represents an important  
377 ecological trait especially in unstable environmental conditions because species are able to find refuge in  
378 more suitable habitats. Alterations in the areolae patterns located within the apical pore fields may  
379 prevent the correct adhesion of erected or pedunculated taxa to the substrate, impairing their ability to  
380 reach the top layer of the biofilm and compete for light and nutrients.

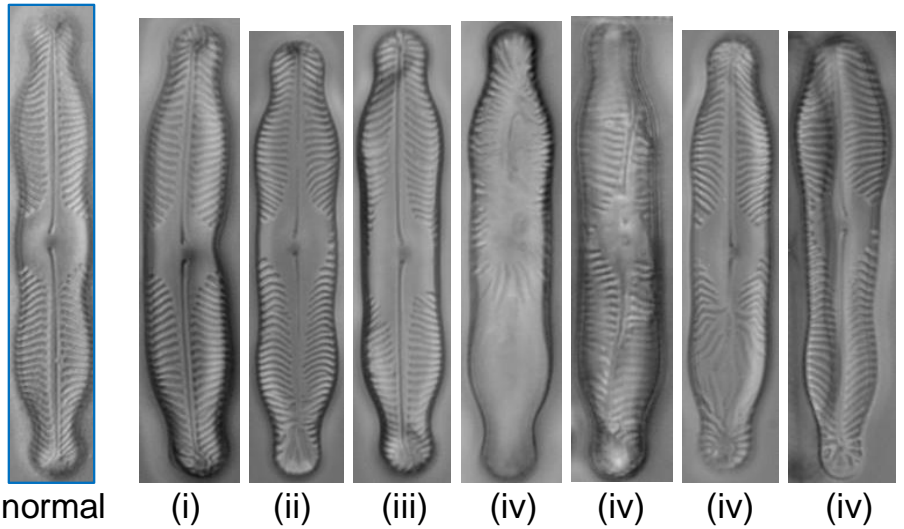
### 381 382 3. THE ECOLOGICAL MEANING OF TERATOLOGICAL FORMS

#### 383 384 3.1 *Types of deformities*

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386 A good fit was observed in certain studies between the abundance of teratologies and the presence of a  
387 contaminant (review in Morin et al. 2012). However, other studies have failed to show a clear  
388 relationship between the frequency of abnormal forms and the level of contamination along a gradient  
389 (e.g., Lavoie et al., 2012; Leguay et al., 2015); this is the “raison d’être” of this paper. Here we discuss  
390 potential avenues to deepen our interpretation of the ecological signal provided by diatoms. Do  
391 deformed cells reproduce normally? Do they consistently reproduce the teratology? These questions are  
392 intimately linked to the various types of teratologies observed. **The type of deformity may therefore**  
393 **be an important factor to consider in biomonitoring because they may not all provide equivalent**  
394 **information** (Fig. 5). Most authors agree to categorize teratological forms based on their type,  
395 summarized as follow: (i) irregular valve outline/abnormal shape, (ii) atypical sternum/raphe (iii)  
396 aberrant striae/areolae pattern, (iv) mixed deformities. Despite the fact that various types of aberrations  
397 are reported, most authors pool them together as an overall % of teratologies (e.g., Roubex et al., 2011;  
398 Lavoie et al., 2012; Leguay et al., 2015; Morin et al., 2008a, 2012) and relate this stress indicator to  
399 contamination. Only a few studies report the proportion of each type of deformity (e.g., Arini et al.,  
400 2013; Pandey et al., 2014; 2015; Pandey and Bergey, 2016).

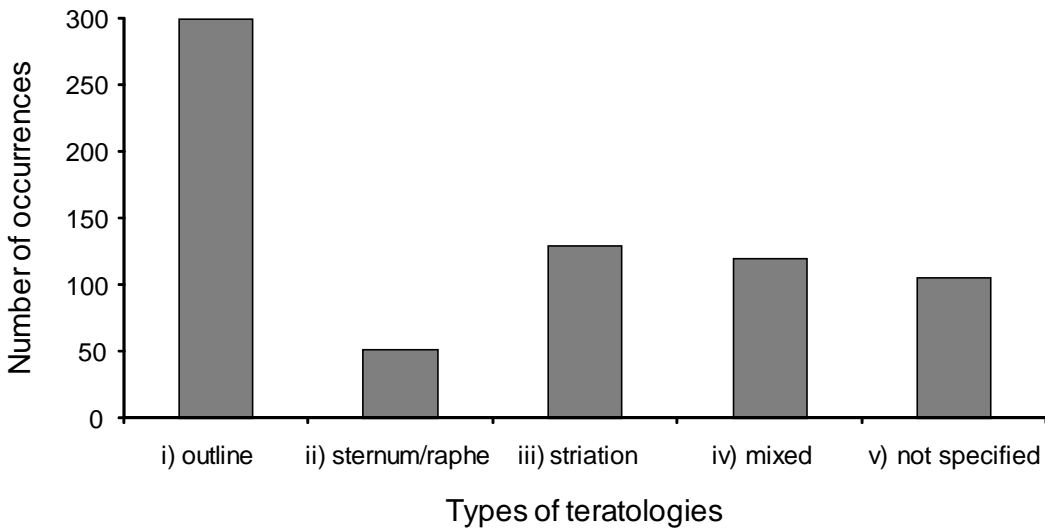
401  
402 Based on a literature review of more than 100 publications on diatoms and teratologies, we created an  
403 inventory of >600 entries concerning various diatom taxa reported as deformed (and the type of  
404 teratology observed) as a response to diverse stresses (Appendix 1). This database is an updated version  
405 of the work presented in Falasco et al. (2009a). We assigned each of the reported teratologies to one of  
406 the four types of aberrations, which resulted in a clear dominance of abnormalities affecting valve  
407 outlines (Fig. 6).

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Fig. 5. Examples of different types (i, ii, iii and iv) and degrees of deformities observed on *Pinnularia* sp. valves in a culture exposed to cadmium. (i) irregular valve outline/abnormal shape, (ii) atypical sternum/raphe (iii) aberrant striae/areolae pattern, (iv) mixed deformities. Should they all be considered equally meaningful for biomonitoring purposes? Scale bar = 10 microns.



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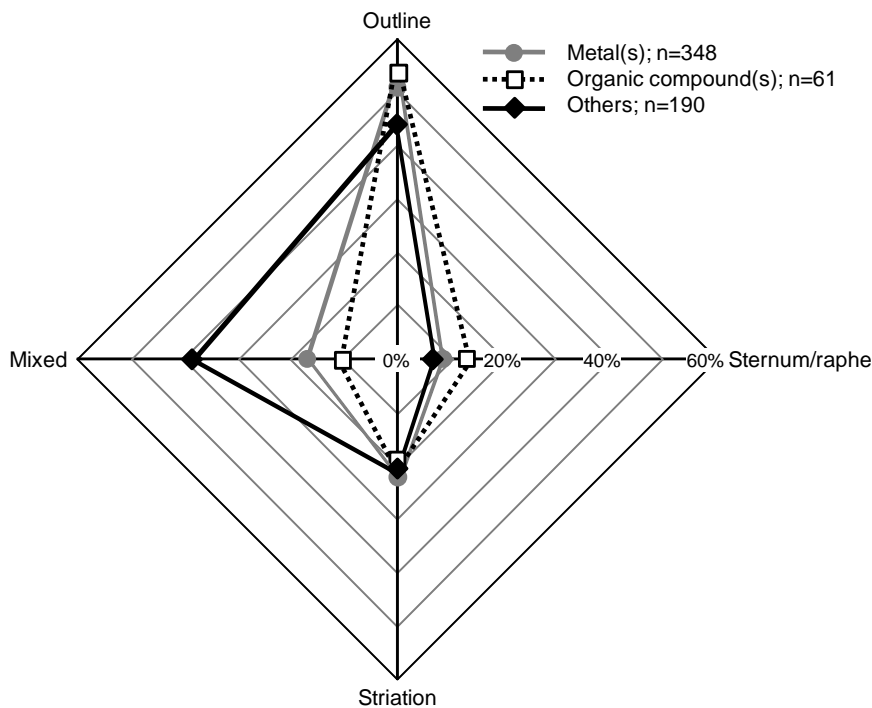
Fig. 6. Types of deformities reported in the literature for various diatom species. The data used to create this graph come from the publications reported in Appendix 1.

### 3.2 Are deformities toxicant-specific?

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As deformities are expected to occur during morphogenesis, different types of deformities may result from exposure to contaminants with different toxic modes of actions. **Are all toxicants likely to induce similar deformities?** From our database, the occurrences of the different types of deformities were grouped into three categories of hypothesized cause (including single source and mixtures): metal(s), organic compound(s), and a third one with all other suspected causes (*a priori* non-toxic) such as

430 crowding, parasitism, and excess nutrients (excluding unspecified causes). The results presented in Fig.  
 431 7 should be interpreted with caution with unequal data available for the different categories (in  
 432 particular, low number of data for organic compounds). Similar patterns in the distribution of  
 433 deformities were found with exposure to organic and inorganic toxicants; in >50% of the cases, solely  
 434 the valve outline was mentioned as being affected. Other types of deformities were, by decreasing order  
 435 of frequency: striation (ca. 20%), followed by mixed deformities (ca. 14%), and sternum/raphe  
 436 alterations (ca. 12%). This is in concordance with other observations indicating that exposures to metals  
 437 led to about the same degree of deformations as exposures to herbicides; in both cases, the highest toxin  
 438 concentrations caused the highest ratio of sternum/raphe deformities to outline deformities (Kahlert,  
 439 2012). In contrast, other than toxic exposure conditions (or unknown) resulted in deformities affecting  
 440 cell outline in 45% of the cases, while 30% were mixed teratologies, 20% affected the striae and less  
 441 than 10% the sternum/raphe system. Thus, the distributions of deformity types for toxic and non-toxic  
 442 exposure were slightly different, which underscores the potential of deformity type to clarify the nature  
 443 of environmental pressures and strengthens the need for describing precisely the deformities observed.



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446 Fig. 7. Deformity occurrence (expressed as %) classified by types and reported causes of stress in field  
 447 and laboratory studies. The data were gathered from the information available in the publications  
 448 presented in Appendix 1. Data were not considered for this graph when the cause of teratology was not  
 449 specified.

450

451 Figure 7 suggests that mixed deformities occur more frequently for environmental stresses (including  
 452 various perturbations such as nutrient depletion) than for contaminant-related stresses. However, timing  
 453 could also be a potential cause of differentiation between the various types of aberrations. Timing here  
 454 can be interpreted in two very different ways. First, it can be related to the chronology of teratology  
 455 appearance in ecosystems or cultures. For example, if an abnormal valve outline aberration occurs early  
 456 during an experiment, then this deformity will be transmitted and multiplied through cell division.  
 457 However, if the individual bearing the abnormal valve shape appears later in time (or if this type of  
 458 deformity does not occur), then other types of deformities may appear and become dominant. On the

459 other hand, the presence of one type of deformity over another could also be associated to the moment  
460 during cell formation at which the stress occurs, i.e., that the contaminant reached the inner cell during  
461 the formation of one structure or another. There is also the possibility that an abnormal outline deformity  
462 is a secondary result from an impact affecting another mechanism of valve formation.

### 463 464 3.3. *Proneness to deformities and tolerance to contamination* 465

466 Are all diatom species equally prone to different types of deformities? From the literature published over  
467 the past ca. 70 years, we present species observed, the type of deformities noted and the tolerance to  
468 contamination when reported (Appendix 1). Based on these data, we observed that the most common  
469 aberration is valve shape (as also presented in Fig. 6) and that this aberration is particularly evident for  
470 araphid species. Deformities in araphid species had ca. 60% of the reported deformities as irregular  
471 shape. This finding suggests that araphid diatoms may be more “prone” to showing abnormal valve  
472 outlines compared to raphid or centric diatoms. Therefore, araphid diatoms may not be good biomarkers  
473 compared to other species especially considering that shape aberration is multiplied by cell division (see  
474 above discussion). However, proneness to different types of deformities differed among long and narrow  
475 araphids: *Fragilaria* species mostly exhibited outline deformity (67%), compared to the robust valves of  
476 *Ulnaria* species (29%).

477  
478 In addition to araphids, *Eunotia* species also have a tendency to show abnormal shapes (>75% in our  
479 database). This suggests that the formation of a long and narrow valve may provide more possibility for  
480 errors to occur or that the araphid proneness to deform may result from the absence of a well-developed  
481 primary and secondary sternum/raphe structure that could strengthen the valve? This argument may also  
482 be valid for *Eunotia* species that have short raphes at the apices, which is supported by irregularities  
483 mostly observed in the middle portion of the valve. Specimens of the *Cocconeis placentula* Ehrenb.  
484 complex (monoraphids) from natural assemblages collected in contaminated and uncontaminated waters  
485 have also frequently been observed with irregular valve outlines in Italian streams (Falasco, Aquatic  
486 Ecosystem Lab., DBIOS, Italy; unpublished results). This genus might be considered as unreliable in the  
487 detection of contamination because it seems to be prone to teratologies (mainly affecting valve outline  
488 which is transmitted during cell division).

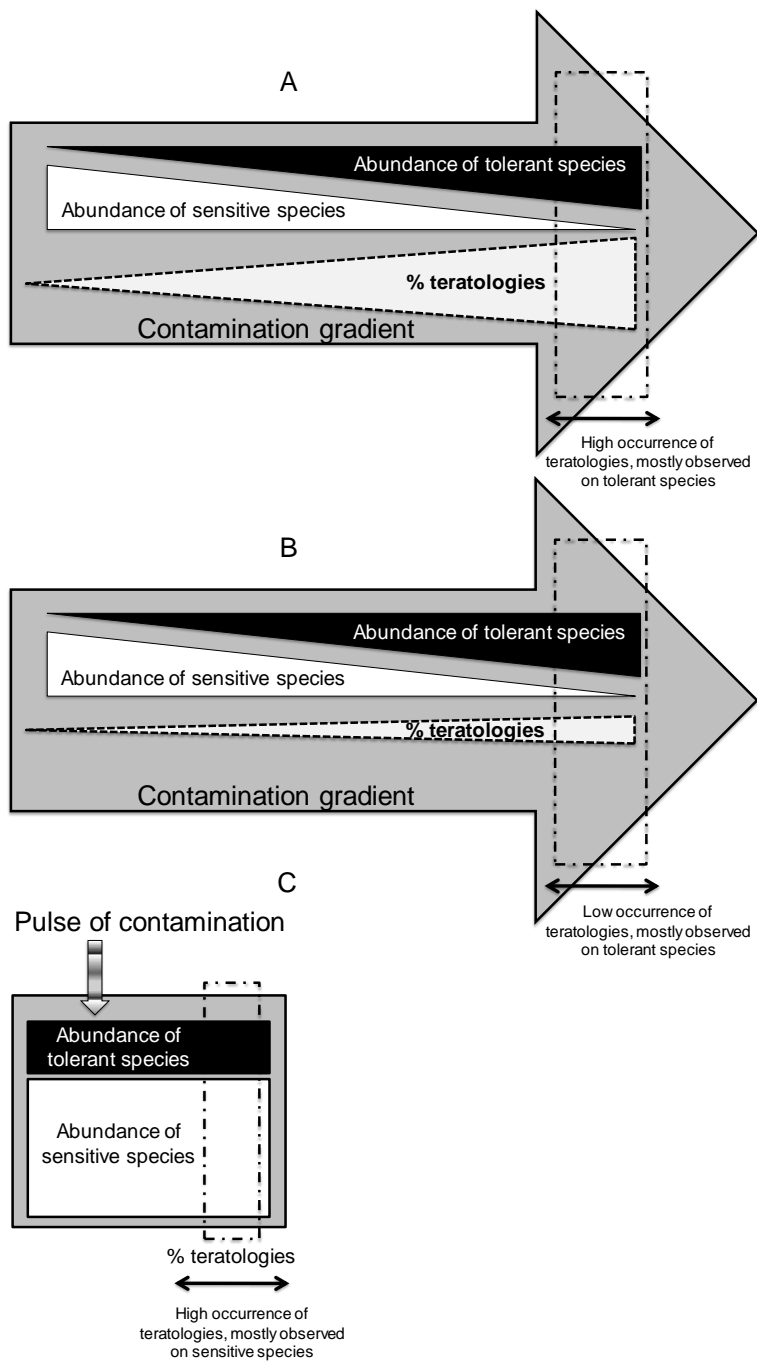
489  
490 A puzzling observation is the presence of deformities affecting only one species among the array of  
491 other species composing the assemblage. The abnormal specimens may all belong to the dominant  
492 species in the assemblage or not. When this situation is encountered for irregular shape teratologies, we  
493 can argue that this is in part due to the transmission of the aberration during cell division. This was the  
494 case at a mine site (with an assemblage almost only composed of two species) where 16% of the valves  
495 showed an abnormal outline and were all observed on species of *Eunotia*, while no teratology was  
496 observed on the other dominant species (Leguay et al., 2015). The same situation was noted in the  
497 previously mentioned example from the French River contaminated by a pesticide where 20–25% of  
498 abnormal shapes were observed on *F. pectinalis* (O.F.Müll.) Gray. On the other hand, when only one  
499 species in the assemblage presents deformities of the sternum/raphe structure and/or the striae, this  
500 suggests a true response to a stress event by a species prone to deformities. This has been observed at a  
501 mine site (high Cu) where deformities reached 8% and were always observed on *Achnanthydium*  
502 *deflexum* (Reimer) Kingston (Leguay et al., 2015).

503  
504 Numerous species are known to be tolerant to contaminants. For example, Morin et al. (2012) provide a  
505 list of diatom species that are cited in the literature as tolerant or intolerant to metals. As explained in

506 their review, species that are able to tolerate toxic stress will thrive and dominate over sensitive species.  
507 Similar observations led to a concept called Pollution-Induced Community Tolerance (PICT) developed  
508 by Blanck et al. (1988). According to this paradigm, the structure of a stressed assemblage is rearranged  
509 in a manner that increases the overall assemblage tolerance to the toxicant. Considering an assemblage  
510 where most species are tolerant, we would expect to observe less teratologies. However, this is not  
511 necessarily the case as **aberrations are commonly encountered on tolerant species**. This observation  
512 is not a surprise because even tolerant and dominant species are still under stress conditions (Fig. 8A). In  
513 this scenario, most teratologies are observed on tolerant species and very few on sensitive species due to  
514 their rarity in the assemblage. However, this is not always the case as some tolerant species are less  
515 prone to deformities than others (Fig. 8B), resulting in fewer deformed valves in highly contaminated  
516 environments. **This raises the question as to whether or not deformities should be weighted as a**  
517 **function of species proneness to abnormalities**. Furthermore, species have been shown to develop  
518 tolerance resulting in a population adapted to certain stressors, which then may or may not show  
519 deformities. For example, Roubéix et al. (2012) observed that the same species isolated from upstream  
520 and downstream of a Cu-contaminated site has different sensitivities to Cu, i.e., that not all populations  
521 of a species have the same tolerance. We should therefore expect variability in the sensitivity to  
522 deformation, even within tolerant species.

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526 Fig. 8. Conceptual schematization representing the interplay between the abundance of sensitive and  
527 tolerant diatom species and percent teratologies under different contamination scenarios. Dashed  
528 rectangles highlight the percentage of deformity expected in diatom assemblages sampled (A and B)  
529 along a contamination gradient of chronic exposure or (C) intermittent (pulse) contaminations.  
530

531 There is also the scenario where diatom assemblages are stressed by intermittent events of  
532 contamination; a spill from a mine tailing pond for example. If such assemblages are dominated by  
533 metal-sensitive species, we would expect to observe more teratologies on these species and very few on  
534 tolerant species as they are rare (Fig 8C). This, of course, is based on the hypothesis that **deformities**

535 **will appear on sensitive species** faster than the time it takes the assemblages to restructure towards a  
536 dominance of tolerant species (which would bring us back to the above-mentioned scenarios; also see  
537 section 5.3).

538

539 We would furthermore expect that tolerance to deformities would not only be species-dependent, but  
540 also environment-dependent. In general, we hypothesize that suboptimal conditions (e.g., pH, nutrients,  
541 light, competition) favour the occurrence of teratological forms, while optimal conditions decrease their  
542 occurrence. Environmental conditions would then set the baseline on how sensitive a diatom assemblage  
543 is to toxic impacts. For example, some samples from pristine forest wetlands/swamps with low pH and  
544 no source of contaminants in the Republic of the Congo showed cell outline deformities (2%) (Taylor,  
545 School of Biological Sciences, NWU, South Africa; unpublished results). The presence of teratologies  
546 was therefore assumed to be attributed to the low pH of the environment or to the fact that these isolated  
547 systems had become nutrient limited. The key message from this section is to acknowledge that  
548 deformities may be found under different stresses (not only contamination by metals or organic  
549 compounds), and also that deformed diatoms are not always observed in highly contaminated  
550 environments.

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## 553 4. ISSUES WITH TERATOLOGY ASSESSMENT

554

### 555 4.1. *Small species and problematic side views*

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557 **Certain abnormalities are more or less invisible under a light microscope, particularly for small**  
558 **species.** There are numerous publications reporting valve aberrations observed with a scanning electron  
559 microscope which would otherwise be missed with a regular microscope (e.g., Morin et al., 2008c). This  
560 is problematic in a biomonitoring context, especially when a contaminated site is dominated by small  
561 species such as *Fistulifera saprophila* (Lange-Bertalot & Bonk) Lange-Bertalot, *Mayamaea atomus*  
562 (Kütz.) Lange-Bertalot or *Achnantheidium minutissimum* Kütz., or by densely striated species like  
563 *Nitzschia palea* (Kütz.) W.Sm.. In these cases, the frequency of deformities may be underestimated.  
564 **Would it be more appropriate to calculate a percentage of teratologies considering only the species**  
565 **for which all structures are easily seen under a light microscope?** In the same line of thought, how  
566 should we deal with specimens observed in girdle view where deformities are often impossible to see?  
567 This situation is of concern when the dominant species tend to settle on their side, such as species  
568 belonging to the genera *Achnantheidium*, *Gomphonema*, and *Eunotia*. It could therefore be more  
569 appropriate for bioassessment purposes to calculate the teratology percentages based on valve view  
570 specimens only. This recognizes that the proportion of aberrations on certain species, often seen in girdle  
571 view, may consequently be underestimated. A separate count of deformities for species regularly  
572 observed side-ways could also be performed only considering valve-view specimens, and the %  
573 teratologies could then be extrapolated to the total valves enumerated for this species. This proposal of a  
574 separate count is based on the likely hypothesis that a deformed diatom has the same probability to lay  
575 in one or the other view as normal specimens.

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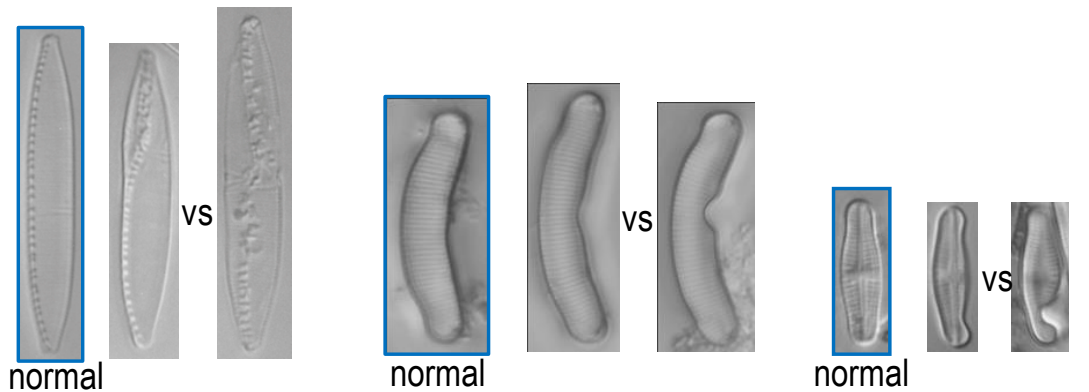
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### 579 4.2. *How to score the severity of the teratology?*

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581 **The severity of teratologies**, i.e. the degree of deviation from the “normal” valve, is usually not  
 582 assessed in biomonitoring (Fig. 9). Would this information be useful to better interpret the magnitude of  
 583 the stress? This question leads to another: how to quantify the severity of valve deformities depending  
 584 on the type of abnormality? The line between a normal variation and a slight aberration is already  
 585 difficult to draw (Cantonati et al., 2014); is it possible to go further in this teratology assessment and  
 586 score the deformities under slight-medium-pronounced deviations from the normal shape/pattern? This  
 587 additional information could be of ecological interest, but might also be very subjective and limited to  
 588 individual studies or situations. Image analysis might help to solve this problem in the future, although  
 589 preliminary tests using valve shape have been inconclusive so far (Falasco, 2009).

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Fig. 9. Normal valve, slightly deformed valve, and markedly deformed valve of *Nitzschia palea*, *Eunotia* sp., and *Achnanthidium minutissimum* exposed to metals. Scale bar = 10 microns.

## 599 5. IMPLICATIONS FOR BIOMONITORING

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### 601 5.1. Deformities as an indicator of unhealthy conditions.

603 The frequency of deformities has been reported as a good biomarker of metal contamination, and in  
 604 fewer studies to organic contamination. In most cases the effects of contamination on diatom  
 605 teratologies were evaluated using percent of deformities regardless of their type. The majority of the  
 606 studies either compared a contaminated site with a reference site or tested experimental conditions with  
 607 a control and one or two contamination levels. As examples, Duong et al. (2008) and Morin et al.  
 608 (2008a) found a significantly higher presence of teratologies in a stream contaminated by metals (Cd and  
 609 Zn) compared to its upstream control. In laboratory experiments using a monospecific diatom culture or  
 610 on biofilm communities exposed to three levels of Cd (control, 10–20 µg/l and 100 µg/l), Arini et al.  
 611 (2013), Gold et al. (2003) and Morin et al. (2008b) observed significantly higher proportions of  
 612 deformed individuals in the contaminated conditions, but the overall difference in % teratologies  
 613 between concentrations of Cd was not statistically significant. These examples underscore the usefulness  
 614 of teratologies as a biomarker of stress. However, linking the magnitude of the response to the level of  
 615 contamination is not as straightforward as comparing contaminated and reference conditions. For  
 616 example, Cattaneo et al. (2004) only found a weak relationship between deformities and metal  
 617 concentrations in lake sediments. Lavoie et al. (2012) were not able to correlate the occurrence of valve  
 618 deformities with a gradient in metal concentrations in a contaminated stream. Leguay et al. (2015)

619 observed the highest proportions of deformities at the most contaminated sites, but significant  
620 correlations were not observed using each metal separately and the confounding effects of metal  
621 contamination and low pH (~3) made the direct cause-effect link difficult to assess. In these last studies,  
622 more aberrant diatom valves were observed at the contaminated sites compared to the reference sites,  
623 but the correlation between teratologies and metal concentrations collapsed in the middle portion of the  
624 contamination gradient. In laboratory cultures, a linear correlation has been observed between the  
625 frequency of deformities and metal concentrations, except for the highest concentration in the gradient  
626 where fewer deformations were noted (Gonçalves, University of Aveiro, Portugal and Swedish  
627 University of Agricultural Sciences, Uppsala, Sweden; unpublished results). This result could be  
628 explained by the fact that deformed cells may be less viable at very high metal concentrations.

629  
630 Using an estimate of metal exposure/toxicity (e.g. CCU, cumulative criterion unit score; Clements et al.,  
631 2000) may result in a better fit between metal contamination (expressed as categories of CCU) and  
632 deformity frequency. Using this approach, Morin et al. (2012) demonstrated that >0.5% of deformities  
633 were found in “high metal” conditions. Falasco et al. (2009b) used a similar approach and also observed  
634 a significant positive correlation between metals in river sediments (Cd and Zn expressed as a toxicity  
635 coefficient) and deformities (expressed as deformity factors). Some metric of integrated information  
636 summarizing (i) the response of diatoms to contaminants (e.g. score based on teratologies) and (ii) the  
637 cumulative stresses (e.g. using an overall “stress value”) seems to be an interesting approach to  
638 establishing a link between contamination level and biomarker response.

#### 639 640 *5.2. Refining ecological signals by weighing teratologies*

641  
642 Water quality assessment with respect to toxic events linked to diatom indices could potentially be  
643 refined by “**weighting the deformities**” as a function of deformation type. Moreover, this assessment  
644 could also be pushed further by considering the severity of the deformity, the proneness of the species to  
645 present abnormal forms and diversity of the species affected. Although abnormal cells are often  
646 classified by types, there seems to be no ecological information extracted from this approach. Here, we  
647 raise the discussion on how (or if!) we could improve biomonitoring by considering the specific  
648 teratologies and their severity by modifying their weight/importance. A systematic notation/description  
649 of the type and severity of deformation and species affected would be required. Thus, “ecological  
650 profiles” of teratologies could be determined, as a function of the species affected and type of deformity.  
651 Indeed, improving our understanding about life cycle processes and the various types of deformations  
652 would greatly enhance the assignment of impact scores for biomonitoring, which is the essence of this  
653 paper.

654  
655 The observation that valve aberrations are routinely found in extremely contaminated conditions led  
656 Coste et al. (2009) to include the occurrence and abundance of deformed individuals in the calculation of  
657 the biological diatom index BDI. In their approach, observed deformities were assigned the worst water  
658 quality profile, meaning that their presence tend to lower the final water quality score. This means that  
659 the severity and type of malformation, and the species involved were not considered; all teratologies  
660 were scored equally. However, based on the discussion presented in section 4, this approach may be  
661 simplistic and valuable ecological information on the characteristics of the deformities lost. For example  
662 in the case of araphid diatoms prone to deformation (even in good quality waters, i.e., Cremer and  
663 Wagner, 2004), the presence of teratologies may not always reflect the true degree of contamination. As  
664 a case example, Lavoie et al. (2012) observed 0.25–1% deformations at a site highly contaminated by  
665 metals and dominated by *A. minutissimum*, while the number of abnormal forms increased up to 4%

666 downstream at less contaminated sites with species potentially more prone to deformation. More  
667 specifically, all aberrations affected valve outline and were mostly observed on *Fragilaria capucina*  
668 Desm.. For this reason, it was impossible for the authors to correlate metal concentrations with  
669 teratologies. In this particular scenario, changing the weight of the deformations based on the type of  
670 deformity recorded and by considering the species (and their proneness to form abnormal valves) would  
671 potentially better reflect the environmental conditions.

672

673 An experiment on the effect of cadmium on a *Pinnularia* sp. (Lavoie, INRS-ETE, Quebec, Canada;  
674 unpublished results) will serve as an example illustrating the potential interest in scoring teratology  
675 severity. In this experiment, a higher percentage of deformed valves were observed after 7 days of  
676 exposure to Cd compared to a control. The observed teratologies were almost exclusively mild  
677 aberrations of the striation pattern. The proportions of deformed valves increased even more after 21  
678 days of exposure, with more severe teratologies of different types (sternum/raphe, striae). In this  
679 experiment, considering the types and severity of the deformities (mild vs severe) would better define  
680 the response to Cd between 7 days and 21 days of exposure, which would bring additional information  
681 on toxicity during longer exposure times. Developing the use of geometric morphometry approaches  
682 could also help to quantitatively assess the deviation to the normal symmetry/ornamentation.

683

684 Also worth discussing is the presence of abnormally shaped valves in high abundances. If mitosis is the  
685 main precursor for the occurrence of abnormal valve shape, then it is legitimate to wonder if these  
686 aberrations really reflect a response to a stressor or if they are the result of an error “inherited” from the  
687 mother cell? If cell division multiplies the number of valves showing abnormal outlines, then this type of  
688 deformity should potentially be down-weighted or not considered for biomonitoring. However, to  
689 identify valves with irregular shapes as a result of contamination versus inherited irregularities is near  
690 impossible without running parallel control studies.

691

692 Finally, the score related to the frequency of deformities could also be weighted by species diversity  
693 estimates. For example, if species diversity in the community is very low (e.g. one species, or one  
694 strongly dominating species and some rare species) there is a potential bias in the assessment of the  
695 response to a stressor. The impact may be overestimated if the species is prone to deformity, and  
696 underestimated otherwise. Therefore, in addition to considering the proneness to deformity, teratology-  
697 based monitoring could also include a metric where the % deformity is combined with information on  
698 species diversity. This should improve ecological interpretations.

699

### 700 *5.3 Biological descriptors complementing a teratology-based metrics*

701

702 This paper has focused on the presence of diatom valve teratologies as an indicator of environmental  
703 stress, specifically for contaminants such as metals and pesticides; this excludes eutrophication and  
704 acidification for which diatom-based indices and metrics already exist (Lavoie et al., 2006; 2014 and  
705 references therein). The teratology metric is gaining in popularity as seen by the number of recent  
706 publications on the subject. However, other biological descriptors or biomarkers have been reported to  
707 reflect biological integrity in contaminated environments. Although it is generally impossible to examine  
708 all metrics due to limited resources and time, the most informative approach would undoubtedly be  
709 based on incorporating multiple indicators.

710

711 One very simple metric to use that does not require any taxonomic knowledge is diatom cell density.  
712 Lower diatom cell counts are expected as a result of altered algal growth under contaminated stress

713 conditions. This has for example been reported in metal-contaminated environments (e.g., Duong et al.,  
714 2010; Gold et al., 2002; Pandey et al., 2014). However, this metric alone does not consistently reflect the  
715 response of diatoms to perturbation because numerous other factors such as water discharge or grazing  
716 pressure have an influence on algal abundance and biomass. Another simple metric to calculate is  
717 diversity. For example, metal loading possibly contributed to lowering diatom diversity in the Animas  
718 River watershed, Colorado (Sgro et al., 2007). On the other hand, diversity is also driven by many other  
719 factors which do not always correlate with ecosystem's health (Blanco et al., 2012). This multilayer  
720 condition has been noticed at sites with different scenarios of contamination (abandoned mine tailings in  
721 Canada, or industrial discharge in France), where assemblages were composed of ~100%  
722 *Achnantheidium minutissimum* (Lavoie et al., 2012; Lainé et al., 2014). In these cases, low diversity was  
723 not exclusively linked to metal contamination but also to low nutrients. Species diversity increased  
724 downstream in both systems which matched with dilution of the contamination; however, this could also  
725 be attributed to cell immigration and to increased nutrient concentrations downstream.

726  
727 Assemblage structure also provides valuable information on ecosystems health as a shift from sensitive  
728 to tolerant species reflects a response to environmental characteristics. This assemblage-level response is  
729 believed to operate on a longer temporal scale as compared to the appearance of teratologies. This has  
730 been observed, for example, in a study with chronic metal exposure where deformed individuals were  
731 outcompeted and replaced by contamination-tolerant species, thus abnormal valves slowly disappeared  
732 from the assemblage (Morin et al., 2014). This suggests that the presence of deformities may be an early  
733 warning of short/spot events of high contamination, while the presence of tolerant species may reflect  
734 chronic exposure. The apparent temporal disparity could in part explain unclear response patterns  
735 observed under natural conditions when documenting teratologies alone as a biological descriptor.

736  
737 Diatom frustule size is considered an indicator of environmental conditions, and selection towards  
738 small-sized individual and or species has been observed under contamination/stress conditions (Barral-  
739 Fraga et al., 2016; Ivorra et al., 1999; Luís et al., 2011; Pandey et al., submitted; Tlili et al., 2011). This  
740 metric is not commonly used in bioassessment, although it has potential in contributing additional  
741 information on ecosystem health. The time required for valve measurements may be one limiting factor  
742 which makes cell-size metrics currently unpopular in biomonitoring studies. Studies also reported  
743 deformities or shape changes in diatom frustules as a result of size reduction (Hasle and Syvertsen,  
744 1996).

745  
746 Assessment of diatom health (live, unhealthy and dead cells) is also an interesting but unconventional  
747 descriptor to consider when assessing a response to contamination (Gillet et al., 2011; Morin et al.,  
748 2010; Pandey et al., submitted; Stevenson and Pan, 1999). It however requires relatively early  
749 observations of the sample. This analysis of fresh material could be coupled with cell motility (Coquillé  
750 et al., 2015) and life-form (or guild or trait) assessments. These biological descriptors, also not  
751 commonly used, have shown relationships with ecological conditions (e.g., Berthon et al., 2011; Passy,  
752 2007; Rimet and Bouchez, 2011). The live and dead status assessment can also be coupled with  
753 teratology observations. For example, live and dead diatoms were differentiated at sites affected by  
754 metals and acid mine drainage, and the results showed a large amount of deformities and high  
755 percentage of dead diatoms (> 15%) (Manoylov, Phycology lab, Georgia College and State University,  
756 Georgia, USA; unpublished results).

757  
758 The presence of lipid bodies or lipid droplets in diatoms can be a descriptor of ecosystem health. Lipid  
759 bodies are produced by all algae as food reserves, and can be stimulated under various conditions (Wang

760 et al., 2009; Yang et al., 2013; Liang et al., 2015; d'Ippolito et al., 2015). This biomarker has shown  
761 good fit with contamination; lipid bodies increasing in number and size under metal contamination  
762 (Pandey and Bergey 2016; Pandey et al., submitted). Lipid analysis does not require taxonomic skills,  
763 and can be quantified using dyes and fluorescence. However, depending on the level of contamination,  
764 the cell may be excessively stressed and the lipid bodies could be oxidized in order to reduce the  
765 overproduction of reactive oxygen species (ROS) (as observed in the green alga *Dunaliella salina*,  
766 Yilancioglu et al., 2014). Moreover, lipid bodies are produced under many environmental conditions  
767 (e.g., lipids, more specifically triacyl glycerol (TAGs), increase under high bicarbonate levels; Mekhalfi  
768 et al., 2014), and the correlation with metal contamination may be subject to fluctuation.  
769

770 Finally, antioxidant enzymes are also good biomarkers of stress (Regoli et al., 2013). Under stress  
771 conditions organisms suffer cellular alterations, such as overproduction of ROS, which can cause  
772 damage in lipids, proteins and DNA. Cells have defense mechanisms against ROS, and once they are  
773 activated, there are several biochemical markers to assess different contaminations. These classical tests,  
774 adapted to diatoms, are associated with the measurement of ROS scavenging enzymes or non-enzymatic  
775 processes such as production and oxidation of glutathione and phytochelatin, or measuring lipid  
776 peroxidation and pigments content. More studies are being developed to find specific biomarkers for  
777 toxicants in order to effectively assess their impact on diatoms (Branco et al., 2010; Corcoll et al., 2012;  
778 Guasch et al., 2016).  
779

780 Considering the number of available diatom-based biological descriptors, we recommend the  
781 development of a multi-metric index for contamination assessment. Keeping in mind the limited time  
782 and resources available (money, analysts, equipment) it would not be reasonable to include all metrics.  
783 In the future, new technologies combining genetic, physiological and environmental measures may  
784 contribute to develop routine biomonitoring tools. As a first step to facilitate future bioassessments, a  
785 library of teratological metrics rated against environmental health will be required. Currently, the  
786 complementary information issued from the combination of certain selected metrics could significantly  
787 enhance the ecological information provided by diatoms, and therefore improve our understanding of  
788 ecosystems status. The assessment of contamination using biological descriptors could also be refined  
789 by combining the response of organisms from different trophic levels. For example, diatom-based  
790 metrics could be combined with invertebrate-teratology metrics such as chironomid larvae mouthpart  
791 deformities.  
792

## 793 6. CONCLUSIONS AND PERSPECTIVES 794

795 Are teratologies alone sufficient to adequately assess a response to contamination? Is this biological  
796 descriptor ecologically meaningful? These are the fundamental questions of this discussion paper. The  
797 answer is undoubtedly *yes* with selected taxa based on the number of studies that have shown good  
798 success in correlating % deformities and contamination (mostly metals and pesticides). However, taxa  
799 prone to shape deformities (e.g., *Fragilaria*, *Eunotia*) under natural conditions must be examined with  
800 scrutiny. Sharing current experiences and knowledge among colleagues has certainly raised numerous  
801 questions and underscores certain limitations in the approach. This paper provides various paths forward  
802 to refine our understanding of diatom teratologies, and hence, increase the sensitivity of this metric in  
803 bioassessments. Many suggestions were presented, and they all deserve more thorough consideration  
804 and investigation. One more opinion to share is that the occurrence of teratologies is a red flag for  
805 contamination, even though teratologies do not always correlate with the level of contamination.  
806 Teratologies, at the very least, are good “screening” indicators providing warnings that water quality

807 measurements are needed at a site. This alone is interesting for water managers trying to save on  
808 unnecessary and costly analyses. Moreover, the general ecological signal provided could suggest the  
809 presence of a stressor that may affect other organisms, and ultimately ecosystem integrity and functions  
810 (ecosystem services). We anticipate that enumerating and identifying diatom deformities can become a  
811 routine part of agency protocols for environmental stress assessment. Most countries are required to  
812 comply with water quality regulations and guidelines that would greatly benefit from such a  
813 biomonitoring tool. Hopefully, this paper will trigger more discussion and research on the subject to  
814 enhance our understanding of the precious ecological information provided by the presence of diatom  
815 teratologies.

816

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