Exploring the Effect of Salinity as a Primary Cause of Teratology in Freshwater Diatoms

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1 Graphical abstract
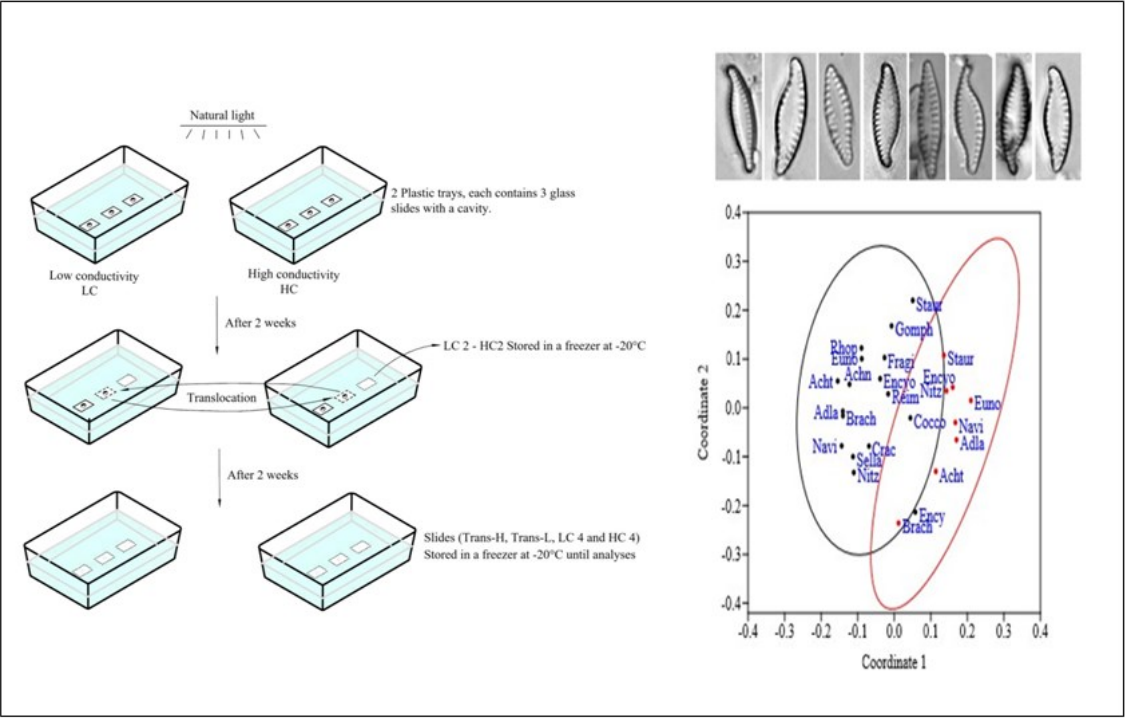
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1   **Abstract**

2   Increased water conductivity levels have been proposed as a key factor to explain the occurrence of

3   teratological forms in freshwater diatom assemblages. The current study aimed to carry out an

4   experiment on a laboratory scale to assess the response of periphytic diatoms to artificially

5   increased salinity levels. The obtained results show that: a) the proportion of aberrant cells

6   increased in high conductivity treatments, b) developed teratologies were preserved in diatom

7   populations even after being translocated to normal conditions, and c) the degree of valve outline

8   deformation in the dominant species was proportional to the induced water conductivity. All these

9   data support previous field observations that linked high electrolyte content and the abundance of

10   aberrant cells among microalgal communities in continental aquatic ecosystems.

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12   **Keywords**: aberrant cells, water conductivity, salt stress, diatom assemblages, phototrophic biofilm.

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1. Introduction

Diatoms are a type of single-celled algae with an important ecological role in the functioning of freshwater ecosystems (Morin et al., 2016). They have been widely used as bioindicators of various environmental conditions, particularly salinity, pH, and nutrients, due to their high diversity, short generation time, sensitivity, and quick response to changes in water quality (Kelly 2003; Smol and Stoermer, 2010; Stevenson et al. 1999). They also play a crucial role in aquatic food webs (Ragueneau et al., 2006; Weitere et al., 2018). . Diatoms are also known to be sensitive to toxic substances (Mccormick and Cairns 1994; Stevenson 2014). In their natural habitat, diatom communities are exposed to multiple anthropogenic inputs that affect their life cycle, so that the resulting valve morphologies developed under unfavorable environmental conditions differ from those inhabiting undisturbed environments. Valves that deviate that ~~differ~~ from normality in a population for a given species, show abnormalities in terms of symmetry, striation pattern, raphe course, and structure, are called teratological forms (Dziengo-Czaja et al. 2008; Falasco et al. 2009a; Gonçalves et al. 2019; Riato et al. 2018; Smol and Stoermer 2010).

Deformation in valve outline, loss of areolae, changes in striation patterns, and disruption of the raphe formation, are the main teratological forms affecting diatom valves, often correlated with physiological and metabolic impairment in the diatom cell (Falasco et al. 2021; Falasco et al. 2009a).

Teratology does not appear to weaken the reproductive capacity or viability of the affected cells (Falasco et al., 2021). However, some forms of teratologies are suspected of being lethal (Arini et al., 2013).

Recovering normal morphology after sexual reproduction in deformed diatoms from long-term cultures indicates that teratologies do not arise from genetic drift (Granetti, 1968). In this regard, the work of Arini et al. (2013) showed that the cadmium-induced teratologies in *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot decreased with decontamination, evidencing that

teratology in diatoms appears in altered environments but progressively disappears along with the return to normal conditions.

The relationship between teratological forms and unhealthy conditions has been investigated in laboratory cultures (Duong et al., 2010; Windler et al., 2014) as well as in the field (Cantonati et al., 2014; Falasco et al. 2009a; Muhr 2014). In the latter case, natural teratology is rare and typically recorded at relative abundances not exceeding 1% (Morin et al. 2012a), so an increase in abundance can be linked to an increase in pollutants or other stressors (Cattaneo et al. 1998; Dziengo-Czaja et al. 2008; Lavoie et al. 2017; Sládeček 1986; Stevenson et al. 1999).

Factors known to be teratogenic for diatoms include high temperatures, light intensity (Antoine and Benson-Evans, 1984; Hill et al., 1995), low current velocity (typical of summer drought conditions (Stevenson 1996), high Ultraviolet (UV) radiation (Cabrol et al., 2004), increased nutrient concentrations (Nicolosi Gelis et al., 2020; Rosemond et al., 2000), and dissolved chemical substances in water, including organic or inorganic substances (Boisson and Perrodin, 2006; Cattaneo et al., 2008; Guasch et al., 2009; Hoagland et al., 1996; Moisset et al., 2015), herbicides (Debenest et al., 2008), and heavy metals (Cerisier et al. 2018; Cunningham et al. 2005; Falasco et al. 2009a; Gold et al. 2003; Morin et al. 2007; Pandey et al. 2018; Pandey and Bergey 2018). Interactions between these factors often occur in aquatic environments and are hard to disentangle (Falasco et al., 2021).

While changes in the composition of diatom communities have been shown to be a good mirror of freshwater ecosystem health thanks to many decades of comprehensive monitoring studies (Medley and Clements, 1998; Sabater, 2000), to date few experimental studies document the effect of abiotic factors on freshwater diatoms.

Olenici et al. (2017) have already revealed conductivity as a major cause for the occurrence of abnormal forms of epilithic diatoms in rivers. In this context, the main objective of the present study was to assess the effect of high salinity levels on freshwater diatoms under laboratory conditions.

64 We focus particularly on the development of teratological forms, assessing the degree of

65 deformation of valve outline, as well as on the dynamics of species richness in these assemblages.

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67 **2. Materials and methods**

68 **2.1. Experimental setup**

69 The experiment was carried out in July 2020 by using plastic trays (length × width × height =

70 46×32×8 cm), filled with 4 L of water from an experimental freshwater pond (Diatom Laboratory,

71 Leon, Spain, 42.6047093° N, 5.5565376° W), to which 25 mL of a periphyton suspension collected

72 from the same pond, and 80 mL of concentrated Alga-Gro® freshwater medium, were added. Two

73 conductivity levels were maintained: the first one as a control treatment (low conductivity: LC:

74 ~460 μS.cm^{-1}: conductivity of water without any additions), and the second as a high conductivity

75 (HC: ~1400 μS.cm^{-1}) treatment by adding marine salt. Water conductivity was daily measured and

76 adjusted. Trays were maintained at a constant water level under natural sunlight for 4 weeks.

77 Figure 3 shows a single trial. The same design was replicated 3 times (3 trials) in the same

78 conditions. Each treatment was replicated three times. The experiment took place in a glass room

79 receiving sunlight with natural intensity and photoperiod throughout the incubation period. The

80 trays were kept at room temperature.

81 Microscope glass slides with a cavity were used as artificial substrata for the settlement and growth

82 of periphytic diatoms. Each water tray contained three slides: two slides were removed from each

83 treatment after two (slides LC$_2$ and HC$_2$), and four weeks (slides LC$_4$ and HC$_4$) respectively,

84 whereas the third slide was translocated from LC to HC (slide Trans-H) and vice-versa (slide Trans-

85 L) after the second week and removed at the end of the experiment (Figures 1–3). Removed slides

86 were placed in 50 mL polypropylene screw-cap tubes (Falcon-BD, Franklin Lakes, NJ, USA) and

87 stored in a freezer at -20 °C until analyses.

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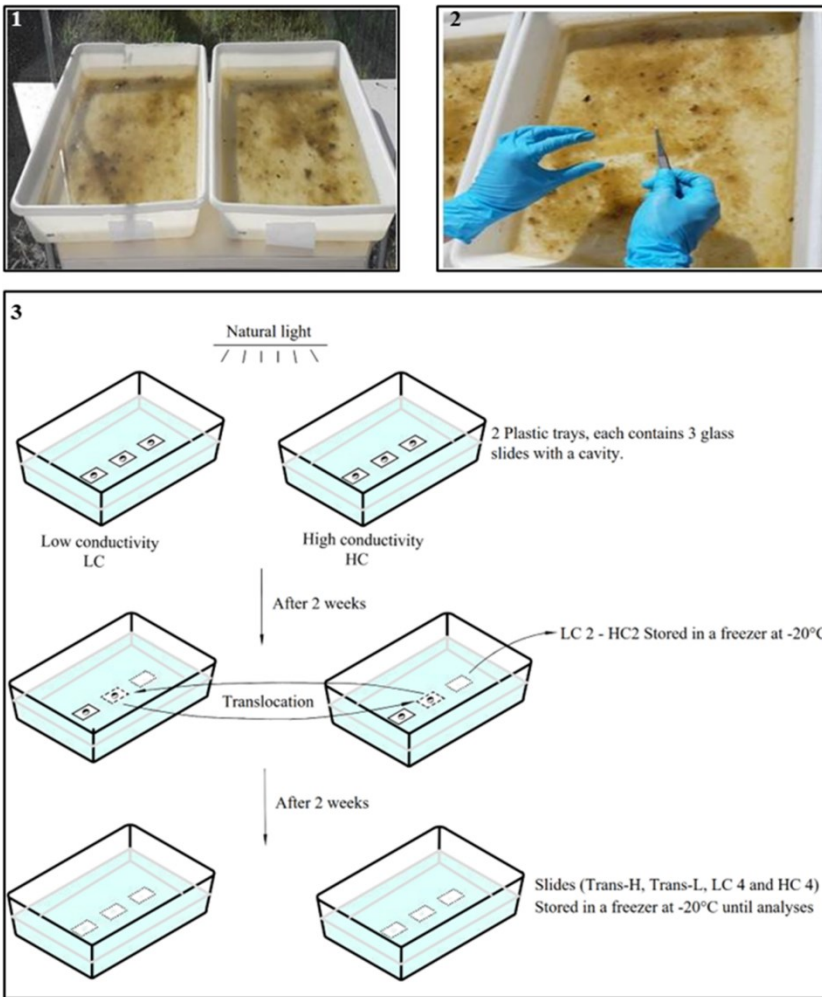
Figures 1, 2. Experiment in trays for attachment of diatoms.

Figure 3. Schematic representation of the experiment consisting in two plastic trays, each contains three glass slides with a cavity, LC2, Trans-H and LC4 / HC2, Trans-L and HC4 respectively for Low conductivity (LC) and High conductivity (HC) trays. Points rectangle are the slides removed from each treatment, traits rectangle are the slides translocated from LC to HC (slide Trans-H) and vice-versa (slide Trans-L). Removed slides were placed in 50 mL polypropylene screw-cap tubes (Falcon-BD, Franklin Lakes, NJ, USA) and stored in a freezer at -20 °C until analyses.

2.2. Identification

Slides were cleaned with hot hydrogen peroxide (30%) and a few drops of hydrochloric acid to remove organic material and dissolve calcium carbonates. The samples were then rinsed several times with distilled water. The cleaned samples were transferred to coverslips to dry for 24 hours. Once the samples were dry, permanent glass slides using high refractive resin Naphrax (RI=1.74) were mounted.

Diatom frustules were identified and counted under light microscopy (1000× magnification, Olympus BX 60 microscope), with oil immersion, using standard references and segregating teratologic forms (Blanco Lanza et al., 2011; Hofmann et al., 2011). The relative abundances of diatom species were finally calculated.

2.3. Data processing

The relative abundance of teratological forms was set as the response variable. Due to the non-normality of this variable, statistical differences between treatments were analyzed by means of the Kruskal-Wallis test (Ostertagová et al., 2014).

Diatom assemblage composition was compared between treatments after 4 weeks using non-metric multidimensional scaling (NMDS), using Spearman rank correlation as distance measure, segregating normal and teratological forms within each genus. Singletons (genera occurring in a single sample) were previously removed. To test for significant differences between treatments, a one-way ANOSIM test (using Euclidean similarity coefficients) was carried between the Cartesian coordinates of the resulting groups.

All statistical analyses were performed with the free Past software, version 3.24 (Hammer et al., 2001).

2.4. Degree of teratologies

To evaluate the degree of valve deformation, geometric morphometry was used to analyze changes in valve outline caused by experimental treatments in *Pseudostaurosira brevistriata* (Grunow) D.M. Williams & Round, the dominant species in the samples.

A total of 141 individuals of *P. brevistriata* were photographed using Optikam digital camera and OptikaView7 software. Images were binarized and segmented using ImageJ software (Abràmoff et al., 2004). Valve outlines were then vectorized with Shape v.1.3 package (Iwata and Ukai, 2002), which uses Elliptical Fourier Analysis (EFA) to describe valve outline. EFA consists of fitting a

128 given number of harmonics to the original valve outline, harmonics which are then analyzed by

129 means of Principal component analysis (PCA).

130 Differences in valve outline between normal and teratologic *P. brevistriata* valves were tested by

131 means of an ANOSIM test using Euclidean distances between the PCA scores.

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133 **3. Results**

134 **3.1. Identification**

135 Diatom composition of all samples was similar with a strong dominance of *Encyonopsis subminuta*

136 Krammer & Reichardt (20.63%), *Achnanthidium minutissimum* (Kützing) Czarnecki (19.86%),

137 *Pseudostaurosira brevistriata* (15.20%), *Staurosira venter* (Ehrenberg) Cleve & Moeller (11.01%),

138 *Nitzschia palea* (Kützing) W. Smith (9.10%), and *Nitzschia dissipata* (Kützing) Grunow (6.06%).

139 Within the typology of deformations described in the literature (Falasco et al. 2021; Falasco et al.

140 2009b), the most widespread ones observed in our samples were the occurrence of irregular valve

141 outlines, in species such as *A. minutissimum, A. pyrenaicum, Brachysira neoexilis, Cocconeis*

142 *euglypta, C.lineata, Craticula subminuscula, E. subminuta, Eunotia sp, Fragilaria gracilis,* several

143 *Nitzschia* species *(N. palea, N. dissipata, N. solgensis, N. frustulum, N. inconspicua, N. amphibia,*

144 *N. filiformis, and N. recta), Navicula tripunctata, N. veneta, Planothidium frequentissimum, P.*

145 *brevistriata, S. venter, S. construens var. binodis, Punctastriata sp., and Ulnaria acus.* Atypical

146 raphes were found in *E. subminuta,* aberrant striae occurred in *F. gracilis* and several *Navicula* (*N.*

147 *tripunctata, N. veneta*, and *N. trivialis*) and disrupted fibulae in *N. palea* and *N. filiformis.*

148 Combined teratologies have been also noted in *E. subminuta* (involving valve outline and in raphe),

149 *F. gracilis, P. brevistriata, S. venter,* and *S. construens var. binodis* (irregular valve outline and

150 aberrant striae), and *N. palea, N. filiformis,* and N. *frustulum* (disrupt fibulae and irregular valve

151 outline) (Table 1). See Figures 4–68 for some deformed diatom species.

Table 1. Diatom species found with the main deformity types.

Species	Code genus	Code species	Type of teratology						
			One teratology				Mixed deformity in		
			Irregular valve outline	Atypical raphe	Aberrant striae	Disrupted fibulae	valve outline and raphe	valve outline and striae	valve outline and fibulae
Achnanthidium minutissimum (Kützing) Czarnecki	Acht	ADMI	+						
Achnanthidium pyrenaicum (Hustedt) H. Kobayasi		ADPY	+						
Brachysira neoxilis Lange-Bertalot	Brach	BNEO	+						
Cocconeis euglypta Ehrenberg	Cocco	CEUG	+						
Cocconeis lineata Ehrenberg		CLNT	+						
Craticula subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	Crac	ESBM	+						
Encyonopsis subminuta Krammer & Reichardt	Encyo	ESUM	+	+			+		
Eunotia sp.	Euno	EUNS	+						
Fragillaria gracilis Østrup	Fragi	FGRA	+		+			+	
Navicula tripunctata (O.F.Müller) Bory	Navi	NTPT	+		+				
Navicula trivialis Lange-Bertalot		NTRV			+				
Navicula veneta Kützing		NVEN	+		+				
Nitzschia amphibia Grunow	Nitz	NAMP	+						

Species		Code								
Nitzschia palea (Kützing) W.Smith		NPAL	+			+				+
Nitzschia dissipata (Kützing) Grunow		NDIS	+							
Nitzschia frustulum (Kützing) Grunow		NIFR	+							+
Nitzschia filiformis (W.M.Smith) Van Heurck		NFIL	+			+				+
Nitzschia solgensis Cleve-Euler		NSOL	+							
Nitzschia inconspicua Grunow		NINC	+							
Nitzschia recta Hantzsch in Rabenhorst		NREC	+							
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	Plan	PLFR	+							
Pseudostaurosira brevistriata (Grun.in Van Heurck) Williams & Round	Staur	PSBR	+						+	
Staurosira venter (Ehr.) Cleve & Moeller	Staur	SSVE	+						+	
Staurosira construens var.binodis (Ehr.) Hamilton		SCBI	+						+	
Punctastriata sp.	Punc	PUCS	+							
Ulnaria acus (Kützing) Aboal	Ulac	UACU	+							
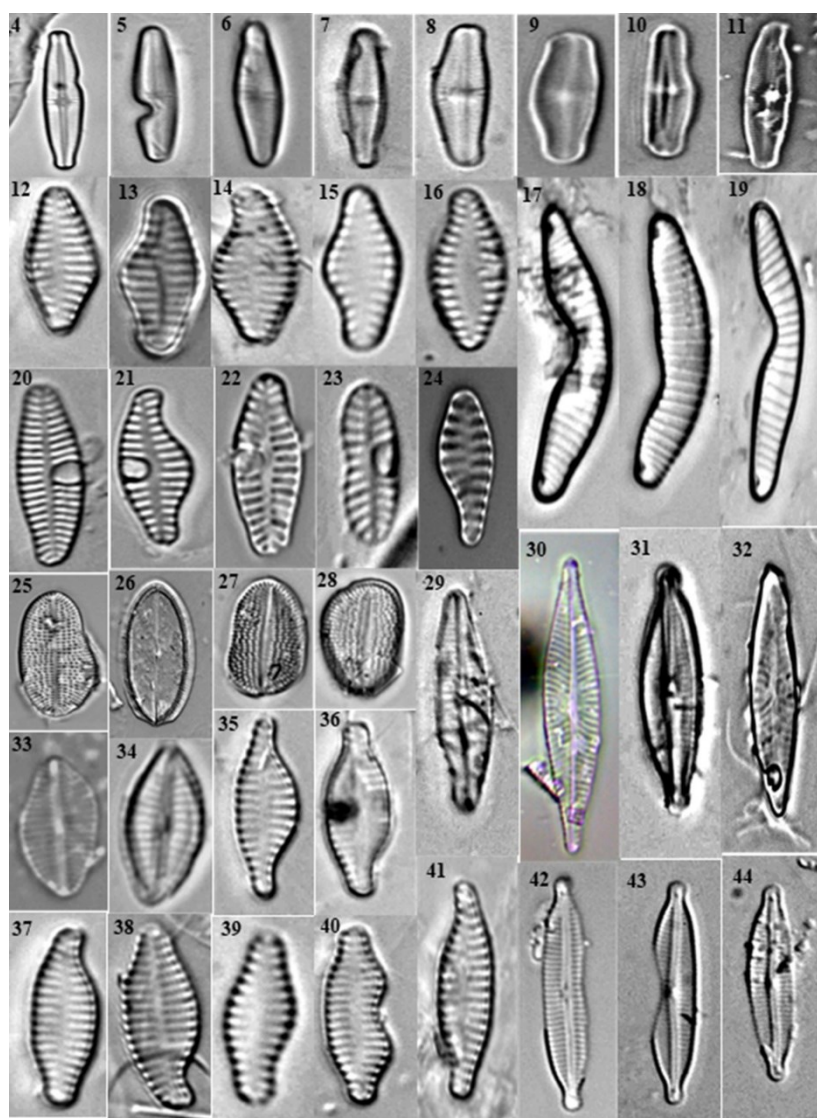
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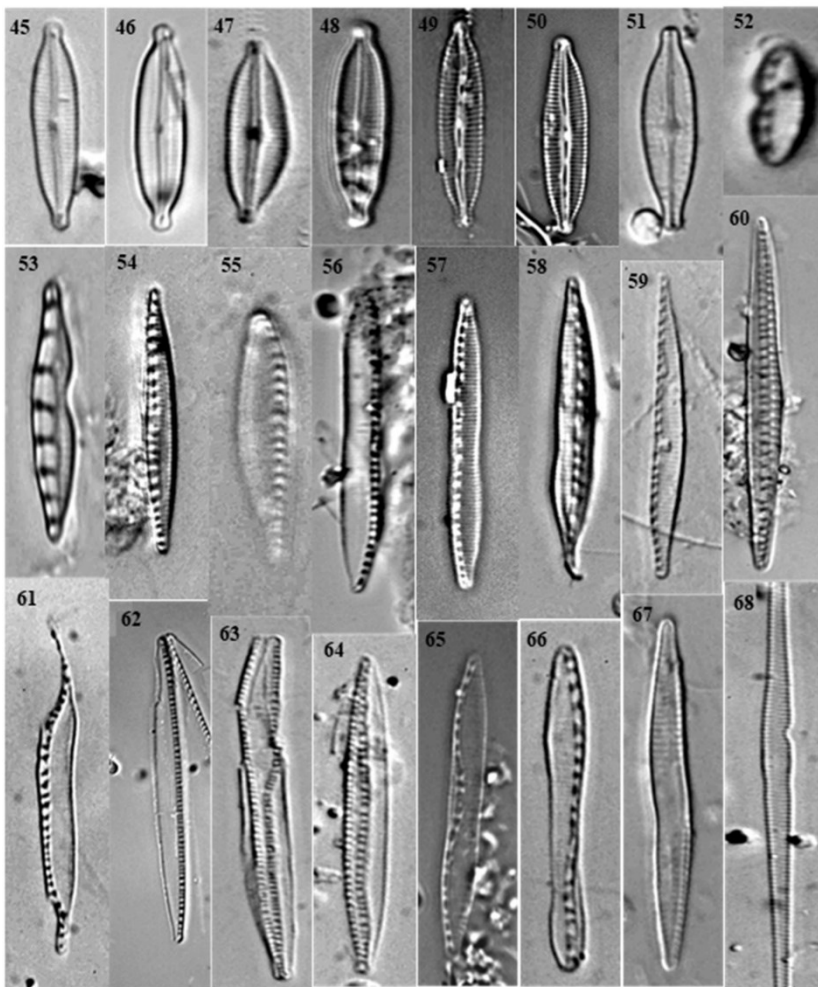
Figures 4–68. Examples of deformed frustules of some diatom species recorded in treatments exposed to a high level of water conductivity (LM microphotographs). Photos are not to scale.

Figs 4–11. *Achnanthidium minutissimum*: deformed valve outlines.

Figs 12–16. *Staurosira venter.*

Figs 12–15. Deformed valve outlines.

Fig16. Mixed teratologies: (deformed valve outline and aberrant striae).

Fig 17–19. *Eunotia sp.* deformed valve outlines.

Fig 20–23. *Planothidium frequentissimum* deformed valve outlines.

Fig 24. *Punctastriatasp*: deformed valve outline.

Fig 25. *Cocconeis euglypta*: deformed valve outline.

Figs 26–28. *Cocconeis lineata*: deformed valve outlines.

Figs 29–32. *Navicula sp. pl.* deformed valve outlines.

Figs 33–34. *Craticula subminuscula :* deformed valve outlines.

**Figs 35–41.** *Staurosira construens var.binodis* (deformed valve outlines).

1 **Figs 42–50.** *Encyonopsis subminuta*.

2 **Figs42–48.** Deformed valve outlines.

3 **Fig 49.** Atypical raphe.

4 **Fig 50.** Mixed teratologies (deformed valve outlines and atypical raphes).

5 **Fig 51.** *Brachysira neoxilis* (deformed valve outline).

6 **Figs 52–66.** *Nitzschia sp. pl*.

7 **Figs 52–62**. Deformed valve outlines.

8 **Figs 63, 66.** Mixed deformities (irregular valve outlines and disrupted fibulae).

9 **Figs 64, 65.** Disrupted fibulae.

10 **Fig 67**. *Fragilaria gracilis* : deformed valve outline.

11 **Fig 68.** *Ulnaria acus*: deformed valve outline.

12

13 **3.2. Teratology**

14 The number of teratological forms was always significantly lower in control treatments, with no

15 statistical differences between LC_2 and LC_4.

16 Figures 69–71 represent the percentage of teratological valves among different treatments. The

17 exposure of the phototrophic biofilm to salt stress induced a significant increase (H=9.46, p=0.023)

18 in the percentage of teratological frustules between treatments LC and HC. Control trays with low

19 conductivity levels (460 ± 0.73 µS.cm^{-1}) harbored low numbers of teratological individuals during

20 the first two weeks (4.86 ± 0.46%), raising to 6.41 ± 0.35% during the fourth week whereas the

21 percentage of teratological forms increased to 11.68 ± 1.87% and 12.52 ± 1.85% in HC (1400 ±

22 40.32 µS.cm^{-1}) treatments, after 2 and 4 weeks, almost doubling the percentage in comparison to the

23 LC treatment.

24 There was a significant difference in the number of abnormal forms comparing LC_2, LC_4, and

25 Trans-H treatments (H=7.2, p=0.027, Figure 69), while no significant differences were found

26 between Trans-L and Trans-H (p=0.83, Figure 70). Statistical analysis revealed also no significant

27 differences (p=0.96) in the number of teratological forms comparing HC_2, HC_4, Trans-L, and Trans-

28 H (Figure 71).
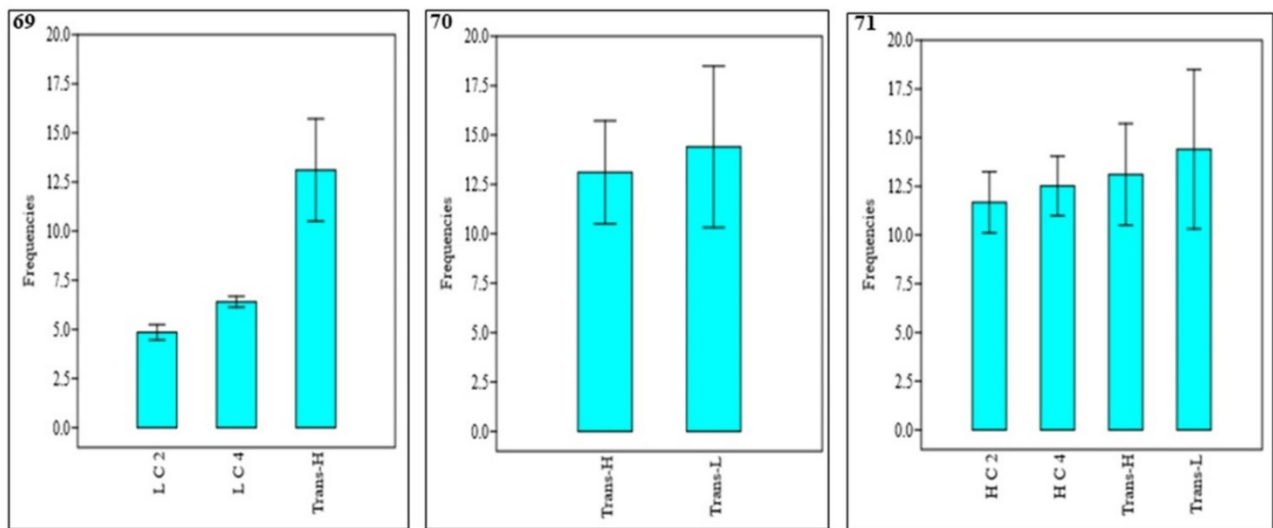
Figures 69–71. Boxplots showing the frequencies of teratological diatom valves across the different treatments. LC_2: Low conductivity after 2 weeks, LC_4: Low conductivity after 4 weeks, HC_2: High Conductivity after 2 weeks, HC_4: High Conductivity after 4 weeks, Trans-H: translocated from LC to HC after 2 weeks. Trans-L: translocated from HC to LC after 2 weeks. Error bars: standard error.

No differences in terms of genera diversity (Shannon's H) were found between LC and Trans-H or between LC and HC levels, and no significant differences were observed either in species richness between low and high conductivity treatments after four weeks of colonization.

The comparison of diatom assemblages using NMDS (Figure 72) allowed clear segregation of normal and teratological individuals throughout the experiment in LC and HC treatments, revealing an evident effect of high conductivity levels on the overall biofilm response. Significant differences were observed (p=0.0001) between the position of normal and teratological individuals in the resulting plot at the end of the experiment.

The NMDS highlighted the different ecological behavior of the teratological forms with respect to the normal ones regardless of treatment, exposure time or substrate translocation status. The resulting graph reveals that actually teratological diatoms have different occurrences and abundances throughout the whole experimental material.
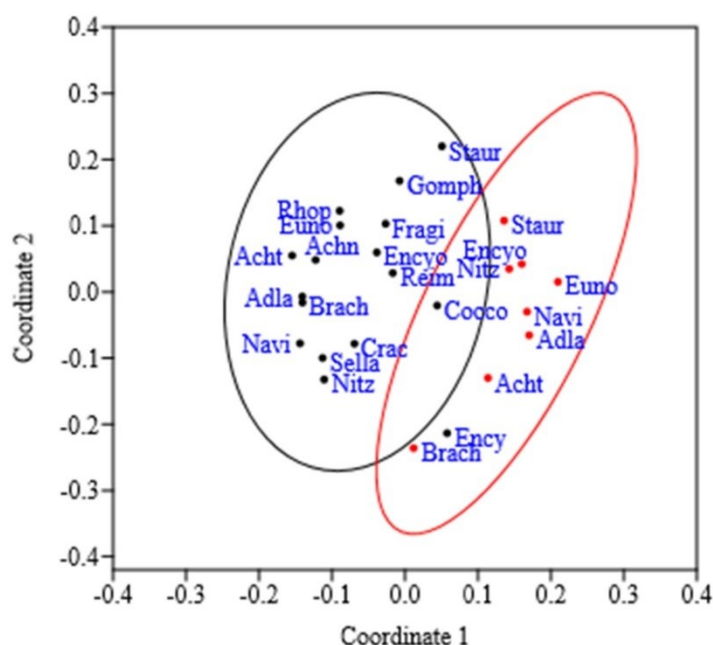
Figure 72. Non-metric multidimensional analysis of diatoms genera, normal (black) and aberrant forms (red). Achn : Achnanthes, Adla: Adlafia, Crac: Craticula, Ency: Encyonema, Euno: Eunotia, Gomph: Gomphonema, Reim: Reimeria, Rhop: Rhopalodia, Sella: Sellaphora. Other code's genera are included in table 1.

3.3. Degree of teratology

Pseudostaurosira brevistriata was the most dominant species among the teratological forms. It was

chosen as a model to quantify the degree of deformation in valve outline:

geometric morphometry revealed few differences in shape between normal and teratological forms

found in LC treatments, but differences in valve outline were evident at the end of the experiment in

treatments exposed to high conductivity (Figures 73–80) (ANOSIM test on PCA scores: p=0.001,

Figures 81, 82).
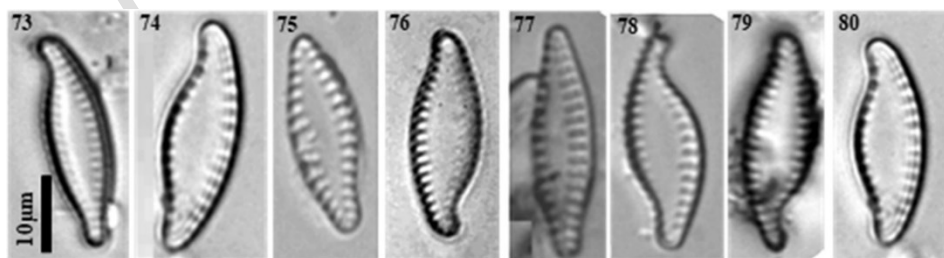
Figures 73–80. LM microphotographs of abnormal frustules of *Psudostaurosira brevistriata* recorded at the end of the experiment in treatments exposed to a high level of water conductivity (HC). Scale bar = 10 μm.
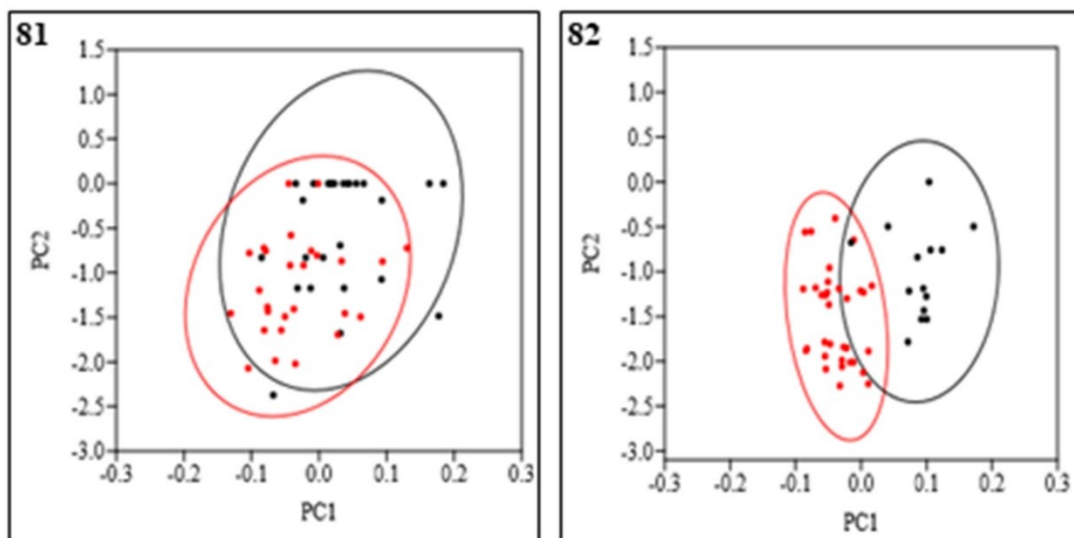
Figures 81, 82. PCA plot of the Elliptic Fourier descriptors obtained in the analyzed *P. brevistriata* populations: deformed (red), and normal (black) valves resulting from low (A) conductivity and high (B) conductivity after four weeks of exposure time.

Discussion

The occurrence of abnormal individuals is among the most striking effects of environmental stress on diatom metabolism. While deformities can be initiated at different stages throughout the diatom life cycle, the processes leading to abnormal cell formation are yet unsolved (Falasco et al. 2021; Lavoie et al. 2017; Morin et al. 2012a). In particular, the deleterious effects of high salinity on photoautotrophs have been described by several researchers (Allakhverdiev et al. 1999; Lyon et al. 2011; Mostaert 1995; Rijstenbil 2005; Schmid 1979; Schultz 1971; Sudhir and Murthy 2004; Vairavamurthy et al. 1985), and the literature gathers extensive research on the acclimation of photoautotrophic organisms to salt stress. For example, hypersaline conditions induce osmotic and ionic stress on cells, this tension disrupts photosynthetic activity, and increases respiration and growth arrest (Sudhir and Murthy, 2004). Salinity causes also indirect damage as a result of oxidative stress (Rijstenbil, 2005).

Noticeably, the altered valve outline was the only kind of teratology observed in control treatments. Although raphe modifications, altered striation patterns, disrupted fibulae, and mixed deformities (several deformities in the same frustule) were additionally observed under salt stress.

At the end of the experiment, Trans-L treatments harbored the highest proportion of teratological frustules (although not significantly different from HC or Trans-H treaetments), even once translocated to low conductivity waters after two weeks of incubation under high conductivity conditions. This indicates that the deformed cells continue to replicate and transfer their teratologies for a number of generations. Hustedt. (1956) had already observed that particular ecological conditions at the time of zygotes' (auxospore) formation may induce changes in frustule morphology that are perpetuated during vegetative reproduction, leading to populations with a morphology quite different from that of the parent population. This new abnormal cell would then split by mitosis and pass on the abnormal shape to all subsequent daughter cells, as also suggested by Stoermer (1967).

It appears from the experiment that 2 weeks of stressful salinity conditions are sufficient to induce a maximum percentage of teratologies. The increase in teratology after 4 weeks compared to 2 weeks is not significant. This can be attributed to the fact that a plateau was reached after 2 weeks of exposure and no further increase in the percentage of teratologies will occur regardless of the increase in time.

Numerous studies (Clavero et al., 2000; Millan et al., 2020; Schmid, 1980; Tuchman et al., 1984) have found a positive correlation between increased salinity in freshwater environments and the number of individuals exhibiting teratological forms. Falasco et al. (2009b) noticed that in long-term cultures, not only nutrients and waste but also salinity can stimulate the development of teratological forms, affecting primarily the production of structures involved in colony formation and cell anchorage to overcome floating difficulties, although teratological forms such as aberrant surface ornamentations (position and shape of areolae) and valve outline can also be formed (Falasco et al. 2009a). Salinity acts on diatoms primarily by osmotic pressure (Stoermer and

Andresen, 2006) by impairing microtubule function (Falasco et al. 2009b), but sometimes the ionic water composition itself can be responsible for morphological variations in algae, as pointed out by Lewin and Roberston (1971), and Schmid (1979). The resulting deformation can be severe and identification becomes difficult even at the genus level (Stoermer and Andresen, 2006).

During her investigations, Schmid (1980) found a close relationship between changes in water salinity and sexual reproduction in *Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer and *Surirella peisonis* Pantocsek. The raphe fissure in these diatoms was fragmented into short parts or tubules as salinity increased. Schultz (1971) also found that a modification in the valve pattern of *Cyclotella cryptica cryptic* Reimann, Lewin & Guillard and *C. meneghiniana* Kützing was related to salinity levels.

Likewise, Håkansson and Chepurnov. (1999) found that *Cyclotella meneghiniana* cells cultured at high salinity levels exhibited greater morphological variability, including differences in the pattern and the number of marginal striae, the number and placement of valve face fultoportulae, and the pattern of mantle fultoportulae satellite pores.

Al-handal et al. (2014) in their study of Lake Sawa (a salt lake in Iraq), observed that water salinity was responsible for teratological traits in *Cocconeis sawensis* Al-Handal & Riaux-Gobin, with the occurrence of abnormalities in valve contour, deflection of the raphe, and distortion of areolae structure.

In our experiment, salt stress didn't induce deformities in some genera, particularly five out of 16 dominant genera (*Achnanthes*, *Encyonema*, *Epithemia*, *Gomphonema* and *Rhopalodia*) wich didn't show any deformity in their frustules even under high conductivity conditions.

On the other hand, species such as *A. minutissimum, E. subminuta, N. palea, P. brevistriata, S. construens var. binodis,* and *S. venter* showed a high percentage of deformities under salt stress. Trobajo et al. (2004) and Vendrell-Puigmitja et al. (2021) noticed also these species-specific responses, but the reasons leading to such taxonomic differences remain unknown. Lavoie et al.

(2017) think that certain genera are more likely to produce teratological forms than others when affected by ″a certain kind ″of disturbance.

The irregular valve outline showed the most prominent form of teratology in our samples, followed by aberrant striae, disrupted fibulae, and atypical raphe. Unlike several works (Jahn, 1986; Trobajo Pujadas, 2007; Trobajo et al., 2011, 2004) we did not notice a change in the length and width of cells throughout the experiment.

The present work demonstrates, particularly, a close relationship between salt stress and valve outline deformity in *P. brevistriata*. Peres-Weerts (2000) had already reported that the percentage of aberrant *P. brevistriata* increased with decreasing water quality conditions, but our study shows also a response in the degree of deformation in valve outline (loss of symmetry, bent, incised, swollen, or notched profile), assessed by means of EFA analysis. The results evidence a close relationship between salt stress and the deformation at the individual level. The degree of deformity is more marked in *P. brevistriata* growing in treatments exposed to salt stress compared to those collected from control trays. Our results are in agreement with Olenici et al., (2017) who detected a deformation gradient in *A. minutissimum* from acid mine drainage sites. They found a positive relationship between the deformation degree in valve outline and dissolved Zinc (Zn) concentrations. Mu et al. (2018) also focused their studies on the degree of teratology in *Halamphora veneta* (Kützing) Levkov when exposed to cadmium (Cd) and lead (Pb) for a period of 96 hours. They found a slight deformation on *H. veneta* cells following cadmium exposure at a concentration of 1.42 mg.L^{-1}, while obvious deformation occurred with cells largely expanded after 96 hours of lead exposure at a concentration of 15.35 mg.L^{-1}.

Cells affected by mixed deformities are poorly viable and are unable to reproduce (Arini et al., 2013). According to many researchers, mixed teratologies are lethal, hence why they are rarely observed in natural biofilms (Falasco et al. 2009b), as well as in laboratory experiments (Arini et al., 2013). This may explain the low percentage of mixed teratologies compared to a single form of teratology (deformity) found in our experiment

It's interesting to note that diatom communities grown in our experiment under various conductivity levels did not differ significantly from one another; this suggests that high conductivity brought on by salt stress did not cause variations in the composition of diatom communities. Our findings are in contrast to those of earlier research that found metal pollution was responsible for changes in the species composition of diatoms (Cantonati et al. 2014; Cunningham et al. 2003; Duong et al. 2008; Morin et al. 2012b; Sgro et al. 2007). Venâncio et al. (2019) noticed that even small increases in salinity may be sufficient to induce structural changes in freshwater communities or to induce changes in trophic relationships. Accordingly, Vendrell-puigmitja et al. (2021) revealed that freshwater salinization, induced by effluent from potash mining caused a shift in the diatom community of the exposed biofilm.

Our results agree, in contrast, with those of (Millan et al., 2020) who found that mineralization and radioactivity did not induce any significant change in diatom communities.

Conclusion

Although our experiment used a simplistic experimental design, it demonstrated that high conductivity induced by marine salt addition has the potential to significantly alter the structure of diatom frustules causing the appearance of teratological forms, this teratology continuing throughout the generations by vegetative reproduction. Irregular valve outlines, aberrant striae, disrupted fibulae, atypical raphes, and some mixed deformities are the most significant deformations observed during our research. The species *P. brevistriata* was a good example used to measure the teratology degree, correlated to conductivity levels. Our study also showed that salt stress did not induce any difference or change in the diatom community composition when comparing control and high conductivity treatments.

These results can be complemented by experimentally establishing a conductivity gradient to establish the salinity thresholds that cause teratology in diatoms.

Future laboratory experiments on morphological changes associated with salinity fluctuations based on axenic cultures (i.e. examining the behavior of each species separately) will be interesting and helpful to refine the data presented in this work. Further studies at the molecular and proteome levels are also needed to understand the mechanism underlying the development of teratologies.

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