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Diatom size plasticity at regional and global scales

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ABSTRACT

Diatom size plasticity at regional and global scales

Studies based on diatom traits are increasing worldwide but most of them are based on database information from other geographical regions whereas data on direct observations and measurements of individuals are lacking. The aim of this study was to understand how diatom cell sizes vary at: 1) a small (regional) scale, among rivers and streams of Central Portugal with different hydromorphological and climatic characteristics (typologies) and 2) a large (global) spatial scale, comparing cell sizes from different areas of the world. For this study, species from 60 sites belonging to four Portuguese river types (Northern Mountain Streams – M; Littoral Rivers - L; Small Northern Rivers - N1 ≤ 100 km^2; Medium-Large Northern Rivers - N1 > 100 km^2) were studied. Only species with a relative abundance of 2.5 % or above were measured (length and width) and the length/width ratio was calculated, resulting in a total of 86 taxa and ≈3600 specimens measured. In parallel, a revision of published information and databases on the sizes of the analysed species was done considering information from Central and Northern Europe, North and South America, Asia, Africa and Antarctica. At the regional scale, 28 of the 36 species that were present in more than one river type showed significant differences in at least one measure studied. At a global scale, in general, diatoms' lengths from Central Portugal differed from Central Europe, Northern Europe and Asia; also, diatoms' lengths from Northern Europe differed from Africa and North America. At the species level, *Achnanthidium minutissimum* from Portugal was different from those of Africa and Asia; *Achnanthidium subatomoides* and *Eolimna minima* were different between Central Portugal and North America and *Eunotia incisa* differed between Central Europe and North America. Our results produced more adjusted information on diatom sizes of Central Portugal, which differed from some of the other world regions studied. Our conclusions indicate that not considering size plasticity in diatoms' ecological studies can lead to biased results and thus further studies are needed to investigate the spatial variability of diatom traits.

Key words: diatoms, cell size, morphological traits

RESUMO

Plasticidade do tamanho das diatomáceas a uma escala regional e global

Os estudos sobre atributos de diatomáceas têm aumentado, porém, a maior parte destes dependem de dados de outras regiões geográficas havendo falta de estudos baseados em medidas e observações diretas dos indivíduos amostrados. O objetivo deste trabalho foi perceber como é que os tamanhos das diatomáceas variam: 1) numa escala espacial pequena (regional), entre rios e ribeiros da região Centro de Portugal e 2) a uma escala espacial maior (global) comparando tamanhos celulares de várias zonas do Mundo. Para isso, foram estudadas espécies encontradas em 60 locais de amostragem pertencentes a quatro tipos de rio portugueses (Rios Montanhosos do Norte – M; Rios do Litoral Centro - L; Rios do Norte de Pequena Dimensão - N1 ≤ 100 km^2; Rios do Norte de Média-Grande Dimensão - N1 > 100 km^2). Apenas espécies com abundância relativa igual ou superior a 2.5 % foram medidas (comprimento e largura) e foi calculado o rácio comprimento/largura, resultando num total de 86 taxa e ≈3600 indivíduos medidos. Em paralelo, foi feito um estudo de revisão recorrendo a informação publicada e bases de dados relacionadas com o comprimento das diatomáceas do Norte e Centro da Europa, da América do Norte e do Sul, da Ásia, da África e da Antártida. À escala regional, 28 das 36 espécies analisadas mostraram diferenças significativas em pelo menos *uma medida estudada. À escala global, em geral, os comprimentos das diatomáceas do centro de Portugal diferem dos do Centro da Europa, Norte da Europa e Ásia; assim como os comprimentos das diatomáceas do Norte da Europa diferem dos de África e do Norte da América. A comparação por espécie à escala global revelou que o comprimento de* Achnanthidium minutissimum *de Portugal foi diferente do da África e Ásia;* Achnanthidium subatomoides *e* Eolimna minima *foram diferentes entre a região Centro de Portugal e o Norte da América e o comprimento da* Eunotia incisa *diferiu entre a Europa Central e o Norte da América. Os nossos resultados produziram uma informação mais ajustada relativa ao tamanho das diatomáceas da região Centro de Portugal que diferiu de outras regiões mundiais incluídas neste estudo. Este trabalho indicou assim que não considerar a plasticidade do tamanho das diatomáceas em estudos ecológicos pode resultar em conclusões erróneas e, por isso, estudos futuros serão necessários para investigar a variabilidade espacial dos atributos das diatomáceas.*

Palavras chave: *diatomáceas, tamanho celular, atributos morfológicos*

INTRODUCTION

Diatoms have been used to assess water quality for decades (e.g. Whitton & Rott, 1996; Prygiel *et al.*, 1999; Rey *et al.*, 2004; Tornés *et al.*, 2007; Tison *et al.*, 2007; Feio *et al.*, 2007; 2009), not only in Europe but also in other continents (Doung *et al.*, 2007). According to the Water Framework Directive (WFD) (European Comission, 2000) diatoms are part of the biological element Aquatic Flora and therefore, obligatory for the determination of the ecological status within the European Union member states.

Autoecological indices are the most common tool for ecological assessment of rivers based on diatoms around Europe, but there are some uncertainties associated to such methods because they depend on taxonomic identification (Tapolczai *et al.*, 2016). Uncertainties such as taxonomic misidentification, nomenclature changes and biogeographic specificity of the indices (Tapolczai *et al.*, 2016) promoted the increase of alternative approaches that relate community traits such as life-forms, ecological guilds, biovolume and cell-size with ecological processes and factors (e.g. Rimet & Bouchez, 2012; Algarte *et al.*, 2014; Elias *et al.*, 2015). In addition, trait-based approaches have the advantage of being able to relate species to the ecological functioning of the ecosystem (Statzner *et al.*, 2001a; Feio & Dolédec, 2012) and are well grounded on ecological theories (Statzner *et al.*, 2001b). According to the habitat templet, traits are the result of a filtering process that selects the best-adapted species to the environment (Statzner *et al.*, 2001b).

Cell and body-size is a trait that has been studied in different groups of organisms. Among other factors, body-size can change with temperature, precipitation, geography (e.g. Blackburn & Hawkins, 2004; Schäuble, 2004; Ramirez *et al.*, 2008) and environmental stress (e.g. Lange *et al.*, 2016). There are evidences that higher temperatures and low precipitation favour smaller specimens in a variety of groups such as insects (e.g. Roberston, 1959; Atkinson, 1994; Stillwell & Fox, 2009), amphibians (e.g. Bizer, 1978; Brady & Griffiths, 2000; Denver *et al.*, 1998; Schäuble, 2004), marine invertebrates (e.g. Williamson *et al.*, 2002; Daufresne *et al.*, 2009; Irie & Fisher, 2009), fish (e.g. Sahin, 2001; Desai & Sing, 2009), mammals (Boyce, 1978) and plants (e.g. Kim, 2007; Hovenden, 2008; Ledesma *et al.*, 2008).

In the case of diatoms, species display a large range of cell-sizes (Round *et al.*, 1990) and cell-size also varies over the life cycle. In pennate diatoms, reproduction is primarily asexual, as cells divide in two similar daughter cells, in which one of them is slightly shorter than the other one, whereas width and depth change relatively less causing a reduction in average cell-size of the population (Round *et al.*, 1990; Mann *et al.*, 1999). Sexual reproduction produces auxospores by gamete fusion reestablishing the original size of the species (Amato *et al.*, 2005).

Explaining the relationship between diatom cell sizes and environmental conditions has been a challenge for diatomists. Some studies showed that there isn't a clear correlation between diatom cell size and organic pollution and nutrients (Lavoie *et al.*, 2006; 2010; Berthon *et al.*, 2011) whereas other studies found a correlation (Carrick & Lowe, 1989; Morin *et al.*, 2001; Lange *et al.*, 2016). For example, Lange *et al.* (2016) showed that a low concentration of nutrients favours smaller taxa, which can be related to their higher surface to volume ratios, that confer them a higher capacity for nutrient uptake (Litchman *et al.*, 2006; Reynolds *et al.*, 2006). Simultaneously, they have lower sinking rates, which is an advantage in water stratification that often happens in lakes (Winder & Sommer, 2011). Other examples of conditions that have been linked with diatom cell size are conductivity (Neustupa *et al.*, 2013), and streamflow (Lange *et al.*, 2016) and grazing (Tall *et al.*, 2006). Previous studies relating temperature with diatom size show contradictory conclusions: some scientists suggest that primary producers are smaller in higher temperatures following the same patterns as other species (e.g. Li *et al.*, 2009; Winder *et al.*, 2009; Moran *et al.*, 2010; Lewandowska & Sommer, 2010) probably because warmer water has less capacity to hold nutrients and oxygen (Sheridan & Bickford, 2009) and as said before smaller diatoms have a higher capacity for nutrient uptake (Litchman *et al.*, 2006; Reynolds *et al.*, 2006); other scientists proved that diatoms are a very important exception to the general rule (Adams *et al.*, 2013).

Despite the increasing number of trait-based diatom studies, most of them are based on database information that might be from other geographical regions whereas data on direct measurements is lacking. Thus, the aim of this study was to check if diatom sizes differ at the regional and global spatial scales. For that, we compared diatom community and species sizes from different river types in the Central Portuguese region (regional spatial scale) and among regions around the world (global spatial scale).

MATERIALS AND METHODS

Study sites

Diatoms measured in this study were collected in the spring of 2017 in 60 sites located in the central region of Portugal and belonging to different river types (Northern Mountain Streams – M;

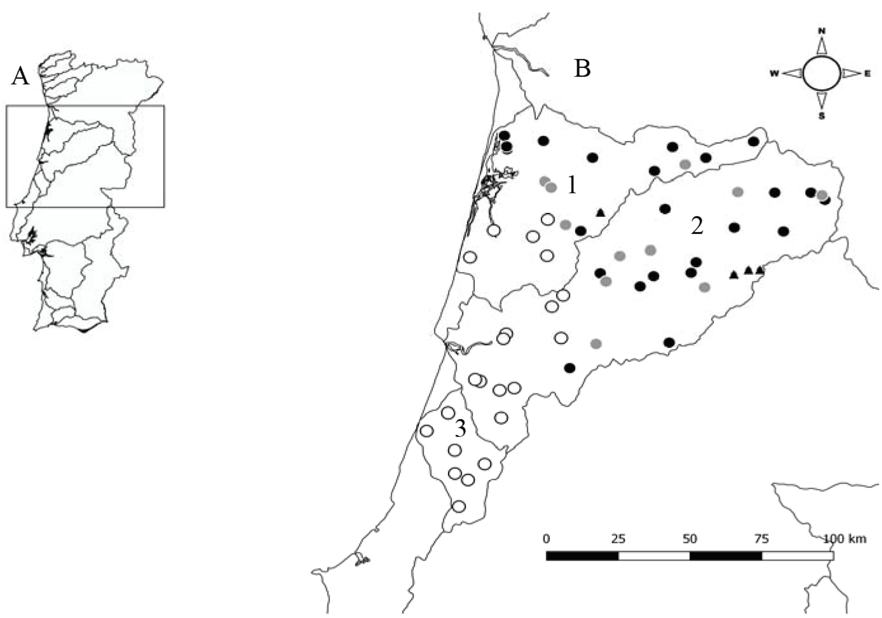

Figure 1. Map of the study sites in the central region of Portugal. A) Continental Portugal. B) Location of sampling sites according to typology: ○ – Littoral Rivers; ● - Large Northern Rivers; ● – Small Northern Rivers; ▲- Northern Mountain Streams. 1- Vouga Basin, 2 – Mondego Basin, 3 – Lis Basin. *Mapa dos locais de amostragem da região centro de Portugal. A) Portugal Continental. B) Localização dos locais de amostragem de acordo com a tipologia: ○ – Rios do Litoral Centro; ● – Rios do Norte de Média-Grande Dimensão; ● – Rios do Norte de Pequena Dimensão; ▲- Rios Montanhosos do Norte. 1 – Bacia Hidrográfica do Vouga, 2 – Bacia Hidrográfica do Mondego; 3 – Bacia Hidrográfica do Lis.*

littoral rivers - L; small northern rivers - N1 ≤ 100 km^2; medium-large northern rivers - N1 > 100 km^2), as represented in figure 1.

All the river types are characterized by low mean annual temperature (11-13 ºC) and high mean annual precipitation (1200-1900 mm per year), except for the Littoral Rivers, which have higher mean annual temperature (15 ºC) and lower mean annual precipitation (900 mm per year) (INAG, 2008a). The highest altitudes are registered in Northern Mountain Streams (506.02 ± 299.75 m), which differ the most from Littoral Rivers (40 m); in Small and Medium-Large Northern Streams altitude ranges between 200 and 600 m. The river types also differed in other aspects such as catchment area dimension and flow regime: the Northern Mountain Streams have the lowest mean catchment area (≈25 km^2) and the highest flow regime (600 to 2200 mm); the catchment area of Small Northern Streams is approximately 33 km^2 and the flow regime is 100 to 1800 mm; Littoral Rivers have a catchment area of approximately 180 km^2 and the lowest flow regime (50-1000 mm) and the medium-large northern rivers have a catchment area of approximately 549 km^2 and a flow regime of 100-2200 mm. Precipitation coefficient and air temperature do not differ much in the river types studied and assume values of approximately 0.30 and 10 ºC respectively (INAG, 2008a; Feio & Pinto, 2009).

Air temperatures registered during the year of 2017 were extremely high (IPMA, 2017) so the mean annual temperature for the sampling sites were higher than expected (19.16-19.41 ºC) (Accuweather, 2018).

Sample collection and processing

Sampling of biofilms and treatment of diatoms was performed according to European standards (European Committee of Standardization, 2003; 2004; 2006). In each site, the diatoms were collected from hard substrate (rocks or stones). The epilithic biofilm was collected from random submerged stones. The upper surface (approximately 100 cm^2) was scraped with a toothbrush. The samples were preserved with formalin (8 % final volume). To ensure that the diatoms were predominantly alive when sampled, an observation of the preserved samples was performed under the light microscope. The samples were then oxidized using concentrated nitric acid and potassium dichromate for 24h (INAG, 2008b). Permanent slides were mounted using Naphrax® (Brunel Microscopes Ltd., UK). Identification of diatoms mainly using the floras of Krammer and Lange-Bertalot (1986; 1988; 1991a; 1991b) and Krammer (2000; 2001; 2002; 2009) followed by enumeration of about 400 valves (AFNOR, 2000) from each slide was performed under the light microscope (Leitz Biomed 19) (100x objective and 1.32 numerical aperture).

In each sampling site, water temperature (ºC), conductivity (µS), pH and dissolved O_2 were measured with a multiparametric meter (WTW Multi 3430 SET F) and the habitat quality assessment score (HQA score) which translates the diversity of habitats found in the river channel, followed the Habitat River Protocol (Raven *et al.*, 2002).

Measurement of individuals

From each sample, 10 valves from each of the diatom species with abundance of 2.5 % or above were photographed with an Olympus DP70 digital camera installed in the light microscope Zeiss Axioplan 2 Imaging (100x objective and 1.40 numerical aperture). This resulted in about 3600 micrographs from 86 *taxa* because some *taxa* were present in more than one sample. Using the images and the respective scales, the length and width were measured and the ratio length/width was calculated.

Abiotic characterization of the sampling sites

To understand which of the abiotic factors considered (water temperature, conductivity, pH, dissolved O_2 and HQA score) contributed the most for the separation of the sampling sites according to river typology, a Principal Component Analysis (PCA) was performed using PRIMER 6 (Clarke & Gorley, 2006).

Diatom size plasticity at a regional scale

Differences between diatom lengths, widths and ratios among the four Portuguese river types from the central region of the country were tested using

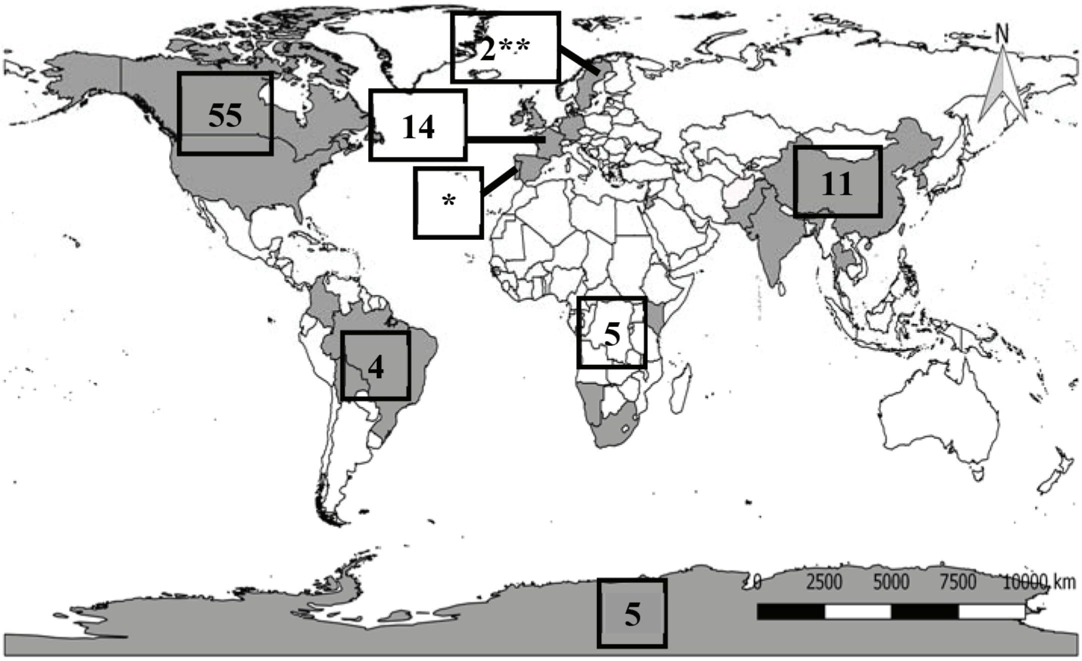

Figure 2. World map showing the regions used to analyse diatom sizes (grey). The numbers in a square represent the number of references used in each geographic region. * - our results; ** - based on a database and a flora. *Mapa-mundo representativo das regiões usadas para analisar as diferenças de tamanho das diatomáceas entre regiões geográficas (cinzento). Os números representam o número de referências usadas para cada região geográfica.* * - os nossos resultados, ** - baseado numa base de dados e numa flora.*

PERMANOVA [PRIMER 6, Clarke & Gorley (2006) with the add-on PERMANOVA+ for Windows, Anderson *et al.* (2008)]. In the first analysis, all the species were considered together and then the tests were carried out for each of the species present in more than one river type.

Preliminarily to all the analyses, a PERMDISP was done to test the homogeneity of the variances. When the assumption was not verified the data was transformed in order to achieve the homogeneity of variances.

Diatom size plasticity at a global scale

To investigate diatom size plasticity around the world, a revision of published information took place (see references in supplementary information available at: http://www.limnetica.net/en/limnetica) which included: diatom floras, scientific papers, and on-line databases from Northern and Central Europe, North America, South America, Asia, Africa and Antarctica. The countries

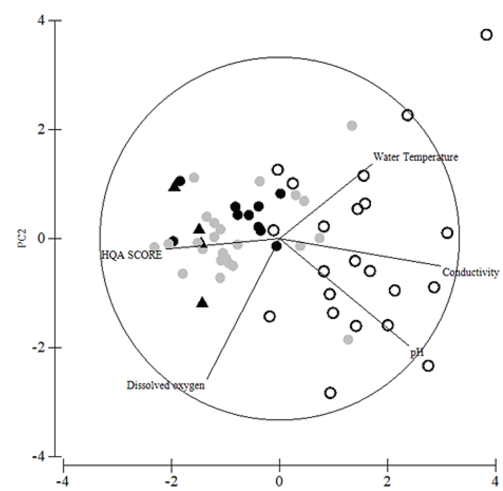

Figure 3. Principal Component Analysis (PCA) showing the abiotic characterization of the sampling sites. ○ – L: Littoral Rivers; ● – N1MM: Medium-Large Northern Rivers; ● – N1m: Small Northern Rivers; ▲- M: Small Mountain Streams. *Análise de Componentes Principais (PCA) para caracterização abiótica dos locais de amostragem. ○ – L: Rios do Litoral Centro; ● – N1MM: Rios do Norte de Média-Grande Dimensão; ● – N1m: Rios do Norte de Pequena Dimensão; ▲- M: Rios Montanhosos do Norte.*

included in the analysed regions are represented in figure 2 in grey. The comparison of diatom sizes found in each global region was based on the maximum and minimum values found in literature ((maximum length + minimum length)/2) and performed using Permutational Multivariate Analysis of Variance, PERMANOVA (software PRIMER 6, Clarke & Gorley, 2006; add-on PERMANOVA+ for Windows, Anderson *et al.*, 2008). Only species represented in at least 2 regions and with at least 2 values per region (including Portugal) were considered in this analysis. For Central Portugal, an average of each river type measurements was used. This analysis was performed considering all the species together and comparing *per species*.

PERMDISP analysis was also performed to test the homogeneity of variances before the size comparisons at a global scale.

RESULTS

Abiotic characterization of the sampling sites

The PCA in figure 3 showed differences between the four river types based on the parameters

Table 1. Comparison of the length of common diatom taxa in more than 1 river type of central Portugal (Northern Mountain Streams – M; Littoral Rivers - L; Small Northern Rivers - N1 ≤ 100 km^2; Medium- Large Northern Rivers - N1 > 100 km^2) that showed at least one significant difference. Pseudo-F values for PERMANOVA pairwise test results in which $0.05 \geq p \geq 0.01$ are represented with * and results in which $p \leq 0.01$ are represented with **. Analysis in which variances are not homogeneous are represented with [a]. *Comparação do comprimento dos taxa de diatomáceas comuns em mais que 1 tipo de rio da região centro de Portugal (rios Montanhosos do Norte – M; Rios do Litoral Centro - L; Rios do Norte de Pequena Dimensão - N1 ≤ 100 km^2; Rios do Norte de Média Grande Dimensão - N1 > 100 km^2) que demonstraram pelo menos uma diferença significativa. Valores Pseudo-F dos testes PERMANOVA nos quais 0.05 ≥ p ≥ 0.01 estão representados com * e os resultados nos quais p ≤ 0.01 estão representados com **. As análises em que as variâncias não são homogéneas estão representadas com* [a].

Taxa	L≠M	L≠N1≤100km²	L≠N1>100km²	M≠N1≤100km²	M≠N1>100km²	N1≤100km²≠N1>100km²
Achnanthidium minutissimum	2.19*	0.5	0.75	2.11*	1.29	1.03
Achnanthidium straubianum		0.76	7.85**			6.13**
Achnanthidium subatomoides	0.22	0.24	1.46	0.63	2.56*	0.94
Achnanthidium subhudsonis[a]						2.28*
Amphora inariensis		3.02**				
Cocconeis placentula var. lineata		3.55**	2.70*			0,57
Eolimna minima	3.52**	9.20E-02	0.12	3.75**	4.16**	0.24
Fragilaria parva			3.84**			
Gomphonema parvulum	0.19	2.12*	2.06*	2.42*	2.44*	0.67
Gomphonema rhombicum				2.8475**	4.1244**	11.054
Gomphonema uniserhombicum					4.39**	
Karayevia oblongella	4.22**	1.55	0.79	5.90**	7.37**	0.32
Navicula cryptotenella			6.80**			
Navicula cryptotenelloides		15.12**				
Nitzschia dissipata		1.16	3.73**			1.35
Nitzschia palea var. debilis		2.52**				
Surirella brebissonii		5.57**				
Sellaphora seminulum		2.26*	0.31			2.01*

analysed. The littoral rivers are segregated from the other three river types due to higher water temperature, conductivity and pH and to a lower HQA score. These variations were largely explained by the first axis (PC1-43 %); while small northern rivers and northern mountain rivers showed larger HQA score and oxygenation than medium-large rivers.

Diatom size plasticity at a regional scale

The mean lengths, widths and length/width ratios measured in this study per species and the micrographs of the common species in the four river types included in this study are represented in the supplementary information (available at: http://www.limnetica.net/en/limnetica). The majority of the species (28 of the 36 species measured in more than one river type) showed significant differences between river types for at least one of the measurements ($p < 0.05$). Tables 1, 2 and 3 represent the *taxa* with significant differences in length, width and ratio, respectively.

Yet, each species varied differently when comparing length, width and ratio between the four river types studied (see in figure 4 the example of *Achnanthidium* and *Gomphonema* species, two widespread genera).

Despite the absence of patterns in diatom size plasticity at the regional spatial scale, in this

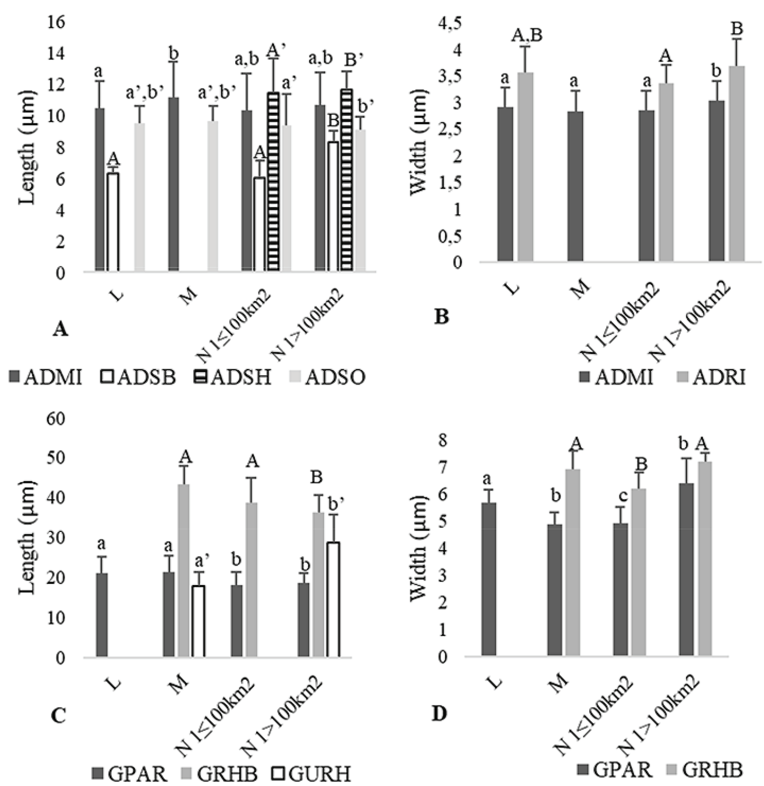

Figure 4. Comparison of mean lengths (A, C) and widths (B, D) of *Achnanthidium* (A, B) and *Gomphonema* (C, D) species that showed at least one significant difference between river types. Different letters of the same type represent significant differences in terms of length and width ($p < 0.05$), each type of letter is associated with the same species. ADMI – *Achnanthidium minutissimum*; ADRI – *Achnanthidium rivulare*; ADSH – *Achnanthidium subhudsonis*; ADSO – *Achnanthidium subatomoides*; GPAR – *Gomphonema parvulum*; GRHB – *Gomphonema rhombicum*, GURH – *Gomphonema uniserhombicum*. *Comparação dos comprimentos (A, C) e larguras (B, D) médias das espécies pertencentes aos géneros* Achnanthidium *(A, B) e* Gomphonema *(C, D) que demonstraram pelo menos uma diferença significativa entre tipos de rio. Diferentes letras dentro do mesmo tipo representam diferenças significativas em termos de comprimento e largura (*p *< 0.05), cada tipo de letra está associado a uma espécie diferente.*

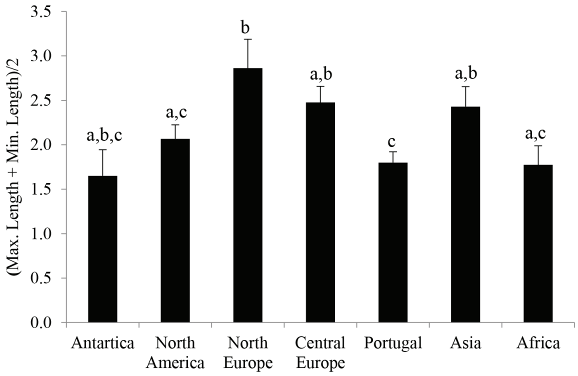

Figure 5. Comparison of the values (Maximum lengths + Minimum lengths)/2 considering the species from different geographic areas around the world. Different letters represent regions with significant differences in terms of diatom size ($p < 0.05$). *Comparação dos valores (Comprimento Máximo + Comprimento mínimo)/2 considerando as espécies todas a nível mundial, letras diferentes representam pares de regiões com diferenças significativas em termos de tamanho das diatomáceas (*p *< 0.05).*

study, lengths and ratios of the species belonging to the genus *Amphora* (*Amphora pediculus* and *A. inariensis*) tended to be larger in Littoral Rivers than in Small Northern Rivers.

Diatom size plasticity at a global scale

Considering all the species together, there were significant differences between some geographic regions (Fig. 5): diatoms in Portugal were significantly smaller when compared with Asia (t = 2.3758; $p = 0.021$) and the other areas of Europe studied - Central Europe (t = 3.2132; $p = 0.004$) and Northern Europe (t = 3.1548; $p = 0.005$). Diatom mean lengths in Northern Europe were significantly larger than in North America (t = 2.1819; $p = 0.024$) and Africa (t = 2.696; $p = 0.015$). South America was excluded from this analysis because the data covered a small number of species.

Per species size comparison revealed that *Achnanthidium minutissimum*, *Achnanthidium subatomoides*, *Eolimna minima* and *Eunotia incisa* showed significant differences in some geographic regions (Fig. 6): lengths of *Achnanthidium minutissimum* from Central Portugal were significantly smaller than those from Africa (t = 2.8708; $p = 0.021$) and Asia (t = 3.0483; $p = 0.019$); lengths of *Achnanthidium subatomoides* in Central Portugal were significantly larger than those registered in North America (t = 3.0952; $p = 0.03$); lengths of *Eolimna minima* from Central Portugal were smaller than those from North America (t = 2.839; $p = 0.026$) and the lengths of *Eunotia incisa* from Central Europe were larger than those from North America (t = 3.4478; $p = 0.03$).

The (maximum length + minimum length)/2 values collected for each region are represented in the supplementary information with the respective references.

DISCUSSION

The comparative study of diatom cell size at the regional and global scales revealed that this biological trait varies at both scales considered. Previous studies also showed the size variation of diatom species. A study based on the diameter of two *Rhizosolenia* species revealed that larger specimens occur in sites with lower temperatures (Wimpenny, 1936). Also, in a more recent study it was shown that the diatom *Brachysira vitrea* decreased size in metal contaminated sites (Luis *et al.*, 2011). Yet, this is the first study analysing this issue with a large number of samples and at the global scale.

Size variations at species level have also been recorded for other aquatic and terrestrial organisms. For example, in anurans it is known that low temperatures may influence growth and development rates in larval and juvenile stages which have follow-on effects on maturity and growth and consequently high latitude and altitude individuals should be generally larger than the low latitude and tropical specimens (Berven, 1982, Zhong *et al.*, 2018). Lower temperatures trigger a similar effect in marine invertebrates (Irie & Fisher, 2009; Daufresne *et al.*, 2009), fish (Desai & Singh, 2009; Sahin, 2009), arthropods (Stillwell & Fox, 2009; Brehm *et al.*, 2018) and other amphibians (Bizer, 1978; Schäuble, 2004).

Considering the Portuguese river types analysed we found significant differences in about 78 % of the species. Yet in most cases, no general trends were identified in size plasticity at this spatial scale: some species were larger in a certain river type while other taxa were smaller for that same river type, in spite of the differences in parameters such as water temperature, conductivity and pH among river types. Different conclusions were drawn in other studies where it was demonstrated that diatom cell size may respond to conductivity (Neustupa *et al.*, 2013,

Table 2. Comparison of the width of common diatom taxa in the 4 river types of central Portugal (Northern Mountain Streams – M; Littoral Rivers - L; Small Northern Rivers - N1 ≤ 100 km^2; Medium-Large Northern Rivers - N1 > 100 km^2) that showed at least one significant difference. Pseudo-f values for PERMANOVA pairwise tests results in which $0.05 \geq p \geq 0.01$ are represented with * and results in which $p \leq 0.01$ are represented with **. Analysis in which variances are not homogeneous are represented with [a]. *Comparação da largura dos taxa de diatomáceas comuns em mais que 1 tipo de rio da região centro de Portugal (rios Montanhosos do Norte – M; Rios do Litoral Centro - L; Rios do Norte de Pequena Dimensão - N1 ≤ 100 km^2; Rios do Norte de Média Grande Dimensão - N1 > 100 km^2) que demonstraram pelo menos uma diferença significativa. Valores pseudo-F dos testes PERMANOVA nos quais 0.05 ≥* p *≥ 0.01 estão representados com * e os resultados nos quais* p *≤ 0.01 estão representados com **. As análises em que as variâncias não são homogéneas estão representadas com* [a].

Species	L≠M	L≠N1≤100km²	L≠N1>100km²	M≠N1≤100km²	M≠N1>100km²	N1≤100km²≠N1>100km²
Achnanthidium minutissimum	0.93	1.13	2.45*	0.13	2.68**	3.23**
Achnanthidium rivulare		1.72	0.96			2.78*
Cocconeis pediculus		2.87*				
Diatoma mesodon				0.77	3.71*	2.32*
Eolimna minima	3**	1.59	0.78	4.24**	3.54**	0.58
Fistulifera saprophila		6.80**				
Gomphonema parvulum	3.98**	3.47**	0.89**	0.13	6.19**	5.65**
Gomphonema rhombicum				4.1224**	11.609	4.9984**
Karayevia oblongella	3.82**	3.45E-02	0.83	1.14	6.36**	0.28
Navicula cryptocephala				3.48**		
Navicula cryptotenella[a]				9.27**		
Navicula cryptotenelloides			21.6**			
Nitzschia inconspicua		3.50**				
Nitzschia palea var. debilis		3.64**				
Planothidium frequentissimum[a]		1.26	2.29*			1.51
Reimeria sinuata[a]		5.53**	0.42			3.17**
Surirella brebissonii		4.58**				
Sellaphora seminulum		3.70**	0.5			4.19**

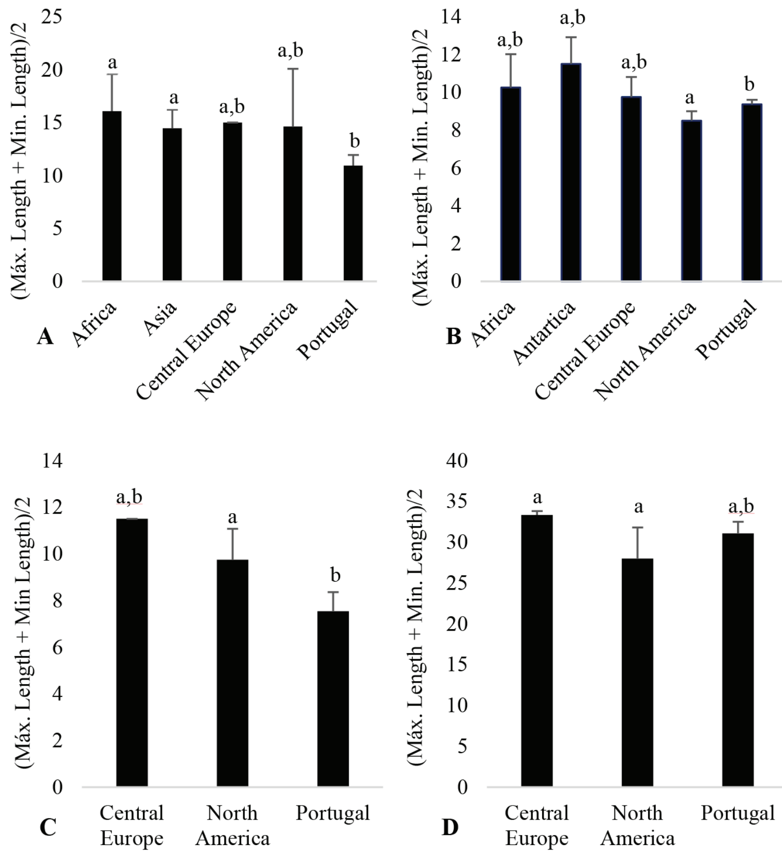

Figure 6. Comparison of the values (Maximum lengths + Minimum lengths)/2 of *Achanthidium minutissimum* (A), *Achnanthidium subatomoides* (B), *Eolimna minima* (C) and *Eunotia incisa* (D) around the world, different letters represent regions with significant differences in terms of diatom size ($p \leq 0.05$). *Comparação dos valores (Comprimento Máximo + Comprimento mínimo)/2 das espécies* Achnanthidium minutissimum *(A),* Achnanthidium subatomoides *(B),* Eolimna minima *(C) e* Eunotia incisa *(D) a nível mundial; letras diferentes representam pares de regiões com diferenças significativas em termos de tamanho de cada espécie (*p $\leq$ *0.05).*

Stenger-Kovács *et al.*, 2018), pH (Neustupa *et al.*, 2013), oxygen and temperature (Walczýnka & Sobczynk, 2017).

These differences may be explained by the fact that cell-size in diatoms can depend on some mechanisms not necessarily connected to what defines the concept of river type such as: their life cycle (Round *et al.*, 1990; Mann *et al.*, 1999). Diatoms can respond to the same condition by progressively reducing their cell size (asexual reproduction) or can restore their size by auxospore formation. Additionally, diatom cell size may respond to grazing (Tall *et al.*, 2006) and other environmental conditions such as salinity (Stenger-Kovács *et al.*, 2018) and anthropogenic pressure (Lange *et al.*, 2016). Thus, the differences observed and the absence of particular patterns may reflect variations not covered in this study. Studies made in controlled laboratory conditions should also contribute to clarify the observed patterns.

At the global spatial scale several differences between regions were registered, including within Europe. As most diatomists, we usually resort to diatom floras which are from other geographical regions. For example, in Portugal (SW Europe) we mostly use diatom floras from Central and Northern Europe. Nevertheless, we found size differences between the diatoms measured by us in Central Portugal and the range of sizes registered in other regions of Europe. In spite of these results, the number of works (publications, floras, on-line databases) found for each species were not many. Therefore, care must be taken with these conclusions and further research should be undertaken based on measurements of diatoms from different areas where the study is taking place to confirm the observed patterns.

Our results indicate that in the potential development of an indirect functional approach for the assessment of rivers based on traits, as those that have been considered for other biological elements such as invertebrates (e.g. Statzner *et al.*, 2005; Statzner & Bêche, 2010; Feio & Dolédec, 2012) or more recently for diatoms (e.g. Lavoie *et al.*, 2006; Berthon *et al.*, 2011; Talpoczai *et al.*, 2017; Pandey *et al.*, 2018; Stenger-Kovács *et al.*, 2018), care must be taken

Table 3. Comparison of the l/w ratio of common diatom taxa in the 4 river types of central Portugal (Northern Mountain Streams – M; Littoral Rivers - L; Small Northern Rivers - N1 ≤ 100 km^2; Medium-Large Northern Rivers - N1 > 100 km^2) that showed at least one significant difference. Pseudo-F values for PERMANOVA pairwise tests results' in which 0.05 ≥ p ≥ 0.01 are represented with * and results in which p ≤ 0.01 are represented with **. Analysis in which variances are not homogeneous are represented with [a]. *Comparação do rácio c/l dos taxa de diatomáceas comuns em mais que 1 tipo de rio da região centro de Portugal (rios Montanhosos do Norte – M; Rios do Litoral Centro - L; Rios do Norte de Pequena Dimensão - N1 ≤ 100 km^2; Rios do Norte de Média Grande Dimensão - N1 >100 km^2) que demonstraram pelo menos uma diferença significativa. Valores pseudo-F dos testes PERMANOVA nos quais 0.05 ≥* p *≥ 0.01 estão representados com * e os resultados nos quais* p *≤ 0.01 estão representados com **. As análises em que as variâncias não são homogéneas estão representadas com* [a].

Species	L≠M	L≠N1≤100km2	L≠N1>100km2	M≠N1≤100km2	M≠N1>100km2	N1≤100km2≠N1>100km2
Achnanthidium minutissimum	2.53*	0.12	1.33	2.34*	3.35**	1.1
Achnanthidium rivulare		2.86**	1.33			1.58
Achnanthidium straubianum[a]		0.18	4.66**			6.69**
Achnanthidium subtomoides	1.78*	1.63	0.36	0.33	2.17*	2.51*
Amphora inariensis		4.22**				
Amphora pediculus[a]		2.55*				
Cocconeis euglypta	1.16	0.98		2.32*		
Cocconeis pediculus		4.19**				
Cocconeis placentula var. lineata[a]		2.73**	4.61**			3.39**
Fragilaria parva			3.42**			
Fistulifera saprophila		6.82**				
Gomphonema parvulum	2.04*	0.12	2.70**	2.53*	5.24*	3.33**
Gomphonema rhombicum				0.02501	5.3645**	3.392**
Mayammaea permitis		1.98*	1.05			2.60*
Navicula cryptotenelloides						
Nitzschia dissipata		1.89*	4.91**			1.73
Planothidium frequentissimum		0.55	1.24			2.01*
Reimeria sinuata		3.59**	1.29			1.64
Surirella brebissonii		10.37**				

in the establishment of reference values for metrics that contemplate diatom size.

We also recommend that measurements of diatoms are performed in ecological studies when size is a relevant feature, as the use of databases based on data from a given region may result in biased conclusions as variations in size occur at both regional and global scales.

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