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Conference Abstract

# Effects of anthropogenic pressures on diatom communities using morphological and molecular approaches

Marco Neto Teixeira[‡], Salomé Almeida[‡], Maria João Feio[§], Andreia Mortágua[‡], Manuela Sales[‡]

‡ Universidade de Aveiro, Aveiro, Portugal
§ Universidade de Coimbra, Coimbra, Portugal

Corresponding author: Marco Neto Teixeira (marcoteixa22@gmail.com), Salomé Almeida (salmeida@ua.pt), Maria João Feio (mjf@ci.uc.pt), Andreia Mortágua (andreiamortagua@ua.pt), Manuela Sales (msales@ua.pt)

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## Abstract

The relevance of molecular composition of diatom assemblages to detect river impairment caused by different intensive land uses (industrial, agricultural, and urbanization) was tested in this study with data from two rivers (Ferreira and Sousa rivers) and 21 sampling sites located in the north of Portugal. The Water Framework Directive (WFD) gives the legal basis for the use of this ecological indicator for water quality assessment (Vasselon et al. 2017 ). However, the morphological identification and count of diatoms using the light microscope requires a high level of expertise, is time-consuming and costly (Valentin et al. 2019). DNA metabarcoding combined with high-throughput sequencing techniques (HTS), offers a promising alternative to classic methodologies, reducing time and costs (Mortágua et al. 2019 ). Thus, here we compared the response of the two approaches in terms of ecological assessments (IPS Ecological Quality Ratios) to the different types of pressures felt in the 21 sites.

Diatoms were sampled at 21 sites located in the North of Portugal in autumn of 2019 (Fig. 1). Samples were submitted in parallel to the molecular and morphological analyses. The eDNA was extracted, PCR amplified (312 bp rbcL DNA barcode), and finally sequenced (Illumina MiSeq). The Mothur software was used to obtain the Operational Taxonomic Units

(OTUs), which were then taxonomically assigned to the species through the R-Syst::diatom version 7.1 (Rimet et al. 2018) reference library. EQR values indicated a good correlation between morphological and molecular methods (Fig. 2). PCA analysis (Fig. 3 ) revealed that in urban, agricultural, and industrial areas there is a greater concentration of nutrients (phosphorus and nitrogen), organic matter, and heavy metals due to the discharge of urban/industrial effluents, while in places considered natural (without any type of anthropogenic pressure) we find low levels of these pollutants and high concentrations of dissolved oxygen (DO). The BEST (BIO-ENV) analysis (Tables 1, 2) shows in the case of the morphological approach, the combination of 4 environmental variables ($NO_3^-$, Li, K, and Cu) is highly correlated with the biological patterns, and in the molecular approach the combination of only 2 of the environmental variables (Li and K), explains the distribution of diatom communities composition and has a slightly higher correlation. The morphological methodology seems to demonstrate a better response to urban pressures, mainly to effluent discharges, while the molecular one demonstrates a more diffuse response with special emphasis on good correlation with variables such as zinc and nitrate, which may also be related to effluent discharges and use of fertilizers in agriculture. However, it is necessary to improve the reference library so that there is a better response of the molecular methodology to the existing pressures.

Table 1.

BEST (BIO-ENV) results from PRIMER showing which environmental variables best explain the biotic patterns observed on morphological approach.

| NO. of variables | Correlation | Variable combination |
|---|---|---|
| 4 | 0.680 | $NO_3^-$; Li; K; Cu |
| 5 | 0.667 | $HCO_3^-$; $NO_3^-$; Li; K; Cu |
| 3 | 0.662 | $NO_3^-$; Li; Cu |
| 5 | 0.659 | COD; $NO_3^-$; Li; K; Cu |
| 5 | 0.657 | COD; $HCO_3^-$; $NO_3^-$; Li; Cu |
| 5 | 0.653 | COD; $HCO_3^-$; Li; K; Cu |
| 4 | 0.651 | COD; $HCO_3^-$; Li; Cu |

Table 2.

BEST (BIO-ENV) results from PRIMER showing which environmental variables best explain the biotic patterns observed on molecular approach.

| NO. of variables | Correlation | Variable combination |
|---|---|---|
| 2 | 0.772 | Li; K |
| 3 | 0.736 | Li; K; Zn |
| 4 | 0.727 | $HCO_3^-$; Li; K; Zn |

| NO. of variables | Correlation | Variable combination |
|---|---|---|
| 3 | 0.725 | $HCO_3^-$; Li; K |
| 4 | 0.721 | $NO_3^-$; Li; K; Zn |
| 3 | 0.712 | $NO_3^-$; Li; K |
| 5 | 0.710 | $HCO_3^-$; $NO_3^-$; Li; K; Zn |

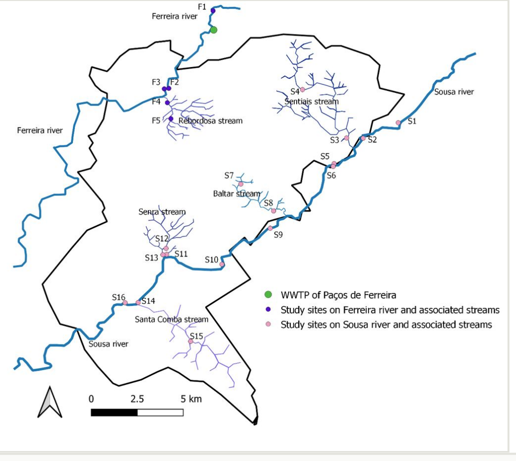


Figure 1. doi

Map of the Hydrographic Network of the Ferreira and Sousa Rivers, and sampled streams, together with the georeferenced location of the sampling sites in the Municipality of Paredes.

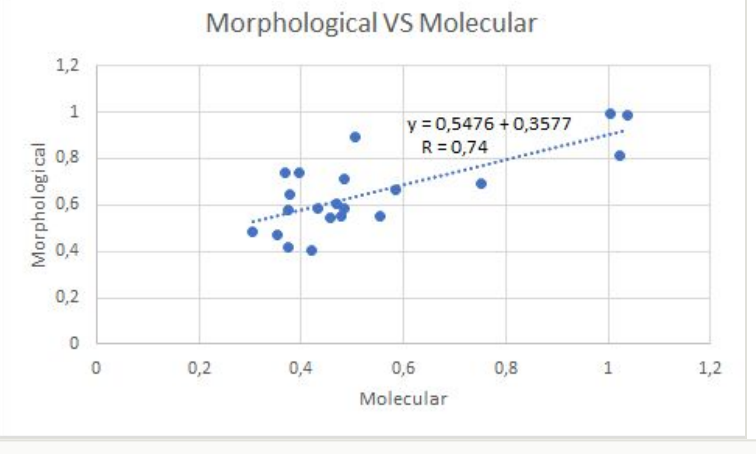


Figure 2. doi

Correlation between the diatom Ecological Quality Ratios (EQR) based on molecular (x axis) and morphological (y axis) EQR for all samples. The linear regression model is represented by the dotted line, and r value is indicated. EQR values are in the range from 0 (bad quality status) to 1 (high quality status).

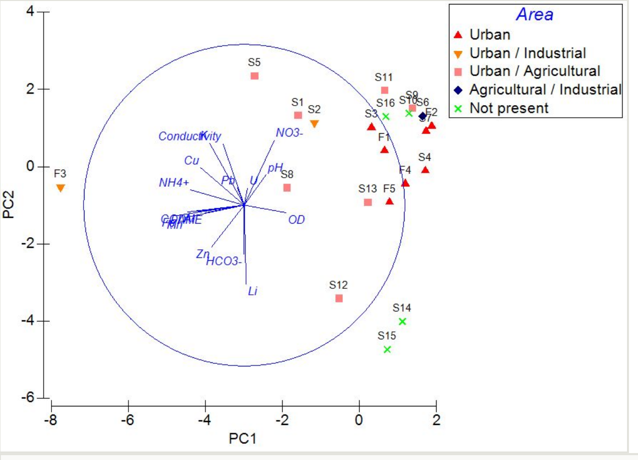


Figure 3. doi

PCA plot based on environmental data of all 21 samples. Vectors representing physical-chemical parameters [Conductivity, pH, FAME, Nitrates ($NO_3^-$), Ammonium ($NH_4^+$), Manganese (Mn), Lead (Pb), Copper (Cu), Zinc (Zn), Lithium (Li), Uranium (U). Potassium (K), Phosphorus (P), Chemical Oxygen Demand (COD), Dissolved Oxygen (OD) and Bicarbonates].

## Keywords

Diatoms; DNA metabarcoding; anthropogenic pressures; WFD; bioassessment

## Presenting author

Marco Neto Teixeira

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