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A predictive diatom-based model to assess the ecological status of streams and rivers of Northern Spain

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ABSTRACT

In this study we developed a predictive diatom-based model to assess the ecological status of streams and rivers of Northern Spain. Diatom samples were collected with standard protocols over stones from 676 sites distributed along existing environmental conditions across Northern Spain, during seven years between 2002 and 2008 (n = 1056 samples). This dataset included a network of 91 reference sites selected by using criteria that confirmed the absence of relevant human pressures according WFD. A multinomial logistic regression using GAAC cluster-derived reference sites group as response variable was performed. The independent variables included obligatory typology factors (WFD System A typology descriptors), and other optional typology B descriptors were included in the model performed on a forward stepwise procedure. The Ecological Quality Ratios (EQRs) were obtained by dividing the observed similarity between the diatoms composition in each sample by the expected median similarity of each type reference diatom community. The model predictions (EQRs) responded significantly to eutrophication and intensive agriculture pressures, but were not related with sewages, hydromorphological alterations and extensive agriculture pressures. These results demonstrated the accuracy of the diatom model in predicting nutrient enrichment in Northern Spanish rivers and streams.

1. Introduction

Predictive models are relatively recent approaches to assess water quality, aiming to evaluate the quality of a stream or river site as the degree of alteration of its biota in relation to the same stream or river type biological reference communities (Reynoldson et al., 1997). There have been multiple approaches to develop predictive models using macroinvertebrate communities, proving to be powerful tools in the assessment of water quality (RIVPACS, Wright, 1995; BEAST, Reynoldson, et al., 1995; AUSRIVAS, Simpson and Norris, 1997; NORTI invertebrates, Pardo et al., 2014). Meanwhile, models based on diatoms have only lately been developed for different regions following similar methodologies as for invertebrates, and also showing significant response to several human pressures (Mazor et al., 2006; Philibert et al., 2006; Feio et al., 2009, 2012; Almeida and Feio, 2012). Diatoms are considered among the best indicators for eutrophication or nutrient enrichment conditions, even better than other organisms such as macrophytes and fish (Hering et al., 2006; Johnson et al., 2006).

During the last 20 years, benthic diatoms have gone from being poorly used to be one of the most used bioindicators together with benthic invertebrates for the assessment of river water quality in Europe (Poikane et al., 2016). Several European studies started to use diatoms as indicators of water quality (Kelly and Whitton, 1998; Kwandrans et al., 1998; Dell'Uomo et al., 1999) in parallel with advances in the field from other worldwide regions (Jüttner et al., 1996; Chessmann et al., 1999). However, significant advances in their use as bioindicators occurred since 2000 with the implementation of the Water Framework Directive (WFD, 2000/60/CE). WFD requirements led to the adjustment of existing assessment systems for water quality and encouraged the design of new classification systems for the ecological status of rivers. The new systems included new approaches for the concept of European reference conditions (Pardo et al., 2011, 2012), the establishment of national and European river typologies, the classification system for the ecological status and ecological classes boundaries, and the mandatory intercalibration exercise between European countries (van de Bund, 2009).

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In Spain, assessment of the ecological status of rivers using diatoms is generally approached with the use of diatom metrics (Gomà et al., 2004; Blanco et al., 2008; Delgado et al., 2010; Martín et al., 2010; Delgado et al., 2012; Álvarez-Blanco et al., 2013), as integrators that summarize the complex ecology of stream diatom communities. In particular the Specific Polluosensitivity Index (IPS, Cemagref, 1982) has been used to assess the ecological status of Mediterranean rivers (Gomà et al., 2004; Blanco et al., 2008) and has enabled the comparison of diatom communities between different catchment areas (Martín et al., 2010). Moreover, the IPS diatom metric is used as the national classification system for all stream and rivers types in Spain (RD 817/2015), in spite of strong regional differences in the Spanish climate and geomorphological characteristics. These environmental differences determine distinctive attributes in geomorphology, biochemistry and hydrology causing variations in physical conditions and chemical composition and, consequently in the biogeography and composition of diatom communities (Stevenson, 2014; Delgado and Pardo, 2014).

A previous study in North Western Spain (Galicia), developed a multimetric diatom index (MDIAT) to classify the ecological status of the rivers of the region (Delgado et al., 2010), a system that was intercalibrated during the first European exercise (Kelly et al., 2009; van de Bund, 2009). Meanwhile, MDIAT only covered the more siliceous and mixed river types in NW Spain, there was a need to develop a more representative and comprehensive WFD classification system in order to cover all existing river types in the studied regions (e.g. from calcareous to siliceous).

The purpose of this study was to develop a predictive model based on diatom assemblages to assess the ecological status of rivers and streams of Northern Spain meeting the scientific requirements of WFD. We assembled biotic and abiotic data from minimally disturbed sites or reference sites where the absence of significant pressure criteria was verified (Pardo et al., 2012,) to develop the predictive diatom-based model NORTIdiat (NORThern Spain Indicator System for diatoms). The model response was tested on stress gradients including multiple stressors to assess the sensitivity of the predictions. Finally, the NORTIdiat was intercalibrated within the European intercalibration exercise based on its comparison with the ICM multimetric index, within the Central Baltic Geographical Intercalibration Group (CB GIG) to which Northern Spanish streams and rivers belong.

2. Material and methods

2.1. Study area

The study area is located in the Northern part of Spain, and covers the river basins managed by four River Basin Authorities (Galicia-Costa, Miño-Sil River basin, Cantabrian basins and Basque basins) (Fig. 1). The area covers 38,450 km^2, from Galicia in the Northwest to the Basque Country in the East. The Cantabrian and "Galicia-Costa" river basins are characterized by small, medium-high elevation gradient streams running from coastal mountains to the sea. Meanwhile, on the Western Atlantic coast the biggest and longest Miño-Sil Basin flows East-Westwards forming long valleys at low altitude. A more detailed description of the study area can be found in Pardo et al. (2014).

2.2. Sampling design

In this study 676 sampling sites distributed along the whole region of study and covering the wide existing environmental gradients in the area were sampled. Each sampling site was visited at least once during the period 2002–2008, generally in summer, except in 2006 and 2008, when several sites (115 and 30 sites, respectively) were visited also in spring. A total number of 1056 samples were collected. The sampling effort per year was not equally distributed along the study period, being samples scarce in 2002 and 2007 (28 and 17 samples per year, respectively) but in the rest of the years ($\geq$146 per year). The maximum number of sites sampled in a year was 466 (in 2003).

2.3. Data collection and laboratory methods

2.3.1. Abiotic data

The same dataset of environmental variables as in Pardo et al. (2014) was used, as diatoms and invertebrates were collected simultaneously during sampling. Briefly, filtered water samples were collected in most sampling visits using standard methods and transported to the laboratory to quantify potential stressors (phosphates as $P\text{-}PO_4^{-3}$ mg/L; nitrates as $N\text{-}NO_3^-$ mg/L; nitrites as $N\text{-}NO_2^-$ mg/L; ammonium as $N\text{-}NH_4^+$ mg/L), and *in situ* Oxygen saturation was measured with a portable oximeter (YSI 556 MPS).

Sampling sites were also characterized according to the obligatory descriptors in the WFD typology System B (latitude [m], longitude [m], altitude [m], catchment area [km^2] and geology represented solely by the percentage of calcareous geology), together with other optional descriptors (catchment slope [%], mean channel slope [%], maximum and mean catchment altitude [m], annual precipitation [mm]) (Table 1). The network of reference sites identified by Pardo et al. (2014) of 108 reference sites was used in this study. However, diatom samples were available in only 91 of these reference sites. For detailed information about selection of reference sites see Pardo et al. (2014).

2.3.2. Diatom samples

In each site, five stones were randomly sampled in the middle of the stream from riffle areas. Diatom samples were pooled by scraping the stone surfaces with a toothbrush in order to remove all the periphyton (Kelly et al., 1998; EN 13946, 2003), and after that were fixed with formaldehyde (4%). Organic matter was eliminated using hydrogen peroxide (33%) and HCl (37%) was added to remove the calcium carbonate. Finally, after rinsing with distilled water, permanent slides were mounted using Naphrax®, a synthetic mounting medium with high refractive index (r.i. = 1.74) to identify diatom species. Diatoms were observed and identified at the lowest taxonomic level, using an optical microscope (Olympus BX41). In each sample a minimum of 400 diatom valves were identified and counted at 1000× magnification (NA 1.25). The identification and nomenclature were based on (Krammer and Lange-Bertalot, 1986, 1988, 1991), Lange-Bertalot (1993) as well as on recent bibliography.

2.4. Data analysis

2.4.1. Group assignment: Model construction and validation

We aimed to identify the natural groupings in diatom assemblages of reference samples. For that we applied Group-Average Agglomerative Clustering (GAAC) to the similarity Bray-Curtis matrix of log-transformed relative abundances of diatoms. GAAC measures the dissimilarity between two clusters by using the dissimilarities average between all two objects combinations (Quinn and Keough, 2002). Four major groups were differentiated at the 29% similarity level, although the smallest group was eliminated as it was only composed of two samples. The largest group was further subdivided in two groups at 36% similarity level. Group consistency was evaluated with an ANOSIM analysis (Clarke 1993) in order to ensure that each group was significantly different from the rest. Complementary, we ran a non-Metric Multidimensional Scaling (MDS) ordination on the same similarity matrix to visualize distinctiveness of groups and their separation on the ordination space. The software PRIMER v.6 (Clarke and Gorley, 2006) was used for the previous analyses.

Multinomial logistic regression using GAAC cluster-derived reference sites group as response variable was performed to predict the diatom groups from abiotic descriptor variables, using 10 environmental typology A and B potential predictors. Multinomial logistic regression can predict nominal variables (4 diatom assemblages) with a set of independent variables. The procedure for automatic forward

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