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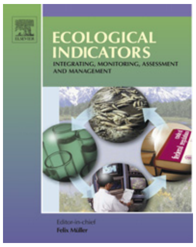

Water quality assessment of Portuguese streams: Regional or national predictive models?

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ABSTRACT

The European Water Framework Directive (WFD 2000) brought the need in European Union countries to establish consistent quantitative methods for the water quality assessment of streams, using aquatic communities. With this work we aimed to develop predictive models using macroinvertebrate communities that could be used in Portugal as an alternative to the more traditional indices and metrics. We used data from 197 reference sites and 174 sites suspected of being impaired, which were obtained in a national survey conducted in 2004–2005 by the Instituto da Água (INAG, Portugal). The spatial scale at which to develop predictive models was an issue to address because the Portuguese territory covers a wide variety of landscapes in a small area. We built three models using the AUSRIVAS methods, a national and two regional (North and South) models that produced acceptable assessments. However, the regional models, predicted more taxa than the National model, were more accurate and had lower misclassification errors when placing sites into pre-defined groups. The regional models were also more sensitive to some disturbances related to water chemistry (e.g., nutrients, BOD_5, oxidability) and land use. The exception was for the northern costal area, which had few reference sites. In the northern costal area the National model provides more useful results than the regional model. The 5-class WFD quality assessment scheme, adapted from the AUSRIVAS bands, appears to be justified because of the good correspondence between the human disturbance level and the classes to which test sites were allocated. Elimination of the AUSRIVAS X band in the WFD scheme has produced a clearer relationship. The predictive models were able to detect a decline in river health, responded to several causes of degradation and provided site-specific assessments.

1. Introduction

The use of bioindicators to assess river health is becoming legislated and mandatory in Europe with the introduction of the Water Framework Directive (WFD) (Directive 2000/60/CE, 2000). Macroinvertebrates were selected as one of the WFD biological quality elements (BQE) because of their ubiquity, easy sampling methods, long aquatic life phases that allow the assessment of changes in river condition through time, and taxonomic diversity with a variety of sensitivity to environmental stress (Hellawell, 1977; De Pauwn and Vanhooren, 1983; Furse et al., 2006). Fundamentally, the BQE stipulated in the WFD do not specify methods but should be developed to meet the need to detect changes in river health, indicate causes of degradation and measure the success of stream rehabilitation.

In Portugal, the most used assessment method based on macroinvertebrates has been the biotic index IBMWP (former

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BMWP; Alba-Tercedor and Sánchez-Ortega) but other alternative methods have also been developed following the multimetric (Pinto et al., 2004) and predictive modeling approaches (Feio et al., 2007a,b). Predictive models, although conceptually simple, are a powerful statistical tool developed for the bioassessment of rivers at various scales (site to nationwide) and used in assessment schemes around the world (Wright et al., 1984; Reynoldson et al., 1995; Parsons and Norris, 1996; Kokeš et al., 2006; Feio et al., 2007a,b). Through predictive models the observed fauna at a test site is compared with the fauna expected/predicted from a set of sites representing the reference condition for a given area (Reynoldson et al., 1997; Simpson and Norris, 2000).

RIVPACS (River InVertebrate Prediction And Classification System, Wright et al., 1984; Armitage et al., 1987; Wright, 1995), developed in the United Kingdom, led the way with broad-scale assessment using the predictive modeling approach. The spatial scale at which to develop predictive models needs addressing in a small but spatially variable country such as Portugal (Mainland Portugal occupies 91,985 km^2). A single model could be used as was done in U.K. (Wright, 1995). Yet, the Portuguese territory has a wide diversity of landscapes and stream types that could represent strong environmental gradients across the country (http://www.iambiente.pt/atlas/est/index.jsp). In Australia, its large size and varied landscape meant regional models were needed (Simpson and Norris, 2000).

The biological assessment method adopted for the WFD needs to align with pre-existing requirements (Directive 2000/60/CE, 2000). Typically, the RIVPACS/AUSRIVAS models produce a site-specific list of the expected taxa and an Observed/Expected ratio (O/E). The O/E scores for each test site are then allocated to a condition band where the deviation of the assemblage from that expected represents the biological condition of the stream. Band A corresponds to sites similar to reference condition and bands B, C and D represent decreasing condition corresponding to increasing levels of degradation. The WFD also requires a similar grading scheme for the assessment system under development, where the range of ecological quality scores could be divided into 5 classes. The first class represents reference condition (high ecological status) and another 4 classes indicate increasing levels of degradation (good, moderate, poor and bad) (Directive 2000/60/CE, 2000; Furse et al., 2006). Thus, the AUSRIVAS system seemed appropriate for adoption in Portugal since it allows the assessment of stream water quality based on changes in macroinvertebrates community structure; gives further information such as the site-specific expected taxa list; produces an ecological quality ratio (O/E) as required by the WFD; simplifies the interpretation of the results through a banding scheme and can be applied regionally.

The objectives of this study were: (1) to develop an AUSRIVAS type biological assessment scheme that uses the 5 WFD classes of ecological status to evaluate the condition of Portuguese rivers (excluding islands) at a national/broad scale; (2) to build regional/local-scale models, and (3) to determine which approach (regional/local scale or national/broad scale) is better suited to biological assessments throughout mainland Portugal.

2. Methods

2.1. Study area

Portugal is located on the west side of the Iberian peninsula with its borders defined by mountains and rivers. The interior and the north of the country are mountainous and lowlands to the south and coastal regions. The highest mountain is Serra da Estrela (2000 m). The larger Portuguese rivers, Tagus and Douro, have their sources in Spain and the largest river entirely in Portuguese territory is the Mondego River. The Portuguese climate is temperate Mediterranean in the south (precipitation below 600 mm yr^{-1}) and Atlantic-humid in north and western coast (precipitation $>$2800 mm yr^{-1}; Atlas do Ambiente). In the NE the precipitation (1000–3000 mm yr^{-1}) is often in the form of snow during winter. The coastal area is densely populated and largely cultivated while the inlands have scattered villages, less industry and agriculture.

2.2. Field sampling

The data used in this work were collected throughout Portugal (excluding islands, Fig. 1). Several teams under the supervision of the Instituto da Água (Portugal), selected and sampled 197 reference sites (good condition or best available for selected stream types, Fig. 1). Sites were selected to represent the 27 stream types established by the Instituto da Água using the WFD System B (with the exception of rivers catchments $>$1000 km^2). According to the WFD system all Portuguese streams were originally grouped according to their hydromorphological the characteristics, geology, altitude and catchment area, latitude and longitude, and additional optional variables slope, runoff, precipitation, mean annual temperature and air temperature range (Directive 2000/60/CE, 2000; Alves et al., 2006). The reference condition for sites was defined by criteria based on previous knowledge, expert judgment and collected information. The reference sites met the common criteria of: (1) good chemical quality (nitrate, nitrite, phosphates, ammonia, pH, BOD_5, COD), i.e., values allocated to the A or B categories for water of multiple uses (INAG, http://snirh.inag.pt/snirh/dados_sintese/qual_ag_anual/classificacao.html); (2) minimal changes in the natural composition of the riparian corridor; (3) no signs of recent changes in the channel morphology and all expected habitats present, and (4) low levels of urbanization and industrial activities in the catchment area. Additionally, 174 sites suspected of being impacted were used to test the method and an additional 16 reference sites were used to validate the method. These sites were also distributed across the country and were sampled using the same procedures as for the reference sites used to develop the models (Fig. 1). A 50 m reach representative of the stream's habitat diversity, including a riffle (whenever that was possible) was defined for each site. Macroinvertebrates were sampled with a hand-net (0.25 m opening and 500 μm mesh size) and each sample comprised six composite collections. Collections were proportional to the area occupied by the most representative habitats (stones, sand and silt, boulders ($>$256 mm), submerged plants and algae) and each collection defined by an area 1 m $\times$ 0.25 m. The composite sample was preserved with formalin (4%) in the field and the invertebrates were later sorted in the laboratory under a

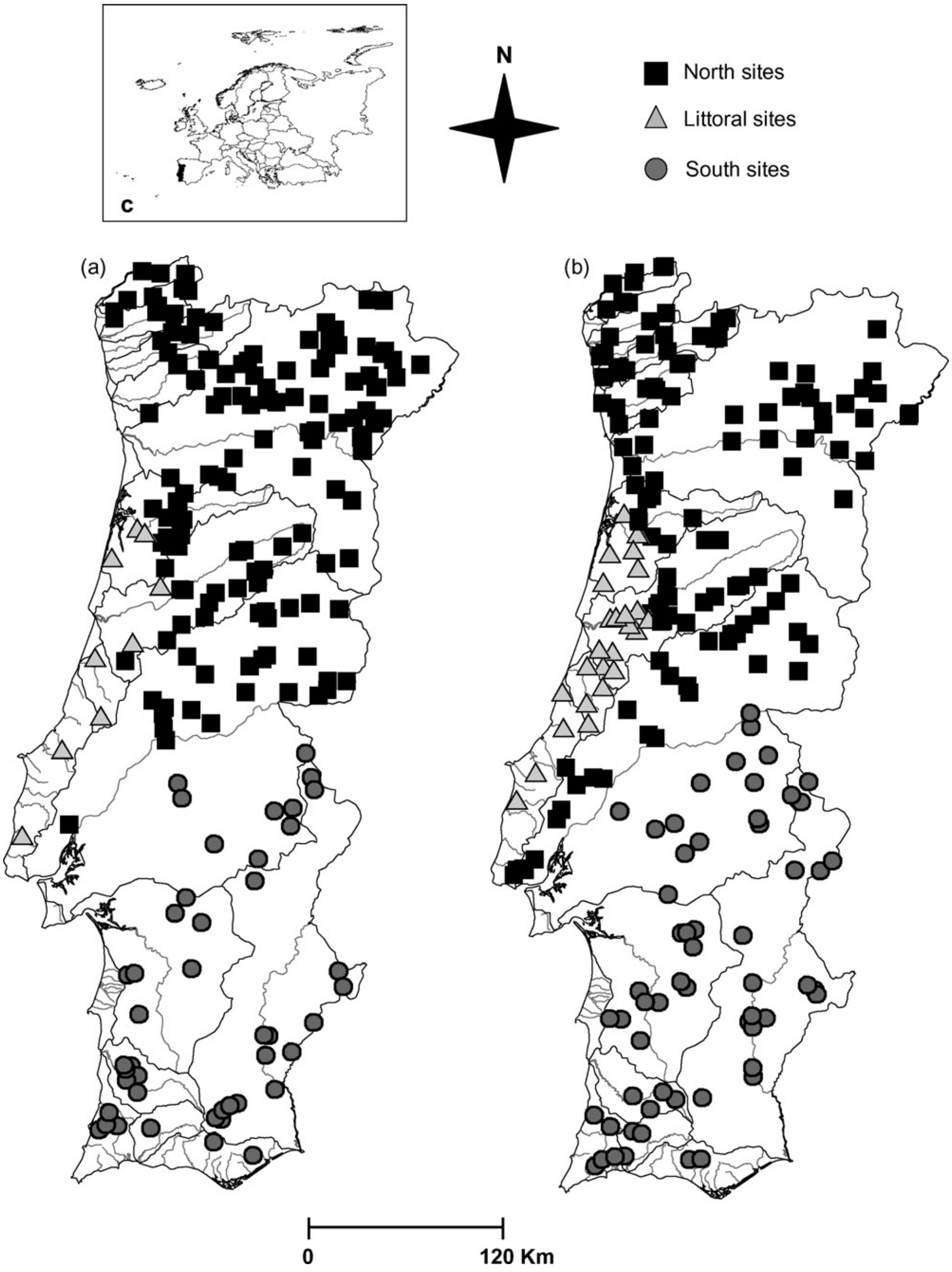

Fig. 1 – Location of all catchments and main rivers in Portugal with respective reference (a) and test sites (b) and localization of Portugal in Europe (c).

stereomicroscope. Identification was done mostly to genus level, except for Chironomidae that were kept at sub-family or tribe level and Oligochaeta identified to family level (sampling methods established by Instituto da Água for the introduction of the Water Framework Directive in Portugal, INAG, 2008). Several programs have shown genus or even family level to be adequate for broad-scale assessment such as that intended here (e.g., Bailey et al., 2001; Hawkins et al., 2000; Marshall et al., 2006; Simpson and Norris, 2000).

Forty-two environmental variables were used to characterize the sites and to build the predictive models (Table 1). Water samples were collected for laboratory analysis for nutrients, alkalinity and oxidability. Environmental measurements of stream morphology, hydrology and physical/chemical characteristics were taken at each site (e.g., discharge, morphological condition, pH, dissolved oxygen). Other related data such as altitude, distance to source, % forest, and % agriculture in the sub-catchment were obtained from cartographic sources (1:25,000 digital military maps, Instituto Geográfico do Exército, Portugal; Atlas do Ambiente Digital: Agência Portuguesa do Ambiente, 2007; Corine Land Cover, 1990).

2.3. *Model development*

Data were collected during the spring of 2004 and 2005 for the Institute of Water (INAG) from three main morpho-climatic

Table 1 – Environmental variables measured or calculated for each site and respective units and transformations applied. In the left column are variables used in Stepwise Discriminant analysis. In the right column are the variables used to characterize the sites and to interpret models results.

Potential predictor variables	Disturbance variables
Latitude (rectangular; log)	Intensive agriculture in the drainage area (%)
Longitude (rectangular; log)	Extensive agriculture in the drainage area (%)
Altitude (m; log)	Natural areas in the drainage area (%)
Mean annual runoff (mm; log)	Dissolved oxygen (%, mg L^{-1})
Mean annual precipitation (mm; $\sqrt{}$)	pH
Coefficient of variation of the precipitation ($\log x + 1$)	Intensive agriculture (in 5 km ratio around the site; %)
Mineralization (category)	Extensive agriculture (in 5 km ratio around the site; %)
Mean annual thermal amplitude (°C)	Nitrates (mg L^{-1})
High mineralization (%)	Nitrites (mg L^{-1})
Average mineralization (%)	Ammonium (mg L^{-1})
Low mineralization (%)	Phosphates (mg L^{-1})
Catchment area (km^2)	N-total (mg L^{-1})
Distance to source (km; log)	P-total (mg L^{-1})
Slope (%; $\sqrt{(\log x + 1)}$)	BOD_5, biological oxygen demand (mg L^{-1})
Conductivity (μS/cm;1/log)	Oxidability (mg L^{-1})
Alkalinity (mg L^{-1} CO3^{2-}; log x)	COD, chemical oxygen demand (mg L^{-1})
Hardness (mg L^{-1} Ca CO3; log x)	Total suspended solids (mg L^{-1}; log)
	Morphological condition (categories: 1–5)*
	Organic contamination and nutrient enrichment (categories: 1–5)*
	Land use (categories: 1–5)*
	Urban area (categories: 1–5)*
	Integrity of the riparian zone (categories: 1–5)*
	Sediments discharge (categories: 1–5)*
	Acidification and toxicity (categories: 1–5)*
	Connectivity (categories: 1–5)*

* Based on Pont et al. (2006).

regions of the country: the north (including the mountains region, with higher altitudes, more precipitation, and schist and granite geology), the south (with river Tejo as an approximate natural northern frontier, the terrain is generally flatter and much drier than the north) and the littoral (the northern costal area, with alluvial plains, limestone, clay and sands). Data from these three main areas of the country were used to build and test the predictive models (Fig. 1). Three predictive models were built: a North (using 103 reference sites), South (using 43 reference sites) and a National model (using 171 reference sites). Construction of a specific model for the littoral region (with its high population density, and the intensive industrial and agricultural activities) was not possible because of the insufficient number of streams that met the pre-defined criteria for reference sites. Therefore, the littoral streams were assessed using a National model. All predictive models followed the AUSRIVAS (AUStralian RIVer Assessment System) model development methods (Simpson and Norris, 2000; Coysh et al., 2000), which are largely based on the British RIVPACS methods (Wright et al., 1984; Armitage et al., 1987; Wright, 1995).

Model construction involved grouping sites with similar macroinvertebrate composition through hierarchical classification (Bray-Curtis dissimilarity measure, flexible UPGMA) of the reference sites using presence/absence biological data with PCORD multivariate analysis package, version 4.20 (McCune and Mefford, 1999). Rare taxa (defined as those that occurred at less than 10 sites, as for the AUSRIVAS models, Simpson and Norris, 2000) were removed from further analyses to reduce unexplained variability caused by their patchy occurrence (Gauch, 1982; Norris and Georges, 1993). Small classification groups (with less than 5 sites) were either deleted from further analysis, or amalgamated with another group of appropriate reference sites after review, as suggested by Simpson and Norris (2000). The allocation of reference sites to groups was also complemented by an ordination (Multidimensional scaling, UP GMA, Primer 6.1.6, Primer-E Ldt, Plymouth, U.K.). When the stress level for 2-dimensional MDS was >0.2, a 3-dimensional MDS was used, as recommended by Clarke and Warwick (2001). SIMPER analysis (Bray-Curtis similarity measure, Primer 6.1.6) was used to check the consistency of groups (the similarity of sites within groups compared to the dissimilarity between groups) and to characterize groups based on taxa common to sites within them.

Seventeen variables were selected (Table 1) and those that best discriminated between the invertebrate classification groups were determined using a Stepwise Multiple Discriminant Function Analysis (MDFA, SAS 9, SAS Institute Inc., Cary, NC). The potential predictor variables were selected not to contain data for nutrient and dissolved substances, periphyton, land use and riparian vegetation related variables because these variables are most likely influenced by human activity or are instantaneous measures that may not provide a good estimate given the potential variability of the data (e.g., water temperature at time of sampling). The variables used in Discriminant analysis were tested for normality using the Kolmogorov–Smirnov test (Minitab Release 12.2, Minitab, Inc., State College, PA). Those not normally distributed were transformed to achieve normality (Table 1). Probabilities of group membership for each reference site and probabilities of occurrence of each taxon were calculated following the AUSRIVAS methods (Simpson and Norris, 2000).

2.4. *Bands of biological condition and WFD classes*

The central 80% of reference site O/E values (between the 10th and 90th percentiles) define Band A (equivalent to reference condition) for the biological condition bands. The lower bands (B, C and D), which represent increasing levels of impairment, are the same width as Band A, although the width of Band D is usually less because it is limited by zero. Therefore, the number of bands depends on the interval used to define Band A. Above the 90th percentile (Band X) sites are considered richer than reference, but note the possibility that an elevated O/E can result from an unnatural change (Simpson and Norris, 2000). Thus, a test site allocated to Band X is not automatically considered to be in better than reference condition.

A second banding scheme was developed to increase the number of bands and achieve the 5 ecological status classes of WFD (High – 1, Good – 2, Moderate – 3, Poor – 4, Bad – 5; Directive 2000/60/CE, 2000). Band A was calculated as described above but the O/E values below Band A to zero were divided in 4 to create the remaining bands. This division resulted in a new banding system with Band A equivalent to Class 1 (equivalent to reference, high ecological status), and 4 classes with increasing degrees of impairment (2 – good, 3 – moderate, 4 – poor and 5 – bad). Sites richer than reference (referred above as Band X) are automatically considered to be in good condition and allocated to Class 1 (High).

2.5. *Model testing and validation*

Sixteen reference sites were set aside and used to validate the models by testing that the model correctly assessed them as in reference condition. Validation sites represented 10 sites from the north and 6 from the south. Moreover, as suggested by Linke et al. (2005), an accurate model will have a regression line of the reference site Observed versus Expected values passing through, or close to, the origin (with a range of -1.5 to 1.5 considered acceptable) and have a slope close to 1 (acceptable range 0.85–1.15).

The North model was tested with 104 test sites, the South model with 47 test sites and 174 test sites were run through the National model, including both North and South test sites. The expected taxa and O/E scores of the test sites were allocated to the series of bands representing different levels of biological condition. The O/E results were compared for all models to determine the quality of each (intercept, slope and variance). Test sites assessments were also analyzed against disturbance variables (Table 1) to provide an indication as to which type of disturbances the models may best detect. The density distribution of the values obtained for each disturbance variable at test sites and the biological condition band were represented in box plots (SYSTAT 8.0) for each model to determine the range of disturbance values for each disturbance type.

3. Results

3.1. *National model*

The National model is based on 157 taxa found in 171 reference sites. The classification analysis (Fig. 2) and MDS images (3D, stress level = 0.18) identified seven groups. Some reference sites were eliminated from these final classification groups because: they were either very different from other reference sites (outliers) or formed very small groups (less than 5 sites) in both cluster and MDS analyses (3 sites); showed low O/E values associated with sampling problems (2 sites) or after review were not considered to be boarder-line regarding reference condition (one site). Another two sites belonged to a small group were integrated in group 2 since the MDS showed their similarity to its sites.

SIMPER analysis revealed that sites within each group had similar macroinvertebrate communities (between 42% and 58%) and that group 7 was the most dissimilar when compared to all other groups (dissimilarities 74–82%). Groups 5 and 6 were most similar to each other. In all groups, the most common taxa were Chironomidae, Oligochaeta and Baetidae. Other most common taxa and relevant abiotic features of each reference group are described in Table 2.

The joint information of SIMPER analysis, geographic distribution and mean values of the abiotic variables of the groups allowed an ecological view of all reference groups (Table 2).

Eight variables were selected to best discriminate between the 7 groups (Table 3). Using these environmental

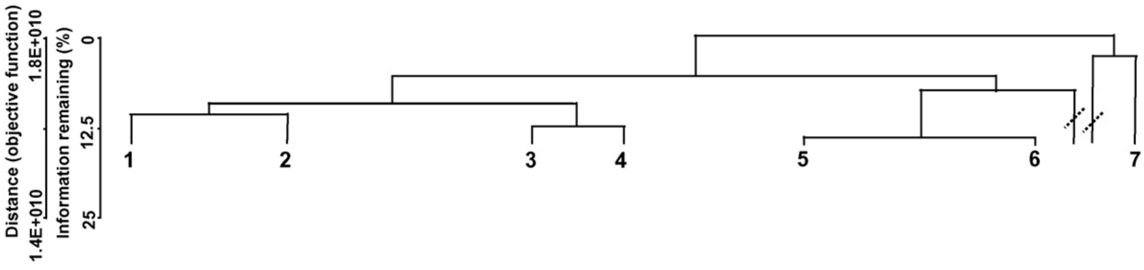

Fig. 2 – Classification of reference sites used in the National model. The numbers (1–7) represent the biological reference groups used in the model. The interrupted lines indicate the reference sites that were not used in the formation of the groups.

Table 2 – Reference-site groups characteristics of the National model, obtained from SIMPER analysis, geographic distribution and mean values of abiotic variables. The most common invertebrates are those taxa that contributed up to 90% of cumulative abundance and were found in >50% of the sites.

Groups	Relevant abiotic features	Most common invertebrates
1	Littoral areas of centre of Portugal; low altitude (140 m); some temporary streams; high mean annual temperature (15 °C); high conductivity (489 µS/cm); high alkalinity (102 mg L^{-1} HCO_3^{2-}); high hardness (109 mg L^{-1} $CaCO_3$); average size (37 km to source and 359 km^2 of drainage area).	*Agabus* Lv., *Ancylus*, *Tipula*, *Gerris*, *Micronecta*, Simuliidae, *Cloeon*, *Isoperla*.
2	South littoral areas and close to the Spanish border, in the Guadiana catchment; low altitudes (172 m); low slopes (1.5%); high mean annual temperature (15 °C); high pH (7.7); high hardness (87 mg L^{-1}).	*Caenis*, Simuliidae, *Ancylus*, Ceratopogonidae, Hydropsychidae, *Oulimnius*, *Habrophlebia*, *Isoperla*, *Leuctra*, *Ecdyonurus*, *Ephemerella*, *Rhyacophila*.
3	Mainly in Douro catchment, close to the border and some sites in the centre (Tejo catchment). Streams of medium size (662 km^2 of drainage area, 48 km of distance to source); high slopes (7%); medium altitudes (222 m).	*Caenis*, *Hydropsyche*, *Bezzia*, *Ecdyonurus*, *Oulimnius*, *Arctocorisa*, *Leuctra*, *Ancylus*, Simuliidae, *Serratella*, *Limnius*, *Habrophlebia*, *Atyaephyra*, *Hydraena*.
4	Sites in mountain areas of the Douro catchment (inlands); highest average altitude (651 m); high slope (10%); the lowest mean annual temperature (12 °C) of all groups.	*Habrophlebia*, *Isoperla*, *Serratella*, *Rhyacophila*, *Ecdyonurus*, *Athripsodes*, *Oulimnius*, *Arctocorisa*, *Graptodytes*, *Siphonoperla*, *Allogamus*.
5	Lower mountain areas of central Portugal and North littoral; average altitude 393 m), low distances to source (14 km); high precipitation (1528 mm); high runoff (787 mm); low pH (6.38); low conductivity (40.5 µS/cm).	*Hydropsyche*, *Ecdyonurus*, Simuliidae, *Leuctra*, *Atherix*, *Oulimnius*, *Caenis*, *Onychogomphus*, *Polycentropus*, *Habrophlebia*, *Ancylus*, *Rhyacophila*, *Habroleptoides*, *Serratella*, *Limnius*, *Epeorus*, *Protonemura*, *Esolus*, *Hydraena*, *Polycelis*, *Cordulegaster*, *Isoperla*, *Siphonoperla*.
6	Centre of Portugal; low altitude (200 m); small streams (91 km^2 drainage area, 17 km distance to source); small slopes (2%); low alkalinity (27.9 mg L^{-1}); low pH (6.7).	Simuliidae, *Leuctra*, *Limnius*, *Serratella*, *Hydraena*, *Onychogomphus*, *Ecdyonurus*, *Caenis*, *Hydropsyche*, *Atherix*, *Elmis*, *Dugesia*.
7	Sites located in the south of Portugal; high mean annual temperatures (16 °C); mainly temporary streams; low altitudes (130 m); low slopes (0.3%).	*Orthetrum*, *Orthotrichia*, Tabanidae, Ceratopogoninae, *Oulimnius*, *Ochthebius*, *Setodes*, *Ancylus*, *Hydrometra*, *Hydropsyche*, *Perla*.

Table 3 – Summary of models characteristics.

	National	North	South
Number of groups	7	6	4
Discriminant error	38%	34%	17%
Discriminant variables (predictor variables)	Latitude Precipitation Catchment area Altitude Hydrological regime Alkalinity Mean annual temperature High mineralization	Slope Precipitation Catchment area Altitude Hydrological regime Hardness Thermal amplitude Coefficient variation of precipitation	Latitude Distance to source Catchment area Thermal amplitude Alkalinity Conductivity
OE regression values			
Slope	0.98	1.04	1.06
Intersection	0.58	0.16	−0.69
r^2	0.545	0.589	0.733
AUSRIVAS bands (maximum OE values)			
Band X	>1.26	>1.22	>1.27
Band A	1.26–0.73	1.22–0.77	1.27–0.72
Band B	0.72–0.20	0.76–0.33	0.71–0.17
Band C	<0.20	<0.33	<0.17
Band D	No Band D	No Band D	No Band D
WFD classes (maximum OE values)			
Class 1	1.26	1.22	1.27
Class 2	0.73	0.77	0.72
Class 3	0.55	0.58	0.54
Class 4	0.37	0.39	0.36
Class 5	0.19	0.2	0.18

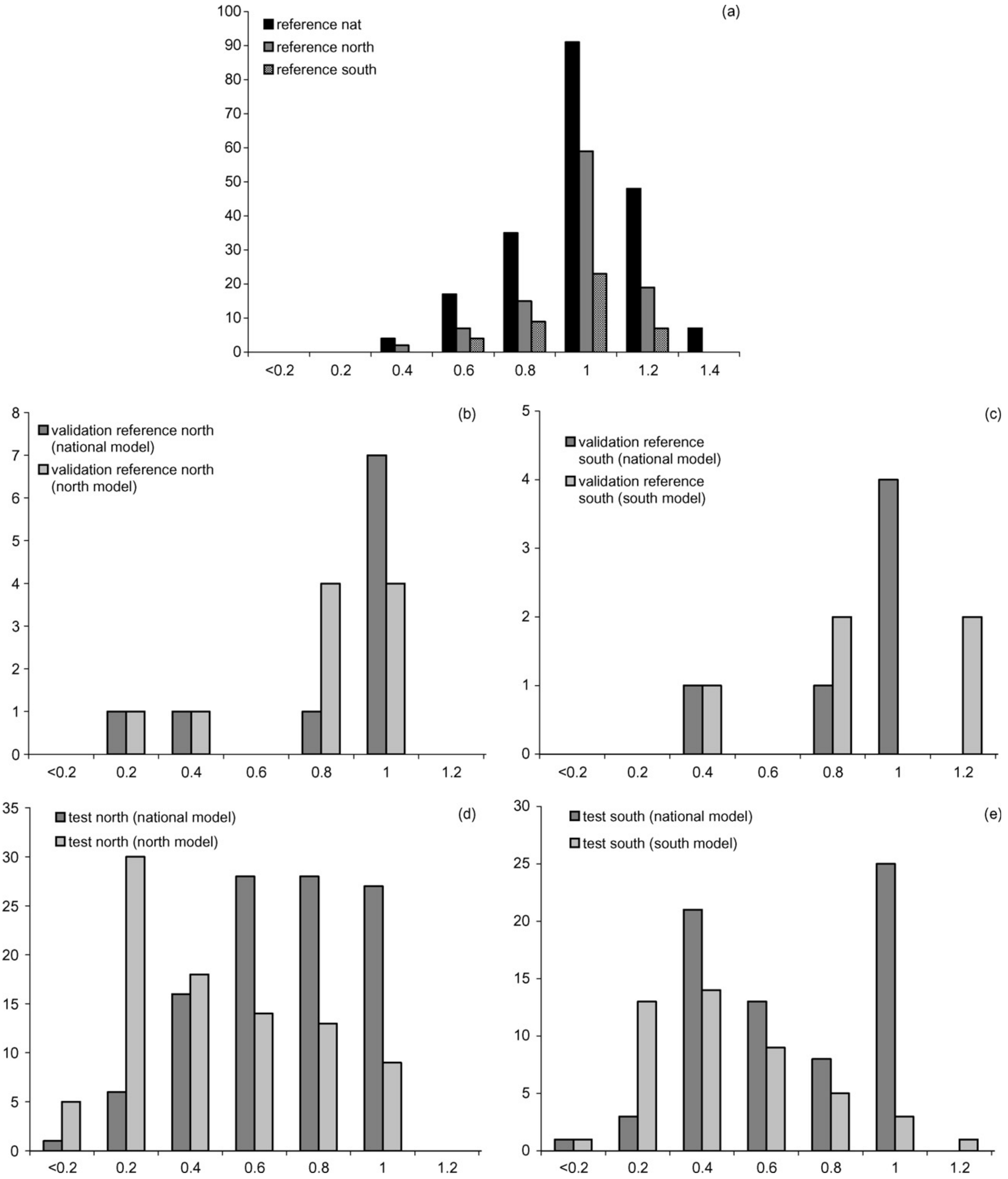

Fig. 3 – Distribution frequencies of the O/E values of all reference sites used in the three models (a); frequencies of the O/E values of validation reference sites of the north and south regions accessed by the National model and the model of the respective region (b); frequencies of the O/E values of test sites of the north and south regions accessed by the National model and the model of the respective region (c).

variables, 62% of the reference sites were correctly assigned to their classification groups based on similarity of the biota (Table 3).

Most reference sites had an O/E value near 1 (observed taxa = expected taxa) and a decreasing number of reference sites had less observed than expected taxa (O/E $<$ 1) or more taxa observed than expected (O/E $>$ 1) (Fig. 3a). The slope of the O versus E regression, the intercept and r^2 were all within the range to indicate a 'good' model (Linke et al., 2005; Table 3).

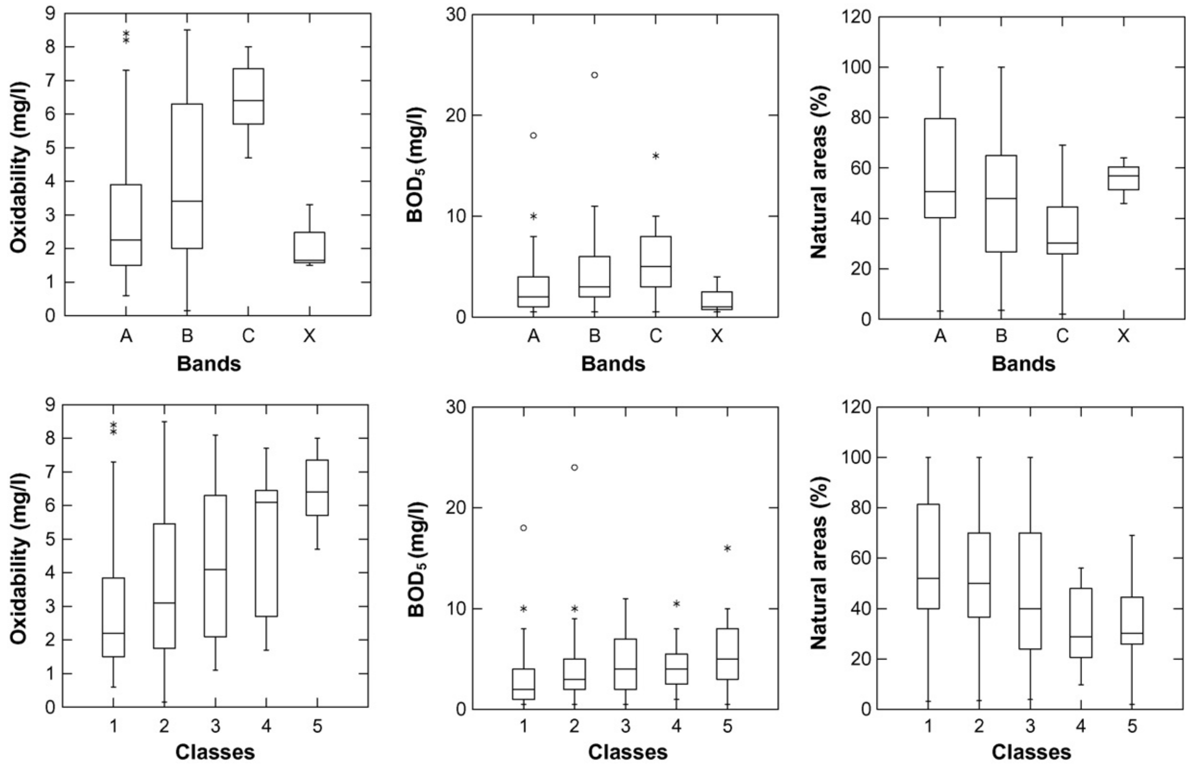

Fig. 4 – (a) Selected disturbance variables and corresponding National model bands (X, A, B and C) for test sites; (b) the same variables for the National model WFD Classes of disturbance. The centre line in the box marks the median value and the length of each box shows the range where the central 50% of the values fall. The box edges are at the first and third quartiles. Empty circles represent outlying values; asterisks are the values between inner and outer fences.

3.2. *National model bands of biological condition and WFD classes*

The mean value for the O/E values was 1.02 and the range between 0.73 and 1.26 defined Band A (10th–90th percentile range) and the width of the other biological condition bands (Table 3). Note that the width of Band A allowed only 2 bands below reference (B and C). The new banding system following the 5 classes of WFD is also described in Table 3.

The best correspondence (continuous decrease of water quality) observed in the box plots are with the variables dissolved oxygen, BOD$_5$, oxidability and percent of natural areas for both banding and classes schemes (Fig. 4). From the boxplots it is clear that in our case Band X (Fig. 4a) is generally represented by sites in good biological condition, and therefore, it is similar to Band A.

3.3. *North model*

The North model is based on 157 taxa found in 103 reference sites. Six reference site groups were defined from the cluster analysis (Fig. 5) and the MDS 3D ordination (stress level 3D = 0.18). Some sites were eliminated from the model at the group classification stage because: they were outliers (two sites);

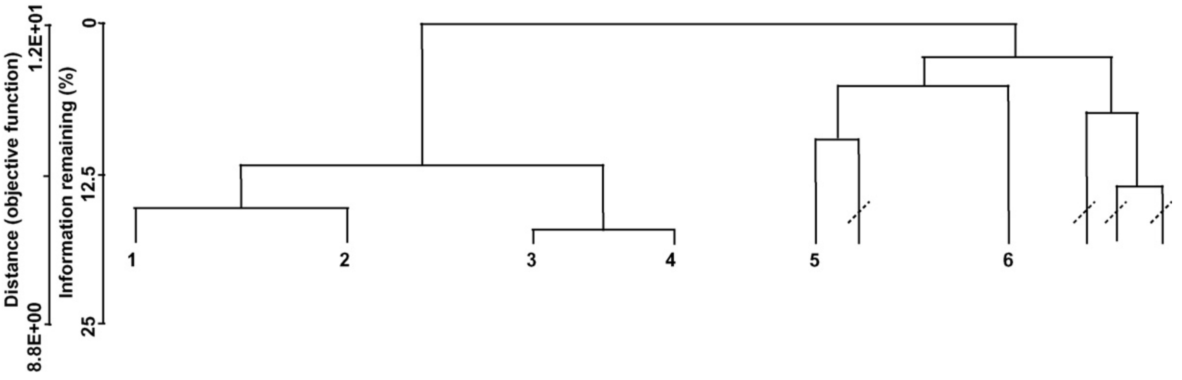

Fig. 5 – Classification of reference sites used in the North model. The numbers (1–6) represent the biological reference groups used in the model. The interrupted lines indicate the reference sites that were not used in the formation of the groups.

Table 4 – Reference-site group characteristics of the North model, obtained from SIMPER analysis, geographic distribution and mean values of abiotic variables. The most common invertebrates are those taxa that contributed up to 90% of the abundance and were found in more than 50% of the sites.

Groups	Relevant abiotic features	Most common invertebrates
1	Sites mainly located in the inlands of Douro catchment and some streams in Tejo catchment (inlands); low mountain areas (mean altitude: 379 m); average/small size (209 km^2 of drainage area; 27 km distance to source); high slope (12%).	*Hydropsyche*, *Polycentropus*, *Rhyacophila*, *Baetis*, *Caenis*, *Serratella*, *Epeorus*, *Habrophlebia*, *Onycogomphus*, *Boyeria*, *Limnius*, *Oulimnius*, *Hydraena*, *Atherix*.
2	Northern coastal catchments, some streams of the centre (Mondego and Tejo catchments); average altitude (312 m); high runnof (1040 mm yr^{-1}); small size streams (51 km^2 drainage area, 12 km distance to source); low slope (2%).	*Baetis*, *Acentrella*, *Caenis*, *Ecdyonurus*, *Ephemerella*, *Leuctra*, *Onychogomphus*, *Cordulogaster*, *Tinodes*, *Athricops*, *Atherix*, Hemerodrominae, *Ancylus*.
3	Inlands of Mondego and Vouga catchments; low mountain areas (309 m altitude); mainly small size streams (78 km^2 of drainage area and 16 km distance to source); low alkalinity (8.5 mg L^{-1}).	*Leuctra*, *Ecdyonurus*, *Serratella*, *Habroleptoides*, *Baetis*, *Caenis*, *Limnius*, *Hydraena*, *Esolus*, *Atherix*, Simuliidae.
4	Small streams (31 km^2 of drainage area and 8 km of distance to source) in upper north region; high altitudes (651 m); low hardness (7.4 mg L^{-1}); low conductivity (23 μS/cm).	*Leuctra*, *Isoperla*, *Siphonoperla*, *Serratella*, *Habrophlebia*, *Ecdyonurus*, *Hydropsyche*.
5	Lowest altitude (187 m), medium size (565 km^2 of drainage area and 52 km of distance to source).	*Baetis*, *Caenis*.
6	Larger streams (1294 km^2 of drainage area and 83 km of distance to source); low altitudes (228 m); the highest alkalinities (69 mg L^{-1}), SST (25 mg L^{-1}) and hardness (39 mg L^{-1}) found in northern streams.	*Baetis*, *Caenis*, *Hydropsyche*, *Ecdyonurus*, *Oligoneuriella*, *Setodes*, *Leuctra*.

or they formed very small groups and could not be integrated in the other groups (18 sites) because they were different from all other sites. These 20 sites were spread through the entire north region and no common feature was found.

SIMPER analysis of the six reference-site groups indicated similarities within groups between 50% and 55% and dissimilarities between groups ranging from 52% (groups 1 and 4) to 68% (groups 1 and 5). Chironomidae and Oligochaeta were the most common taxa in all groups. Table 4 resumes the most relevant abiotic features and most common invertebrates of all reference groups. The 8 variables best discriminated between the 6 classification groups (Table 3) and correctly assigned 66% of the reference sites into their classification groups. The slope of the O versus E regression, the intercept and r^2 were all within the range to indicate a 'good' model (Linke et al., 2005; Table 3). The frequency distribution of the O/E values of the reference sites run through the North model revealed that most of the sites have a ratio near 1 and the distribution was narrow (Fig. 3a).

3.4. *North model bands of biological condition and WFD classes*

The mean O/E value obtained for the reference sites was 1.03 and the band width 0.45 allowing only three bands (Table 3). The North model using 5 condition classes resulted in the intervals described in Table 3.

The graphs of both banding and WFD classification schemes revealed good correspondence of the increasing class and the increasing level of impact with variables dissolved O_2, COD, oxidability, N-total, sediments discharge, % of intensive and extensive agriculture (in 5 km radius) and % of natural areas (Fig. 6). Additionally, the banding scheme showed also good correspondence with % extensive agriculture in the drainage basin, nitrates and nitrites and the WFD classes with P-total.

3.5. *South model*

A total of 126 taxa found in the final 43 reference sites were selected to build this model. Four groups were defined using both analyses of the classification and MDS analyses. The first three groups were selected from the dendrogram (Fig. 7). The fourth group resulted from the examination of the 2D MDS (stress = 0.15) where those sites were clearly grouped (Fig. 8). Therefore, the cut level of similarity used differed slightly from the previous three groups (Fig. 7). Choosing a higher similarity to the cut of level for the other groups resulted in poorer discriminations. Some reference sites were also eliminated after the cluster and MDS analyses revealed that they were different from the other groups (6 sites) or having a too few taxa to be considered reference (one site 139, 14 Taxa) and 2 sites were added to group 3 after ordination revealed their macroinvertebrate assemblages were similar.

SIMPER analysis indicates similarities within groups between 55% and 65% and dissimilarities between groups ranging from 49% (groups 1 and 2) to 80% (groups 3 and 4). In all groups Chironomidae, Oligochaeta and Baetidae were present in almost all sites. Table 5 resumes the abiotic and biotic features of all reference groups.

The stepwise MDFA selected 6 environmental variables that best discriminated between the 4 reference-site groups (Table 3) and 83% of reference sites were correctly assigned to the reference-site groups using these variables (Table 3). The distribution of the reference site O/E values was quite narrow, as for the other two models. The slope of the O versus E regression, the intercept and r^2 were all within the range to indicate a 'good' model (Linke et al., 2005; Table 3).

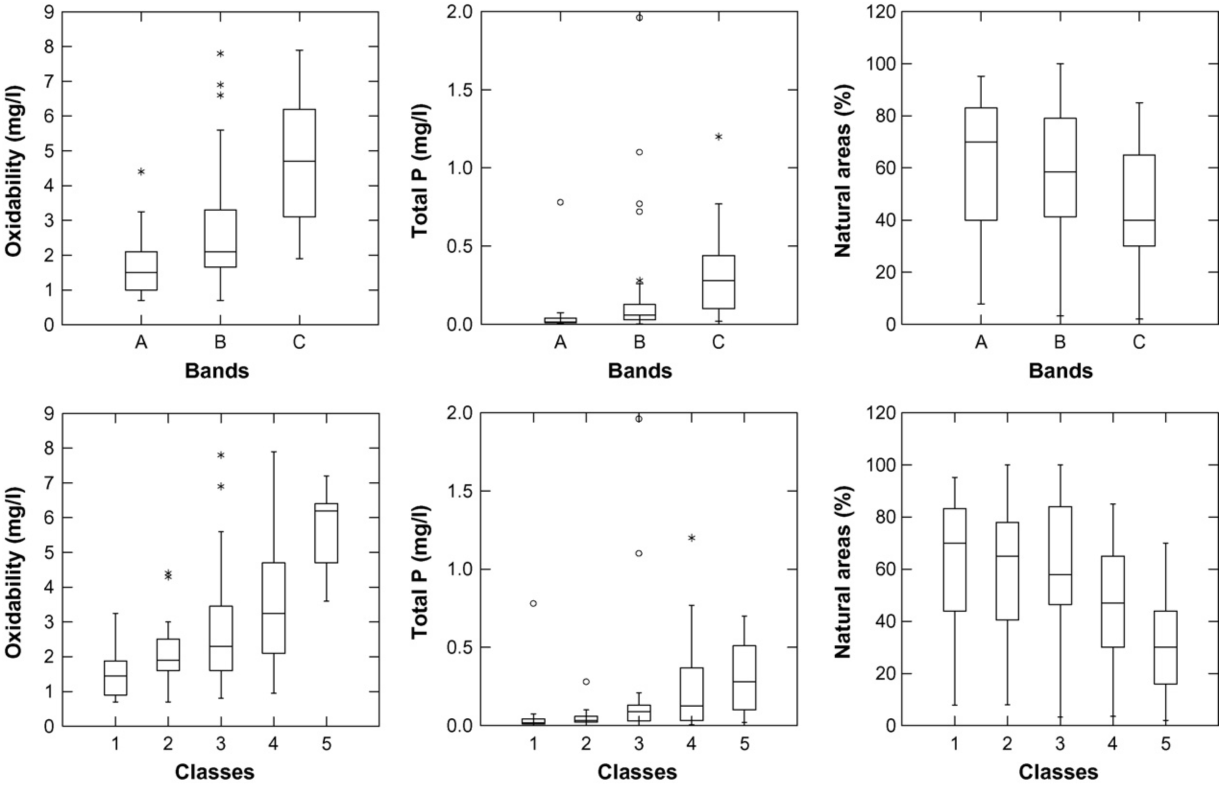

Fig. 6 – (a) Selected disturbance variables and corresponding North model band allocation (A, B and C) for test sites; (b) the same variables for the North model WFD Classes of disturbance. The centre line in the box marks the median value and the length of each box shows the range where the central 50% of the values fall. The box edges are at the first and third quartiles. Empty circles represent outlying values; asterisks are the values between inner and outer fences.

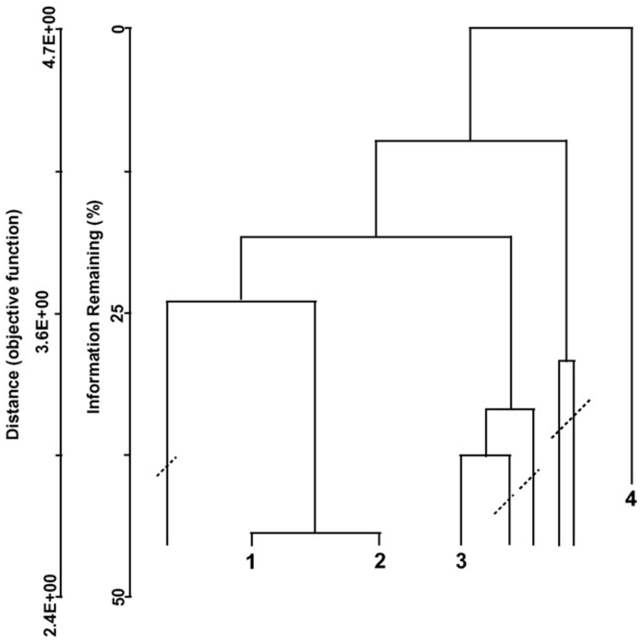

Fig. 7 – Classification of reference sites used in the South model. The numbers (1–4) represent the biological reference groups used in the model. The interrupted lines indicate the reference sites that were not used in the formation of the groups.

3.6. *South model bands of biological condition and WFD classes*

The mean O/E value for the South model was 1.02 with the Band A interval of 1.27–0.72, which allowed only two more bands (Table 3). The division of the interval of O/E values after reference Band A /Class 1 – high status (1.27–0.72) resulted in the classes described in Table 3.

There was a good correspondence between the South model bands with several types of disturbances: dissolved O_2, BOD_5, COD, oxidability, total suspended solids, P-total, sediments discharge, extensive agriculture (in 5 km radius) and natural areas (Fig. 9). The WFD classes showed good correspondence only with BOD_5, extensive agriculture (in 5 km radius) and natural areas.

3.7. *Comparison between models*

The predictive variables selected for the three models, while not identical, could all be categorized as geographic location (latitude, altitude), climatological (precipitation, hydrological regime, mean annual temperature), stream size (catchment area, distance to source) or chemical characteristics (alkalinity, hardness and mineralization) (Table 3). The National and North models had greater misclassification errors (38% and 34%, respectively) than the South model (17%), had similar discriminant variables selected and number of reference

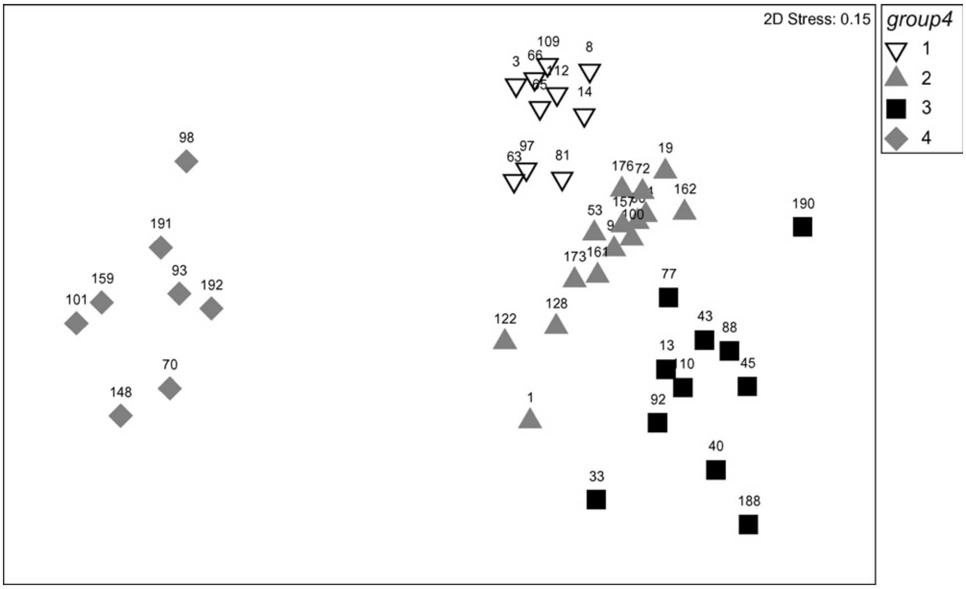

Fig. 8 – Ordination (2D) of all reference sites used to build the South model.

groups (7 and 8), probably because of the large overlap in sites. The South model used less predictor variables and has fewer groups (4) (Table 3).

3.8. *Model validation*

The assessment of the validation reference sites (i.e., the 16 reference sites not included in the model construction) were similar in the proportion of sites assessed as in reference condition and the small differences are likely to result from the different number of sites assessed (Table 6). The regional models had narrower 10–90 percentile bands than the National model (Fig. 3b and c).

Using the AUSRIVAS assessment scheme, the assessments of the 174 test sites run through the National model resulted in 2 sites assessed as Band X, 81 into Band A, 84 into Band B and 7 into Band C. The North model assessed 104 test sites: 17 in Band A, 66 in Band B and 21 in Band C. The South model assessed 47 sites resulting in 1 to Band X, 7 into Band A, 29 into Band B and 10 into Band C. The results for test sites assessed using both the National and North models (Fig. 3d) produced 51% sites in the same band, in 47% were assessed in one band of difference, and in 2% the difference was two bands. The North model attributed a poorer condition to 49% of the sites and the remaining 51% had corresponding assessments to the National model. The comparison of the South and National models assessments of the common test sites (Fig. 3e), showed that 38% of the assessments were in the same band and in remaining sites there was a difference of one band with the majority of sites (except 4 of the 28) being assessed as having poorer quality (lower band) in the South model. In only one site there was a difference of two bands. So, regional models seem to be more conservative in their assessments than the National model which might be preferred as a safer approach in water quality assessment.

Table 5 – Reference groups characteristics of the South model, obtained from SIMPER analysis, geographic distribution and mean values of abiotic variables. The most common invertebrates are those taxa that contributed up to 90% of the abundance and were found in more than 50% of the sites.

Groups	Relevant abiotic features	Most common invertebrates
1	South littoral sites in small streams; low distances to source; small catchment areas (mean values: 15 km and 59 km^2, respectively); the lowest values of alkalinity (40.7 mg L^{-1}) and hardness (88.4 mg L^{-1}) for the southern groups.	*Ancylus*, Limoniidae, *Dugesia*, *Rhyacophila*, *Hydropsyche*, *Stenophylax*, *Plectronemia*, *Caenis*, *Ephemerella*, *Habrophlebia*, *Habroleptoides*, *Leuctra*, *Isoperla*, *Hydraena*, *Esolus*, *Oulimnius*, *Elmis*, *Stelnemis*.
2	Sites located in plains, temporary streams; almost no slope (0.7%); high conductivity (566 μS/cm), alkalinity (88 mg L^{-1}) and hardness (132 mg L^{-1}).	Ceratopogonidae, Limoniidae, *Hydropsyche*, *Hydroptila*, *Isoperla*, *Tyrrhenoleuctra*, *Hemimelaena*, *Ecdyonurus*, Leptophlebidae, *Oulimnius*, *Hydraena*.
3	North of the South region, in river Tejo catchment; higher altitudes in the south region (168 m), mostly small streams (76 km^2 catchment area, 17 km distance to source).	*Oulimnius*, *Anacaena*, *Helophorus*, *Hydraena*, *Deronectes*, *Agabus*, *Caenis*, *Cloeon*, *Choroterpes*, *Habrophlebia*, *Arctocorisa Notonecta*, *Chalcolestes*, *Physella*, *Ancylus*.
4	Southern region of the country in waters with high harness (141 mg L^{-1}) and conductivity (429 μS/cm).	*Orthretum*, *Ochtebius*, *Setodes*, *Oulimnius*, *Dysticus*, Tabanidae, Ceratopogonidae, *Orthotrichia*, *Hydropsyche*, *Ancylus*, *Hydrometra*.

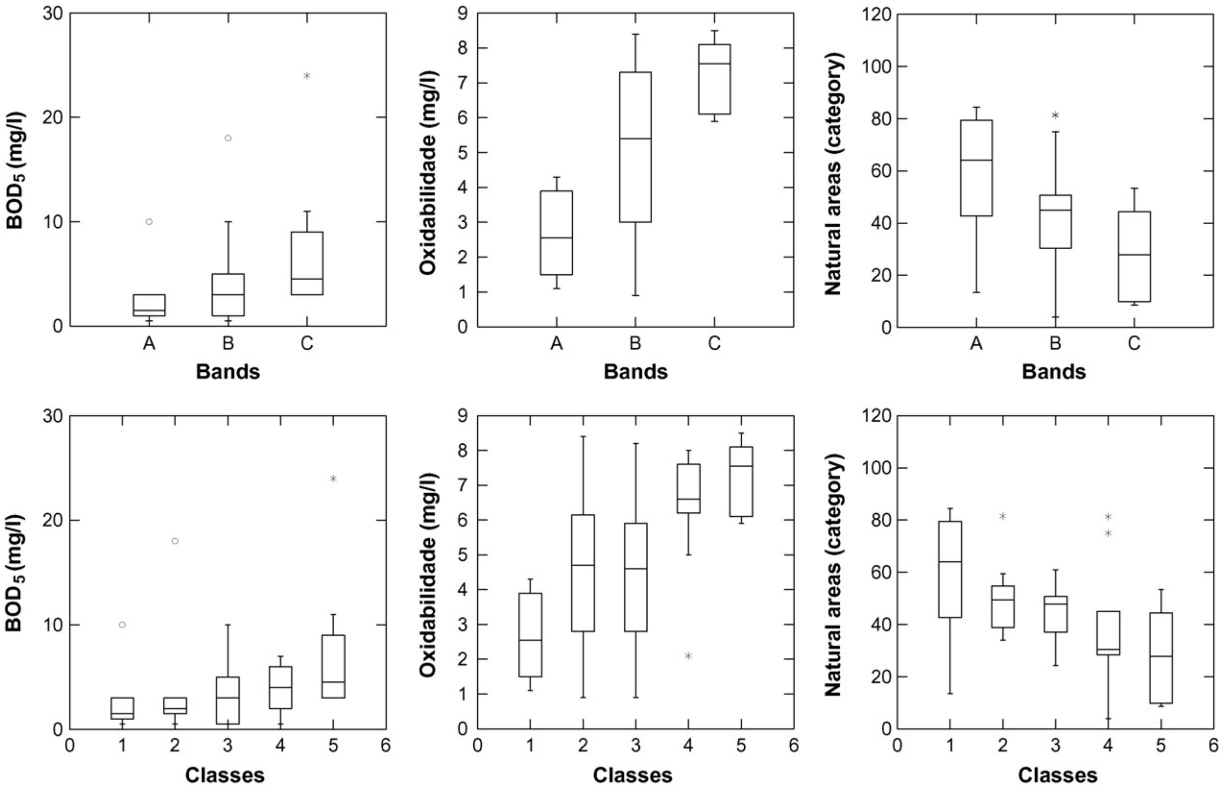

Fig. 9 – (a) Selected disturbance variables and corresponding South model band allocation (A, B and C) for test sites; (b) the same variables for the South model WFD Classes of disturbance. The centre line in the box marks the median value and the length of each box shows the range where the central 50% of the values fall. The box edges are at the first and third quartiles. Empty circles represent outlying values; asterisks are the values between inner and outer fences.

Table 6 – Comparison between the assessments provided by the National, North and South models of the validation reference sites.

Reference sites	Region	Assessment band and O/E value					
		National model		North model		South model	
2	N	A	0.87	A	0.81		
11	S	A	1.49			A	1.08
25	N	A	1.13	A	0.80		
30	N	A	1.02	A	1.14		
31	S	A	0.95			A	0.77
37	N	A	1.31	B	0.75		
52	N	A	0.89	A	0.83		
69	N	A	1.20	A	0.92		
85	S	A	1.19			A	1.26
87	N	A	1.06	A	0.89		
102	S	B	0.53			B	0.51
118	N	A	1.03	A	0.87		
124	N	B	0.26	B	0.33		
127	S	A	0.74			A	0.87
171	N	A	1.71	A	1.01		
175	S	A	1.63			A	1.02
Average O/E			1.06 1.05 (North only) 1.09 (South only)		0.84		0.92

All models indicate sensitivity to similar disturbance variables (low oxygen, high chemical and biological oxygen demand, land use, oxidability) using both banding and WFD classes schemes (examples in Figs. 4, 6 and 9). The North model also indicates a relationship between the model assessment classes and the concentrations of N and P (Fig. 6).

4. Discussion

In Europe, the projects AQEM and STAR (Hering et al., 2006; Furse et al., 2006) developed tools and protocols for the assessment of rivers throughout Europe using all biological elements and hydromorphological features. Most of these procedures were considered in the construction of the Portuguese national protocols reported here. However, while the sampling device used (hand-net), the multi-habitat approach and the sampling period (low-water) were similar to those of the AQEM/STAR protocols other aspects differed, including the sampling reach or the sampling processing which were set for the Portuguese protocols by a group of national experts in accordance to streams characteristics and survey goals. Also, the STAR project focused on a multimetric approach for data analysis (Hering et al., 2006), and here we describe a predictive modeling approach as followed by other European countries such as U.K. (RIVPACS; e.g., Wright et al., 1984) or Czech Republic (PERLA; Kokeš et al., 2006). Even though the process of model construction can be considered complex when compared to the application of metrics, the models philosophy is conceptually simple and a comparative study by Reynoldson et al. (1997) found the predictive models to be more precise and accurate than the multimetric approach.

In Australia, AUSRIVAS predictive models were developed at regional scales because of the large size of the continent and different landscapes with strong environmental gradients (Simpson and Norris, 2000) while in United Kingdom a National model was developed (Wright et al., 1984; Wright, 1995). Yet, size is not the only factor to consider regarding the area covered by a single model. If very different regions require very different sampling methods this may also prevent the use of a single model to cover all regions because model predictions will be sensitive to the methods. This could happen in Portugal, where regions with large rivers (catchment area >1000 km^2) cannot be sampled using the same kick-sampling methods used in smaller streams to collect macroinvertebrates. Those rivers were not considered in the present study and the sampling methods used were common to all streams. However, the future use of a single National model for Portugal would need to consider these possible limitations, a problem common to all methods.

The type of predictive variables selected for the three models (geographic location, climatological, stream size and chemical characteristics) are also common predictors used for macroinvertebrate models elsewhere (e.g., Hawkins et al., 2000; Simpson and Norris, 2000; Reynoldson et al., 2001; Feio et al., 2007a,b). In our case, they reflect the known north–south, east–west environmental gradients of the Portuguese territory and are clearly reflected in the distribution of macroinvertebrate communities through the country. Elements of these gradients are also present at the regional scale (north, south). Even within the south there are mountain areas with very different climates.

The quality of all the models was considered good using the approach proposed by Linke et al. (2005) to evaluate the quality of predictive models based on the Observed/Expected regression but the better discriminant errors and r^2 values of the North and South models indicates that they may perform better than the National model (Table 3).

The National and North models performed similarly in the discrimination of reference sites groups (only 4% difference after cross-validation, Table 3). On the other hand, the difference between National and South models (17% more in the National model) indicates that the variables selected for the South model provide better predictions of group membership for new sites and this will have some influence on the taxa predicted. This difference could be attributed to the smaller number of groups in the South model (4 instead of 7) and the small sample size (6 sites).

The reference site validation showed that all but one validation site (Table 6) were assessed the same in the regional models as the National model. Thus, both National and regional models are working well on the prediction of the assemblages since the expected taxa were close to those actually observed. However, the National model bands were wider, which means that a bigger range of reference conditions are accepted, resulting in the inclusion of more sites into Band A. Also, the National model predicted less taxa than the North model and since the most common and less sensitive taxa (e.g., Chironomidae, Baetidae, Oligochaeta) are always present in the predictions of all models, it is therefore likely to be less sensitive than the North model.

The South model assessed the reference sites in the same bands of the National model which results from a similar range of reference conditions included in Band A (band widths of 0.53 and 0.55, respectively). However, the South model predicted more taxa than the National model again suggesting a regional model would be more sensitive than a National model.

Both North and South models attributed poorer quality to test sites compared to the National model assessments (Fig. 3d and e), also indicating greater sensitivity. The following examples help to clarify the differences in the model assessments.

Test site 250 (data not shown) was assigned to Band A by the National model and to Band B by the North model. Fewer taxa were predicted by the National model (8) than by the North model (22). All the taxa predicted by the National model were found and none of them were very sensitive (mainly Diptera, Baetidae, one Gastropoda, one Coleoptera and Oligochaeta) while only few taxa predicted by the North model (e.g., several genera of Trichoptera, Coleoptera, Ephemeroptera, Plecoptera), were actually found in the sample even though they would be expected in a stream in that area (personal observations) and, the riparian vegetation, and some morphological characteristics were degraded. This might justify the Band B of the North model, which seems to assess the loss of environmental quality better than the National model.

Site 255 (data not shown) was assessed as equivalent to reference (Band A) by the National model but assigned to Band B by the South model. The chemical and physical data revealed some organic enrichment (e.g., $BOD_5 = 10\ mg\ L^{-1}$, $COD = 62\ mg\ L^{-1}$, oxidability = $8.20\ mg\ L^{-1}$), a high proportion of the catchment under agriculture (65%), and also some degradation of the riparian corridor and channel morphology (categories 4 and 3, respectively). Thus, it is likely that the site has suffered from human impact. The difference in bands between the models is likely to have resulted from more taxa predicted to occur at this site by the South model (29 compared with 15 by the National model) many of which were absent from the sample. Australian combined season models that were found to predict more taxa than single season models were also regarded as more sensitive (Marchant et al., 1997). Thus, Band A may have been assigned by the National model simply because the criteria to achieve an A band was less demanding than for the South model.

The National model is the only possible option to access northern-coastal sites, because the small number of reference sites in the area (Fig. 1), therefore, it is also important to check its performance for this area. The coastal test site 279 was attributed to Band B (data not shown) because three taxa predicted were missing from the sample: *Agabus*, *Ancylus* and Baetidae. Yet, other taxa were found that were not predicted, some of them sensitive to water quality such as the Ephemerellidae *Serratella* (Alba-Tercedor and Sánchez-Ortega, 1988). The chemical analysis revealed moderate levels of organic contamination, with elevated levels of nitrites ($0.28\ mg\ L^{-1}$), and moderate degradation of morphological conditions (such as bank modifications, presence of dams and lost natural habitat diversity) and land-use degradation (some agriculture and a village nearby). Therefore, Band B might be accurate for this site. Comparatively, site 297 was assessed as much poorer (only Diptera, Baetidae and Oligochaeta were collected in the sample) and was assigned to Band A by the National model, because most of the taxa expected were observed. In this case the chemical and physical assessment of the site indicated poor condition, with elevated levels of BOD_5 ($6.0\ mg\ L^{-1}$), COD ($31\ mg\ L^{-1}$), nitrites ($8.2\ mg\ L^{-1}$) and ammonia ($1.64\ mg\ L^{-1}$) in the water and degraded riparian corridor and morphological condition. Thus, the site was unlikely to have been correctly assessed by the National model.

The independent data from these sites revealed a possible lack of sensitivity of the National model for the littoral reference sites, which might be because of an insufficient representation of this type in the reference sites. Inclusion of more sites from this region could allow the formation of a specific reference group in the classification step and result in more sensitive predictions of expected fauna would be expected, leading to more accurate results.

The problem of determining reference conditions in regions that have been extensively modified has been considered by several authors (e.g., Reynoldson and Wright, 2000; Verdonschot and Nijboer, 2000; Chessman and Royal, 2004; Stoddard et al., 2006). The collection of more reference sites is a possibility but may not be possible because of the difficulty finding acceptable sites in this highly developed area of Portugal. A promising alternative for the use of reference sites that could be applied in the littoral region of Portugal is that of environmental filters (Chessman and Royal, 2004). Filters are major components of the habitat patterns in streams (such as riverbed composition or flow regime) that exclude taxa of a regional pool from sites where the ranges of environmental conditions are incompatible with their preferences and tolerances. This way the taxa at a site can be predicted and thus its condition assessed by comparing the potential assemblage with that observed.

Overall test sites in the littoral region showed a lower physical–chemical quality of the streams than the sites in the less developed north and south regions. Organic contamination associated with destruction of natural areas (mainly for construction or urban areas, agricultural practices and deforestation) are the main disturbances verified in all Portuguese territory (MAOT, 2002) and the banding system of all models was able to detect these ecological effects of these human disturbances (Figs. 4a, 6a and 9a).

The Water Framework Directive (Directive 2000/60/CE, 2000) requires a quality classification of water bodies in 5 classes (from high to poor quality) and that those classes should correspond to increasing degrees of physical and chemical disturbances (Directive 2000/60/CE, 2000; Furse et al., 2006). Based on the O/E values of test sites the new banding/classification system showed a similar good correspondence between the progressively higher class attributed (i.e., better condition) and the level of physical and chemical degradation of streams measured by the selected disturbances variables (Figs. 4b, 6b and 9b). This indicates that the O/E values below reference can be divided into more bands than provided by the AUSRIVAS system because they correspond to intermediate levels of disturbance for the same variables (Figs. 4b, 6b and 9b).

Predictive models could also be a useful tool to observe the improvement in river condition following rehabilitation. However, hysteresis, time lags between a pressure change and the corresponding change in the communities, non-linear responses, points of no return and climate changes, may create confounding effects in the evaluation of systems recovery (Lyytimäki and Hildén, 2007; Jeppesen et al., 2005; Monteith et al., 2005). Therefore, these factors should be considered when analyzing cause–effect relationships in a recovery process and future developments in predictive modeling may consider predictions in climate change scenarios. We also suggest when applying these models, it is useful to sample a number of reference sites as well as test sites to aid in the detection of external factors such as climate-change induced drift from the reference condition.

5. Conclusions

This work showed that the distribution of macroinvertebrate communities in Portugal can be predicted from a small set of geographical, climatic and hydrological variables.

We produced three models, one National and two regional, that passed all tests for accuracy, predictability and validation. However, the regional models (North and South) performed better than the National model and these are preferred for the assessment of test sites from each area because: more taxa are predicted; they have lower discriminant errors; better predictabilities or accuracies (based on regression between observed and expected values of reference sites) and the assessments of water quality seem to be more accurate according to all information available from the sites.

For regions with few reference sites, such as the littoral area, the National model is an alternative although it requires further refinements. Also, in the future, a different approach for establishing the expected taxa with which to compare those observed, such environmental filters not requiring reference sites, could be tried for this region.

The five class WFD quality assessment scheme, adapted from the AUSRIVAS bands, appears to be justified because of the good correspondence between the human disturbance level and the classes to which test sites were allocated. Elimination of the AUSRIVAS X band in the WFD scheme has produced a clearer relationship (Fig. 4). The AUSRIVAS methods that use macroinvertebrates as a WFD Biological Quality Element are able to detect changes in river health and respond to several causes of degradation.

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