RIVER RESEARCH AND APPLICATIONS
River Res. Applic. **31**: 457–469 (2015)
Published online 15 January 2015 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/rra.2860

A LANDSCAPE-BASED REGIONALIZATION OF NATURAL FLOW REGIMES IN THE EBRO RIVER BASIN AND ITS BIOLOGICAL VALIDATION

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ABSTRACT
Flow prediction in ungauged basins is an important task for water resources planning and management, and remains a fundamental challenge for hydroecological research. Based on a previous classification of streams and rivers in the Ebro River basin (Spain), where six natural flow-regime types were identified, we apply a new predictive approach of the flow regime type based on climatic and physiographic descriptors. We used a set of easily available environmental variables as discriminant parameters: annual precipitation, annual evapotranspiration, annual air temperature, elevation, catchment area, drainage density and geology. A stepwise landscape-based classification procedure consisting of several stepwise discriminant analyses and canonical discriminant analyses allocated a set of sites with poor or no natural flow data into the flow types defined. Misclassification rates obtained by cross-validation ranged between 1.12% and 11.9%. Additionally, the ecological soundness of the proposed regionalization was tested by the concordance between macroinvertebrate communities and the proposed classification using NMDS and ANOSIM. NMDS resulted in a clear separation of sites into five NFR classes with available macroinvertebrate data, and ANOSIM found significant differences in macroinvertebrate communities among classes.

KEY WORDS: natural flow regimes; landscape-based models; environmental flows

Received 30 September 2013; Revised 15 September 2014; Accepted 23 October 2014

INTRODUCTION

By the late 1990s, there was a growing agreement among scientists and water managers that for the purposes of protecting freshwater biodiversity and maintaining the ecosystem goods and services provided by rivers, natural flow variability, or some semblance of it, must be maintained (e.g. Arthington *et al*., 1992; Richter *et al*., 1996; Poff *et al*., 1997; King and Louw, 1998). This new paradigm was articulated in the principle of the 'natural flow regime' (hereafter NFR; Poff *et al*., 1997), which defends the variation in flows as a cornerstone to sustain ecosystems (and associated biodiversity) and that the natural variation in any river is defined by climatic, geologic and land cover controls on precipitation and runoff. Because these controls vary geographically, natural flow regimes do so as well.

In terms of water resources management and related to the principle of the NFR, the environmental flow concept is gaining affection among scientists and managers (e.g. Annear *et al*., 2004; Gippel *et al*., 2009; Gippel, 2010; Kendy *et al*., 2012). The environmental flow concept refers to the quantity, timing and quality of water flows required to sustain freshwater and estuarine ecosystems and the human livelihoods and well-being that depend on these ecosystems (Brisbane Declaration, 2007). In this direction, Arthington *et al*. (2006) expose a method that describe the guidelines for designing environmental flows at the regional scale in different NFR types. Based upon this approach, Poff *et al*. (2010) presented the ELOHA framework (Ecological Limits Of Hydrologic Alteration) that assess the environmental flow needs to develop and implement environmental flow standards at the regional scale. By comparing ecological condition along flow-alteration gradients, ecologically relevant flow standards can be developed and calibrated for each hydrologic river type. This methodology requires hydrologic and ecological information at both impaired and unimpaired sites.

The problem appears when few good quality hydrological data, or even no data at all exist. In such situations, flow prediction in ungauged basins becomes a challenge for hydrologists all over the world. A variety of approaches can be used to provide estimates of hydrological indices at ungauged sites. They range from purely physically based to purely empirically based methods. Physically based ones are those that aim to estimate streamflow by utilizing a conceptual understanding of the physics describing various parts of the hydrological cycle, such as interception,

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evaporation and storage (e.g. Clark *et al*., 2008). Empirically based approaches are those that seek to estimate hydrological indices by quantifying relationships between observed hydrological indices and catchment characteristics. The availability of GIS landscape data and the ease of computation, compared to the calibration of model parameters, make these models very attractive for water managers and riverine scientists (e.g. Chiang *et al*., 2002; Alcazar and Palau, 2010; Bejarano *et al*., 2010; Kennard *et al*., 2010; Li *et al*., 2010; Bao *et al*., 2012; Liermann *et al*., 2012; Ahiablame *et al*., 2013; Pruski *et al*., 2013; Booker and Woods, 2014).

In Spain, many authors have obtained stream classifications at regional scale from landscape criteria following the standards of the Water Framework Directive (WFD) (e.g. Bonada *et al*., 2002; Munné and Prat, 2004; Sánchez-Montoya *et al*., 2007). However, the WFD classification System B, which is the most broadly used in Spain and Europe, does not consider key aspects of the NFR for achievement of good ecological status of rivers, as seasonal flow patterns, extreme flow conditions or flow predictability. For example, Centro de Estudios y Experimentación de Obras Públicas (CEDEX) (2005) classified streams and rivers at a national scale by using mean annual streamflow, specific mean annual streamflow, mean catchment slope, altitude, latitude, conductivity, mean annual temperature, annual temperature range and stream order. Other classification systems are based on estimated flow data by the SIMPA model (Álvarez *et al*., 2005), which is available for the entire territory, instead of real measurements at gauged stations (e.g. Alcazar and Palau, 2010; Bejarano *et al*., 2010; Centro de Estudios y Experimentación de Obras Públicas (CEDEX), 2005; Centro de Estudios y Experimentación de Obras Públicas (CEDEX), 2010).

On the other hand, Solans and Poff (2013) developed a classification of NFRs in the Ebro River basin (NE Spain) based on real flow data and considering a wide spectrum of natural flow features: 54 hydrologic parameters that represent four facets of the NFR (magnitude, variability, frequency and duration). The flow regimes identified were: Continental Mediterranean-pluvial, Nivo-pluvial, Continental Mediterranean-pluvial with groundwater dominance, Pluvio-oceanic, Pluvio-nival-oceanic and Mediterranean. This classification, however, was restricted to few sites with at least 20 years of unimpaired flow data. Nonetheless, a clear affinity between NFRs and geographic location was evidenced, suggesting a link between natural flow type distribution and climatic and physical variability in the basin (see Solans and Poff, 2013). The aim of present work is, therefore, to extend the NFR classification proposed in Solans and Poff (2013) to the whole Ebro River basin, through a new predictive approach based on climatic and catchment physical characteristics. Predictions are carried out by several discriminant function analyses that are undertaken in a stepwise manner to the different NFR types already defined in the basin. Annual precipitation, evapotranspiration and air temperature; elevation, catchment area and slope, density drainage and geology were used as discriminant parameters. Cross-validation and visual evaluation of the distribution of the NFR types across the basin were used to validate the results.

On the other hand, one critical aspect of any environmental classification is its ecological soundness. If we want to develop such a tool, in order to better manage and protect aquatic ecosystems, it is crucial to integrate the biological component into the classification process (Dodkins *et al*., 2005; Turak and Koop, 2008; Snelder *et al*., 2012), or by serving as a benchmark against which we can test the validity of a particular classification (e.g. Bonada *et al*., 2002). These biological validations have been widely applied in a range of aquatic ecosystems and covering a variety of classification approaches (Hawkins *et al*., 2000; Bonada *et al*., 2002; Snelder *et al*., 2004; Sánchez-Montoya *et al*., 2007). Stream macroinvertebrate communities play a key role in shaping the structure and functioning of the ecosystem (Wallace and Webster, 1996; Covich *et al*., 1999), and exhibit a wide range of responses to different environmental factors, associated with both natural and human induced environmental changes (Vinson and Hawkins, 1998; Hussain and Pandit, 2012). So they are particularly well suited for this purpose. We applied a non metric multidimensional scaling ordination and an analysis of similarities using stream macroinvertebrate data from reference sites (i.e. with minimal anthropogenic alteration) to visually interpret the distribution of sites according to macroinvertebrate community similarity within and between NFR types, and to test for differences in community structure.

STUDY AREA

The Ebro River basin is the second largest in the Iberian Peninsula and one of the largest in the Mediterranean region. It drains approximately 85 400 km^2 along the southern-facing slopes of the Cantabrian Range and the Pyrenees, the northern-facing slopes of the Iberian Massif and the western-facing slopes of the Catalan Ranges, emptying into the Mediterranean Sea (Figure 1).

The Ebro River basin receives both Atlantic and Mediterranean climate influences (Confederación Hidrográfica del Ebro (CHE), 2005). Atlantic climate is present in the northwestern corner of the basin, the western half of the Pyrenean Range and the northern part of the Iberian Range. There is a transition zone to Mediterranean climate represented by the Western-Central Pyrenees and the central Iberian Range. The Mediterranean climate characterizes the Catalan Ranges and the south-east corner of the Iberian range. Mean annual precipitation in the basin is 622 mm, ranging from over

DOI: 10.1002/rra

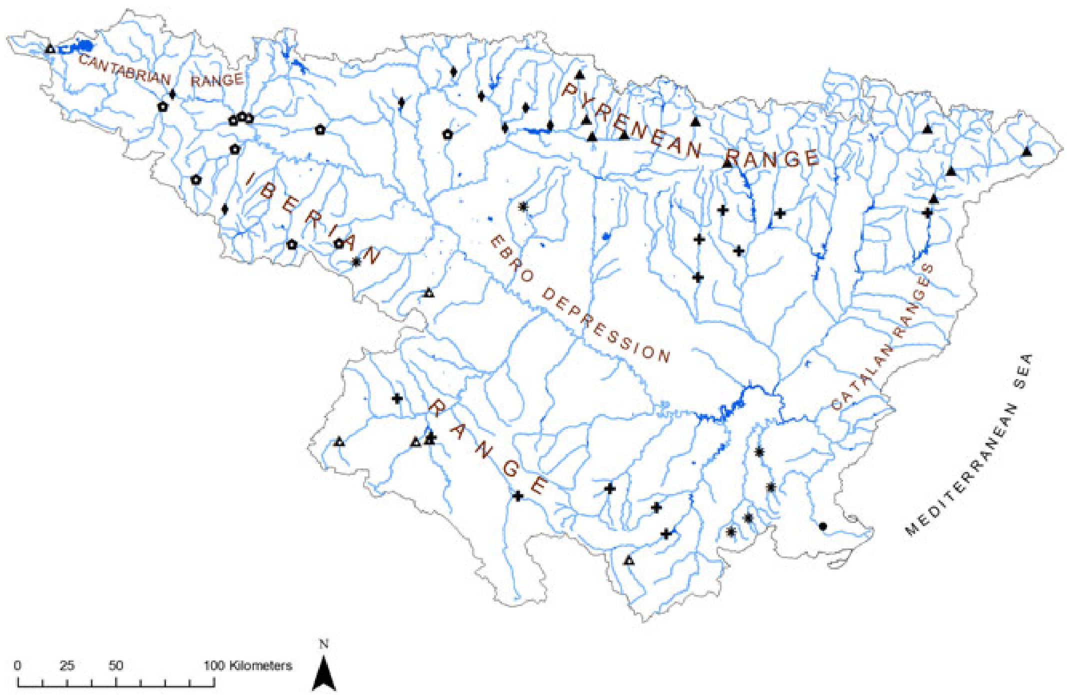

Figure 1. Ebro River basin with the main geographical features, and distribution of NFR types obtained from Solans and Poff (2013). Flow regimes are as follows: A: Continental Mediterranean-pluvial (E); B: Nivo-pluvial (▲); C: Continental Mediterranean-pluvial with a groundwater-dominated flow pattern (:); D: Pluvio-oceanic (X); E: Pluvio-nival-oceanic (K); and F: Mediterranean (k). This figure is available in colour online at wileyonlinelibrary.com/journal/rra

2500 mm in the Pyrenees and the Cantabrian Range, to less than 300 mm in the inner Ebro Depression.

Geologically, the Ebro River basin has an endorheic origin, and, as a consequence, it is formed by evaporitic rocks in most of the central and lower parts of the basin. Large-scale sedimentation during the Secondary and the Tertiary periods explains the presence of limestones and karstic formations in the Iberian, Catalan and Pyrenean ranges. Conglomerates are also present, mostly located in the eastern and western parts of the Ebro Depression.

Solans and Poff (2013) identified six distinct NFR types in the Ebro River basin: Continental Mediterranean-pluvial (type *A*), Nivo-pluvial (type *B*), Continental Mediterranean-pluvial with groundwater dominance (type *C*), Pluvio-oceanic (type *D*), Pluvio-nival-oceanic (type *E*) and Mediterranean (type *F*) (see Figure 1). Pluvio-oceanic (type *D*) and pluvio-nival-oceanic (type *E*) flow regimes are present at the north-western sector of the basin. The two annual flow patterns are characterized with a regular flow from December to April and differ for the peak flow timing, with *D* having a December peak and *E* an April peak. The *E* flow regime suffers the effects of snow cover in winter and the corresponding melt in spring, which delays the winter peak.

Through the Pyrenean Range we can find the nivo-pluvial flow regime (type *B*). This regime is characterized by low winter flows and high spring peaks due to snow melt and spring precipitation occurring simultaneously. Sites at lower altitudes than *B*, and similar hydro-climatic conditions but no snowfall during winter, produce a continental Mediterranean-pluvial flow regime (type *A*). The *A* type has flow peaks directly related to precipitation maxima. It is also present in the central and southern sectors of the Iberian Range. The *A* flow regime is characterized with a consistent baseflow with two maxima, autumn and spring, and a permanent summer flow. At the headwaters of the central and southern Iberian Range a low, variable groundwater-dominated flow pattern is present, the continental Mediterranean-pluvial with groundwater dominance flow regime (type *C*). Finally, the Mediterranean flow regime, with a strong equinoctial character (type *F*) is found in the southeastern part of the basin. The type *F* annual pattern has two main peaks, one in early winter and another in spring, linked directly to the equinoctial precipitation. This area experiments the lowest low flows, the highest maximum flows, the highest flashiness and the highest frequency of low flow spells and dry periods in the basin (see Solans and Poff, 2013; for details).

METHODOLOGY

Selection of sites

We used 51 sites with unimpaired flow data previously classified in Solans and Poff (2013) as *training sites* (Figure 1). Another set of 128 sites with poor (<20 years) or no natural flow data were selected as *test sites.* Test sites were located

DOI: 10.1002/rra

in high and middle parts of the Ebro River basin, and climate and watershed characteristics were similar to those of the training sites.

Obtaining of variables

Catchment characteristics and climate were used as discriminant variables to allocate the test sites into the different flow regime types. Raster files (100 m × 100 m raster cell) of: altitude, mean annual precipitation, mean annual temperature, mean annual potential evapotranspiration and mean annual hydrologic balance (precipitation minus evapotranspiration); and feature files of: superficial geology, streamflow network at 50.000 and 100.000 spatial scales and subcatchment areas of Ebro sites, were obtained from the Ebro Water Authorities. Surficial geology data were previously classified into four classes (A, B, C and D) according to the infiltration capacity, with A representing the maximum and D no infiltration capacity. Geographic Information System tools were used to calculate the slope raster map (100 m × 100 m) and also the variables taking part in the analysis. In total, 32 parameters related to climate and watershed properties were obtained at the subcatchment scale to carry out the regionalization of natural flow regimes at the Ebro River basin (Table I).

Regionalization process

The present regionalization process consists in classifying some stream sites with poor or no unimpaired flow data into some a priori known NFR types. This process was undertaken in two phases: (i) selection of the classification variables for each NFR type. This part was carried out with training data; and (ii) allocation of new sites into the NFR types, with training and test data. Both phases were performed in a stepwise manner due to the high cohesion among site groups (NFR types). Figure 2 describes one step of the classification process, from variable selection to site allocation.

Selection of classification variables. The selection of classification variables for the different NFR types consisted

Table I. Climatic and physiographic parameters taken into account for carrying out the hydrologic regionalization of natural flow regimes in the Ebro River basin

Variable code	Definition
Area	Subcatchment area (km^2)
meanHeight	Mean altitude of the subcatchment (m)
minHeight	Mínimum altitude of the subcatchment (m)
maxHeight	Maximum altitude of the subcatchment (m)
Height_range	Altitude range of the subcatchment (m)
St_meanHeight	Standard deviation of mean subcatchment altitude (m)
meanSlope	Mean slope of the subcatchment (°)
maxSlope	Maximum slope of the subcatchment (°)
St_meanSlope	Standard deviation of the mean subcatchment slope (°)
Pmin	Minimum value of the mean annual precipitation in the subcatchment (mm/year)
Pmax	Maximum value of the mean annual precipitation in the subcatchment (mm/year)
Pmean	Mean value of the mean annual precipitation in the subcatchment (mm/year)
St_Pmean	Standard deviation of the mean annual precipitation in the subcatchment (mm/year)
Pmax_Pmin	Ratio between Pmax and Pmin
ETPmin	Minimum value of the mean annual potential evapotranspiration in the subcatchment (mm/year)
ETPmax	Maximum value of the mean annual potential evapotranspiration in the subcatchment (mm/year)
ETPmean	Mean value of the mean annual potential evapotranspiration in the subcatchment (mm/year)
St_ETPmean	Standard deviation of the mean annual potential evapotranspiration in the subcatchment (mm/year)
BHmin	Minimum value of the mean annual hydrologic balance in the subcatchment (mm/year)
BHmax	Maximum value of the mean annual hydrologic balance in the subcatchment (mm/year)
BHmean	Mean value of the mean annual hydrologic balance in the subcatchment (mm/year)
St_BH	Standard deviation of the mean annual hydrologic balance in the subcatchment (mm/year)
Tmin	Minimum value of the mean annual temperature in the subcatchment (°C)
Tmax	Maximum value of the mean annual temperature in the subcatchment (°C)
Tmean	Mean value of the mean annual temperature in the subcatchment (°C)
St_Tmean	Standard deviation of the mean annual temperature in the subcatchment (°C)
d50	Drainage density 1: Ratio between the river network length at 1:50.000 scale and subcatchment area (km/km^2)
d100	Drainage density 2: Ratio between the river network length at 1:100.000 scale and subcatchment area (km/km^2)
geo_A	Percentage of area in the subcatchment with superficial geologic materials of infiltration class A
geo_B	Percentage of area in the subcatchment with superficial geologic materials of infiltration class B
geo_C	Percentage of area in the subcatchment with superficial geologic materials of infiltration class C
geo_D	Percentage of area in the subcatchment with superficial geologic materials of infiltration class D

DOI: 10.1002/rra

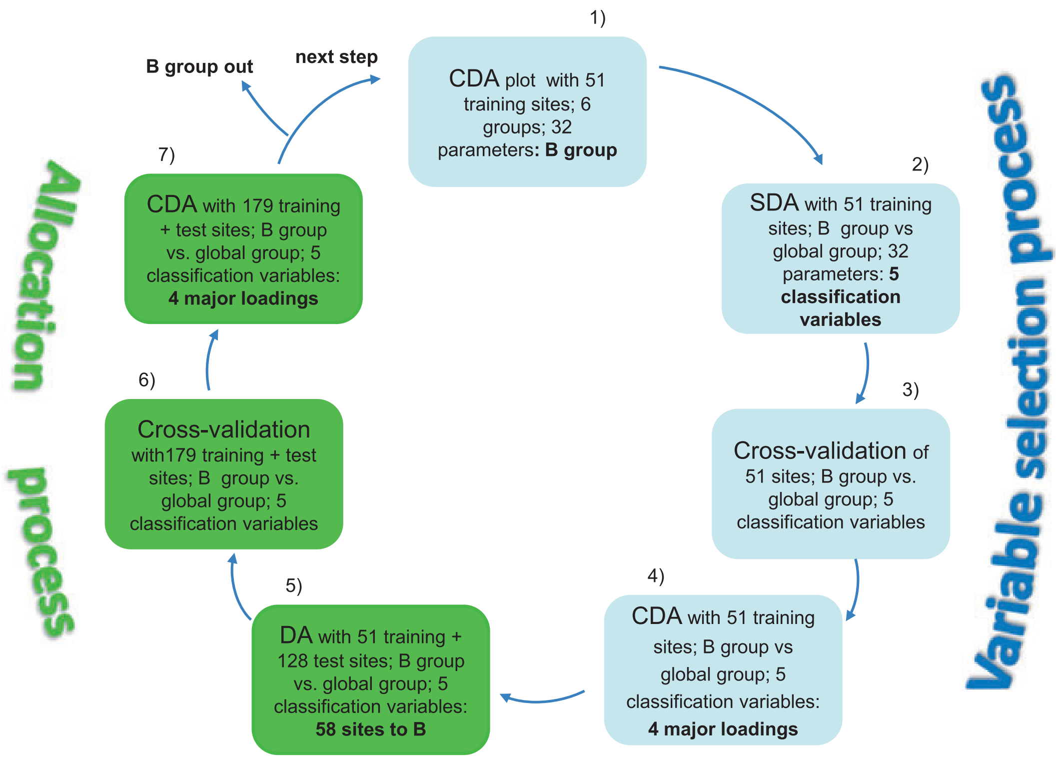

Figure 2. Description of the regionalization process for the NFR type *B*. The blue parts correspond to the variable selection process, and the green parts correspond to the allocation process. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

in applying several *stepwise discriminant analyses* (hereafter SDA) and *canonical discriminant analyses* (hereafter CDA), in a stepwise manner.

At each step:

(1) A CDA plot based on the 32 parameter-set (Table I) was constructed to visually identify the group (or NFR type) most different from the rest (outgroup).
(2) A SDA applied to the two obtained groups, the outgroup and the group composed by the rest of sites (global group), extracted the significant discriminant (or classification) variables useful to discriminate both groups.
(3) Validation of the classification variables obtained in (2). According to Manly (1994), any SDA must be treated with caution and carefully combined with *cross-validation*. Therefore, a cross-validation process using only the classification variables obtained in point 2) estimated the expected error for the two-group classification.
(4) Moreover, we plotted the two-group CDA by using exclusively the classification variables obtained in 2). This second CDA permitted to extract the univariate contribution of each classification variable towards group separation, known as loadings or structure coefficients (Rencher, 2002). Besides, the two-group CDA plot permitted to verify visually the discriminant power of the classification variables. Box-plots of major loadings were constructed to visually assess the strongest physical and climatic differences between flow regime types. Box-plots were constructed with row data.

Once the classification variables for a NFR type were identified, all sites within this type were removed from the data set before proceeding with a new flow type (a new step). It was necessary to add a step to separate eastern flow regime types (*A* and *C*) from western (*E* and *D*), due to the high cohesion between the four site groups.

At each step, pre-treatment of data included transformation, when required, to meet normality assumptions, and standardization of variables. Also the first canonical correlation coefficient and Rao's approximation test were computed at each step. Rao's approximation test evaluates the significance of canonical correlation coefficients. Stepwise discriminant analysis, canonical discriminant analysis and cross-validation were carried out using the SAS 9.1 statistical software package, with SAS Function Codes: PROC STEPDISC, PROC CANDISC and PROC DISCRIM, respectively.

Classification process. Classification of new (test) sites into existing NFR types was carried out through *Discriminant analysis* (DA), following a stepwise process in the same order than variable selection process.

DOI: 10.1002/rra

At each step:

(5) A DA with training and test sites was performed using the classification variables obtained in 2). DA consists in two parts: first, the classification function of each group (outgroup and global) is obtained from training data; and second, test sites are allocated into the group with the lowest distance between the site and the corresponding classification function.
(6) Validation of allocation process. Cross-validation of both groups, the outgroup and the global group, was performed using the classification variables obtained in 2).
(7) A two-group CDA plot was constructed using the training and test sites, and the classification variables obtained in 2). The two-group CDA plot was constructed to assert the discriminant power of classification variables and to identify the major loadings. Box-plots of the major loadings were built using row data and they were compared with box-plots obtained in section *Selection of classification variables*.

At each step, pre-treatment of data included transformation to meet normality assumptions, when required, and standardization of variables. First canonical correlation coefficient and Rao's approximation test were computed at each step. The classification and validation processes were developed using the PROC DISCRIM function in SAS 9.1 software package.

Biologic correspondence with NFR classification

Aquatic macroinvertebrate data (family level mean percentage abundance for the 2007–2009 period) were used to biologically test both the hydrologic classification obtained in Solans and Poff (2013), and the posterior regionalization across the basin. We assigned 42 least impaired sites with invertebrate data (from the Ebro's biological reference network) to a natural flow regime type based on their geographical location. Only five NFR types were represented: *A*, *B*, *D*, *E* and *F*. The 42 sites were included in an ordination analysis (non metric multi-dimensional scaling, NMDS). NMDS ordination places the samples in an arbitrary dimensional space such that their relative distances represent their corresponding pair-wise similarities: nearby sites have similar communities and viceversa. A visual assessment of the positioning of sites grouped by NFR types was performed. In addition, biotic differences between hydrological types were tested by means of analysis of similarities (ANOSIM; Clarke, 1993) using Bray–Curtis distances. Each test in ANOSIM produces an R-statistic, which contrasts the similarities of sites within a flow type with the similarities of sites among types (when the R value is close to one, similarities between sites within a type are higher than those between sites from different types, and values close to zero indicate no differences among types). The number of Monte Carlo permutations was set at 9999. We also performed pair-wise ANOSIM comparisons among hydrological types to distinguish among possibly contrasting effects. All analyses were undertaken using the package 'vegan' version 2.0.6 (Oksanen *et al*., 2013) for the R software, version 2.15.3 (R Core Team, 2013). The data 'autotransform' tool was selected for the NMDS (see MetaMDS function in vegan, Oksanen *et al*., 2013).

RESULTS

Selection of variables

Table II shows the classification variables identified at each step and the misclassification rates obtained by cross-validation. Table II also shows the squared canonical correlation between the first canonical component and the different groups (NFR types) at each step. Canonical correlations are high (0.63–0.82) in almost all cases, which means strong associations among individual sites within the clusters. Differences between NFR types are significant according to Rao's approximation test.

Allocation of sites

Table II shows the number of sites allocated at each group and the misclassification error rates obtained by cross-validation: Fifty-eight sites were allocated into group *B*, with a misclassification error rate of 1.12% (2/179). Once sites of group *B* were removed from the analysis, 12 sites were allocated into group *F*, with a misclassification error rate of 5.36% (6/112). At the next step, 24 sites were allocated into the eastern Ebro sector (*A* and *C* types), and 34 sites into the western Ebro region (*D* and *E* types), with a misclassification error rate of 4.26% (4/94). Within the western sector, the transformed variable Pmean allocated 17 sites into each group, *D* and *E*, with a 5.77% (3/52) misclassification error rate. Finally, in the eastern sector, 12 sites were allocated into each flow regime, *A* and *C*, with 11.90% of misclassification error.

Table II also shows the squared canonical correlations between each discriminant function and the NFR types. Rao's test shows significant differences among all pairs of site groups.

The distribution of the natural flow types obtained through the hydrologic regionalization was consistent with the pattern obtained in Solans and Poff (2013) (see Figure 3).

Major loadings

Major loadings were studied for both variable selection and allocation processes, in order to assess the robustness of variable contribution to group separation (see Figure 2). Major loadings are highlighted in bold in Table II. At each step, the same variables were identified as major loadings in both processes. The correlations between each variable and the

DOI: 10.1002/rra

Table II. Results of variable selection and site allocation procedures at each step of the regionalization analysis. The steps are ordered downward following the chronological order in the process. Classification variables; n° of sites allocated into the group; misclassification rates; squared canonical correlation and F statistic based on Rao's approximation test of canonical correlation correspondent to the first discriminant function. Major loadings are highlighted in bold and the correspondent loadings (correlation between the variable and the first discriminant function) are indicated between brackets (t = training sites/T = training + test sites)

			Cross-validation		Squared canonical correlation		Pr > F	
Procedure step	Classification variables	No of sites allocated	Variable select.	Allocat.	Variable select.	Allocat.	Variable select.	Allocat.
Nivo-pluvial (group B)	**maxSlope** (t = 0.69;T = 0.79) **Tmin** (t = −0.63;T = −0.98) **Tmean** (t = −0.77;T = −0.79) **St_Tmean** (t = 0.75;T = 0.81) geo_A	58	0%	1.11 % (2/179)	0.82	0.8	<0.0001	<0.0001
Mediterranean (group F)	meanSlope maxSlope ETPmax ETPmean **Tmax** (t = 0.51;T = 0.4) **geo_D** (t = −0.58;T = −0.65)	12	11.9% (5/42)	5.36% (6/112)	0.62	0.63	<0.0001	<0.0001
East/West Ebro basin	Area maxHeight meanSlope St_Pmean **ETPmax** (t = 0.70;T = 0.71) d50 geo_B	24 to East/ 34 to West	5.55% (2/36)	4.26% (4/94)	0.82	0.77	<0.0001	<0.0001
Pluvio-oceanic (group D) /Pluvio-nival-oceanic (group E)	**Pmean** (t = 1;T = 1)	17 each	11.11% (2/18)	5.77% (3/52)	0.67	0.63	<0.0001	<0.0001
Continental Mediterranean-pluvial with (C)/without (A) groundwater dominance	**d100** (t = 0.64;T = 0.82) geo_A	12 each	11.11% (2/18)	11.9% (5/42)	0.57	0.5	0.0019	<0.0001

first canonical component are indicated within brackets in Table II. Univariate contribution of variables to group separation is steady despite using different site samples (training sites; training + test sites). When comparing box-plots of major loadings, it was observed that differences between flow regime types were consistent in both processes for all the variables (see Figure 4). It was observed that *B* catchments have lower mean annual temperatures (Tmean, Tmin) than the other regions. On the other hand, the standard deviation of the mean annual temperature (St_Tmean) and maximum slopes (maxSlope) in *B* catchments are the highest. Regarding group *F*, the maximum value of mean annual temperatures (Tmax) in *F* subcatchments is, in general, slightly higher than in the other regions. The main difference that determines the *F* flow type, however, is the low proportion of impermeable soil (D soil class) in their drainage catchments, with respect to other areas. In 75% of *F* catchments, superficial impermeable soil materials barely represent 20% of the total area. The main climatic difference between the west and east sectors of the Ebro basin, is the higher maximum value of mean annual potential evapotranspiration (ETPmax) in the eastern sector. Inside the western sector, results for Pmean show a clear threshold between *D* and *E* flow regions around 1000 mm/year. Finally in the eastern sector, regions of type *A* carry a slightly higher flow network density than regions of type *C*.

Macroinvertebrate communities and hydrological groups

The ordination of macroinvertebrate communities (NMDS) resulted in a clear separation of the 42 sites in the five hydrological types using the available biologic data (*A*, *B*, *D*, *E* and *F*)

DOI: 10.1002/rra

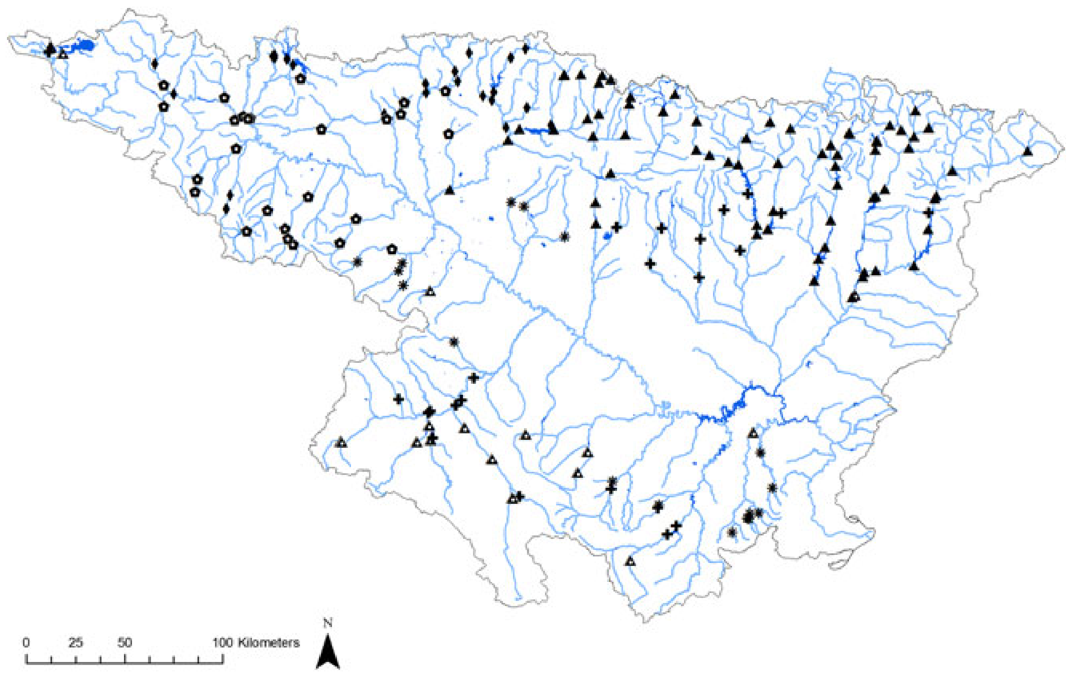

Figure 3. Clustering of the six flow regimes in the Ebro River basin based on first a hydrological classification of 51 sites with natural flow regimes (Solans and Poff, 2013), and a posterior landscape-based regionalization of 128 sites with poor or no natural flow data. Flow regimes are as follows: A: Continental Mediterranean-pluvial (E); B: Nivo-pluvial (▲); C: Continental Mediterranean-pluvial with a groundwater-dominated flow pattern (:); D: Pluvio-oceanic (X); E: Pluvio-nival-oceanic (K); and F: Mediterranean (k). This figure is available in colour online at wileyonlinelibrary.com/journal/rra

(Figure 5). Types *B* and *F* were the most clearly differentiated along the first NMDS axis, occupying right-side and left-side positions in the 2D ordination space, respectively. Types *A*, *D* and *E* occupied intermediate positions with strong overlap along this first axis. The second axis, in contrast, differentiated type *A* (lower positions) from types *D* and *E* (upper positions). Types *F* and *B* showed central positions with much overlap along this second axis

Analysis of similarities (ANOSIM) revealed significant, global differences in macroinvertebrate communities among the NFR types (Global $R = 0.251$, $p = 0.0002$). Pairwise comparisons showed significant differences ($p < 0.05$) between groups, with the exception of pairs *D-A*, *D-E* and *E-F* (Table III). The widest differences in invertebrate communities were found between pairs *B-F* and *B-E*, both with an ANOSIM R of 0.47.

DISCUSSION

In a previous hydrological classification of natural flow regimes in the Ebro River basin, it was suggested a close link between NFR types and climatic and physiographic differences across the Ebro River basin (see Solans and Poff, 2013). Extrapolation of hydrologic information from sites with available data to places with poor or no data is known as hydrologic regionalization. This study presents a hydrologic regionalization of the six natural flow regime types identified in Solans and Poff (2013) across the Ebro River basin through a stepwise landscape-based classification procedure.

The distribution of flow regime types obtained in the present regionalization matched closely that of Solans and Poff (2013). In addition, the process identified robust statistical differences between flow regime types regarding physiographic and climatic conditions: discriminant functions in both variable selection and allocation processes were significant for all cases, and the squared canonical correlations between the discriminant functions and the flow groups (i.e. association strength between the functions and the site groups) were medium or high in all cases. The consistent squared canonical correlation values, in both variable selection and allocation processes, indicates consistency in both within-group and between-group differences. Besides, the same major loadings were identified in both processes, with similar correlation coefficients values, which emphasize the robustness in univariate contribution of variables to group separation.

Box-plots of major loadings in both processes were consistent, which also demonstrates the strong physical and climatic differences between NFR types. These differences, in turn, are consistent with actual variation on main climatic and physical features in the Ebro River basin. For example, low mean annual temperatures, high variation in mean annual temperatures and steep slopes found in *B* regions are

DOI: 10.1002/rra

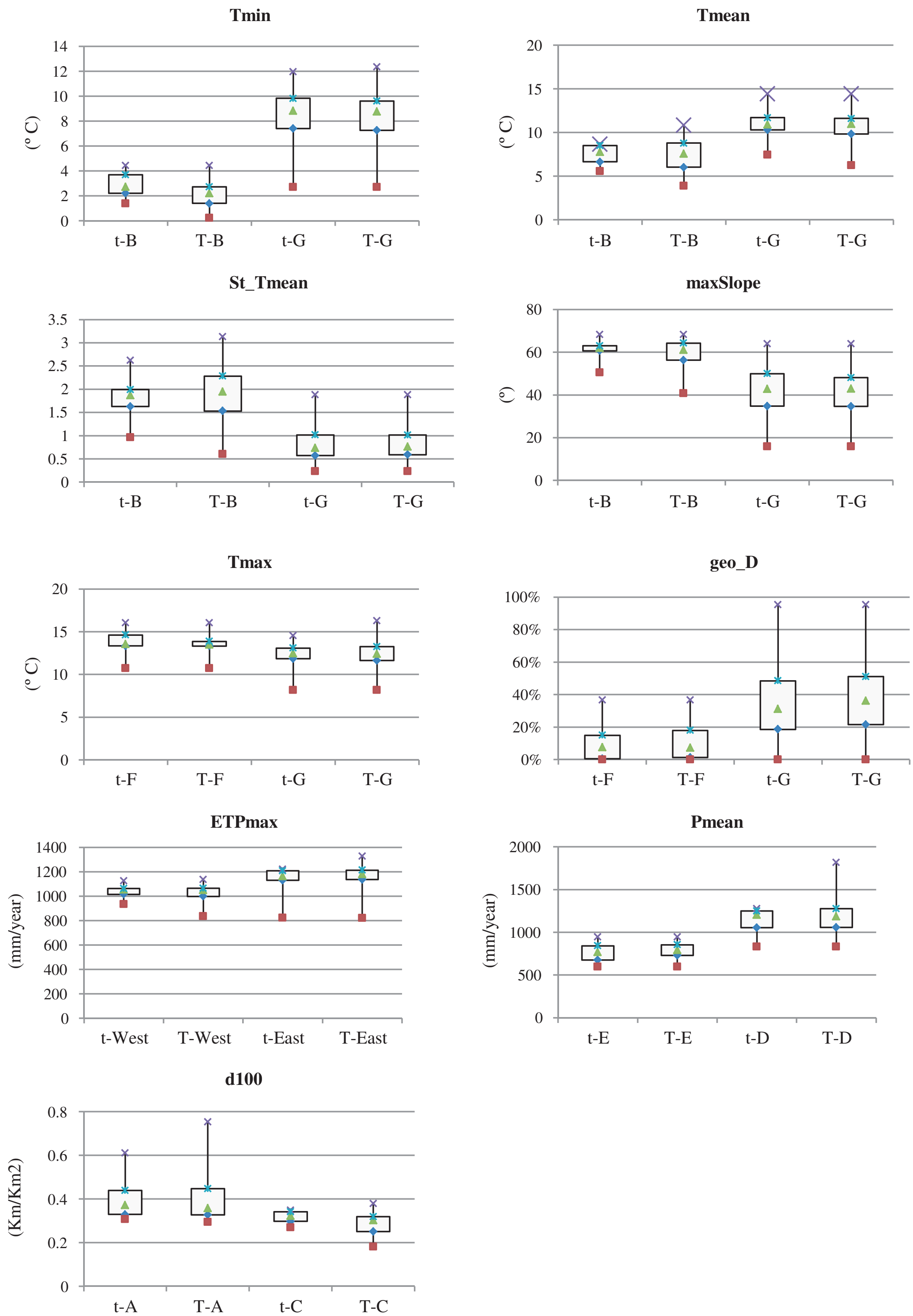

Figure 4. Box-plots of major loadings for the different assessment steps of regionalization analysis. Initials indicate A, B, C, D, E, F, West, East and General groups, and variable selection (t) and site allocation (T) processes. The variables correspond to raw data. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

DOI: 10.1002/rra

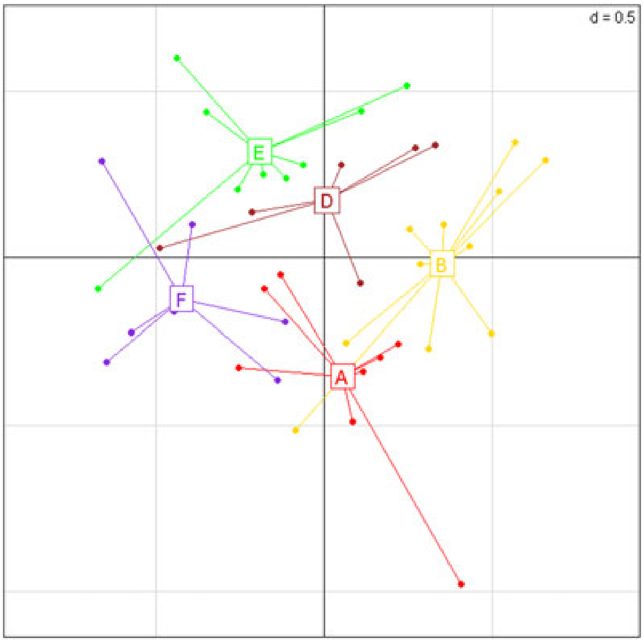

Figure 5. Non-metric multidimensional scaling (NMDS) plot for the 42 biological sites from Ebro River basin representing five NFRs: A, B, D, E and F. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

characteristic of high mountain environments, where this group is located. A low proportion of impermeable soil found in *F* regions is a distinctive feature of the south-east sector of Ebro basin, which is especially rich in karstified carbonate lithologies (see section 2. *Study Area*). The lower mean annual potential evapotransporation in the western Ebro zone points to a climatic transition from oceanic in the western part, with regular storms along the year, to mediterranean in the eastern sector, characterized with low rainfalls and arid summers (Confederación Hidrográfica del Ebro (CHE), 2005). Another example is the higher mean annual precipitation registered in regions with *D* flow type in comparison with *E* flow type. Effectively, sites of type *D* are located in the highest parts of Cantabrian and Pyrenean Ranges, and on the Demanda Mountain, in the Iberian Range, which are the wettest regions of the Ebro basin. Finally, the lower flow network density found in regions with *C* flow type than A flow type is consistent with the karstic compositions present in many of the headwaters of the central and southern Iberian Range, where *C* sites are located. It is feasible to assume that the river network is poorly developed in these areas, because of the high infiltration rates compared to other zones (Buchanan *et al*., 2013).

Table III. Results of pairwise analysis of similarities (ANOSIM) among A, B, D, E and F flow regime groups. When R is close to 1, similarities within-group are higher than between-group, and when R is close 0 there are no differences between groups

Groups	*R* Statistic	*p*
D, E	0.02	0.365
D, B	0.22	0.043
D, F	0.20	0.038
D, A	0.04	0.287
E, B	0.47	0.000
E, F	0.11	0.118
E, A	0.27	0.012
B, F	0.47	0.000
B, A	0.23	0.007
F, A	0.18	0.039

Other regionalization approaches have been previously developed in the Ebro River basin. Centro de Estudios y Experimentación de Obras Públicas (CEDEX) (2010) developed a hydro-regionalization across all major catchments in Spain with the purpose of developing a program of environmental flow regimes. They created hydro-regions based on inter-annual and intra-annual variability of minimum and maximum annual streamflows estimated by SIMPA model (Álvarez *et al*., 2005). They obtained twelve regions for the Ebro River basin, with two of them covering almost the whole area. The first one comprises the areas of types *B*, *D*, *E* and part of *A* (left Ebro River side). The other one covers the zones of *C* and the right side of *A* flow regime types. Centro de Estudios y Experimentación de Obras Públicas (CEDEX) (2005) did also a classification of fluvial segments across Spain based on mean annual streamflow and specific mean annual streamflow estimated by SIMPA model (Álvarez *et al*., 2005), mean catchment slope, altitude, latitude, conductivity, mean annual temperature, annual temperature range and stream order. This classification found ten stream types in the Ebro River basin and three of them were the most frequent. The classes found do not correspond either to our flow regime types. One of them corresponds to rivers located in low to medium altitude Mediterranean mountains, with highly mineralized waters, and covers areas of *A*, *E* and *F* flow regime types. A second major class includes rivers located in medium to high Mediterranean mountains and encompasses *B*, *C* and *E* flow regime zones. The third major class gathers rivers of intermediate altitude wet mountains with highly mineralized waters and comprises *B* and *D* flow regime type regions.

The use of only one aspect of the natural flow regime (discharge or variability) and the inclusion of other non-hydrological variables in the classification process could be the main causes of this mismatch. Nevertheless, it is worthy to acknowledge the different spatial scale of both classifications.

In order to biologically validate the hydrologic classification of the Ebro River basin, we used stream macroinvertebrate communities, whose responses to a number of hydrological factors (that constitute the different NFRs) are well documented in the literature (Poff and Ward, 1989;

DOI: 10.1002/rra

Poff *et al.*, 1997; Extence *et al.*, 1999; Hart and Finelli, 1999; Belmar *et al.*, 2013). Not surprisingly, we found that differences in macroinvertebrate community composition were greatest between classes at both extremes of the flow-stability gradient (*B-F*), from the nivopluvial flow regime (type *B*) to the extreme low-flows, dry periods and high flashiness characterizing type *F*. Similar results have been reported in other catchments in the Iberian Peninsula (Sánchez-Montoya *et al.*, 2007; Belmar *et al.*, 2013). Other NFR types that were efficiently discriminated were types *B* (Pyrenean nivopluvial regimes) and *E* (high Cantabrian range, with abundant precipitation and regular flow with an April peak). When other pair-wise differences were examined, more subtle, but still significant, biological differences were detected, with the exception of pairs: *A-D*, *D-E* and *E-F*. Nevertheless, although ANOSIM found no significant differences in similarity between these pairs, the relative positioning of sites in the ordination was clearly distinct, showing very little overlap. Thus, we consider that these results support the general hypothesis that streams with similar NFRs host similar macroinvertebrate assemblages (Resh *et al.*, 1988; Poff, 1996). In addition, they demonstrate that a landscape-based regionalization can work consistently with existing ecological differences in the basin. In New Zealand, for example, Snelder *et al.* (2011) report that flow-ecology relationships (represented by habitat availability) vary among major river types defined by morphology and flow regime. Although it is well known that the composition and distribution of macroinvertebrates in streams are governed by numerous physical, chemical and biological factors (see Hussain and Pandit, 2012; for a recent review) which could be responsible of the observed patterns, we can conclude that the present hydrological classification (and so the morpho-climatic surrogates) can explain most of the observed variability in macroinvertebrate community composition in the studied area.

Numerous examples of hydrologic regionalizations have been developed using different physiographic and climatic variables and a variety of statistical procedures. For example, Chiang *et al.* (2002) found clear relationships between some hydrologic parameters and watershed elevation and precipitation in southeastern USA by using multiple regression analyses. Kennard *et al.* (2010) applied a classification tree model based on a combination of catchment, substrate, vegetation and climate variables at the continental-scale of Australia, and it correctly classified 62.1% of stream gauges into their a priori flow-regime classes. Liermann *et al.* (2012) also used classification and regression trees to predict hydrologic class as a function of climatic and physical drainage basin characteristics for rivers throughout Washington State. They found precipitation variables, elevation and winter temperatures as the principal discriminators of hydrologic groups. The lack of importance of physical basin attributes, other than elevation, in predicting flow class could be due to the scale (Washington State) and resolution (flow segment) of the cited study. This is consistent with other studies that place climate as a first-order driver of flow regime at a broad scale, with basin attributes affecting more localized flow patterns. For example, Bejarano *et al.* (2010) found a hierarchical importance of physiographic factors to discriminate flow regimes in the Ebro River basin based on flow estimations from SIMPA model (Álvarez *et al.*, 2005). Biogeography and precipitation regime were the main environmental discriminators, followed by geological characteristics and basin size, and finally, by elevation and slope of the fluvial segment.

CONCLUSIONS

The present study describes a landscape-based regionalization process in the Ebro River basin to extend flow regime information from areas with available flow data to areas with poor or no natural flow data. Flow prediction in ungauged basins is an important task for water resources planning and management, and remains a fundamental challenge for hydroecological research.

We developed a stepwise, landscape-based approach capable of allocating consistently new sites into some pre-existing NFR types (obtained from real unimpaired hydrologic data) that can serve as management units. Moreover, this study shows biological differences between five over the six different flow regime types found in the basin.

We propose the present hydrologic regionalization approach as a tool to predict unknown natural flow information in highly regulated basins with important natural flow gaps, or in ungauged sites. The present approach is transferable at any region with sufficient climatic and watershed data, and even at broader scales. Despite the difficulty in obtaining sufficient real unimpaired flow data and biological reference data at many regions, both kinds of data are essential to give robustness to the process. According to McManamay *et al.* (2012), hydrological data should be integrated in the landscape-based approach when its purpose is to predict hydrologic information. For example, simulation techniques can provide unimpaired flow data, assuming the partial loss of accuracy in the hydrological classification. The biological validation of natural flow regime classifications should be also compulsory when the purpose of those classifications is the management of water resources according to ecosystem requirements. The general scarce data on freshwater macroinvertebrates at the lowest levels (genus, species) makes difficult to elaborate accurate assessments about indicator or characteristic communities in freshwater ecosystems. However, the present work shows promising relationships between macroinvertebrate communities at

DOI: 10.1002/rra

family level and physiographic differences at the regional scale in the Ebro basin. Family level data, although also difficult, is frequently available or more feasible to obtain at many regions.

In addition, the hydrologic regionalization presented here provides fundamental information to carry out assessments of anthropogenic or climate change-related hydrological alterations in these ecological and economically essential freshwater ecosystems.

ACKNOWLEDGEMENTS

The first author wants to thank Ebro's Authorities for his support and for the facilitation of the data, essential for the development of present work. The first author also wants to thank Diego García de Jalón for his advice during the development of the present study. The authors want to thank Dr. Peter Gell and two anonymous referees for improving the quality of the paper with their comments on earlier versions.

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