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A multimetric model to assess the biological quality of the Cabe River, NW Spain

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ABSTRACT

The Water Framework Directive (WFD) establishes the need to define specific reference conditions for the assessment of river ecosystems. This paper presents a multimetric index for assessment of biological quality in a river ecosystem using benthic macroinvertebrates. The study area corresponds to the headwater of the Cabe River, Northwest Spain, upstream of the irrigation dam at Ribasaltas, a water catchment for irrigation in the Lemos Valley area. Because Lemos Valley is an important irrigation district located in an area with key environmental features, defining the quality objectives that must be used to assess the impact of agriculture becomes essential. In our research, reference stations were chosen on the basis of a number of strict conservation criteria, and the macroinvertebrate community present in those stations was analysed. Following the verification of the homogeneity of environmental features in the study area, we selected the biological indices and metrics that provided the strongest correlations between the condition of the macroinvertebrate community and the overall environmental quality. After the most suitable metrics were selected, the weight of each metric in the final multimetric index was determined on the basis of the greater or lesser ability of each metric to define biological quality. The correlation between biological metrics and quality, and the ability of the selected metrics to define quality were determined on the basis of their performance at a number of control stations with different levels of disturbance. Finally, the quality thresholds for the proposed multimetric index were established such that the quality thresholds of the index matched the quality levels defined in the WFD. Copyright © 2013 John Wiley & Sons, Ltd.

KEY WORDS multimetric; macroinvertebrates; biological quality; quality thresholds; reference conditions

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INTRODUCTION

The possibility of conducting an integrated assessment of the sustainability of our agricultural systems based on the use of water resources is essential to achieve effective resource management and planning and particularly to ensure the protection of increasingly limited resources (Clark, 2002). Such an integrated approach comprises not only the physicochemical quality or the amount of water but also the quality of water as a sustaining part of a biotic organization (Norris and Hawkins, 2000). Scarcity and misuse of fresh water pose a serious and growing threat to sustainable development and protection of the environment. Technically and economically inefficient water distribution networks for urban or agricultural uses contribute to increased deterioration in the quality of inland waters and negatively affect the biological status of river systems (Tafangenyasha and Dube, 2008). These factors, together with the fact that agriculture is the largest user of water, determine the need to apply restrictions, particularly relative to the environment, to change the dynamics of water use.

The use of bioindicators to assess the quality of water in river ecosystems is widespread throughout the world, and the benefits of using integration tools have been envisaged in the legislation of many countries (Resh, 2008). The European Union acknowledged the importance of integration tools with the approval of the Water Framework Directive, WFD (OJEU, 2000). In the WFD, biological indicators are the core of the system for quality monitoring and assessment. Actually, the WFD has coined the concept of ‘ecological status’, which has dramatically changed the way in which governments regard the biological indicators of water quality. Water is no longer considered a mere production factor but an important ecological and social asset that greatly contributes to sustaining the ecosystem functions (Van der Brugge and Rotmans, 2007). This approach intends to make the use of water and of its associated space compatible, which involves good ecosystem structure and functioning and ensures the conservation of communities over time, i.e. it ensures good ecological status.

The knowledge gained on river ecosystems must allow for the determination of their level of disturbance and the design of efficient monitoring methods (Højberg *et al.*, 2007). The

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protocols for the assessment of ecological status are based on the combination of biological, hydromorphological and physicochemical indicators that provide structural and functional data on the health of these systems. In order to determine the ecological status of an ecosystem, the reference conditions or undisturbed conditions specific to the area must be determined first. To provide a reasonable level of confidence for the values of the indicators used to assess the reference conditions, a network of sites with undisturbed conditions must be created. In addition, the analysis tools used to assess an aquatic environment must represent the health of the environment, such that impacts can be better diagnosed and the most suitable management and protection models that allow for the conservation of the environment over time can be proposed.

The use of multimetric indices to assess the biological quality of rivers is currently widespread because these indices integrate the functional and structural variability of the biotic components of a river ecosystem in a single metric (Segnini, 2003). Multimetric indices combine the individual scores of a number of metrics and indices used to determine quality on the basis of any biological group that occurs in a river ecosystem, such as fish, diatoms or macroinvertebrates, in a final score. The combination of metrics must be characteristic of each index and is usually adapted to each region or even to specific sub-basins of a river. For an index to be characteristic of a specific region or basin, the characteristics of the area must be similar in terms of temperature, precipitation, geology and riparian vegetation (Barbour and Paul, 2010).

Today, there is broad consensus that benthic macroinvertebrates are most suitable for the assessment of the biological quality of river ecosystems (Bonada *et al*., 2006). Compared with fish, which is affected by constant restocking, benthic macroinvertebrates are ruled only by biological control models or environmental factors. Actually, biotic interactions such as predation or competition become less relevant in the benthic macroinvertebrate community because of the frequency and intensity of physical events in the river system (Prat *et al*., 2013). Within environmental factors, the impact of the natural environmental variability in a region is usually minor compared with anthropogenic factors. The commonest anthropogenic alterations in our rivers are organic pollution, and flow diversion and regulation. Organic pollution is the most frequent factor in the drainage system of Galicia, where pollution is caused mainly by occasional wastewater discharges from human settlements, or agricultural and farming activities (Carballo *et al*., 2009). Although easily emendable (Sanchez-Hernandez *et al*., 2012), organic pollution in Galicia affects the distribution patterns of macrobenthos.

The objective of this paper is defining a multimetric model to assess the biological quality of the Cabe River, Northwest (NW) Spain. To do so, this paper defines the reference conditions for the Cabe River upstream of Ribasaltas irrigation dam, a water catchment used to irrigate the Lemos Valley irrigation district, NW Spain. The reference conditions are defined by establishing quality thresholds for a number of biological indices and metrics derived from the analysis of the macroinvertebrate community upstream of the disturbance. The correct discrimination of the variability in the composition of benthic macroinvertebrates, caused by pollution and human impacts on riverbeds, provides specific data of the biogeographical distribution of the community. These data can be used to define a target threshold of biological quality as a function of communities under natural conditions. After analysing the response of the communities and adjusting the basis for the indices of biological quality, all the indices are combined into a multimetric index suitable for the assessment of the study area.

STUDY AREA

The Cabe River is a right-bank tributary of the Sil that drains a basin of 353 km^2 up to the irrigation dam at Ribasaltas (Figure 1). The Cabe River rises at an elevation of 960 m above sea level in Foilebar, in the Loureira Mountains, and flows southwestward for 500 m incised into a N/S fracture, receiving Antiga from the left. For the next 350–400 m, the Cabe River flows into the flat-bottomed Lemos graben and receives its two main tributaries along this river stretch. The basin is drained by three natural drainage channels: rivers Mao, Saá and Cabe. All three rivers have a remarkable length and a marked change in slope at about 13-km distance from their sources. Thus, the stretch of the Cabe River is 30 km long; river Mao, a right-bank tributary of the Cabe River that rises in San Salvador beside Oribio mountain range, is 31 km long; and river Saá, a left-bank tributary of the Cabe that rises in Cima das Pías and drains Pobra de Brollón, is 21 km long.

The study area is characterized by a humid Mediterranean climate, with areas with cumulative annual precipitation below 900 mm and annual average temperature above 14 °C. During the summer, water is generally scarce because of irregular rainfall, which is in contrast with the need of water for agriculture. In this context, irrigation must be used as a complement in order to obtain competitive crops (Neira *et al*., 2005).

Demographically, the study area is characterized by continuous population decline and by highly scattered population, as most of inland Galicia. Monforte de Lemos concentrates the population migrated from rural areas (Cuesta *et al*., 2005). Most of the population are engaged almost exclusively in agriculture and farming, whereas manufacturing is scarcely developed, and the service sector concentrates in the most important towns.

The Cabe River and some stretches of the Mao River have been declared as sites of community importance (SCI) according to the criteria established in Directive 92/43/EEC (OJEU, 1992). The Saá River was not a part of the initial SCI

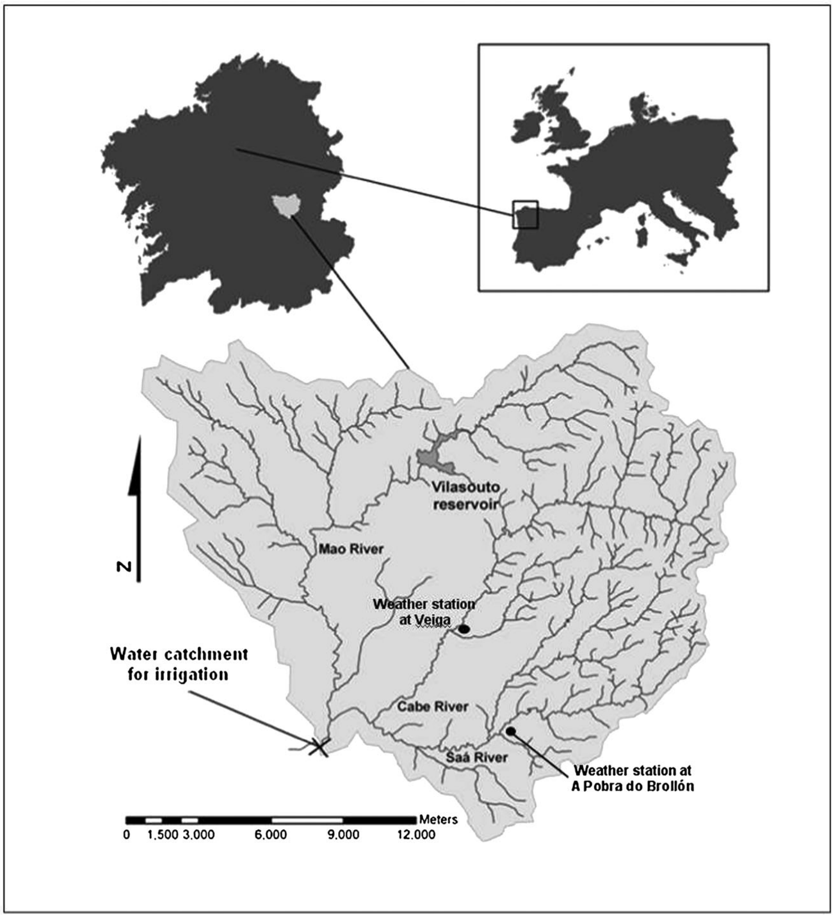

Figure 1. Location of the study area with location of the weather station at A Pobra do Brollón and the weather station at Veiga.

and was eventually excluded from the enlargement of this SCI. Among the types of habitat of community interest present in the study SCI are alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae* and *Salicion albae*). Also, the following animal and plant species of community interest protected by the Habitats Directive are present in the study area: Odonata *Macromia splendens* and *Oxygastra curtisii*, and the stag beetle (*Lucanus cervus*). In addition to brown trout (*Salmo trutta*), four endemic continental fish types are present in the Cabe River basin, namely, northern straight-mouth nase (*Pseudochondrostoma duriense*), bermejuela (*Achondrostoma arcasii*), northern Iberian chub (*Squalius carolitertii*) and European eel (*Anguilla anguilla*). There is strong presence of herpetological fauna, with 13 species of amphibians and at least 13 species of reptiles because of the co-occurrence of typically Mediterranean elements, and taxa of Eurosiberian origin or endemic to the NW corner of Spain (Cuadrado *et al*., 2011).

In 1966, the colonization of the Lemos Valley irrigation district was declared to be of great national interest (Cuesta *et al*., 2005). As a result, irrigation development was implemented in 5300 ha of five municipalities of the southern part of the province of Lugo, NW Spain, by using run-of-river flows from the Cabe River and regulated water from Vilasouto dam in river Mao. For the Cabe River, 2500 ha were irrigated using two irrigation channels derived from the irrigation dam at Ribasaltas, a left-bank channel and a right-bank channel.

Cuadrado *et al*. (2010) characterized the Ribasaltas irrigation dam as dividing two quantitatively and qualitatively different areas: an upstream area that combines forestry, and traditional agriculture and farming, and a downstream area that combines irrigated agriculture and urban land. Downstream of Ribasaltas dam, the habitat is impaired by qualitative disturbances related to the environment and quantitative disturbances related to the decrease in discharge. Moreover, this area shows a high risk of naturalization of non-native species due to illegal releases by urban population of Monforte de Lemos (Andreu and Vilá, 2007; Cuadrado *et al*., 2010).

MATERIALS AND METHODS

To assess the biological quality of the Cabe River, the results of sampling surveys performed from April 2006 to August 2009 at 30 sampling stations that were representative of the conditions of the study area were analysed. Sampling surveys were performed twice a year, one in April, during spring, and one in August, during summer. Sampling dates were delayed or brought forward in order to make the flow regime suitable to type of sampling. During each sampling, a sample of the macroinvertebrate community and a sample of water for physicochemical analysis were taken. In addition, temperature and dissolved oxygen were measured *in situ* with an OXI 92 oximeter (WTW GmbH, 2004). Once a season, the hydromorphological conditions were verified using a variety of quality indices.

The multihabitat approach for benthic macroinvertebrates described in *USEPA Rapid Bioassessment Protocols* was used (Barbour *et al.*, 2006). The multihabitat approach is a standardized semi-quantitative multihabitat sampling protocol for bioassessment that has been often used in both USA (Danehy *et al.*, 2012) and Europe (AQEM consortium, 2002). Each sampling station corresponded to a selected reach of 100 m in length in which the most frequent types of habitat in the water body were represented. To characterize river habitats, the USEPA protocol was used. The USEPA protocol consists in a visual-based identification of the coverage of all the types of habitat present in the selected reach. On the basis of this estimate, benthic invertebrates were sampled systematically according to the proportional distribution of microhabitats within a sampling reach (Barbour and Paul, 2010).

Sampling was performed by collecting 20 kicks in 100 m. A kick is a stationary sampling performed by using hands and feet to scrape the substrate for 0·5 m upstream of the net. Sampling was performed using a square kick-net with 0·25-m frame width, 0·5-m frame length and 500-μm opening mesh. The content of the net was placed on a tray, from which stones and large pieces of detritus were carefully removed. The rest of the material was placed into a container, fixed with 5% formaldehyde solution and subsequently labelled.

Laboratory processing of macroinvertebrate samples comprised sample sorting, counting and identification to family level. Identification to family level reduced identification times and minimized errors caused by the presence of intolerant genera or species found in small proportions in impaired environments (Barbour *et al.*, 2006). Laboratory processing data were used to analyse the sampled community or the total taxocenes found at each sampling station. The taxocenes sampled at each station and survey were used to analyse macroinvertebrate communities and their response to the application of biological quality indices, whereas total richness, obtained by jointly considering data from all surveys, was used to compare data from different sampling stations.

Selection of reference stations

The indicators of anthropogenic stressors generated in a basin are conservative variables that directly affect the overall quality of river ecosystems. The selection of reference stations was based on the degree of anthropogenic disturbance and stress at each station, which was determined using ten descriptors (Table I) related to human impacts on river ecosystems. Every descriptor was assigned a score of 1 if the condition stated in the descriptor was MET or 0 if the condition stated in the descriptor was NOT MET. The sum of scores given to each descriptor was termed Site Quality Index, SQI.

Descriptors 1–4 in Table I, i.e. the index of riparian forest quality, QBR from its Catalan abbreviation (Munné *et al.*, 2003), the index of river habitat, IHF from its Spanish abbreviation (Delgado *et al.*, 2012), the index of riparian vegetation, IRV (Gutiérrez *et al.*, 2001), and the index of naturalness of the river channel, NRC, are morphological indicators of the naturalness of river ecosystems and are closely related to biological condition (OJEU, 2000).

Descriptor 5, river without regulation, fills a gap in the WFD regarding the environmental impacts of the amount of flow. Krysanova *et al.* (2006) claimed that allocating resources of river ecosystems to a given use breaks the naturalness of the ecosystem and, consequently, stations affected by human regulation of water flow do not meet the naturalness condition.

Descriptor 6 regards human interventions in basins. Tong and Chen (2002) claimed that land use changes, which can be detected with Geographical Information Systems, contribute to the deterioration of the quality of rivers. Some authors related land cover to suspended particulate matter (Sylaios *et al.*, 2005), whereas other authors related land use to the biological quality of the system (Tafangenyasha and Dube, 2008). In this paper, a Geographical Information System was used to identify the naturalness of the selected reference stations based on the naturalness of the floodplain. The ArcGis 9.2 software (ESRI, 2006) was used for this purpose.

Descriptors 7–10 assessed the physicochemical characteristics of the environment. The Canadian Council of Ministers of the Environment Water Quality Index physicochemical quality index (Lumb *et al.*, 2006) was used to jointly assess ten variables representative of organic pollution: total phosphorus, nitrates, nitrites, ammonia, ammonium, BOD_5, faecal streptococci, total coliforms and faecal coliforms. The selected variables were assessed as a function of a number of target values. Descriptors 8, 9 and 10 were temperature, dissolved oxygen and water pH, respectively. The target values set to assess whether the descriptors met the established condition matched the ideal thresholds for growth and development of *Salmo trutta fario*, L., a salmonid present in the Cabe basin whose ranges of tolerance to these variables are well known (Cuadrado *et al.*, 2010).

Reference stations were selected as follows: first, stations with an SQI below 9 were excluded; then, the final reference stations were chosen from the remaining stations by removing those with deficient biological condition. To this end, we

Table I. Criteria for the selection of reference conditions.

Descriptor	Observations
Riparian Forest Quality Index of riparian forest quality (QBR) (Munné *et al.*, 2003).	This index assesses the quality of riparian forests and their degree of impairment through four independent measures: degree of cover, structure of vegetation, quality of the cover and naturalness of the river channel (NRC). The condition is MET if $\mathrm{QBR} \geq 95$.
Habitat Diversity Index of River Habitat (IHF) (Delgado *et al.*, 2012).	The results of IHF alone do not necessarily express a specific quality level but determine a good biological quality of the analysed ecosystem. The established condition is MET with levels above 85.
Naturalness of vegetation Index of Riparian Vegetation (IRV) (Gutiérrez *et al.* 2001)	The IRV index assesses the conservation status of river basins using vegetation as a bioindicator of their naturalness. The IRV index complements QBR data and the condition is MET if the value of the index is above 8.
Naturalness of River Channel $\mathrm{NRC} = \frac{\sum\left(0,5\tilde{n}\ \mathrm{Channeled}_{\mathrm{length}}\right)}{\mathrm{Total}_{\mathrm{length}}}$	The disturbance of the NRC is assessed from the ratio of reach affected by canalizations to the total length of the evaluated reach (NRC). The canalization is multiplied by two if both banks are affected. The condition is MET if $\mathrm{NRC} < 0{\cdot}2$.
River without regulation	When a sampling station is subjected to flow regulation by the presence of dams upstream, the established condition is NOT MET.
Naturalness of river basin	Once the floodplain was defined, the land uses present in the area were studied. The ArcGis 9.2 software was used to create polygons that delimited land uses on the basin, which were classified into three categories: urban, agriculture and natural. The percentage of floodplain occupied by each land use was calculated. The condition was MET if natural land use is >85%, agriculture is >15% and urban is 0%.
Lack of organic pollution Water Quality Index (CCME WQI) (Lumb *et al.*, 2006)	The CCME WQI index was used to jointly assess ten variables that are representative of organic pollution: total phosphorus, nitrates, nitrites, ammonia, ammonium, BOD_5, faecal streptococci, total coliforms and faecal coliforms according to the defined target values. The condition is MET if the value of the CCME WQI index is above 85.
Water temperature	MET = water temperature is between 9 and 17 °C.
Dissolved oxygen	MET = dissolved oxygen is between 7 and 12 mg O_2/litre.
pH	MET = pH is between 6 and 8.

CCME WQI, Canadian Council of Ministers of the Environment Water Quality Index.

analysed the sampled community and the total community at every station and removed the stations with significantly lower values.

Selection of metrics and determination of quality thresholds

When choosing a method for the assessment of the biological quality of river ecosystems, two major issues must be considered: (1) the accuracy of the method to produce results that conform to reality as closely as possible (Karr and Chu, 2002) and (2) the ease of use, which helps obtain results rapidly (Mazor *et al.*, 2006).

Most of the metrics and indices used to assess the biological quality of water based on macrobenthos use the tolerances of macroinvertebrates to a given perturbation, usually organic pollution. However, the variability of metrics is often unclear. When disturbances are strong, the response of macroinvertebrates reveals alterations in community structure, but when disturbances are not very intense or frequent, the analysis of metrics might not reveal changes. Consequently, analysing a variety of indices and metrics for the study area becomes essential to choose the indices and metrics that best conform to reality.

In order to obtain reliable information for different degrees of perturbation, the selected set of biotic metrics and indices (Table II) was applied to reference stations and to a number of control stations chosen from the original 30 stations. Such a procedure helped find correlations between metrics and recorded environmental data, such that only the metrics that were strongly correlated with environmental changes were used in the final index. Once the accuracy of the selected metrics and indices was tested, the metrics and indices with the best performances were chosen and combined in the multimetric index analysed in this paper.

To define quality thresholds, only data of reference stations were considered. The mean values of each metric for each reference station were used to estimate quality ranges according to the criteria defined in proposal COM/93/0680 for a Council Directive on the ecological quality of water (Table III).

Table II. Metrics considered for incorporation into the multimetric index.

Metric	Description
Total number of taxa (S)	Total number of families obtained from a survey of the community.
Number of EPT taxa (S_{EPT})	Total number of Ephemeroptera, Plecoptera and Tricoptera families, which are most sensitive to pollution.
Percent EPT (%EPT)	Percentage of EPT: ratio of total EPT individuals in the sample to total individuals in the sample.
Simpson's diversity index (λ) (Simpson, 1949)	Probability that two individuals randomly chosen from a sample belong to the same species. This index is strongly influenced by the importance of the dominant species (Moreno, 2001).
Shannon–Wiener's diversity index (H') (Shannon, 1949)	H' expresses the uniformity of the values of importance across all the species in the sample. It measures the mean uncertainty level for predicting the species to which an individual randomly chosen from a sample belongs.
Hill's number (N_1) (Hill, 1973)	N_1 is an index of dominance that considers the representativity of the most important species without assessing the contribution of the rest of species.
Iberian Bio-Monitoring Working Party (IBMWP) (Alba-Tercedor *et al.*, 2002)	This index combines the total number of taxa and a value of intolerance. The IBMWP considers the family taxonomic level. The final score is obtained by adding the values of intolerance for each family, which are in the range 0–10. The value of the index increases with the increase in the number of intolerant families.
Iberian Average Score per Taxon (IASPT) (Alba-Tercedor *et al.*, 2002)	The IASPT is calculated by dividing the final score of the IBMWP index by the number of families (S). It represents the mean value of intolerance of the community.
Extended Biotic Index (EBI) (Woodiwiss, 1978)	The EBI index is based on the analysis of macroinvertebrates according to their sensitivity to organic pollution in descending order. Pure waters have the highest score (15), whereas polluted waters have the lowest score (0–1).

Table III. Criteria used to allocate quality levels based on the proposal for a Council Directive on the ecological quality of water (93/0680), later incorporated into the Water Framework Directive (OJEU, 1994).

Degree of divergence from reference conditions	Disturbance	Quality level
>0·95	Minimum impact	Very good
0·8–0·95	Slight impact	Good
0·6–0·8	Important impact	Moderate
0·3–0·6	Serious impact	Deficient
<0·3	Severe impact	Poor

Biogeoclimatic characterization

The combination of metrics included in the multimetric index must be characteristic of every index and of the region for which it is developed. This means that the characteristics of the area must be similar in terms of temperature, precipitation, geology and riparian vegetation. Accordingly, it becomes necessary to verify that the study area has similar biogeoclimatic characteristics.

Precipitation was analysed using data from two weather stations representative of the characteristics of the study area: the weather station at A Pobra do Brollón (07°23′ W 42°33′ N), located at 401-m elevation, and the weather station at Veiga (07°24′ W 42°35′ N), located at 400-m elevation (Figure 1). To compare temperature, we used data from two additional weather stations, the station at Monforte, located at 300-m elevation, and the station at Vilar de Caurel, located at 670-m elevation.

Likewise, we verified that the geological characteristics of the study area did not result in differences in the physicochemical characteristics of water for the reference stations considered using 1:50 000 national geological maps. Finally, the vegetation series of the basin were analysed in order to find correlations between the altitudinal gradient and the vegetation series observed.

Design of a multimetric index

Once the final stations and the metrics that would be included in the final multimetric index were selected and the quality thresholds for each metric defined, each metric was assigned a different weight in the final score of the index. The weight assigned to each metric within the combined index was determined according to the ability of each metric to effectively explain deviations from the reference situation. For the weighting process, there has been taken into account the experience of other authors (Munné *et al.*, 2003; Barbour and Paul, 2010; Cao and Hawkins, 2011) according to the capacity of each metric to define the biological quality. Currently, the absolute value of metrics is rarely used; rather, the relative value of metrics against the reference conditions (between 0 and 1) is used, such that the value of the multimetric index is in the range 0–1. To do so, a linear relation is defined for each metric, taking as limits of the quality thresholds the maximum value, with a valuation of 1, and the minimum value with a valuation of 0.

RESULTS AND DISCUSSION

Selection of reference stations

After having calculated the SQI value for the 30 stations analysed in this paper, only ten stations met the condition of complying with nine out of ten descriptors of anthropogenic stress on rivers (Table IV). Conditions 7, lack of organic pollution, and 6, naturalness of river basin, were not met at 14 and 13 stations, respectively. Contrastingly, all 30 stations complied with descriptors 8, 9 and 10.

For the final selection of stations, we determined the biological condition of the ten selected stations by using boxplots that represented the number of families per station for each sampling survey (Figure 2) and the total number of families per station for all the samplings (Figure 3).

Station MO-07 was removed from the selection of reference stations because its values were considerably lower than the values obtained for the rest of stations. Station

Table IV. Site Quality Index (SQI) values for the sampled stations, grouped by river.

River	Station	SQI	River	Station	SQI	River	Station	SQI
Cabe	CE-01	4	**Saá**	**SA-01**	**10**	**Mao**	MO-01	6
	CE-02	**10**		SA-02	8		MO-02	8
	CE-03	**10**		SA-03	8		MO-03	8
	CE-04	8		SA-04	6		**MO-04**	**9**
	CE-05	**9**		SA-05	7		MO-05	7
	CE-06	7		**SA-06**	**9**		**MO-06**	**10**
	CE-07	**9**		SA-07	4		**MO-07**	**9**
	CE-08	8		SA-08	4		MO-08	8
	CE-09	8		SA-09	6		MO-09	8
	CE-10	7		**SA-10**	**10**		MO-10	8

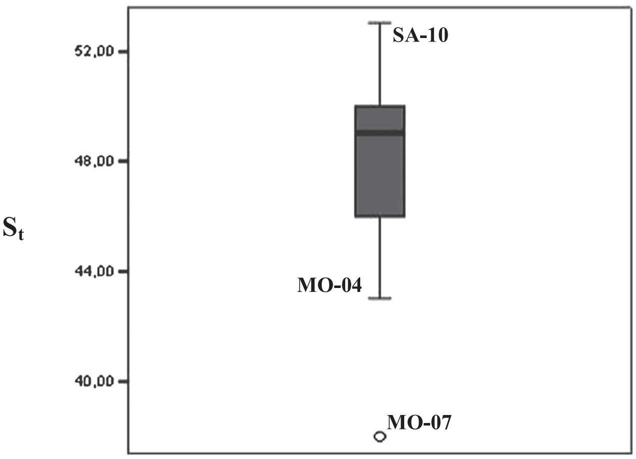

Figure 3. Total taxonomic richness (S_t) of stations classified as stations of low human pressure. The following metrics are represented: mean (black), values of the first and third quartiles of data (grey), maximum and minimum values (lines) and deviated values (circles).

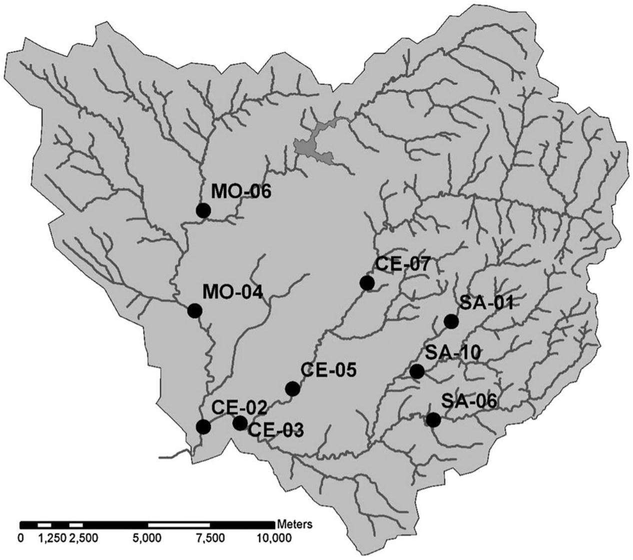

Figure 4. Location of reference stations.

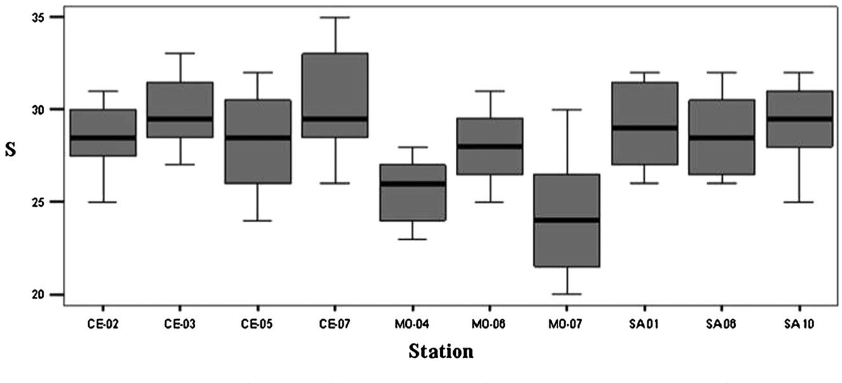

Figure 2. Taxonomic richness per sampling (S) at stations classified as stations of low human pressure. The following metrics are represented: mean (black), values of the first and third quartiles of data (grey), maximum and minimum values (lines).

MO-07 showed low anthropogenic stress and good physicochemical quality, and also alterations in flow that assumedly caused a decrease in diversity of macroinvertebrate families to values below the average for the stations in the region. Holomuzki and Biggs (2006) demonstrated that changes in the ecology of populations present in flow-regulated environments result from a reduction in the variability of microenvironments and resources caused by flow regulation. Therefore, the final number of reference stations was nine, distributed as shown in Figure 4.

Selection of metrics and establishment of quality thresholds

The metrics that best fitted the real environment situation are as follows: total number of taxa (*S*), number of EPT taxa (S_{EPT}), Hill's number (N_1), Iberian Bio-Monitoring Working Party (IBMWP) and Iberian Average Score per Taxon (IASPT). Total number of taxa (*S*) is the best and most simple expression to establish the *a priori* richness of an area. Using S_{EPT} is more reliable than using %EPT because %EPT is affected by the increase in the number of individuals that belong to other groups, which shows frequently when the number of individuals achieved by

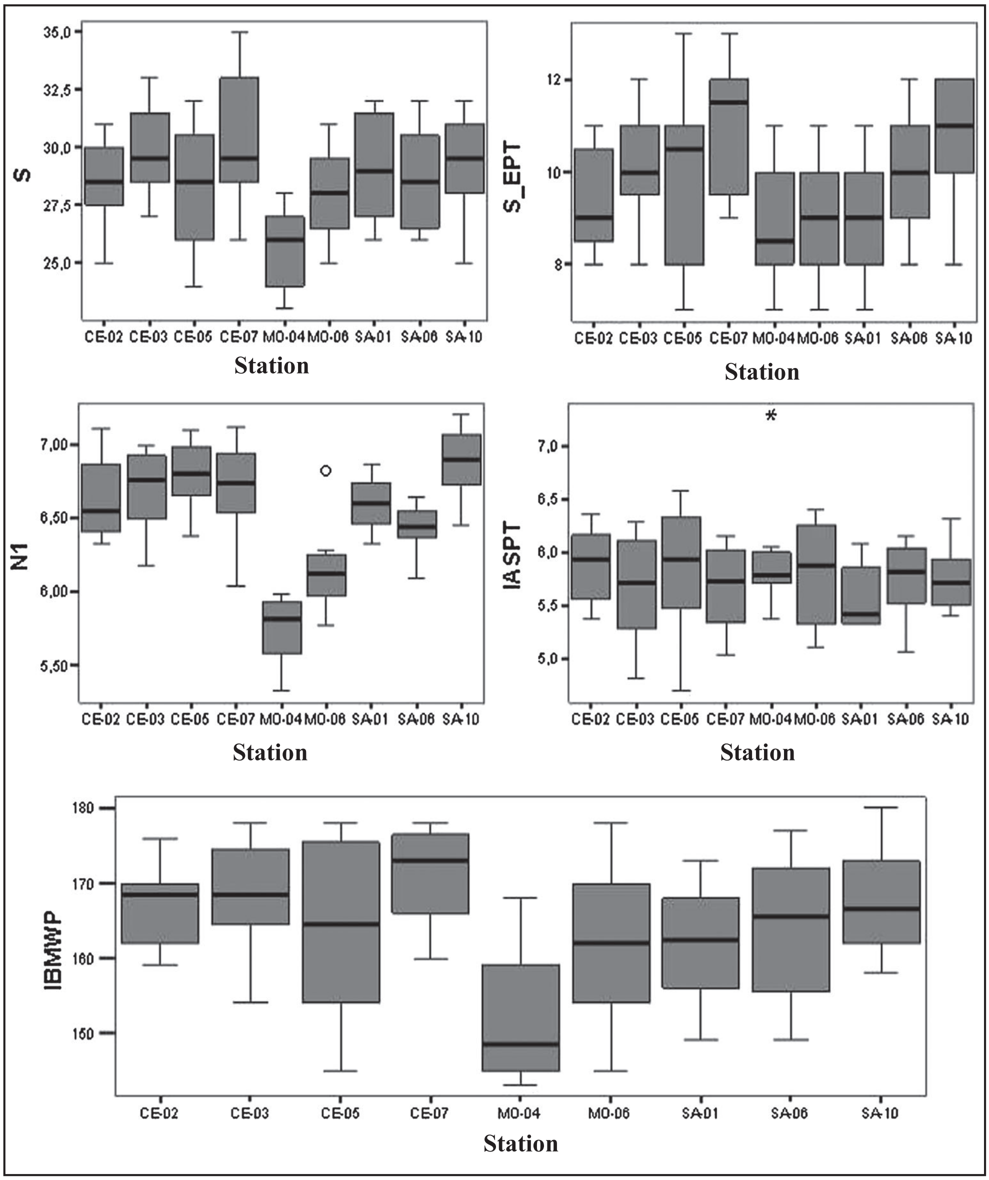

Figure 5. Boxplots for number of families, number of Ephemeroptera, Plecoptera, Tricoptera families, Hill's number, value of Iberian Average Score per Taxon (IASPT) index and value of Iberian Bio-Monitoring Working Party (IBMWP) index for each reference station and sampling. The following metrics are plotted: means (black area), values of the first and third quartiles of the data (grey area), minimum and maximum values (lines), values deviated (circles) and extremely deviated (asterisks) from the mean.

some Oligochaeta families is analysed. From among the analysed diversity indices, Hill's number (N_1) is least affected by changes in the uniformity and number of rare species in the samples. N_1 is higher than H' when quantifying diversity in samples subjected to random variations in the number of species. Hill's number is more simple (it depends on a single variable), coherent (units in number of species) and easy to interpret (Moreno, 2001). IBMWP and IASPT were chosen for a number of reasons, among which the specific design of these indices for bioassessment of Spanish continental water, the strongest environmental correlation of IBMWP compared with EBI in the analysed stations and their complementarity. Although IBMWP and IASPT are a function of one another, IASPT is less sensitive to sampling technique and effort and to seasonal variation, and shows a strong correlation with the stress gradient (Sandin and Hering, 2004). In addition, the IASPT index is capable of detecting variations in water quality caused by slight signs of pollution that could be masked in the IBMWP index (Cao and Hawkins, 2011).

For every station, the selected indices were calculated on the basis of the taxocenes collected during each sampling and station separately. The following metrics were used: total number of families (S), total number of Ephemeroptera, Plecoptera and Tricoptera families (S_{EPT}), Hill's number (N_1), IASPT and IBMWP. As stated earlier in this paper, quality thresholds were established using only data from the selected reference stations, such that the mean value of biological quality was obtained under natural conditions or with minimum anthropogenic disturbance. The scores for every station were used to analyse the quality thresholds required for the application of these indices and to verify whether there were differences between stations. As expected, the application of quality indices did not reveal significant differences among the nine analysed stations because stations within a region are characterized by a similar macroinvertebrate community (Figure 5).

Table V summarizes the average values obtained for every index and station during the sampling surveys conducted, as well as the mean of each index for the whole sampling area. Results reveal a richness of macroinvertebrates in the study area that contributes to high values of IBMWP compared with IASPT values.

The diversity index, N_1, showed the highest fluctuations for station quality, mainly because this index can be affected by factors such as the presence of very abundant species like some diptera or oligochaeta. For this reason, less-strict quality thresholds must be defined for this index. Consequently, values above 5 corresponded to good environmental quality and were assigned 100% of the score associated with this metric in the final multimetric index. Values of N_1 below 5 were assigned 0% of the score.

Table V. Results of the selected metrics for reference stations and results for the total number of stations.

Station		S	S_{EPT}	N_1	IASPT	IBMWP
CE-02	Mean	28·50	9·38	6·64	5·88	167·00
	Stand. Dev.	1·93	1·19	0·30	0·36	5·61
CE-03	Mean	29·88	10·13	6·69	5·66	168·38
	Stand. Dev.	2·03	1·25	0·29	0·51	7·76
CE-05	Mean	28·25	9·88	6·79	5·84	163·88
	Stand. Dev.	2·82	2·10	0·24	0·63	12·62
CE-07	Mean	30·38	11·00	6·70	5·67	171·13
	Stand. Dev.	3·07	1·51	0·34	0·42	6·60
MO-04	Mean	25·63	8·88	5·75	5·96	152·00
	Stand. Dev.	1·77	1·36	0·25	0·58	8·99
MO-06	Mean	28·00	9·00	6·16	5·80	161·88
	Stand. Dev.	2·00	1·41	0·32	0·52	11·53
SA-01	Mean	29·13	9·00	6·60	5·58	161·88
	Stand. Dev.	2·42	1·31	0·18	0·31	8·11
SA-06	Mean	28·63	10·00	6·43	5·75	164·00
	Stand. Dev.	2·26	1·31	0·17	0·36	9·99
SA-10	Mean	29·25	10·75	6·88	5·75	167·63
	Stand. Dev.	2·38	1·39	0·25	0·31	7·48
Total	Mean	28·63	9·78	6·51	5·76	164·19
	Stand. Dev.	2·55	1·55	0·42	0·45	9·98

IASPT, Iberian Average Score per Taxon; IBMWP, Iberian Bio-Monitoring Working Party.

To facilitate the application of the indices, the results of biological quality for the selected metrics were adjusted to the integer value immediately below the obtained value. The quality thresholds for each metric (Table VI) were calculated from these data according to the criteria summarized in Table III.

Biogeoclimatic characterization

To demonstrate that precipitations in the study basin were at least similar, we compared monthly precipitation data from the selected weather stations, A Pobra do Brollón and Veiga. Precipitation data from both stations were compared on a monthly basis. In addition, we compared total precipitation over the last 45 years. Both comparisons suggest that precipitation is similar at both stations. Actually, the analysis of total precipitation over the last 45 years (Figure 6) reveals a

Table VI. Biological quality thresholds for richness of families, S; richness of Ephemeroptera, Plecoptera and Tricoptera families, S_{EPT}; Hill's number, N_1, Iberian Bio-Monitoring Working Party, IBMWP, and Iberian Average Score per Taxon, IASPT.

Quality level	S	SEPT	N_1	IBMWP	IASPT
Very good	>26	>8	>5	>150	>5·5
Good	22–26	7–8		130–150	4·5–5·5
Moderate	17–22	5–7		95–130	3·5–4·5
Deficient	8–17	2–5	<5	50–95	2–3·5
Poor	< 8	<2		<50	<2

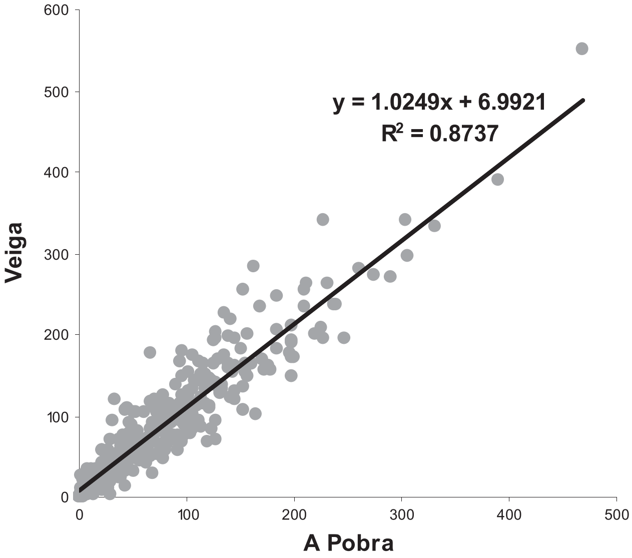

Figure 6. Comparison of total monthly precipitation in Veiga and A Pobra do Brollón weather stations.

value of slope = 1 and a goodness of fit of $R^2 = 0{\cdot}8737$ for the comparison of both stations.

Annual average temperature in the study area for 1964–2009 was in the range between 12·2 °C at Vilar do Caurel station, in the northeast part of the basin, and 13·9 °C at the Lemos graben, in the southwest part of the basin. Additionally, we compared the average daily value of maximum and minimum temperatures, in the weather stations of A Pobra de Brollon and Veiga, for the same period, the latest 45 years. R^2 values of 0·8123 for maximum temperatures, and of 0·7801 for minimum temperatures, are obtained. According to these results, there are no significant differences in the characteristics of the basin caused by high temperature gradients.

The geology of the basin is characterized by the presence of Quaternary detrital material, particularly Holocene alluvial deposits. Such deposits generally cut older deposits, such as glacises of Plesitocene age or sedimentary terraces. In addition, the study basin shows small intrusions of Tertiary deposits of green and red clay in the innermost areas of the basin. Yet, the geological characteristics of the basin were considered similar because no substantial differences were found.

With regard to vegetation along watercourses, the study area shows forests of *Alnus glutinosa* mixed with *Salix atrocinerea*, *Fraxinus* sp. and *Acer pseudoplatanus*. Above 700-m elevation, *Alnus glutinosa* is replaced with *Betula alba*. Other vegetation in the area includes the acidophilic Galician-Portuguese *Quercus robur*: *Rusco aculeati-Querceto roboris sigmetum* series as a potential vegetation series. This series shows degradation caused by constant fires and reforestation, which has brought about the presence of heathland with *Ulic-Ericetum cinerae* as a result of forest fires

Table VII. Original ranges for every index and unitary value allocated to each index.

S		S_{EPT}		N_1		IASPT		IBMWP	
Original	Unitary	Original	Unitary	Original	Unitary	Original	Unitary	Original	Unitary
>26	1	>8	1	>5	1	>5·5	1	>150	1
22–26	$S_u = 0{\cdot}056S - 0{\cdot}445$	7–8	$S_{EPT\text{-}u} = 0{\cdot}17S_{EPT} - 0{\cdot}33$			4·5–5·5	$IA_u = 0{\cdot}21\mathrm{IA} - 0{\cdot}57$	130–150	$IB_u = 0{\cdot}01IB - 0{\cdot}5$
17–22		5–7				3·5–4·5		95–130	
8–17		2–5		<5	0	2–3·5		50–95	
<8	0	<2	0			<2	0	<50	0

IASPT, Iberian Average Score per Taxon; IBMWP, Iberian Bio-Monitoring Working Party.

Table VIII. Weight allocated to each index in the final multimetric index: richness of families (*S*), richness of Ephemeroptera, Plecoptera and Tricoptera families (S_{EPT}); Hill's number (N_1), Iberian Bio-Monitoring Working Party (IBMWP) and Iberian Average Score per Taxon (IASPT).

Weight in the multimetric index	S	S_{EPT}	N_1	IASPT	IBMWP
	0·2	0·1	0·1	0·2	0·4

and the replacement of *Quercus robur* with *Pinus pinaster* as a result of reforestation. The current and potential distribution of vegetation does not show important differences within the study area.

The biogeoclimatic characteristics of the basin are not a cause of significant variations in the macroinvertebrate community.

Design of the multimetric index

To design the final multimetric index, we used the relative value of the selected metrics instead of the absolute value. Accordingly, the value of metrics was in the range 0–1. To make the application and display of data easier, the original ranges of values of the analysed indices were associated with their unitary value. To obtain more accurate unitary values, a linear equation was created, and the exact unitary value was calculated from the original value on the basis of the equation (Table VII). As expected, the final multimetric obtained after the weight of every index was calculated was in the range 0–1.

As stated earlier in this paper, the weight of each metric within the final index was allocated on the basis of the ability of each metric to explain deviations from the reference situation. Table VIII shows the weights allocated to every index. The IBMWP index was allocated the greatest weight because, according to the quality thresholds calculated, the IBMWP index provided the best measure of the biological quality of an ecosystem (Prat *et al.*, 2013).

CONCLUSIONS

The study basin, with an area of 353 km^2, presents a high number of reference stations, nine, particularly considering the difficulties to find undisturbed stations. Such a large number of reference stations are especially relevant if compared with the quality level defined for such stations on the basis of particularly strict conditions aimed at fully discriminating anthropogenic stressors in the system. The study area presents an excellent conservation status, particularly in the areas that are most distant from rural settlements. The study area has been categorized as an SCI not only because of the presence of threatened fauna but also because of the excellent conservation status of riparian forests, which is confirmed in this research.

From among the biological characteristics observed in the study area, the great richness of macroinvertebrates is particularly remarkable. The biological analysis revealed great presence of families very sensitive to pollution, and also a large number of identified families, which resulted in very high values of the quality indices. Such results suggest the appropriateness of modifying the ranges of the IBMWP index because, originally, values above 100 equalled undisturbed conditions, but the real conditions of the study area did not fit those ranges (Munne and Prat, 2009). The results for other indices such as IASPT revealed that the value of intolerance to pollution for the mean sampled taxon was 5·75 out of 10, which resulted from the richness of families in the area.

Selecting a specific set of indices to define ecological status, which includes an index for assessment of environmental biodiversity, weighting each index according to their correlation with the definition of the ecological status in homogeneous environmental conditions and eventually establishing quality thresholds specific to the study area, is the most accurate and efficient method to assess and further monitor the biological quality of a river ecosystem (Buffagni and Furse, 2006).

The development of multimetric indices for the assessment of river ecosystems is acceptable only in cases like this case study, in which the possibility of assessing in the future the biological quality of an important irrigation district located in an area with important natural assets becomes particularly relevant. To safeguard biodiversity, the development of strategies for the protection of the natural environment that are supported by solid mechanisms of impact identification becomes essential. In addition, when preserving threatened biodiversity is a priority, it becomes essential to create a specific index to provide a specific and combined measure of environmental quality.

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