



## Original Articles

## Freshwater biodiversity loss in urbanised rivers

David Gutiérrez-Rial<sup>a,\*</sup>, Benedicto Soto González<sup>b</sup>, David García Vázquez<sup>b</sup>, Gonzalo Méndez-Martínez<sup>c</sup>, Manuel Ángel Pombal Diego<sup>d</sup>, Josefina Garrido González<sup>a</sup><sup>a</sup> Department of Ecology and Animal Biology, Faculty of Biology, University of Vigo, Campus Lagoas Marcosende s/n, 36310 Vigo, Spain<sup>b</sup> Department of Plant Biology and Soil Science, Faculty of Biology, University of Vigo, Campus Lagoas Marcosende s/n, 36310 Vigo, Spain<sup>c</sup> Department of Marine Geosciences and Territorial Planning, Faculty of Biology, University of Vigo, Campus Lagoas Marcosende s/n, 36310 Vigo, Spain<sup>d</sup> Department of Functional Biology and Health Sciences, Faculty of Biology, University of Vigo, Campus Lagoas Marcosende s/n, 36310 Vigo, Spain

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## ABSTRACT

Since the second half of the 20th century, when monitoring programmes were implemented as a measure to improve the water quality of rivers, numerous advantages have been achieved. One of the most remarkable advances was the integration of bioindicators as a suitable and quick tool to complement the assessment based on the evaluation of physical and chemical parameters. This paper evaluated which of the already used water quality indices based on macroinvertebrates would be more suitable to assess the ecological status of 6 rivers (Barxas, Deva, Tea, Manco, Louro and Furnia rivers) within the Miño River international basin (NW Spain and N Portugal) applying the methodology proposed by the Water Framework Directive (WFD). In addition, the relationship between the water quality and the land uses within a buffer zone of 100 along the watershed of each river was studied. Our findings demonstrate that the IBMWP (Iberian Biomonitoring Working Party) is the most suitable index to evaluate the water quality of rivers from this geographical zone. Nevertheless it is necessary to use other more sensitive index like EPT and PT indices to identify any potential pressures that might be concealed by IBMWP. The tributaries of the Miño River generally presented a good ecological status according to the IBMWP. Nevertheless, the Louro River had the lowest score for all the indices being the worst preserved among all within the basin. On the contrary Barxas, Deva and Furnia rivers showed the higher values. The results indicated that the most urbanised river was the Louro River (13 %) followed, to a lesser extent, by Manco River (7 %) and Tea River (2 %). Consequently, the Louro River had the worst water quality (poor quality) and a less diverse benthic macroinvertebrate community, in which more generalist taxa such as Chironomids, Ceratopogonids, or Crustaceans were found. On the other hand, the rivers with the best water quality were the Barxas, Deva, and Furnia, all of them with a negligible proportion of artificial cover surface (<1 %) within the delimited buffer zone. This would suggest a negative influence of the proportion of artificial areas over diversity and quality indices.

## 1. Introduction

Freshwater ecosystems are one of the most diverse ecosystems in the world. These ecosystems represent the suitable habitat for almost 10 % of the known species, despite covering less than 1 % of the planet's surface (Acero Triana et al., 2021; Dudgeon et al., 2006). Moreover, aquatic ecosystems provide a wide variety of goods and services for humans, many of which are irreplaceable (Covich et al., 2004; Faghini et al., 2021). To take advantage of the resources obtained from these ecosystems, human settlements have been established close to rivers and lakes for centuries. This results in high population densities,

intense use of resources and pollution hotspots in the surrounding areas of rivers. All these factors led to the ongoing degradation of the freshwater ecosystems and the loss of species (Weijters et al., 2009).

Indeed, freshwater ecosystems services and resources are threatened by climate and human dependent factors such as nutrient, hydrological, morphological, thermal and other toxic and chemical stressors (Birk et al., 2020). Land-use change is the main cause of habitat and biodiversity loss worldwide, having caused declines in abundance, diversity and health of both species and ecosystems (Davison et al., 2021). During the last 300 years, the terrestrial biosphere has transitioned from being mostly wild to being mostly anthropogenic (Ellis et al., 2013). In fact,

\* Corresponding author.

E-mail address: [dagutierrez@uvigo.gal](mailto:dagutierrez@uvigo.gal) (D. Gutiérrez-Rial).<https://doi.org/10.1016/j.ecolind.2023.111150>

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only in Europe up to 80 % of land is used for settlements, infrastructure and production systems (Feld et al., 2016).

Urbanisation and agriculture are considered an intensive land use that strongly affect the biotic communities of both lentic and lotic ecosystems via flow modification, pollution by fine sediment, pesticide fluxes or punctual pollution episodes (Feld, 2013). These anthropogenic modifications cause changes in the condition of aquatic ecosystems (Allan, 2004). Urbanisation increases peak flows, pollutants and nutrients through increases in impervious surfaces and waste water treatment plant effluent resulting in altered channel form and reduced water quality (Walsh et al., 2005). All these alterations modify the physical and chemical characteristics of rivers which is linked to reduction in ecological function, reflected in biodiversity loss and impairment of ecosystem services provided by streams (Stepenuck et al., 2002).

Thus, anthropogenic stress caused by land use modifications influence on biodiversity, impacting biodiversity patterns. This has driven the scientific community and politicians to develop monitoring programmes with the aim of guarantee the sustainability of biodiversity and ecosystem services (Feld et al., 2016). In this regard, biomonitoring represents a useful tool for assessing anthropogenic disturbances on exposed communities (Nash, 1996). For this reason, benthic macroinvertebrates have been widely used as indicators of water quality in rivers management (Armitage et al., 1983). They are affected not only by natural changes but also by chemical and physical factors caused by human activities (Zamora-Muñoz and Alba-Tercedor, 1996). The response of these ecological indicators to land use changes was documented by several authors (Allan, 2004; Blann et al., 2009; Lenat, 1988)

but the effect of land use on stream benthic macroinvertebrates should receive more efforts by scientific community.

All these advantages convert benthic macroinvertebrate as one of the proposal tools within the Water Framework Directive (WFD) for biomonitoring water quality and the ecological status of European rivers (European Commission, 2000). However, despite all the advances made in relation to the management of freshwater ecosystems, there are some aspects than need to be improved. For example, the design of biomonitoring programmes, referred to the number and location of sampling points (Van Hoey et al., 2010). In this sense, geoprocessing tools (Nama et al., 2022) attending topics like land use should play an important role rather than criteria based on political or socio-economic aspect. This is particularly relevant in the case of transboundary rivers where the management depends on some administrations or governments usually with different criteria (Fabian et al., 2018).

Considering this context, the objective of this work is to evaluate the ecological status of the main water bodies of the Miño River international basin applying the WFD methodology. The specific objectives are, on the one hand, to analyse the water quality of the rivers by applying different biotic indices trying to detect the most suitable for this geographical zone. On the other hand, to check whether the degree of urbanisation of each sub-basin could be related to the degradation of these freshwater bodies and also with the changes in the assemblage and structure of benthic macroinvertebrates communities.

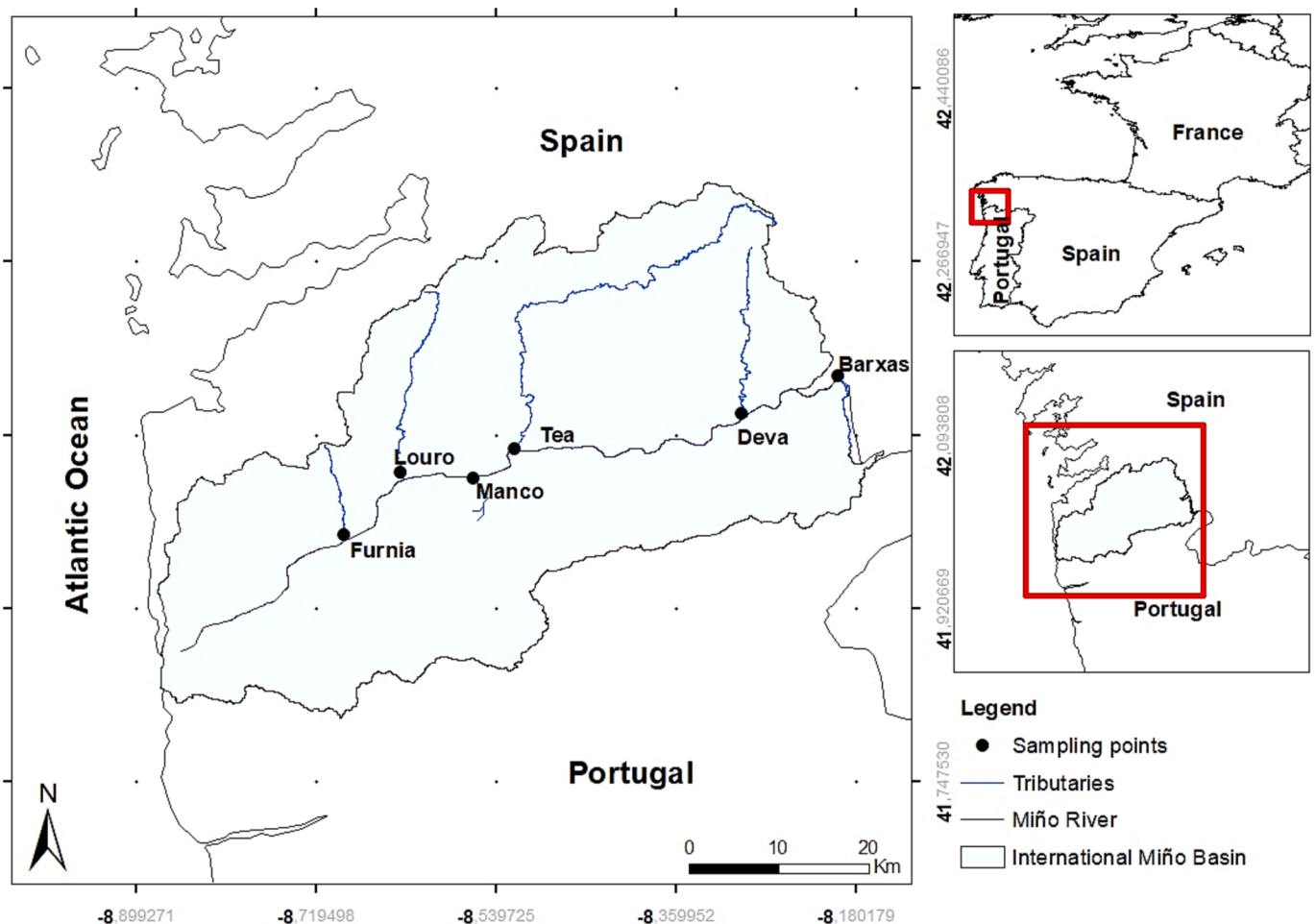


Fig. 1. Left: Study area and location of the sampling points within the Miño River international basin. Upper right: situation of the study area in western Europe. Middle right: situation of the study area within the Iberian Peninsula.

## 2. Methodology

### 2.1. Study area

This work was developed in the Miño River international basin, one of the most important areas for biodiversity conservation in the north-west of the Iberian Peninsula. For this study, four Spanish and two Portuguese tributary rivers were selected, and a sampling point was established in each river, as close as possible to its mouth in the Miño River. The Spanish rivers were Deva, Tea, Louro and Furnia, all of them within the Miño-Sil Hydrographic Demarcation (Fig. 1). Barxas and Manco were the selected rivers on the Portuguese side, both included in the Minho-Limia River basin District (Fig. 1). The Barxas, Deva, Tea and Manco rivers are classified as Cantabrian-Atlantic siliceous rivers (R-T21). The Furnia River is classified as a Cantabrian-Atlantic coastal river while the Louro River is included in the Cantabrian-Atlantic small siliceous axes typology (R-T31) according to the EU Water Framework Directive Plan (Directive, 2000).

The Miño River international basin is characterized by a rugged relief with mountains that can reach significant elevations, separated by deep valleys with rivers at the base. The altitude varies from sea level in the coastal areas to more than 1,000 m in the eastern mountains of the region (1,314 m.a.s.l. in Arcos de Valdevez). Nevertheless, more than 50 % of the territory is within the range of 0–300 m.a.s.l. and the average slope in the mountain sides is 25.5 %.

According to the Corine Land Cover Soil use (Land Monitoring Service, 2018 by Euop.Environ.Agency, EEC), 67 % of the territory within the Miño River international basin is covered by forests, made up mainly of coniferous and broad-leaved trees and, to a lesser extent, shrubby zones. Crops represent the next most abundant land use (30 %) and artificial areas cover the remaining territory (3 %).

Finally, the study area has a particular climatic condition since it is considered a transition zone between the Mediterranean and the Oceanic climate. According to the Koppen-Geiger climate classification (Peel et al., 2007), the Miño River international basin is characterized by a temperate climate with dry and warm summers and rainy winters (Cfb).

### 2.2. Sampling methodology

For this study, four sampling campaigns were developed, one per season in the years 2018 (summer and autumn) and 2019 (winter and spring). On the one hand, samples of benthic macroinvertebrates were taken according to the methodology established by the Water Framework Directive (Directive, 2000) described in Boonsoong et al. (2009). Benthic macroinvertebrates were captured by using entomological hand nets with a mesh size of 500 µm. The samples were pooled and kept in a 4 % formaldehyde solution until all the individuals were identified at the family level using a stereomicroscope, as well as some specialized identification keys (Barrios Barcia et al., 2012; Tachet et al., 2000).

Moreover, at each sampling point, the pH, water temperature, electrical conductivity, redox potential, and dissolved oxygen were measured *in situ* by using the multiparametric sensor Hanna® HI98194. In addition, water samples were also collected to take to the food safety and sustainable development laboratory of the Scientific and Technological Support Centre for Research at the University of Vigo to analyse the Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD) through combustion method and the main inorganic anions ( $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$  and  $\text{Cl}^-$ ), determined by thermocatalytic decomposition method.

### 2.3. Data analysis

Once all the individuals were identified, different biological indices were calculated. On the one hand, to assess the ecological status of the studied zones and the average sensitivity of the families found, the IBMWP (Iberian Biomonitoring Working Party) and IASPT (Iberian

Average Score per Taxon) indices were calculated, following the protocol described in Alba-Tercedor (1988). On the other hand, the composition of the macroinvertebrate community was analysed by using some diversity indices. Richness (S) was calculated like the number of different identified taxa belonging to a sample. Abundance (N) referred to the total number of macroinvertebrates per sample. EPT index was calculated as the number of taxa belonging to orders Ephemeroptera, Plecoptera and Trichoptera, PT index was the number of taxa included in the last two groups. Finally, the diversity indices of Shannon-Wiener ( $H'$ ), Simpson (D) and taxa evenness (e), were calculated with R Software and the specific packages betapart (Baselga, 2010) and vegan (Dixon, 2003). Finally, the relative abundance of individuals grouped according to their feeding strategy was calculated for each river in the 4 seasons following the criteria of Cummins and Klug (1979). This index was calculated by dividing the amount of individuals of each feeding group by the total number of individuals in each sample.

Additionally, the surface occupied by the different land-uses in each basin was calculated with the QGIS Geographic Information System to analyse the relationship between the water quality and the land use within a 100 m buffer zone along each river. This was carried out to study if the proportion of artificial areas within this buffer zone might have any negative influence on the diversity of macroinvertebrates found in each of the rivers and consequently on the water quality.

## 3. Results

In total, 14,810 individuals belonging to 93 different taxa were identified. In general, insects were the most abundant group (73 %) followed by crustaceans (21 %) and, to a lesser extent (6 %), oligochaetes. The abundance of all the identified taxa can be consulted in the Table S1. The total abundance of macroinvertebrates in each river in the 4 seasons and the value of the biological indices calculated based on the fauna data are shown in Table 1. The highest values of the diversity indices corresponded to the rivers Barxas, Deva and Furnia and Manco while the lowest was reported in the Louro River.

Firstly, the highest value of richness was found in the summer sample from the Barxas River (39), followed by the autumn samples from the Deva (38), Barxas (36) and Furnia (33) rivers, respectively. The lowest richness values were found in the Louro samples, in winter (14), spring (17), summer (18) and autumn (20). The result of the remaining biodiversity indices did not show a marked trend. But highest values of the Shannon-Wiener, Simpson and evenness indices were found in the Deva, Barxas and Furnia rivers, except for winter values in which the Manco River raised the highest score.

The same trend was observed in the EPT and PT indices, which reflect the presence of less tolerant taxa to pollution. In most cases, the lowest values were found in the Louro River and, on the contrary, the highest diversity of Trichoptera, Ephemeroptera and Plecoptera was found in the Barxas (19), Furnia (16) and Deva (18) rivers. Consequently, the IASPT index was also higher in these rivers. This reflects the presence in these rivers of more sensitive taxa compared to the Louro, Manco or Tea rivers, which included a greater number of tolerant taxa in the macroinvertebrate community assemblage, with a lower score for the IBMWP index.

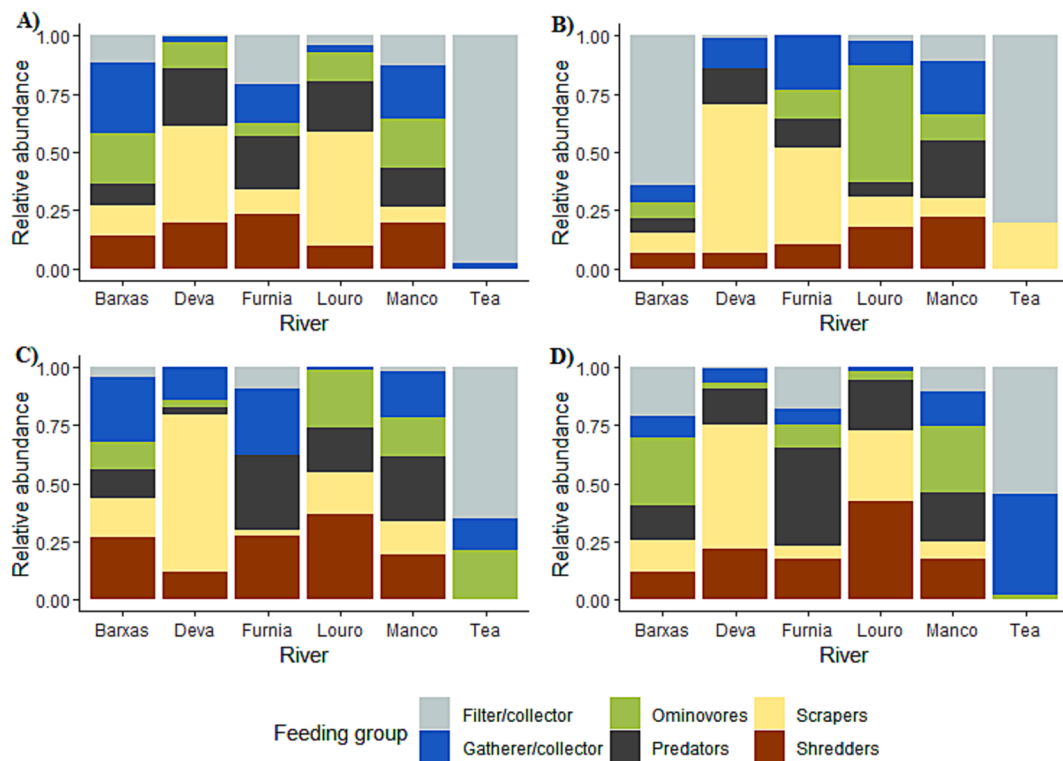
The relative abundance of macroinvertebrates classified according to their feeding strategy is shown in Fig. 2. Foragers-gatherers were the most abundant feeding group, especially in the Deva, Barxas, Tea and Manco rivers. Afterward, shredders were dominant in the Furnia River, while filter-gatherers and omnivores reached the highest abundances in the Tea and Louro rivers, respectively. To a lesser extent, predators were the next abundant group, present in all rivers, but with greater abundance in the Louro River in winter. Finally, the smaller group corresponded to the scrapers in all the rivers.

The variation of physical and chemical parameters among different sites is shown in the Table 2. The highest concentrations of phosphate or chloride were reported in this river. The remaining rivers were

**Table 1**

Diversity indices calculated based on the benthic macroinvertebrate community for the 4 seasons. **N**: abundance (N° of individuals/sample). **S**: Richness (N° of taxa/sample). **D**: Simpson index (value 0 means no diversity; value 1 means maximum diversity). **H'**: Shannon-Wiener index (values lower than 2 means low diversity). **e**: evenness index (value 1 means that all the taxa are represented by equal; 0 value means that a few taxa are very much dominant). **IBMWP**: Iberian Biomonitoring Working Party index. **IASPT**: Iberian Average Score Per Taxon. **EPT**: Ephemeroptera, Plecoptera and Trichoptera index (Number of taxa belonging to the previous orders). **PT**: Plecoptera and Trichoptera index (Number of taxa belonging to the previous orders).

Winter										
	N	S	D	H'	e	IBMWP	IASPT	EPT	PT	
Barxas	379	26	0.72	1.94	0.59	165	7.17	16	11	
Deva	603	27	0.86	2.40	0.73	164	6.56	13	8	
Tea	980	27	0.64	1.60	0.49	132	5.74	12	9	
Louro	139	14	0.82	2.08	0.79	73	5.62	5	2	
Manco	294	29	0.87	2.58	0.77	172	6.37	15	10	
Furnia	345	24	0.82	2.31	0.73	154	7.00	12	9	
Spring										
	N	S	D	H'	e	IBMWP	IASPT	EPT	PT	
Barxas	604	29	0.85	2.35	0.70	170	6.54	13	9	
Deva	533	32	0.90	2.68	0.77	176	6.09	12	7	
Tea	1055	32	0.83	2.27	0.65	183	5.90	12	7	
Louro	368	17	0.69	1.54	0.54	91	5.69	5	3	
Manco	279	27	0.85	2.40	0.73	163	6.27	10	4	
Furnia	436	31	0.88	2.56	0.75	196	6.76	16	12	
Summer										
	N	S	D	H'	e	IBMWP	IASPT	EPT	PT	
Barxas	1224	39	0.75	2.07	0.56	246	6.83	19	14	
Deva	357	27	0.90	2.59	0.79	170	6.54	12	9	
Tea	530	25	0.82	2.13	0.66	246	6.83	9	5	
Louro	1772	18	0.17	0.50	0.17	96	5.65	5	1	
Manco	447	29	0.86	2.37	0.70	154	5.92	11	9	
Furnia	958	31	0.82	2.27	0.66	189	6.52	12	9	
Autumn										
	N	S	D	H'	e	IBMWP	IASPT	EPT	PT	
Barxas	513	36	0.90	2.73	0.76	212	6.38	17	13	
Deva	694	38	0.92	2.83	0.78	205	6.21	18	13	
Tea	196	30	0.82	2.31	0.68	159	6.11	10	8	
Louro	672	20	0.63	1.50	0.50	113	6.27	4	2	
Manco	541	32	0.72	1.82	0.53	181	6.24	12	6	
Furnia	891	33	0.85	2.39	0.68	205	6.41	13	9	

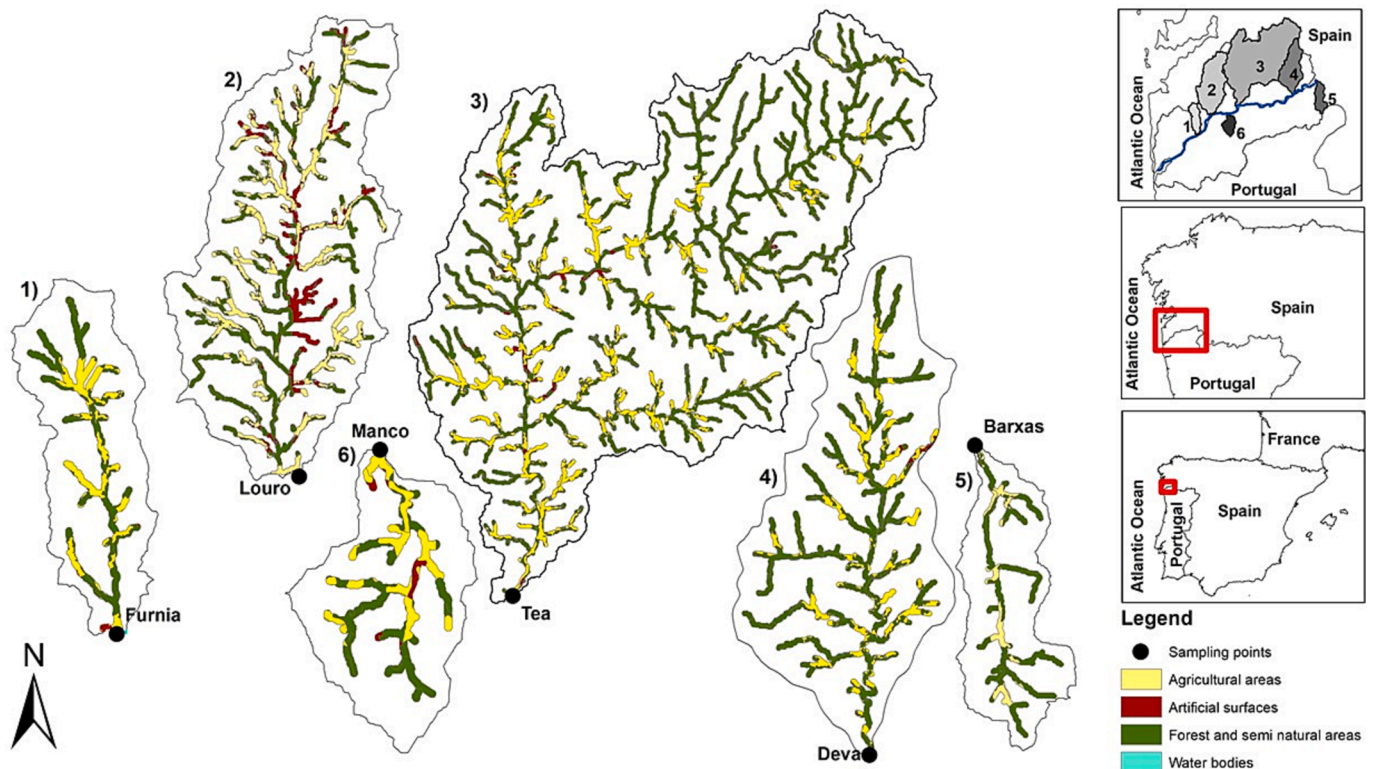


**Fig. 2.** Relative abundance of macroinvertebrates classified in the different feeding groups for each river in winter (A), spring (B), summer (C) and autumn (D). The feeding strategy of each of the taxa was classified according to Cummins and Klug (1979).

**Table 2**

Physical and chemical parameters measured *in situ* and in the laboratory for each river in the four seasons. T°: Temperature (°C); pH; EC: electric conductivity ( $\mu\text{S cm}^{-1}$ ); DO: dissolved oxygen (ppm); ORP: redox potential (V); Cl<sup>-</sup>: chloride ( $\text{mg L}^{-1}$ ); NO<sub>2</sub>: nitrite ( $\text{mg L}^{-1}$ ); NO<sub>3</sub>: nitrate ( $\text{mg L}^{-1}$ ); PO<sub>4</sub>: phosphate ( $\text{mg L}^{-1}$ ); SO<sub>4</sub>: sulphate ( $\text{mg L}^{-1}$ ); COD: Chemical Oxygen Demand ( $\text{mg L}^{-1}$ ); TOC: Total Organic Carbon ( $\text{mg L}^{-1}$ ).

Winter												
	T°	pH	EC	DO	ORP	Cl <sup>-</sup>	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	COD	TOC
Barxas	16.10	6.93	51.00	10.25	163.0	4.63	0.05	2.08	0.12	2.23	NA	0.33
Deva	13.04	6.08	37.00	12.10	219.0	5.41	0.05	1.80	0.05	1.82	NA	0.17
Tea	11.90	7.13	40.00	12.70	140.0	7.14	0.05	2.34	0.05	2.00	NA	0.72
Manco	14.40	7.00	58.00	11.80	185.0	8.69	0.05	3.53	0.05	2.01	NA	0.10
Louro	14.08	7.00	88.00	10.48	114.0	12.88	0.05	5.77	0.62	5.36	NA	0.27
Furnia	13.30	5.96	63.00	11.60	196.0	11.09	0.05	4.88	0.05	2.73	NA	0.21
Spring												
	T°	pH	EC	DO	ORP	Cl <sup>-</sup>	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	COD	TOC
Barxas	8.00	7.53	35.00	8.99	167.5	4.31	0.05	1.90	0.05	1.42	0.80	1.35
Deva	9.60	5.82	34.00	8.20	301.0	4.95	0.05	1.77	0.05	1.39	0.80	0.91
Tea	11.42	7.23	47.00	7.88	124.9	6.80	0.05	2.79	0.05	1.96	0.80	1.36
Manco	11.60	7.20	53.00	7.81	248.0	7.96	0.05	2.77	0.05	1.90	0.80	1.77
Louro	12.17	6.71	82.00	7.77	152.5	11.20	1.74	5.35	0.23	4.86	3.20	2.97
Furnia	12.28	7.34	59.00	7.80	135.1	10.15	0.05	4.32	0.05	2.46	0.80	1.09
Summer												
	T°	pH	EC	DO	ORP	Cl <sup>-</sup>	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	COD	TOC
Barxas	18.90	6.70	50.00	11.20	306.7	6.67	0.05	3.23	0.05	3.22	5.20	3.09
Deva	17.40	7.03	54.00	11.75	280.0	8.06	0.05	3.53	0.05	3.86	5.60	3.10
Tea	19.08	6.86	69.00	6.95	223.9	10.99	0.05	2.01	0.05	3.39	6.80	6.96
Manco	17.40	7.30	72.00	11.70	280.0	11.14	0.05	5.46	0.05	NA	2.40	3.78
Louro	18.02	7.18	90.00	7.59	273.0	13.65	0.05	3.29	0.05	5.62	6.80	6.34
Furnia	15.35	6.30	69.00	10.74	186.4	13.04	0.05	5.49	0.05	2.62	5.20	2.61
Autumn												
	T°	pH	EC	DO	ORP	Cl <sup>-</sup>	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	COD	TOC
Barxas	9.12	6.70	37.00	12.45	122.7	5.75	0.05	2.78	0.05	15.62	1.60	1.79
Deva	10.38	6.38	36.00	11.34	231.7	6.11	0.05	2.78	0.05	3.94	2.40	2.06
Tea	11.40	6.31	43.00	11.15	234.5	6.93	0.05	3.28	0.05	3.50	1.60	1.57
Manco	12.50	6.32	53.00	11.77	227.5	8.75	0.05	3.57	0.05	2.24	2.00	1.21
Louro	12.90	6.80	85.00	11.04	146.2	12.57	0.05	4.70	0.05	6.77	2.80	1.95
Furnia	13.40	6.22	53.00	11.90	194.2	10.01	0.05	3.45	0.05	2.19	2.40	1.56



**Fig. 3.** Land use according to Corine Land Cover Classification within a buffer zone of 100 m created along the watershed of each river.

characterized by higher values of dissolved oxygen.

The differences between the sampling points are also reflected in the assembly and composition of the macroinvertebrate's communities found in each of the rivers (Table S1). In the Louro River, the majority groups corresponded to taxa with lower ecological requirements such as Libellulidae, Lestidae, Potamanthidae or Physidae. Conversely, the remaining rivers were more linked to taxa with greater ecological demands. Consequently, individuals belonging to Ephemeroptera, Plecoptera and Trichoptera were more frequent, mainly in the Barxas, Deva and Furnia rivers, but were also present, to a lesser extent, in the Manco and Tea rivers.

The land use within the buffer zone along the drainage network of the different rivers is shown in the Fig. 3. The proportion of each of the four land uses included in level 1 of the Corine Land Cover classification (agriculture, artificial surfaces, forest and seminatural areas and water bodies) within 100-meter buffers are shown in Fig. 4. Forests and seminatural areas were the predominant land use in all rivers, except in the Louro, where the proportion did not reach 50%. Agricultural areas were the second most abundant land use in all rivers. However, the most significant difference was the proportion of artificial areas, which was higher than 15% in the Louro River but less than 2% in the others rivers.

#### 4. Discussion

The impact of urbanisation and industrialisation on rivers became evident in the early 20th in some parts of Europe. At the beginning of the century, the water quality of rivers deteriorated significantly in areas such as the Ruhr Basin in Germany and the Moldau river in the Czech Republic due to heavy and fast industrialisation (Brüggemeier, 1994) and extensive settlements without adequate water purification processes (Spolecnosti and Jansky, 2002). These changes were mainly caused by nutrient pollution caused by inputs of nitrogen and phosphorous (Fowler et al., 2013; Sutton et al., 2011), morphological changes of rivers, flow regulation and the introduction of alien species (Grizzetti et al., 2017). In this situation, chemical monitoring was introduced in most of the large rivers of Europe (Gunatilaka and Diehl, 2001). But one of the most important advances was reached in the second half of the 20th with the implementation of biomonitoring programmes based on indicators such as diatoms (Pajunen et al., 2020), macrophytes (Husák et al., 1989) or benthic macroinvertebrates (Cairns and Pratt, 1993).

As previous researchers have demonstrated (Lenat, 1988; Lock et al., 2011; Ofenböck et al., 2004), the use of macroinvertebrates as

bioindicators is an useful tool to evaluate water quality in river monitoring programmes. In our case, the IBMWP (Iberian Biomonitoring Working Party) and IASPT (Iberian Average Score Per Taxon) indices were the most suitable to assess the water quality, as previously observed by Leunda et al. (2009), Munné and Prat, (2011) and Sánchez-Montoya et al. (2007) in rivers from other regions of the Iberian Peninsula.

According to the IBMWP reference values (Alba-Tercedor, 2002) for each river typology, the only river that did not reach Class I (IBMWP > 100) was the Louro River (Table 1), with scores classified in the Class II (100–60) defined as rivers with acceptable water quality. In the other rivers, IBMWP scores fall always in the quality class I defined for unpolluted rivers. The variability of IASPT highlighted the influence of seasonality on this index as was observed previously by Zamora-Muñoz et al. (1995). In any case, IASPT is in concordance with the IBMWP in the quality assessment of the rivers Furnia, Deva and Barxas that reached the highest quality class independently on the sampling period, defined as “clean waters”. In the other rivers IASPT ranged from values corresponding to class I to class II, which indicated “dubious water quality”. Thus IBMWP was considered a more suitable biological index to avoid seasonality as several authors found in previous studies (Armitage et al., 1983; Munné and Prat, 2011; Zamora-Muñoz et al., 1995)

The physical and chemical parameters (Table 2), did not show any type of alteration. Most of the variables fell within the limits established by the national regulatory agencies although one exception was found. The concentration of phosphates in the Louro River during winter reached a high value (0,62 mg L<sup>-1</sup>). This exceeded the limit allowed by the Spanish authorities for this river typology. Moreover, this river also exhibited higher levels of COD, sulphates and TOC in comparison to the other rivers. Previous studies carried out in this river found inputs of illegal wastewater discharges from urbanised and industrial areas, one of the main sources of contamination (Eltaweil et al., 2021; Santos et al., 2013).

The low score of EPT and PT indices indicated this river is being subjected to any perturbation. Although differences associated with seasonality were found, higher scores for these indices generally corresponded with higher IBMWP values. Thus, sensitive taxa such as Perlidae, Perlodidae, Capniidae, or Philopotamidae were not found in the Louro River but were present in rivers with higher values of the other indices (Table S1). This support that a single metric could be misinterpreted, but using multimeric indices is more effective because provide an integrated analysis of the biological community (Karr, 1999; Ofenböck et al., 2004). Taxa belonging to the groups within the EPT and

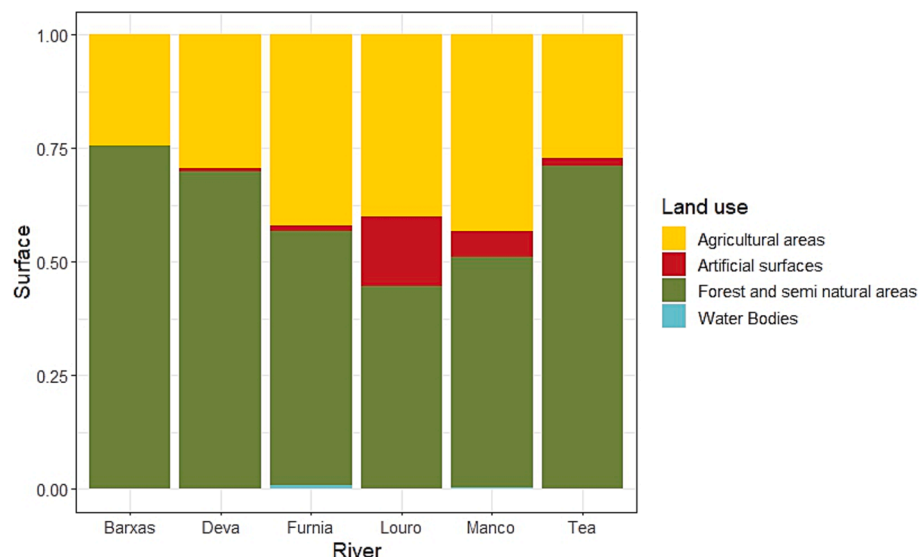


Fig. 4. Surface proportion of the different soil uses within the 100-meter buffer calculated along the drainage network of each river.

PT indices are recognized to be very sensitive to perturbations. Consequently, in case of impairment the loss of richness within these groups would be well reflected (Altieri et al., 2022; Wallace et al., 1996).

Our results also evidenced that both diversity and water quality have a marked seasonal variation. In natural water courses the cyclical pattern of the samples reflects macroinvertebrate life cycles in response to the seasonal changes of habitat characteristics such as water temperature (Hieber et al., 2005), discharge (Armitage et al., 2001) or food availability (González et al., 2003). Most taxonomic groups appear in a specific season, so that the Diptera groups such as Limoniidae, Culicidae or Anthomyiidae cannot be found in winter, while Ephemeroptera are specially abundant in spring (Sánchez-Montoya et al., 2007; Vivas et al., 2002).

But these changes do not only affect the composition of the community; seasonality also changes the trophic structure of the community (Fig. 2). In all cases, the relative abundance of each feeding group changes between seasons. Generally, in temperate streams herbivores (scrapers and shredders) and detritivores (filters and gatherers collectors) represent the most abundant groups (Cortés-Guzmán et al., 2021) in temperate streams. However, during dry periods (summer and autumn) the abundance of predators increases (Fig. 2); this is usually associated with the greater stability of the river and with the fate that predator species usually have the capacity to attach themselves to the substrate where they live (Tamaris-Turizo et al., 2018). Moreover, the reduced volume of water favours the colonization of many prey taxa along the river so the availability of resources for predators increases (Gasith and Resh, 1999).

Our results showed an important pattern in terms of weak response of biodiversity to land use at small scale considering the transformation of the riparian forest. Thus, we analysed the differences between the land use to which each of the rivers are exposed within an area that covered 100 m towards each side of the channel of the entire drainage network (Fig. 3). The results obtained showed that the Louro River has a high proportion of artificial areas (15 %), much higher than in the remaining rivers where this land use did not reach 2 % (Fig. 4).

Urban development lead to enlarge impermeable surfaces and the capability of rainfall detention declines sharply and runoff increases (Shi et al., 2007). This involves impacts on rivers, such as water withdrawal, increased sediment loads, and direct habitat destruction, especially in areas for building (Chin and Gregory, 2009; Williams-Subiza and Epele, 2021). In urbanised areas, riverine habitats are characterized by accelerated run-off peaks, increased nutrients and other contaminants, erosion channel degradation and the main consequence is a decline in biodiversity of benthic communities (Hanh Nguyen et al., 2023). Nevertheless not all macroinvertebrates are equally affected by these disturbances. Mayflies, stoneflies, and caddisflies are the first organisms to disappear. These are replaced by more tolerant taxa (Atyidae, Chironomidae, Ceratopogonidae, Acari, Elmidae), as in the case of the Louro River where the EPT and PT indices were lower than in the remaining rivers (Table 1).

Our results also showed differences in the trophic structure of the communities, as evidenced by the high proportion of omnivores, found mainly in the Louro River and, to a lesser extent, in the Manco and Tea rivers. In altered systems, low resource heterogeneity and high primary productivity reduce competition for basal resources, increasing the abundance of generalist and more tolerant species (Sroczynska et al., 2020). The ultimate result is a decrease in the link between top and intermediate species, creating a simplified food web where omnivores and predators dominate while specialist feeders are replaced (Baumgartner and Robinson, 2017). Similar results were observed by Layer et al. (2010), who showed that greater anthropogenic disturbance in aquatic ecosystems not only results in lower biodiversity, but also enhances the abundance of ant species with a more generalist feeding. Moreover, in specific periods such as droughts when the streamflow is low, there are species with the ability to change their feeding strategy to omnivore habits (Blanchette et al., 2014).

## 5. Conclusions

Aquatic macroinvertebrates are widely recognized as one of the most useful tools to assess water quality in rivers. This study confirms their efficacy in the NW of Iberian Peninsula, whereby the IBMWP index is the most suitable to make a quick assessment of the ecological status of running waters. However, the use of a single index seems not appropriate since it is insufficient to indicate all potential river disturbances. For this reason, other indices based on the presence/absence of the most sensitive taxa represent a complementary tool, as they can reflect disturbances that may have been masked for the other indices. The best complementary indices for biomonitoring programmes in rivers are EPT and PT. Moreover, certain diversity indices like Shannon-Wiener, Simpson, or evenness are not recommended for assessing water quality.

From the results of this work, we can conclude that the tributaries of the transboundary section of the Miño River are generally in good ecological condition. However, there are differences between the rivers. The IBMWP, EPT and PT indices show that the Louro River is the worst preserved river of the whole Miño River international basin. On the contrary the best-preserved rivers are Barxas, Deva and Furnia rivers. These rivers showed higher scores for quality indices and had major biodiversity of most sensitive taxa (ephemeropterans, plecopterans and trichopterans). Finally, this work aims to motivate further studies on the relationship between land use and water quality indices. Our results suggest a negative relationship between the percentage of artificial zones within the sub-basin of each river and the score of the different indices. This is evident if only a buffer zone of 100 m along the drainage network of each river is considered. Authorities should take these results into account and increase efforts to preserve the areas surrounding the watercourses, especially the riparian forests.

## CRedit authorship contribution statement

**David Gutiérrez-Rial:** Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Formal analysis. **Benedicto Soto González:** Conceptualization, Methodology, Investigation, Supervision, Visualization, Writing – review & editing. **David García Vázquez:** Conceptualization, Methodology, Investigation. **Gonzalo Méndez-Martínez:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing. **Manuel Ángel Pombal Diego:** Conceptualization, Methodology, Investigation, Supervision. **Josefina Garrido González:** Conceptualization, Methodology, Investigation, Supervision, Validation, Project administration, Funding acquisition, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

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