Integrating species richness and environmental quality in Mediterranean River Ecosystems through multivariate analyses: A case study of the Besòs Basin

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Sara Giménez Villanueva

Directors:
Dr. Sònia Sanchez-Mateo

Tutor:
Dr. Sara Dallarés Villar

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Fundació RIVUS, Av. Sant Julià 241, Granollers (08403), Barcelona (Spain).

Universitat Autònoma de Barcelona

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Authors:

Sara Giménez Villanueva, Sonia Sanchez-Mateo[ab], Sara Maria Dallarés Villar[c]

Affiliations:

[a] Fundació RIVUS, Av. Sant Julià 241, Granollers (08403), Barcelona (Spain).

[b] Department of Geography, Universitat Autònoma de Barcelona (Bellaterra), Barcelona, Spain; e-mail: sonia.sanchez.mateo@uab.cat

[c] Department of Animal Biology, Plant Biology and Ecology, Universitat Autònoma de Barcelona (Bellaterra), Barcelona, Spain; e-mail: Sara.Dallares@uab.cat

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Integrating species richness and environmental quality in Mediterranean River Ecosystems through multivariate analyses: A case study of the Besòs Basin

Sara Giménez Villanueva

e-mail address: gimenezv.sara@gmail.com

Fundació RIVUS, Av. Sant Julià 241, Granollers (08403), Barcelona (Spain).

Abstract

Mediterranean rivers are highly vulnerable to anthropogenic pressures and hydrological variability, making them priority systems for ecological monitoring. The Besòs River Basin, situated within the densely urbanized Barcelona Metropolitan Region, is heavily impacted by human activities. This study assessed faunal richness and environmental quality across the basin using six years of data (2019–2024) recorded by the *Observatori RIVUS*, from eleven monitoring stations. Species richness was quantified for total, protected, and invasive alien species, while ecological status was evaluated through physicochemical, hydromorphological, and biological indicators.

Multivariate analyses revealed clear longitudinal gradients in biodiversity and environmental quality. Stations located in the upper and upper-middle sections supported higher species richness and better ecological status, whereas the ones located in the middle and lower sections showed eutrophication, organic pollution, elevated conductivity, and a higher presence of invasive species. While avifauna dominated all stations, the other groups of fauna also highlighted key ecological gradients. The headwater station B0_LB0 emerged as a reference site, showing the highest ecological quality and distinct faunal composition, while B7_LB19 exhibited the lowest ecological condition, followed by stations located in the mid-lower and lower sections, including B8, B9, and B10_LB22.

These findings demonstrated that long-term, integrative ecological monitoring provides a consistent framework for assessing the status of the river basins in the Mediterranean region. The study highlights the good condition of the well-preserved headwaters, the need to prioritize conservation efforts in the middle and lower reaches, and the importance of urban basins as a key focus for restoration initiatives.

Key words:

Species richness, Ecological quality, Mediterranean rivers, Anthropogenic pressures, Long-term monitoring

1. Introduction

River water is a vital natural resource, yet freshwater systems are widely regarded as highly vulnerable to pollution, land cover change, urbanization, industrialization, among other pressures. Both anthropogenic activities and natural processes can easily degrade the quality of surface waters, impairing their ecological integrity and usability, although human activities play a primary role and are expected to further impact freshwater systems through anthropogenic climate change (Vörösmarty et al., 2010; Ouyang, 2005; Arenas-Sánchez et al., 2021).

The systematic collection of data and long-term monitoring of aquatic systems are crucial for understanding the ecological dynamics of river basins, detecting environmental changes, and safeguarding river ecosystems (Wang et al., 2014). Such knowledge not only facilitates the early identification of ecological alterations but also supports decision-making in management and conservation. In this context, monitoring plays a central role within the framework of the Water Framework Directive (WFD) (European Commission, n.d.), which promotes adaptive management strategies based on ecological status of the ecosystems.

Scientific progress in understanding aquatic systems has benefited greatly from long-term and integrative approaches. Due to their high dynamism, aquatic ecosystems often exhibit faster ecological processes than terrestrial systems. Moreover, aquatic communities can act as bioindicators of cumulative regional pressures (Hampton et al., 2019). For example, species richness is a key descriptor of aquatic animal communities, as it is influenced by a wide range of environmental factors. The interaction among these factors creates well-known gradients in stream species richness (Céréghino et al., 2003).

However, the degradation of water quality due to various contaminants has led to shifts in species composition and a decline in the overall health of aquatic communities within river basins (Ouyang, 2005). This highlights the importance of long-term and comprehensive monitoring to inform management and restoration of aquatic biodiversity and ecosystem function.

Given the current vulnerability of aquatic ecosystems to environmental degradation in such an anthropogenic context, it is especially important to understand how these dynamics are influenced by regional climatic conditions. This is particularly true in the Mediterranean region, where human activity is deeply integrated into the landscape.

The Mediterranean climate is characterized by marked seasonal differences, with dry summers and mild winters (Feio et al., 2014; Bonada & Resh, 2013). Rivers influenced by this climate (hereafter referred to as Mediterranean rivers) exhibit flow regimes that reflect the precipitation patterns typical of the region. As a result, they support unique freshwater ecosystems from both hydrological and a biological perspective (Bonada & Resh, 2013).

Mediterranean river communities differ from those in temperate regions, showing inter-annual fluctuations in species richness, community composition, and trophic structure (Ferreira et al., 2006). These ecosystems also support highly endangered biodiversity, a condition that is strongly linked to a long history of human disturbances in the region (Zeder, 2008).

Among the most significant pressures are seasonal hydrological variation and chemical pollution, primarily from agricultural runoff and urban wastewater, which together represent major drivers of biodiversity change in Mediterranean rivers (Arenas-Sánchez et al., 2021), particularly under water stress scenarios such as river drying (Carceller et al., 2002).

Understanding the response of freshwater communities to these stressors is essential for informing effective conservation and management strategies. In particular, biological quality elements, such as species richness, can serve as valuable indicators for assessing ecosystem health and guiding restoration efforts (Heino, 2002; Céréghino et al., 2003; Carmezim, Pennino, Martínez-Minaya, Conesa, & Coll, 2022).

These broader considerations are particularly relevant when assessing riverine biodiversity in specific Mediterranean regions, such as the Besòs River basin in Catalonia. This basin has a long history of environmental degradation and is subject to intense anthropogenic pressures since the intense demographic and industrial expansion of the 1960s, which can strongly influence its ecological integrity and biodiversity (Benages-Albert & Vall-Casas, 2014).

Despite the high levels of degradation and significant anthropogenic impacts affecting the basin, in 2006, the European Union included parts of the Congost, Mogent, and Besòs rivers in the Natura 2000 network (site code ES5110025), making it one of the few urban rivers with such a designation (Consorci Besòs Tordera, 2025). This area serves as an ecological corridor connecting other Natura 2000 sites: to the southeast, the Serres del Litoral Septentrional (ES5110011), and to the north, the Montseny Massif (ES5110001) and Gallifa–Cingles de Bertí (ES5110008) (Generalitat de Catalunya, Departament de Territori i Sostenibilitat, 2014).

In the upper parts of the basin, particularly in the Montseny area where the headwaters are located, the rivers are small but have a vigorous flow due to the steep gradient. These waters are characterised by low mineralisation and low temperature. In this area, the riparian forest is well conserved and plays a key role in river dynamics, contributing significant amounts of organic matter and reducing sunlight penetration into the channel (Carceller et al., 1995).

From their respective headwaters, the tributaries, such as the Mogent, Congost, Tenes, riera de Caldes, and finally the Ripoll, gradually converge to form what is known as the Besòs River, although it is not characterized by a single, clearly defined main course. These inputs increase the flow and alter the physicochemical characteristics of the water. The gradient decreases, mineralisation and nutrient concentrations increase, and the river becomes wider. Due to reduced riparian forest density and the presence of human activity, sunlight penetration into the water also increases (Carceller et al., 1995).

In the lower reach, corresponding to the main course of the Besòs River, water temperature undergoes significant daily fluctuations, the gradient is almost non-existent, and the fluvial landscape is heavily urbanised and channelised (Carceller et al., 1995).

Moreover, this area supports a diverse mosaic of environments, within which a total of 44 habitats of community interest have been identified, 10 of which are considered priority habitats, according to the terrestrial habitat map of the counties of Barcelona (Diputació de Barcelona, 2022). The high habitat heterogeneity present in the Besòs River basin is a key factor in the development of ecologically diverse environments, supporting the establishment and maintenance of high species richness in both flora and fauna.

Despite the basin's high degree of urbanisation and industrialization, particularly in the middle and lower reaches of the basin, it continues to host remarkable and unique biodiversity, making it a key area for conservation within a metropolitan context. Furthermore, these natural spaces provide direct benefits to human health, aligning with the One Health paradigm, which emphasises the interconnection between environmental, animal, and human health. In this regard, preserving and studying the biodiversity of the Besòs River Basin not only supports ecological conservation but also enhances collective well-being and urban resilience in the face of current environmental challenges.

Significant efforts have been made to restore and preserve the ecological integrity of the Besòs basin since the establishment of the *Consorci Besòs Tordera* in 1988. Since then, environmental management and monitoring systems have been implemented, playing a decisive role in the ecological improvement of the riverine environments. At that time, many stretches of the river were severely degraded due to intense urban and industrial pressure, resulting in extremely poor environmental quality. This context of degradation marked the beginning of a long-term restoration process that has gradually led to significant improvements. Today, aquatic fauna can be found along the entire course of the river, including its final stretch near the mouth, a scenario that would have been unimaginable just a few decades ago (Consorci Besòs Tordera & Moya-Angeler, 2018).

Building on these efforts, the *Observatori RIVUS*, launched in 2019 by the *Fundació RIVUS* as an interdisciplinary research initiative, conducts active and systematic monitoring of river ecosystems in the Besòs basin. Its origins lie in the *Observatori de la Tordera*, established in 1996, which pioneered the use of bioindicators for the implementation of the Water Framework Directive (WFD) in Catalonia. The long-term monitoring methodology developed in the Tordera basin was later extrapolated to the Besòs basin. Through the collection of long-term biological, hydrological, hydromorphological, and physicochemical data, the *Observatori RIVUS* aims to rigorously assess the ecological status of the basin in accordance with WFD standards (Fundació RIVUS, 2025).

In this context, the present study addresses the need to continue improving our understanding of both the current ecological status and species richness across the Besòs River basin. This knowledge is essential for addressing emerging challenges, guiding future conservation priorities, and identifying areas where ecological restoration and management actions should be focused.

The primary aim of this work is to assess the status of the Besòs basin by examining species richness, placing particular emphasis in native species, and the environmental quality of riverine ecosystems. This objective is intended to be achieved through the using multi-annual data collected from several monitoring stations across the catchment.

To achieve this, the study is structured around several specific objectives: (i) to characterise faunal richness at each station, distinguishing between total richness, exotic and invasive species, and protected species listed under Decree 172/2022, as well as (ii) to explore similarities among stations based on faunal composition; (iii) to assess the ecological status of the stations using key bioindicators and physicochemical parameters; and (iv) to determine whether environmental gradients explain biodiversity distribution, and which variables most strongly influence faunal composition.

2. Methods

2.1. Study site

The Besòs River basin, located in Catalonia in the north-eastern region of the Iberian Peninsula, flows into the Mediterranean Sea along the coast of the *Barcelonès* area, between the municipalities of Barcelona and Sant Adrià de Besòs. Covering an area of 1,038 km² and comprising a total river network length of 181.9 km, the basin lies between the Prelitoral and Litoral mountains ranges and runs mainly through the Vallès Depression (Carceller et al., 1995; Benages-Albert & Vall-Casas, 2014).

In Figure 1 we can see that the basin is composed of six main river courses: the Mogent, the Congost, the Tenes, the Riera de Caldes, the Ripoll, and the Besòs (Benages-Albert & Vall-Casas, 2014). Numerous tributary watercourses are not included in the analysis due to a lack of sufficient data, as the *Observatori RIVUS* focuses its assessment of river system quality exclusively on the main courses of each of the six sub-basins, rather than on their smaller tributaries. As a result, the sub-basin of the Riu Sec or the Riera de Sant Cugat is not identified in Figure 1.

This basin displays a typically Mediterranean hydrological regime, with very low summer flows, around 2 m^3/s at the mouth, that can increase more than a thousand-fold during intense autumn storms. Despite its relatively small size, it offers a high diversity of natural habitats, structured into three clearly distinct environments: the mountainous areas; the Vallès plains, characterised by a strong urban presence; and the river mouth, which is fully integrated into the urban fabric of the metropolitan area of Barcelona (Consorci Besòs Tordera, 2025). These three zones correspond broadly to upper, middle and lower stretches of the territory, respectively. While transitional areas between the upper and middle zones can still be identified, often marked by a gradual change in topography and land use, this gradation is far less evident between the middle and lower stretches, where urbanisation is so continuous that the shift in landscape becomes almost imperceptible.

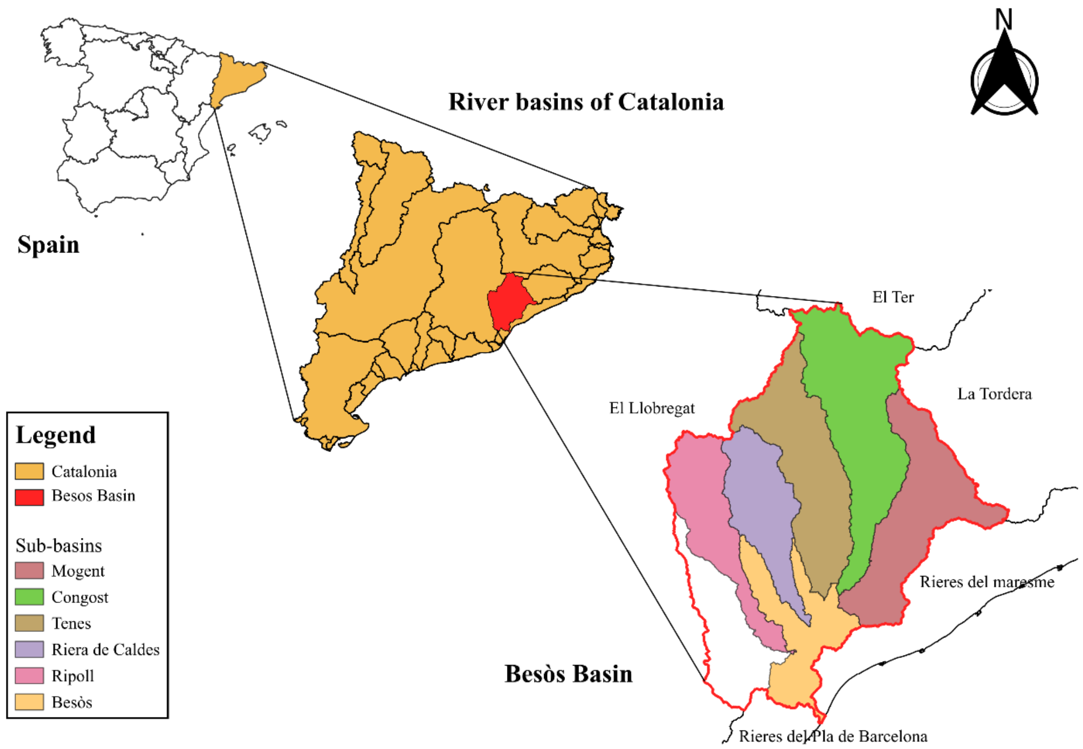

Figure 1. Location of the Besòs River Basin and its sub-basins. In the enlarged image (bottom right corner), the different sub-basins of the Besòs are shown, each represented with a different color. The surrounding major river basins adjacent to the Besòs Basin are also labelled: *El Llobregat, El Ter, La Tordera, Rieres del Maresme, and Rieres del Pla de Barcelona*.

Today, the Besòs basin is the most densely populated in Catalonia, with over two million people living around its river courses, a fact that highlights the social dimension of this aquatic system (Pavón, 2024; Consorci Besòs Tordera, 2025; Benages-Albert & Vall-Casas, 2014).

2.2. Data collection

The data used in this study were collected through the *Observatori RIVUS* , over a six-year period, from 2019 to 2024, across a set of sampling units. Data collection was carried out annually through different seasonal campaigns, with each year including between one and four campaigns depending on the specific monitoring programme in question. The frequency and timing of these campaigns vary according to the particular monitoring line they belong to. Detailed information on these periodicities can be found in the annual reports available on the *Fundació RIVUS* website (Fundació RIVUS, 2025).

Monitoring of the different parameters at the *Observatori RIVUS* is conducted using stations, transects, and river stretches, depending on the methodological requirements of each monitoring program. For the purposes of this study, only the stations were considered as sampling units, as they represent the reference units used by the research teams across the various faunal monitoring programmes (view table 2) to initiate transects and record the corresponding data.

Since its establishment, the *Observatori RIVUS* has set up 32 monitoring stations across the Besòs River basin, 11 of which are common to all monitoring programmes and have therefore been used in this study. The locations of these monitoring stations are shown in Figure 2 and Table 1.

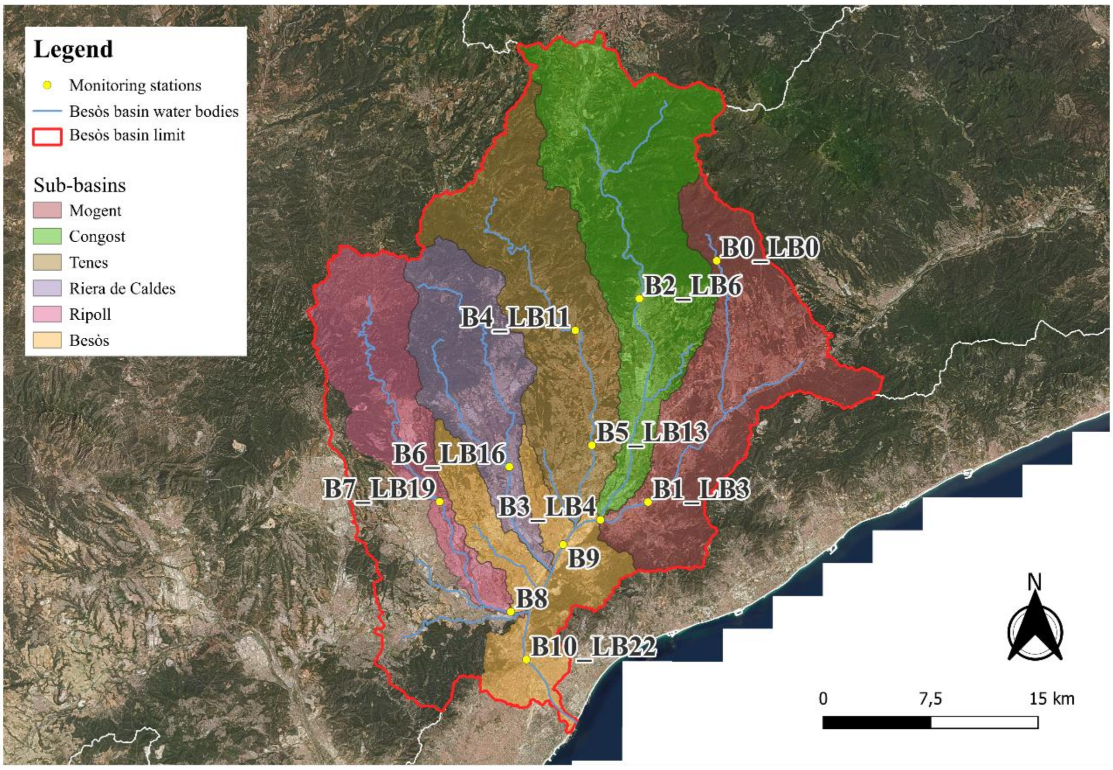

Figure 2. Location of the monitoring stations in the Besòs River Basin. River courses are shown with blue lines, stations are marked with yellow dots along with their corresponding code, and the different colors indicate the different sub-basins as shown in the legend.

Table 1. Sampling station details within the Besòs River Basin, categorized by sub-basin. Information includes station and waterbody codes, associated municipalities, whether the site lies within the Natura 2000 protected area, and the longitudinal river section (upper, middle, lower) of each monitoring station

Sub-basin	Station code	Waterbody code	Municipality	Natura 2000	Section
Mogent	B0_LB0	1100100	Cànoves i Samalús	No	Upper
	B1_LB3	1100110	Vilanova del Vallès	Yes	Middle
	B3_LB4	1100110	Montmeló	Yes	Middle
Congost	B2_LB6	1100040	La Garriga	Yes	Upper-Middle
Tenes	B4_LB11	1100140	Santa Eulàlia de Ronçana	No	Upper-Middle
	B5_LB13	1100140	Lliçà de Vall	No	Middle

Caldes	B6_LB16	1100200	Palau Solità i Plegamans	No	Middle
Ripoll	B7_LB19	1100240	Sabadell	No	Middle
	B8	1100250	Montcada i Reixac	No	Lower
Besòs	B9	1100180	Martorelles	Yes	Middle
	B10_LB22	1100300	Santa Coloma de Gramenet	No	Lower

2.3. Data analysis

2.3.1. Data monitoring programmes

All data analysed in this study comes from the monitoring programmes carried out by the *Observatori RIVUS*, except in specific cases where complementary data were obtained through the SDIM21 viewer provided by the Catalan Water Agency (ACA).

The *Observatori RIVUS* monitors the indicators and parameters established under the Water Framework Directive (WFD) for the assessment of the ecological status of river ecosystems. In addition, it also develops its own monitoring lines (Table 2).

Table 2. Monitoring programs, indicators, and other parameters of the *Observatori RIVUS* and those considered under the Water Framework Directive (WFD). Source: Adapted from Monitoring of socioecological indicators in the Besòs River Basin. 2019 Report, L'Observatori de la Tordera i del Besòs (ICTA-UAB).

Parameters	Indices or metrics	Monitoring program
Biological	Kilometric Abundance Index (IQA)	Avifauna
		Herpetofauna
		Ichthyofauna
		Bats
	Presence/Absence	Otter
	Specific Polluosensitivity Index (IPS)	Diatoms
	Iberian Biological Monitoring Working Party Index (IBMWP), Index of Average Score Per Taxon (IASPT)	Macroinvertebrates
	Catalan Index of Biological Integrity - version 2b (IBICAT2b)	Ichthyofauna

Physicochemical	Total Organic Carbon (TOC), salinity (chloride concentration and conductivity), eutrophication (ammonium, nitrates, and nitrites), temperature, pH, alkalinity, sulfates, chlorides, calcium, magnesium, sodium, potassium, iron, and manganese.	Macroinvertebrates
Hydromorphological	River Habitat Index (IHF)	Macroinvertebrates
	Riparian Forest Quality Index (QBR)	Macroinvertebrates and Riparian vegetation

2.3.2. Selected variables

Of all the parameters collected through the *Observatori RIVUS* various monitoring programmes, only those used to assess the status of water bodies, according to Agència Catalana de l'aigua (2019), were included.

Richness data

In this study, species richness was used as an indicator of the faunal composition of the basin. The calculation of diversity indices, such as the Shannon or Simpson indices, has not been considered, as the different methodologies used across the monitoring programmes do not permit reliable comparisons of species abundances. Some schemes are based on call abundance indices; others on kilometre-based abundance indices; and in cases such as the otter (*Lutra lutra*), only qualitative indicators such as marking intensity are available. This methodological heterogeneity makes a robust analysis of diversity in the basin not feasible within the context of the present study.

The faunal richness considered is based on the monitoring programmes for ichthyofauna (fish), herpetofauna (amphibians and reptiles), avifauna (birds), and mammals (including only otters and bats). The diversity of macroinvertebrates was not taken into account, as the methodological protocol for evaluating ecological quality establishes macroinvertebrate identification at the family level, not at the species level.

For objective 1, the characterization of stations, species richness was analyzed separately, distinguishing between the following categories:

- **Species listed in Decree 172/2022**, a regional regulation issued by the Government of Catalonia that establishes the Catalogue of Threatened Native Fauna of Catalonia (Catàleg de la fauna autòctona amenaçada de Catalunya). This catalogue includes species and subspecies of protected native wildlife considered to be under threat (Generalitat de Catalunya, 2022).
- **Invasive alien species**, as defined in the Spanish Catalogue of Invasive Alien Species (Catálogo Español de Especies Exóticas Invasoras (EEI)), regulated under national law by Royal Decree 630/2013. This catalogue includes non-native species for which scientific and technical evidence indicates a serious threat to native species, habitats, ecosystems, agronomy, or economic resources linked to

the use of natural heritage (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2013).

However, for the rest of the objectives, invasive alien species were excluded from the faunal community composition analyses because they do not positively contribute to native community structure or ecosystem functioning and may distort ecological patterns. Additionally, studying invasive species was not a primary focus of this research; the emphasis was on native species richness, which justifies their exclusion.

It should be noted that station B8 did not become a common monitoring station until 2020. Therefore, the species richness recorded at this station in 2019 was significantly lower and, to avoid underestimation, the value "ND" (no data) was assigned.

Regarding herpetofauna richness, it must be noted that the *Observatori RIVUS* was unable to obtain data for the year 2023. Consequently, any observed decrease in richness for that particular year should be interpreted because of the lack of available data, rather than a genuine decline in biodiversity.

Explanatory variables

For the construction of the database, several explanatory variables were selected, corresponding to physicochemical, biological and hydromorphological indicators. These variables were those considered in Agència Catalana de l'Aigua (2019). Specifically, only those indicators directly used by the Catalan Water Agency (ACA) for the assessment of the ecological status of water bodies have been included.

Among the physicochemical indicators, nitrites have been excluded, as they are not used by the ACA due to their rapid oxidation or reduction, as well as sulphates and chlorides, which are considered jointly with conductivity.

Regarding hydromorphological indicators, the IHF index has been excluded, as the ACA (Catalan Water Agency) does not consider it a quality element for the assessment of biological quality in water bodies (Agència Catalana de l'Aigua, 2019).

Finally, regarding the biological indicators, the three considered in this study (IPS, IMBWP, and IBICAT2b) are recognised as quality elements for assessing biological status in river water bodies by the ACA, as outlined in the report mentioned earlier.

Thus, the explanatory variables considered include the hydromorphological index QBR and several physicochemical parameters, such as pH, conductivity, dissolved oxygen (mg/L), ammonium, nitrates, and phosphates; as well as biological indicators: IPS, IBMWP and IBICAT2b (view Table 4 in section 3.1).

2.3.3. Data curation and sampling considerations

For the explanatory variables, mostly in the physicochemical factors, depending on the parameter and the analytical method, the concentration may be below the limit of detection (LOD) of the laboratory equipment. In such cases, results are reported using the symbol "<" followed by a numerical threshold, which indicates the lowest concentration that can be reliably detected by the instrument.

In order to perform the statistical analyses, values reported as being below the detection limit were treated as equal to the detection threshold. For instance, a result of <0.2 was considered as 0.2 mg/L for that campaign.

In cases where no value is available (NO DATA), efforts were made to retrieve the data from the SDIM21 viewer of the Catalan Water Agency (Agència Catalana de l'Aigua, n.d.), by identifying a representative sampling point within the same water body as the stations monitored by the *Observatori RIVUS.*

If no data were available for that year, the value "ND" (no data) was assigned, and the annual mean was calculated only from the available values from the remaining campaigns.

2.3.3. Statistical methods

To provide an overall characterisation of the available data, species richness was summarised and classified into three categories: (i) total richness, (ii) richness of invasive alien species (EEI), and (iii) richness of protected species listed under the Decree 172/2022.

Subsequently, the ratio of exotic invasive to protected species was then determined by dividing the percentage of exotic invasive species by that of protected species. These values were represented using bar plots in Microsoft Excel.

To analyse the similarity between sampling stations based on faunal composition, a Non-metric Multidimensional Scaling (NMDS) analysis was conducted using RStudio software (R Core Team, 2023), based on presence (1) / absence (0) matrices of the total species richness (excluding EEI) at each station over the six-year period. The Jaccard index, suitable for binary data, has been used to calculate dissimilarity matrix among stations. A stress value below 0.1 was considered to indicate a good fit and reliable dimensionality reduction.

Alongside the NMDS, a hierarchical cluster analysis was performed using the same Jaccard distance matrix and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) linkage method, which is well-suited for binary presence-absence ecological data. UPGMA provides a conservative clustering approach that captures average similarities in species composition between stations, making it appropriate for detecting gradual ecological gradients.

The resulting dendrogram was visually inspected and a cut-off height of h = 0.45 was applied to define distinct clusters. The clusters were overlaid on the NMDS ordination to assess the spatial coherence of the groupings.

Following the NMDS ordination, descriptive analyses were performed to assess faunal composition across the main taxonomic groups (avifauna, mammals, ichthyofauna, and herpetofauna). For this purpose, the number of species within each group was summed and subsequently converted into percentages using Microsoft Excel. Some species were highlighted either because they act as key bioindicators, represent the only records within their group (in the case of *Lutra lutra*), are the more frequent, or are closely tied to river systems. Piscivorous birds and herpetofauna are strongly dependent on aquatic habitats, and their occurrence responds sensitively to ecological conditions in the catchment. Likewise, ichthyofauna, which live in aquatic environments, provide integrative information about it. This procedure allowed for a more detailed characterisation of the assemblages and facilitated the interpretation of the clustering patterns.

Secondly, a Principal Component Analysis (PCA) was performed using only the set of environmental variables defined in Section 2.3.2., with the objective of characterising the ecological condition of the stations. Axes explaining between 75% and 90% of the total variance, and with eigenvalues greater than 1, were ideally considered as reference thresholds.

A Multiple Factor Analysis (MFA) was conducted to explore the relationship between faunal composition and environmental quality, using the same presence/absence matrix applied in the NMDS analysis. MFA is based on principal component analysis (PCA), generating factorial axes that summarize the main sources of variability while balancing the contribution of each variable group. In this context, MFA complements the prior PCA by adding the faunal composition of each station and allowing comparison of the relative impact of different types of data.

Prior to the analysis, all species that were either present (1) or absent (0) across all monitoring stations were removed, as they exhibited no variance and thus did not contribute to the ordination. The dataset was structured into four groups: river section (categorical), bioindicator variables (quantitative), physicochemical variables (quantitative), and faunal presence/absence (categorical). The “river section” variable was included as a group given its apparent influence on faunal distribution observed in the NMDS results. The MFA was performed using the FactoMineR package in R (Husson, Josse, Le, & Mazet, 2017), specifying the nature of each group. The number of dimensions retained was determined based on eigenvalues greater than 1 to ensure that only dimensions explaining substantial variance were included in the interpretation.

To explore the relationships among the studied indices, we calculated Pearson correlation coefficients. A correlation matrix was first computed including all environmental variables, and the results were visualized using a heatmap with the *corrplot* package in R (Wei & Simko, 2017). Pairwise correlations between the ecological quality indices QBR, IPS, and IBMWP were assessed using Pearson’s correlation coefficient. For each pair of variables, correlation coefficients (r) and their associated p-values were obtained with the *cor.test* function, also in R.

3. Results

3.1. General data description

Table 3. Species richness recorded at the different monitoring stations. The table presents: (i) total species richness; (ii) richness of alien invasive species (EEI); and (iii) species included in Decree 172/2022

Station code	Species Richness		
	Total	EEI	Decree 172/2022
B0_LB0	52	0	36
B1_LB3	70	4	40
B3_LB4	80	5	49
B2_LB6	72	4	43
B4_LB11	71	2	44
B5_LB13	81	6	48
B6_LB16	71	4	41
B7_LB19	67	3	41
B8	53	3	31
B9	77	7	42
B10_LB22	57	5	33

Table 3 shows that the maximum total species richness is 81 species, recorded at station B5_LB13, while the minimum is 52 species, found at B0_LB0.

The highest number of invasive alien species (EEI) is 7, observed at B9, whereas B0_LB0 registers 0 EEI. Regarding protected species listed under Decree 172/2022, the highest count is 49 species at B3_LB4, and the lowest is 31 species at B8.

On average, the Besòs basin shows 4 EEI species per station, compared to an average of 41 protected species per station.

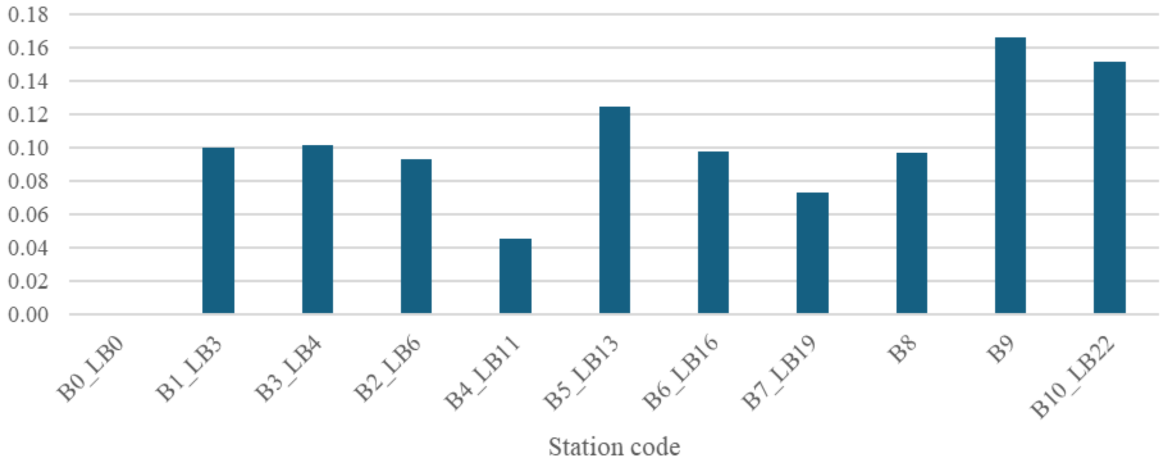

Figure 3. Ratio of invasive alien species (EEI) relative to protected species (Decree 127/2022) across sampling stations. Values represent the ratio between the percentage of EEI and the percentage of protected species. Ratios above 1 indicate a predominance.

Figure 3 shows that the relative proportion of invasive alien species (EEI) to the total number of protected species, it can be observed that none of the monitoring stations show a predominance of EEI.

The lowest ratios are found at stations B0_LB0, B2_LB6, and B7_LB19, with B0_LB0 showing a zero value for EEI, and values increasing progressively across these stations.

The highest ratios are recorded at B9, followed by B10_LB22, and then B5_LB13.

Table 4. Average values of the different selected parameters across the monitoring stations over the 6-year period (2019–2024). QBR = Riparian Forest Quality Index; IBICAT 2b = Catalan Index of Biological Integrity (version 2b); IBMWP = Iberian Biological Monitoring Working Party index; IPS = Specific Pollution-sensitive diatom Index; pH = hydrogen ion concentration; Oxygen = dissolved oxygen (mg/L); Conductivity = electrical conductivity (µS/cm); Ammonium = NH_4^+ (mg/L); Nitrates = NO_3^- (mg/L); Phosphates = PO_4^{3-} (mg/L).

Station code	QBR	IBICAT 2b	IBMWP	IPS	pH	Oxygen mg/L	Conductivity	Ammonium	Nitrates	Phosphates
B0_LB0	90.00	0.60	159.50	16.11	7.42	9.63	200.13	0.08	0.25	0.07
B1_LB3	30.00	0.56	48.42	9.96	7.27	6.53	1832.08	0.15	1.40	0.42
B3_LB4	39.17	0.47	48.17	7.47	7.70	7.98	1413.42	0.26	0.56	0.72
B2_LB6	48.33	0.67	63.67	11.86	8.05	8.18	969.08	0.31	1.33	0.49
B5_LB13	25.00	0.75	68.17	9.80	8.22	8.31	2407.83	0.16	1.22	0.32
B6_LB16	26.67	0.35	44.08	8.17	8.05	9.00	1267.75	0.09	1.55	0.91
B7_LB19	13.75	0.78	61.42	9.97	8.00	7.38	1442.58	0.09	0.70	0.65
B8	12.50	0.80	35.42	7.22	8.51	8.81	1352.42	0.13	1.65	0.83
B9	6.67	0.38	35.00	6.80	7.97	9.99	1823.50	0.31	1.38	0.31
B10_LB22	4.17	0.40	36.58	6.25	7.62	7.57	1537.83	5.00	1.03	0.51
B4_LB13	51.67	0.94	75.33	12.17	8.08	8.33	1285.00	0.09	1.40	0.39

The average values over the six-year period at each of the 11 selected stations are shown in Table 4. Each of the ten parameters has its own units.

The station B0_LB0 in the upper reaches shows the highest values for bioindicators such as QBR, IPS, and IBMWP, in contrast to the lowest values observed at lower reach stations B9 and B10_LB22. Regarding physicochemical factors, B0_LB0 also shows lower values for parameters associated with poor environmental quality, such as ammonium, nitrates, and phosphates, and higher dissolved oxygen. Of particular note is the ammonium value at B10_LB22, which is five times higher than at the other stations and indicates high organic pollution.

3.2. Faunal composition of the basin

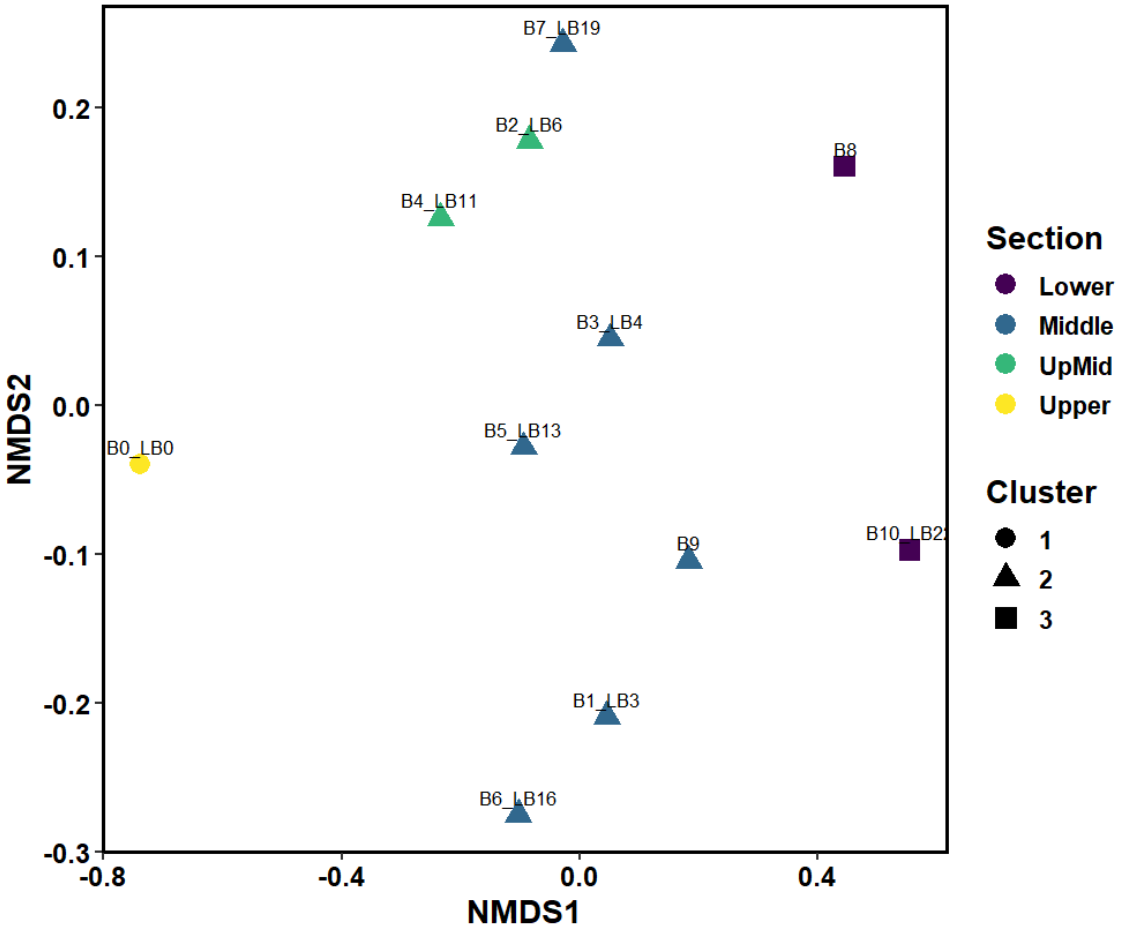

Figure 4. NMDS ordination plot based on Jaccard distances, showing the distribution of monitoring stations according to faunal composition. Shapes represent clusters identified through hierarchical clustering (UPGMA), while colours indicate river sections (Upper, Upper-Middle, Middle, Lower). Cluster 1 (circles) includes the only Upper section station; Cluster 2 (triangles) groups Upper-Middle and Middle stations; Cluster 3 (squares) corresponds to stations in the Lower section. The ordination suggests that faunal composition varies along the river's longitudinal gradient.

The NMDS showed a good fit, with a stress value of 0.088 (<0.1), indicating that the two-dimensional representation adequately reflects the dissimilarities in species presence.

Figure 4 shows the hierarchical clustering (UPGMA) applied to the same Jaccard distance matrix identified three distinct groups of stations (Clusters 1, 2, and 3), which were superimposed on the NMDS plot using different shapes. These clusters partially correspond to the river's longitudinal gradient.

Stations in the Upper and Lower sections stand out for having a distinctive and highly differentiated faunal composition compared to the others. Regarding the Upper-Middle stations, their faunal composition more closely resembles that of the Middle section stations rather than the Upper ones.

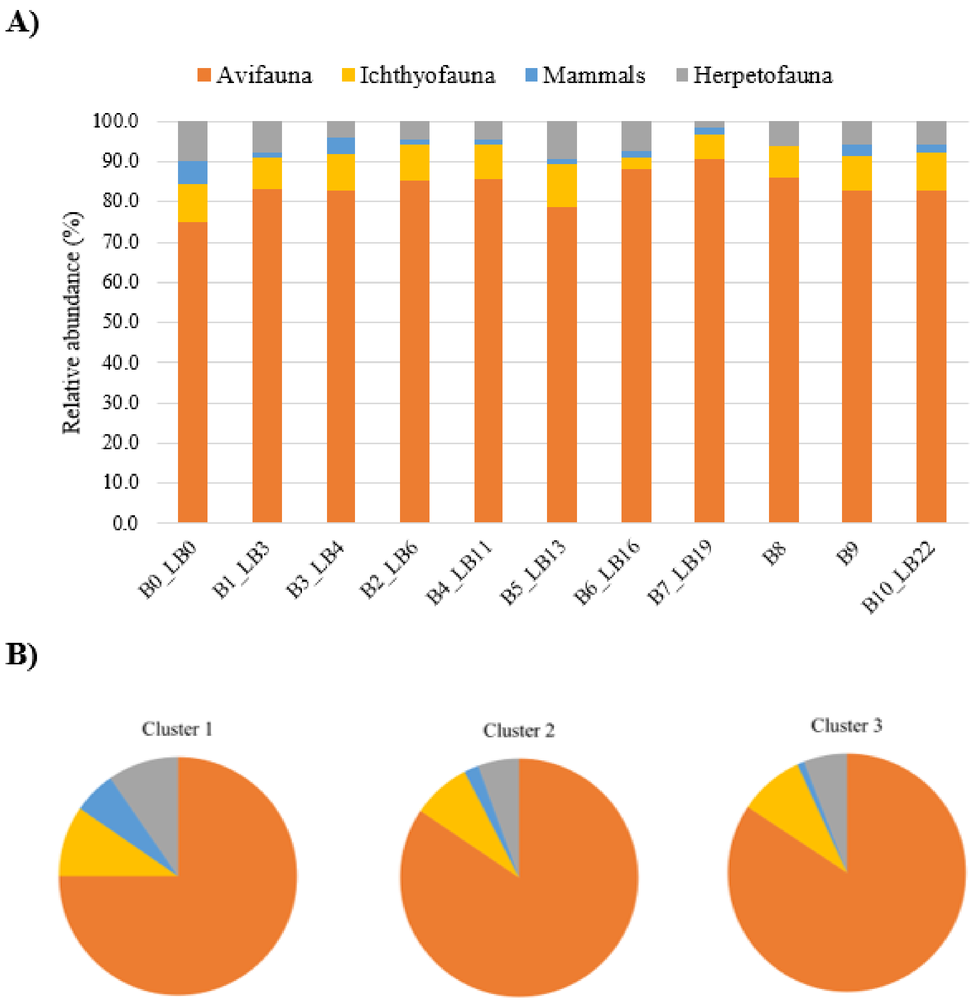

Figure 5. Relative abundance of faunal groups across sampling stations (A) and aggregated by clusters (B). A common pattern of faunal composition is observed, characterised by the dominance of avifauna at all stations, while clusters differ mainly in the contribution of secondary groups. The clusters are those previously defined in the hierarchical UPGMA cluster analysis.

In figure 5 we observe that although the faunal composition did not differ substantially among stations, a consistent pattern emerged in which avifauna clearly dominated across all sites, representing over 80% of the records. In figure 5B, Cluster 1 showed a comparatively more balanced composition, with higher relative contributions of ichthyofauna, mammals, and herpetofauna. In contrast, Cluster 2 displayed a highly homogeneous pattern strongly dominated by avifauna, while Cluster 3 presented a similar dominance but with slightly greater proportions of ichthyofauna and herpetofauna. The differences among clusters are primarily driven by the relative contribution of secondary groups, whereas avifauna remains consistently prevalent.

For the piscivorous bird species such as *Alcedo atthis* we observe that it is present at all stations except B0_LB0 and B10_LB22. Other piscivorous birds, including *Phalacrocorax carbo*, *Ardea cinerea*, and *Egretta garzetta*, were likewise frequently absent from station B0_LB0.

Only three mammal species were studied, among them the Eurasian otter (*Lutra lutra*), which was absent at stations B8 and B9. The other two species recorded belong to chiropterans.

Regarding herpetofauna, a key fluvial species, the salamander (*Salamandra salamandra*), was only observed at station B0_LB0, the sole site included in Cluster 1. Two amphibians, the common midwife toad (*Alytes obstetricans*) and the Iberian green frog (*Pelophylax perezi*), were present throughout the catchment. Notably, at station B7_LB19, only *Pelophylax perezi* was recorded. The spiny toad (*Bufo spinosus*), a relatively common and least-concerned species in Catalonia, was observed only at stations B0_LB0 and B4_LB11, corresponding to the upper and upper-middle sections of the basin, indicating its preference for less impacted habitats.

Among fish, the mountain barbel (*Barbus meridionalis*) and the catalan chub (*Squalius laietanus*) were widely distributed, except for *Squalius laietanus* at station B6_LB16. Overall, differences in ichthyofaunal composition among river sections were minor. The European eel (*Anguilla anguilla*) was mainly restricted to downstream stations, consistent with its catadromous life cycle, though it was also found at middle and upper-middle sections such as B2_LB6 and B3_LB4.

3.3. Ecological quality status of the basin

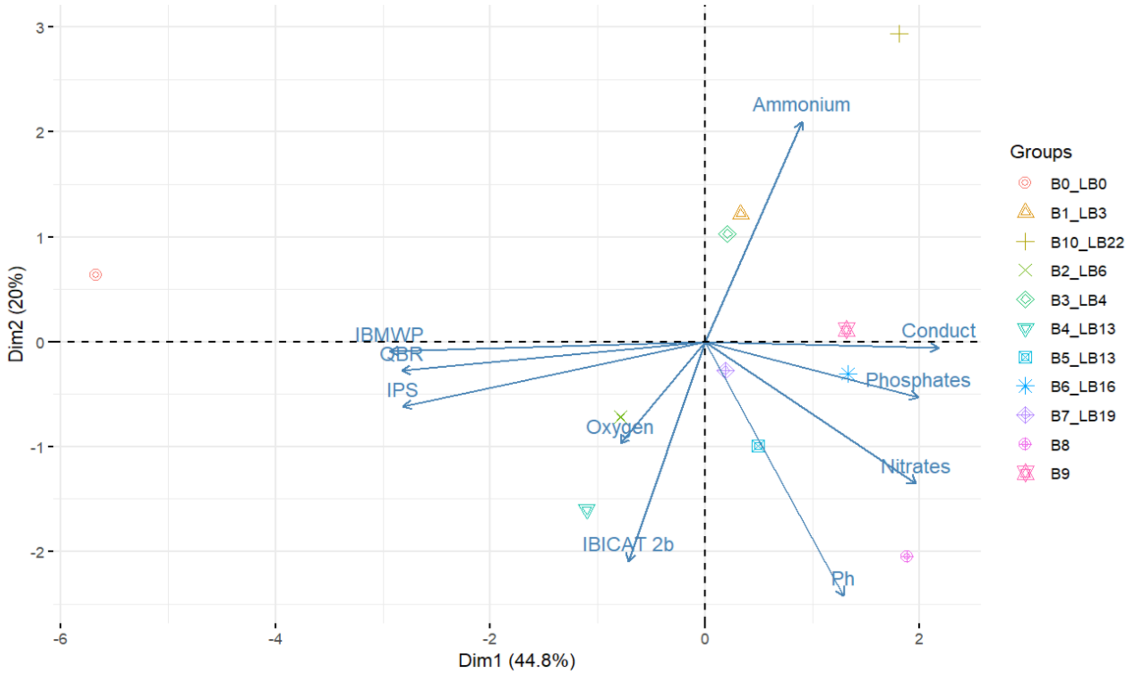

Figure 6. Principal Component Analysis (PCA) biplot based on physicochemical, hydromorphological and biological variables across all monitoring stations. Arrows indicate the direction and strength of each variable's contribution to the ordination space. Stations are represented by different symbols and colours according to their identity (see legend). The plot reveals patterns of association among variables and stations, highlighting contrasts in ecological quality and environmental conditions.

For PC1, stations located on the left of the plot are associated with high values of the biological indices IPS, IBMWP, and QBR, as well as elevated levels of dissolved oxygen. In contrast, stations on the right are characterised by higher concentrations of dissolved ions, phosphates, and nitrates. Along PC2, stations with higher ammonium concentrations are found towards the top of the plot, while those with higher pH values and better IBICAT 2b scores are located towards the bottom.

Figure 6 shows the Principal Component Analysis (PCA) based on the first two components with eigenvalues greater than 1, which together explain 64.84% of the total variance (PC1: 44.83%, PC2: 20.01%).

Station B0_LB0 stands out for exhibiting high values in the IPS, IBMWP, and IBICAT 2b indicators. Stations B2_LB6 and B4_LB13, also located in headwater areas like B0_LB0, similarly display elevated values in these indicators, as well as in dissolved oxygen concentration (mg/L).

In contrast, station B8, situated in a lower stretch of the river, along with B10_LB22, shows elevated concentrations of phosphates and nitrates. Nevertheless, B8 still maintains a high value of IBICAT 2b. Conversely, station B10_LB22 exhibits significant ammonium concentrations.

Stations B6_LB16 and B9 also tend to display high concentrations of phosphates, nitrates, and elevated ion levels in the water.

The other monitoring stations do not show clear relationships with the various indicators considered.

3.4. Biodiversity patterns and environmental status of the basin

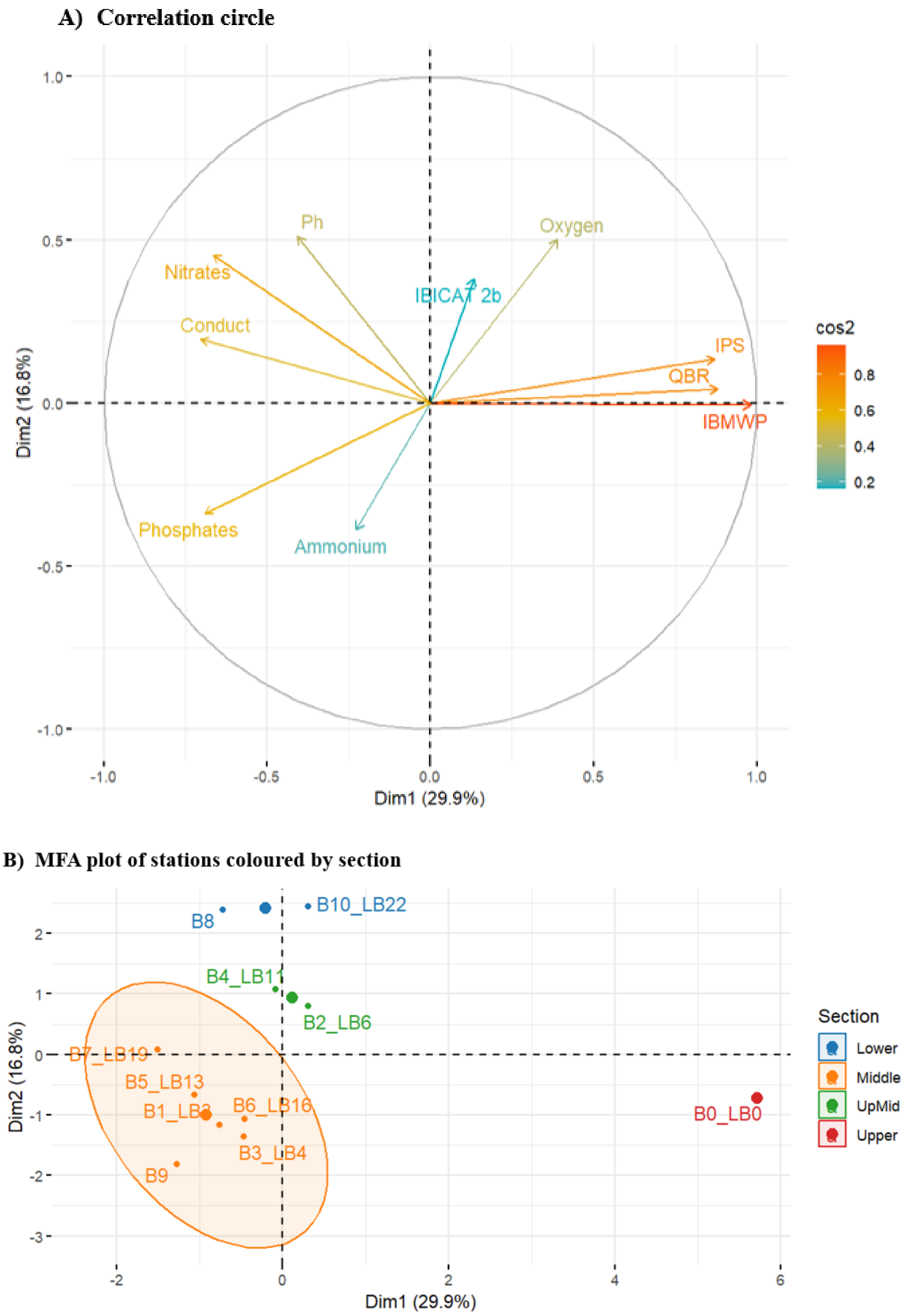

Figure 7. Multiple Factor Analysis (MFA) among stations on the Besòs basin. (A) Correlation circle of PCA showing the variables grouped by bioindicators and physicochemical parameters, with variables colored according to their squared cosine (cos²), indicating their quality of representation. (B) MFA ordination of stations colored by section, illustrating their distribution based on section, bioindicators, physicochemical parameters, and faunal composition.

The Multiple Factor Analysis (MFA) explained 46.7% of the variability of the data in the first two axes, with the first axis explaining 29.9% and the second axis 16.8% of the total variability (Figure 7).

Group contributions to Dimension 1 were relatively balanced: Section (26.9%), Fauna (25.9%), Bioindicators (23.8%), and Physicochemical (23.4%). On Dimension 2, Section (45.0%) and Fauna (28.5%) were dominant, with Physicochemical parameters also contributing substantially (23.8%).

The first dimension, primarily separate sites according to river section and biological indicators. It contrasted stations with high values for IBMWP, QBR, and IPS, positioned on the right side, with those showing higher conductivity, nitrate, and phosphate concentrations on the left side. The second dimension was associated with oxygen concentration, pH, and the IBICAT 2b index on the positive side, while ammonium was opposed (Figure 7A).

In Figure 7B, we can see that stations located in the upper sections were clearly positioned on the positive side of the first dimension while the ones located in middle sections clustered on the negative side of first dimension. Lower and Up-Mid sections occupied intermediate positions.

The correlation circle suggested a strong association between biological indices (IBMWP, QBR, IPS) and good ecological conditions, while conductivity, nitrates, and phosphates were aligned with the opposite gradient. Oxygen and pH were more closely related to Dimension 2.

As the IBMWP, QBR, and IPS variables appeared to behave similarly, a Pearson correlation analysis was conducted. The results showed strong positive associations in all cases. Specifically, QBR and IPS were highly correlated ($r = 0.91$, $p < 0.001$), QBR and IBMWP also showed a strong positive correlation ($r = 0.89$, $p < 0.001$), and IPS and IBMWP presented the strongest relationship ($r = 0.92$, $p < 0.001$). These findings indicate that the three indices vary closely together also in a statistically significant manner.

In contrast to the other bioindicators, the IBICAT2b index did not exhibit strong correlations with QBR, IPS, or IBMWP, suggesting that it captures different ecological information. This distinct behaviour is also reflected in the Multiple Factor Analysis (MFA): IBICAT2b shows a relatively low squared cosine value ($\cos2 \approx 0.2$; see Figure 7A), which indicates that this variable is poorly represented on the first two dimensions of the MFA. In practical terms, a low cos2 implies that the variance of IBICAT2b is not well explained by the main MFA axes, and therefore its contribution to the common multivariate structure is limited.

4. Discussion

4.1. Drivers of biodiversity in Mediterranean rivers

As previously highlighted, the main drivers shaping biodiversity patterns in Mediterranean rivers, particularly under hydrological stress conditions such as river intermittency and drying events, increasingly exacerbated by climate change, are the combined impacts of agricultural runoff and urban wastewater discharges (Arenas-Sánchez et al., 2021; Carceller et al., 2002). In the case of the Besòs basin, which has experienced a long history of environmental degradation linked to intense anthropogenic pressures since the period of demographic and industrial expansion (Benages-Albert & Vall-Casas, 2014), continuous and long-term ecological monitoring becomes essential.

Detailed assessment of ecological status and faunal community composition provides the scientific foundation for designing adaptive management strategies aimed at conserving these ecosystems, which are highly vulnerable due to the convergence of multiple stressors within densely urbanized landscapes.

4.2. Biodiversity structure of the basin

Based on the monitoring carried out in the Besòs basin between 2019 and 2024, encompassing eleven sampling stations distributed across different sub-basins, the analysis identified patterns in community biodiversity structure and overall ecological status. These patterns were strongly associated with the longitudinal gradient of the river system, showing principal differences between upper, middle, and lower sections of the basin. In particular, middle and lower sections showed greater anthropogenic impacts due to their location between the Vallès plains and the metropolitan area of Barcelona (Consorci Besòs Tordera, 2025).

Regarding the relative proportion of invasive alien species (EEI) to the number of protected species, considering invasive alien species as indicators of environmental degradation and protected species as proxies of good ecological quality, results indicate that no monitoring station was dominated by invasive alien species. However, the highest occurrence of EEI was recorded in stations located in the middle and lower sections of the basin, such as B8, B9 and B5_LB13. These sites also coincided with those exhibiting a lower ecological status, as reflected both in biotic indices and physicochemical parameters in the PCA analysis.

In terms of protected species, station B0_LB0, characterized by high ecological conditions and outstanding ecological quality, hosts the highest proportion of protected species. In contrast, station B9, located in the lower river section and exhibiting much lower ecological quality (see Figure 6 and 7), shows the lowest proportion of species protected under Decree 172/2022.

These findings suggest that the prevalence of invasive alien species in the middle and lower sections of the basin is linked to degraded environmental conditions, which might facilitate their establishment. While protected species reflect legal protection rather than environmental degradation, the occurrence of EEI can serve as a meaningful indicator of ecosystem degradation processes.

4.3. Ecological status of the basin

Multivariate analyses (PCA, NMDS and MFA) consistently revealed a strong gradient in ecological quality along the basin. Stations in the middle and lower sections clustered together and were characterized by degraded conditions, reflected in high concentrations of phosphates, nitrates, ammonium and dissolved salts, indicative of eutrophication and organic pollution (Agència Catalana de l'Aigua, 2019). In contrast, stations from the upper and upper-middle sections (B0_LB0, B2_LB6 and B4_LB11) showed better ecological status, associated with high dissolved oxygen and elevated values in biotic indices (IBMWP, QBR, IPS, IBICAT2b), typical of river stretches with greater flow dynamics and lower anthropic pressure.

The MFA, which integrates both environmental quality metrics and faunal composition, improved the resolution of these gradients, separating more clearly the middle from the upper-middle stations (Figure 7). This separation is likely driven by the incorporation of a broader set of variables that capture additional sources of variability, thereby improving the resolution of ecological gradients and allowing the analysis to better discriminate between stations in terms of their similarity patterns. It highlighted subtle differences not evident in the PCA alone: B7_LB19 emerged as the most degraded site overall, while B8 and B10_LB22, despite their poorer status in the PCA and NMDS, presented slightly better faunal composition when integrated with environmental variables. Conversely, B2_LB6 and B4_LB11 grouped within Cluster 2 in the NMDS, reflecting higher similarity to other stations compared to the distinct position of B0_LB0.

Among all sites, B0_LB0 consistently emerged as the most well-conserved station, displaying the highest ecological status. Both PCA and NMDS ordinations positioned it apart from the rest, reflecting its distinct faunal composition and highest ecological status. It presented higher values in all biotic indices, high dissolved oxygen concentrations, a key element for sustaining aquatic life and a fundamental driver of redox processes, and a heterogeneous community structure. The absence of invasive species and the presence of key species, such as the salamander (*Salamandra salamandra*), the otter (*Lutra lutra*), and the spiny toad (*Bufo spinosus*), further confirm its role as a reference site within the study area, as also recognized by the *Observatori RIVUS*.

By contrast, B7_LB19 represented the lowest ecological quality, hosting onlyone species of herpetofauna, *Pelophylax perezi*, the most common amphibian species recorded at all stations, highlights the degraded status of this site. This station is located in Sabadell, the largest and most densely populated municipality among the studied sites, with 221,564 habitants and a population density of 5,863 habitants/km² (Idescat, 2024), a context that helps to explain the strong anthropogenic pressures affecting its ecological integrity.

Stations B8 and B9 also reflected lower ecological quality: B8 lies in a lower section, while B9 is located at the downstream end of the middle reach. Notably, these were the only stations where the European otter was not detected, underlining how human pressures and altered prey communities can restrict the distribution of top predators (Clavero, Prenda, & Delibes, 2003). Faunal analyses complemented the environmental gradients detected in multivariate ordinations. The otter confirmed its value as a top-predator bioindicator, since its presence was associated with well-structured trophic webs, while its absence in B8 and B9 pointed to disturbed conditions (Clavero, Prenda, & Delibes, 2003; Tolrà, Ruiz-Olmo, & Riera, 2024).

Station B10_LB22, located at the lowest point of the basin, exhibited the second-highest ratio of invasive alien species, together with elevated ammonium concentrations (Table 4). Both PCA and MFA analyses consistently indicated poor ecological conditions at this station. This pattern is consistent with its geographical position, where strong anthropogenic pressures are expected (Carceller et al., 1995). Consequently, prioritizing management efforts to the last three stations located in the lower sections of the basin (B8, B9, and B10_LB22) would be justified.

Also, for amphibians that contributed valuable information to the ecological assessment. Their dual life cycle, spanning aquatic and terrestrial environments, together with their permeable skin, makes them particularly susceptible to pollutants (Hopkins, 2007). Their distribution across the basin supports the ecological gradients detected in the MFA. The presence of salamanders (*Salamandra salamandra*) exclusively at B0_LB0 supports the interpretation of this station as the most well-conserved because this species is sensitive to habitat degradation, including the introduction of non-native fish and the loss or deterioration of water bodies, which can rapidly affect reproduction and population persistence (Ministerio para la Transición Ecológica y el Reto Demográfico [MITECO], 2009). Conversely, the fact that B7_LB19 only hosted *Pelophylax perezi*, the most common amphibian species recorded at all stations, highlights the degraded status of this site.

Birds emerged as the best-represented vertebrate group in the Besòs basin, reflecting the variety of habitats present, from riparian woodlands and reedbeds to urban areas, and their potential as indicators for future ecological monitoring (Diputació de Barcelona, 2022).

It is worth noting, that the distribution of all the taxonomic groups studied across the basin are not homogeneous, as the presence or absence of certain species also depends on the specific habitat requirements that determine whether a given site can support them.

4.3. Synthesis and management implications

The integration of multivariate approaches such as PCA, NMDS, and MFA provided a complementary perspective on the ecological status and faunal composition across the Besòs basin. While PCA highlighted strong gradients of degradation associated with nutrient enrichment and organic pollution due to anthropogenic pressures, and NMDS revealed patterns of similarity between stations in terms of faunal composition, MFA revealed more detailed differences between stations, particularly distinguishing sites such as B8 and B10_LB22, which, despite showing degraded conditions in the PCA, presented comparatively slightly better faunal assemblages when integrating multiple datasets. These results emphasize the importance of applying integrative analyses to capture the complexity of ecological patterns in Mediterranean river systems and to avoid simplistic interpretations based on single metrics.

Taken together, the findings respond to the initial objectives by showing that faunal richness and composition, when examined in relation to environmental quality, are powerful descriptors of ecosystem status in Mediterranean systems. The detection of clear longitudinal gradients, the identification of sites of both higher conservation value (such as B0_LB0) and heightened vulnerability (such as B7_LB19), and the correspondence between faunal distribution and physicochemical stressors provide key insights for adaptive river management. In this regard, long-term monitoring under the framework of the WFD remains essential to safeguard biodiversity in Mediterranean basins under strong anthropogenic pressures such as those affecting the Besòs basin.

Beyond the ecological perspective, these results highlight the relevance of maintaining and expanding conservation initiatives, as this basin continues to function not only as a critical biodiversity reservoir within an urbanized landscape but also as a living indicator of the effectiveness of restoration efforts, supported by initiatives such as those led by the *Fundació RIVUS* through the *Observatori RIVUS*, which ensure continuity in monitoring and contribute to the preservation of riverine ecosystems.

4.5. Limitations and Future Directions

This study also highlights several aspects that could guide future monitoring efforts in the Besòs basin. First, some of the biotic indices used (e.g., IBMWP, IPS, QBR) were found to be highly correlated (see section 3.4). This suggests that monitoring programs could potentially reduce redundancy by prioritizing a single representative index, thereby optimizing resource allocation. The resources saved could then be redirected to improve the standardization of faunal sampling methods, which would allow the calculation of robust diversity indices, which are more valuable insights in the long term, as faunal proportions offer a powerful way to capture ecological composition and dynamics.

Second, the addition of further monitoring stations in the upper reaches of the basin would strengthen comparisons with the B0_LB0 reference site and help refine the detection of longitudinal gradients. Where complete harmonization of faunal sampling methods across taxonomic groups is not feasible, more focused studies within individual groups could be particularly informative. Birds, for example, are especially well represented in the Besòs basin, and future work could focus on their phenology, as breeding and seasonal dynamics may provide complementary perspectives on ecosystem health. Amphibians could also be considered, as they have been shown to be good predictors of the condition of the different monitoring stations due to their dual life cycle, makes them sensitive to a wide range of stressors. Concerning mammals, the development of complementary research initiatives focusing on species other than otters and bats would contribute to a more comprehensive understanding of this group.

5. Conclusions

This study demonstrates that faunal richness and composition, when examined together with environmental quality indicators, are reliable descriptors of ecological status in Mediterranean rivers. The results reveal strong longitudinal gradients in the Besòs basin, with the upper and upper-middle sections maintaining higher ecological quality and biodiversity, while middle and lower sections are more affected by eutrophication, organic pollution, and the presence of invasive alien species. The distinct role of B0_LB0 as a reference site, contrasted with the vulnerability of B7_LB19, underlines the importance of identifying both conservation priorities and critical points of degradation.

Beyond describing ecological patterns, the integration of PCA, NMDS, and MFA proved essential for capturing complex gradients and avoiding simplistic interpretations. These findings highlight the need for long-term monitoring under the Water Framework Directive and for maintaining conservation initiatives such as those led by the *Fundació RIVUS*. Ultimately, the Besòs basin illustrates both the challenges of managing biodiversity in heavily urbanized Mediterranean basins and the opportunities to enhance ecological resilience through sustained monitoring and adaptive management.

Although this basin is marked by a relatively low ecological quality ecological status, the Besòs basin represents a unique opportunity for ecological restoration and renaturalization due to its strategic location within one of the most densely populated areas of Catalonia. Enhancing its ecological condition could not only promote biodiversity conservation but also provide sustainable ecosystem services, contributing to improved urban health and human well-being.

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