Biodiversity and ecological quality of Angueira River (NE Portugal): effects of natural and anthropogenic impacts

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ABSTRACT

Freshwater ecosystems are, at a global level, strongly threatened by several pressures, mostly of anthropic origin, with emphasis on water pollution and eutrophication, fragmentation and degradation of aquatic and riparian habitats, overexploitation of resources, introduction of exotic and invasive species and extreme hydrological changes resulting from climate change.

The objective of the present study was to evaluate the biodiversity and ecological integrity of watercourses in the Angueira River basin, located in the northeast of Portugal, focusing on natural variability and anthropogenic impacts. In this way, several metrics were determined, during the spring of 2023 and 2024, in 14 sampling locations, using standardized methodologies within the scope of the European Water Framework Directive. Water quality, hydromorphology, biological communities, namely benthic macroinvertebrates and fish, were the main abiotic and biotic elements evaluated. The results revealed a decline in biodiversity and ecological quality in different areas along the river, mainly under the influence of physical barriers (weirs) and rural settlements. It was also observed that prolonged drought phenomena, particularly in smaller tributaries and in the final stretch of the Angueira River, contributed to the lower biodiversity and ecological quality of aquatic and riparian ecosystems. In contrast, in reference sites, mostly located in the upper reaches of the basin, diverse and native communities of macroinvertebrates and fish were found, although the presence of invasive alien species (e.g. red crayfish and signal crayfish, mosquitofish), widespread throughout the river network. In fact, the synergistic effects resulting from the dispersion of exotic species, water pollution, degradation of the riparian ecotone, the high presence of physical barriers and the prolonged droughts in recent years, had negative impacts affecting the ecological equilibrium of the Angueira River. In this context, the study highlights the need for conservation and management efforts to protect and restore the Angueira River basin. Priority recommendations include pollution control measures, invasive alien species control and aquatic and riparian habitat restoration to ensure long-term sustainability for both human well-being and for the aquatic and riparian environments.

Keywords: *ecological integrity, impacts, invertebrates, fish, conservation*

RESUMO

Os ecossistemas dulçaquícolas estão, a nível mundial, fortemente ameaçados por várias pressões, maioritariamente de origem antropogénica, com destaque para a poluição e eutrofização da água, a fragmentação e degradação dos habitats aquáticos e ribeirinhos, a sobre-exploração de recursos, a introdução de espécies exóticas e invasoras e as mudanças hidrológicas extremas decorrentes das alterações climáticas.

O objetivo do presente estudo consistiu na avaliação da biodiversidade e integridade ecológica de cursos de água da bacia do Rio Angueira, situada no nordeste de Portugal, com foco na variabilidade natural e nos impactes antropogénicos. Desta forma, foram determinadas diversas métricas, na primavera de 2023 e 2024 em 14 locais de amostragem, através do recurso a metodologias padronizadas no âmbito da Diretiva-Quadro da Água, vigente na Europa, relativamente à qualidade da água, da hidromorfologia e das comunidades biológicas, nomeadamente de macroinvertebrados bentónicos e peixes. Os resultados revelaram um declínio na biodiversidade e qualidade ecológica, particularmente nas zonas do curso do rio sob influência de barreiras (açudes) e de aglomerados rurais. Observou-se ainda que os fenómenos de seca prolongada, nomeadamente nos afluentes de dimensão inferior e no troço final do rio Angueira concorrem para uma menor biodiversidade e qualidade ecológica. Por sua vez, nos locais de referência, maioritariamente situados no troço superior da bacia, foram encontradas comunidades nativas de macroinvertebrados e peixes, embora a presença de espécies invasoras (e.g. lagostim-vermelho, lagostim-sinal, gambúsia) esteja disseminada por toda a rede hídrica. Com efeito, os efeitos sinérgicos resultantes da dispersão de espécies exóticas, poluição da água, degradação do ecótono ripário, elevada presença de barreiras físicas e das secas prolongadas nos anos mais recentes têm afetado negativamente o equilíbrio ecológico no rio Angueira. Neste sentido, o estudo realça a necessidade de serem desenvolvidos esforços de conservação e gestão para proteger e restaurar a bacia do Rio Angueira. Entre as recomendações prioritárias incluem-se medidas de controle da poluição, restauro do habitat aquático e ribeirinho e controlo de espécies invasoras, no sentido de garantir a sustentabilidade a longo prazo tanto para o bem-estar humano quanto para o meio ambiente aquático e ribeirinho.

Palavras-chave: *integridade ecológica, impactes, invertebrados, peixes, conservação*

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1. INTRODUCTION

Healthy rivers, from their headwaters to their deltas, are the lifeblood of our planet, providing essential resources for human survival and development. They support agriculture, industry, transportation, and recreation, while also harbouring a wealth of biodiversity (Millennium Ecosystem Assessment, 2005). However, the ecological integrity of these vital waterways is increasingly threatened by anthropogenic pressures and the looming spectre of climate change. Water pollution, stemming from industrial effluents, agricultural runoff, and untreated sewage water, introduces a cocktail of contaminants into rivers, disrupting delicate ecosystems and posing risks to human health (Letchinger, 2000; Pink, 2006; EPA, 2009). Eutrophication, fuelled by excess nutrients, triggers algal blooms that deplete oxygen levels, suffocating aquatic life (Grizzetti et al., 2017). River regulation and channelization, often implemented for flood control or navigation, alter natural flow regimes and fragment habitats, impacting biodiversity and ecosystem function (Cortes et al., 2019). The introduction of invasive species further compounds these challenges, disrupting native communities and altering ecological dynamics. Climate change is projected to exacerbate these existing pressures, with rising temperatures, altered precipitation patterns, and more frequent extreme events like floods and droughts (Huntington, 2006; Oki & Kanae, 2006). These changes will have cascading effects on water quality, biodiversity, and the overall resilience of freshwater ecosystems.

1.1. Water quality assessment

Assessing the health of rivers needs a multi-faceted approach that encompasses both abiotic and biotic factors. Physical and chemical parameters, such as temperature, dissolved oxygen, pH, conductivity, and nutrient concentrations, provide crucial information about water quality and its suitability for supporting life (APHA, 2005). These parameters can be influenced by natural processes and human activities, and their monitoring is essential for detecting pollution and identifying areas to restoration (Florescu et al., 2010).

Bioindicators, including algae, macrophytes, benthic macroinvertebrates and fish, offer valuable insights into the ecological status of rivers. Many of these organisms respond to environmental stressors, and their community composition and abundance can reveal the cumulative impacts of various pressures (Adams, 2002). Benthic macroinvertebrates are widely used due to their sensitivity to pollution and their diverse functional roles in the ecosystem. Metrics like diversity indices (e.g. Shannon-Wienner – H') and biotic indices (e.g., Iberian Biological Monitoring Working Party - IBMWP) provide quantitative measures of ecological status based on macroinvertebrate communities (Alba-Tercedor et al., 2002). Fish communities also serve as important bioindicators, reflecting long-term habitat conditions and the effects of pollution and habitat degradation. The Fish Biotic Integrity Index for wadable Rivers of Portugal (F-IBIP) is also used to assess the ecological quality based on fish community attributes (Oliveira et al., 2007).

The European Union's Water Framework Directive (WFD) provides a comprehensive framework for protecting and improving the quality of water bodies across Europe (Directive 2000/60/EC). The WFD emphasizes an integrated approach to water management, requiring member states to assess and classify the ecological status of their water bodies based on a combination of physical, chemical, and biological elements. This holistic approach recognizes the interconnectedness of various environmental factors and the need to address multiple pressures to achieve, at least, the "good ecological status" (Filipe et al., 2019).

1.2. Major impacts on aquatic ecosystems

Anthropogenic activities exert a profound influence on aquatic ecosystems. Pollution from various sources, including agriculture, urban areas, and industry, introduces contaminants and excess nutrients into rivers, leading to a cascade of negative effects (Grizzetti et al., 2017). Eutrophication, triggered by nutrient enrichment, can cause algal blooms, oxygen depletion, and fish kills. Toxic pollutants can accumulate in sediments and biota, posing risks to both aquatic life and human health. Habitat degradation, through activities like channelization, dam construction, and riparian vegetation removal, alters flow regimes, reduces habitat complexity, and disrupts natural ecological processes. The introduction of invasive species further threatens native biodiversity by competing for resources, altering food webs, and disrupting ecosystem dynamics (Anastácio et al., 2019; Oliva-Paterna et al., 2021).

Climate change is poised to exacerbate these existing pressures on freshwater ecosystems. Rising temperatures can directly affect aquatic organisms' physiology and survival, particularly those adapted to cooler conditions (Huntington, 2006). Changes in precipitation patterns and flow regimes can alter habitat availability, disrupt migration patterns, and increase the frequency and severity of droughts and floods (Oki & Kanae, 2006). These climate-induced changes interact with existing anthropogenic pressures, creating complex and unpredictable challenges for freshwater biodiversity and ecosystem functioning (Dudgeon et al., 2006).

1.3. Conservation and management

Conservation and restoration efforts are essential to safeguarding the ecological integrity of rivers. Pollution control measures, such as reducing agricultural runoff and improving wastewater treatment, are crucial for maintaining water quality and protecting aquatic life. Habitat restoration, including riparian vegetation planting and instream habitat improvement, can enhance biodiversity and ecosystem functioning, providing critical refuges for native species. Additionally, managing invasive alien species is necessary to protect native fish populations and maintain ecosystem balance. Effective water resource management requires an integrated approach that considers the multiple uses and values of rivers. For these reasons, monitoring programs are essential for tracking changes in water quality and ecological status, enabling adaptive management strategies that respond to evolving conditions. Other important aspect can be the collaboration among stakeholders, including governmental agencies, local communities, and researchers, crucial for the successful implementation of conservation and management actions. By incorporating scientific knowledge and addressing the challenges of climate change, sustainable water management can be achieved, ensuring its ecological integrity for future generations (Geist, 2011).

1.4. The Angueira River Basin

The Angueira River basin, nestled in northeastern Portugal, is a tributary of the Douro River, one of the Iberian Peninsula's major waterways. The basin showcases a mosaic of land uses, including agriculture, forestry, and urban areas, each contributing to the complex tapestry of pressures on its aquatic ecosystems. The basin also encompasses protected areas, underscoring its ecological significance and the need for careful stewardship. Despite its natural value, the Angueira River faces a range of anthropogenic pressures. Pollution from agricultural runoff and urban wastewater discharges threatens water quality, while habitat degradation due to riparian vegetation removal and the presence of weirs alters the physical structure of the river and its tributaries. The looming threat of climate change adds another layer of complexity, with potential impacts on water temperature, flow regimes, and the distribution of aquatic species.

1.5. Study objectives

The main objective of this dissertation was the evaluation of biodiversity and the integrity of aquatic and riparian ecosystems of River Angueira basin, located in northeastern Portugal. Specific objectives were assessed, based on the WFD methodologies, mainly related with:

1) **water quality**, based on several physical and chemical variables.
2) **hydromorphological quality**, i.e. aquatic and riparian habitats.
3) **Biodiversity and biota quality**, namely macroinvertebrate and fish communities.

After the evaluation of ecological integrity, several **management measures** were proposed for the conservation of Angueira River basin.

2. METHODOLOGY

2.1. Study Area

River Angueira basin is located in northeastern Portugal, and belongs to River Sabor network, one of the main tributaries of Douro River basin. The study area is very sensitive in terms of natural values and is included in the Special Conservation Zone ZEC - PTCON0021 and the Special Protection Zone ZPE - PTZPE0037 of the Sabor and Maçãs Rivers. The ZEC and ZPE of the Sabor and Maçãs Rivers are part of the Natura 2000 Network and unique habitats, and several threatened species of fauna and flora can be found. Priority habitats are identified in the study area, such as the Habitat 91E0* - Alluvial forests of alder (*Alnus glutinosa*) and ash (*Fraxinus excelsior*), as well as fish species (Leuciscidae), like Northern straight-mouth nase (*Pseudochondrostoma duriense*), and panjorca-do-esla (*Achondrostoma asturicense*), and mammals, like the Pyrenean desman (*Galemys pyrenaicus*) and the European otter (*Lutra lutra*), with high value in terms of conservation. The flow of the main rivers and streams in the region is ensured by the local occurrence of aquifers and by precipitation ranging between 500 and 1000 mm. Despite the good ecological state of the Maçãs and Angueira rivers, several pressures are identified in the watercourses of this area, such as the river fragmentation (high presence of weirs), riparian degradation and more recently climate change, mainly reflected in extended dry periods during summer and autumn seasons. In relation to groundwater, the outlook is also favorable, as they show good chemical and qualitative status. In terms of land use, native hardwoods (*Quercus suber, Quercus rotundifolia*, among others) and introduced forest stands (*Pinus pinaster* and *Eucalyptus* sp.) can be highlighted (Flordata, 2022).

Fourteen sampling sites were selected and distributed along the longitudinal axis of River Angueira (A1 to A9) and Ferreiros Stream (F1 to F5) (**Figure 2.1**). The sampling sites were selected along the longitudinal gradient, but also considering the impact of human activities, such as the presence of weirs, pollution and eutrophication of the water and degradation of riparian vegetation. The sampling sites were grouped into 4 typologies: 1) Upper Angueira, which included sites A1 to A3, i.e., and 2) Median Angueira, which comprised sites A4 to A6; 3) Lower Angueira, including A7 to A9, ands 4) Tributary stream, designed F1 to F5, including a stream crossing a ZEC zone (Minas de Santo Adrião). The portfolio of photos for all sampling sites can be observed in **Annex I**.

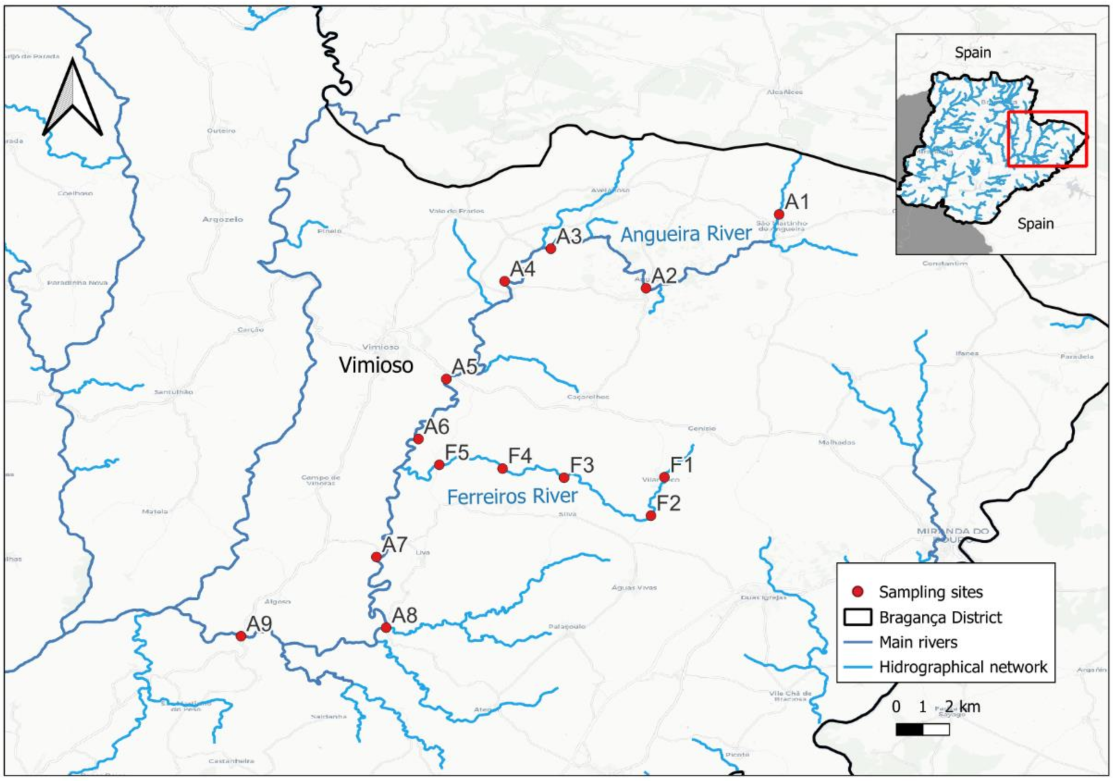


Figure 2.1. Map of the watercourses in River Angueira basin and the 14 sampling sites.

Based on the river typology for macroinvertebrate communities (INAG, 2008a), four different zones can be identified in the River Angueira basin: **1) Northern Rivers of Small Dimension (N1 ≤ 100 Km²)**, represented by the upper part of the main river, such is the example of sampling site A1, near São Martinho de Angueira (**Figure 2.2**).

Figure 2.2. Site A1 Upper Angueira (near S. Martinho de Angueira village).

2) Northern Rivers of Median-Large Dimension (N1 > 100 Km²), represented by the intermediate zone, such is the sampling site A4, near São Joanico (**Figure 2.3**).

Figure 2.3. Site A4 Median Angueira (near São Joanico).

3) Alto Douro Rivers of Median-Large Dimension (N2), represented by the lower part of the main river, and the representative sampling site A9, near Algoso (**Figure 2.4**).

Figure 2.4. Site A9 - Lower Angueira (Algoso, Vimioso).

4) Alto Douro Rivers of Small Dimension (N3), represented by the tributary, Ferreiros stream, and the representative sampling site F5, near Vila-Chã (**Figure 2.5**).

Figure 2.5. Site F5 - Ferreiros Stream, tributary of Angueira river (Vila-Chã, Vimioso).

2.2. Water quality: Physical and chemical variables

Several physical and chemical variables were measured. Some variables were immediately measured ***in situ***, using multiparametric portable probes (HACH HQ 2200 ©) (**Figure 2.6**): 1) Dissolved Oxygen (mg O_2/L; % saturation); 2) Temperature (°C); 3) Total Dissolved Solids (TDS mg/L), 4) Electrical Conductivity EC25 (µS/cm), and 5) pH.

Figure 2.6. *In situ* measurement of water quality in River Angueira (Spring 2024).

Other variables were determined in the laboratory, namely: 1) Total Acidity (mg HCO_3^-/L) and 2) Oxidability (mg O_2/L). The physical and chemical variables were measured in the spring season of two successive years, 2023 and 2024. The water quality followed different analysis methods and units for general physicochemical parameters (**Table 2.1**). The procedure for collection, conservation and water sample transport for general physical-chemical elements followed the 2017 RELACRE guide.

Table 2.1. Analysis methods and units for general physicochemical parameters.

Elements	Parameters	Measurement	Units
Thermal Conditions	Temperature	in situ	ºC
Oxygenation Conditions	Dissolved Oxygen	in situ	mg O_2/L
	% Saturation rate O_2	in situ	% saturação O_2
	Oxidability	In laboratory	mg O_2/L
Salinity	Electrical conductivity	in situ	µS/cm, 20 ºC
Acidification state	pH	in situ	Sorensen scale
	Alkalinity/Acidity	In laboratory	mg HCO_3^-/L
Nutrient Conditions	Total Dissolved Solids	in situ	mg/L

The reference analytical methods for natural surface waters were complied with Decree-Law no. 236/98 of 1 August, Decree-Law no. 83/2011 of 20 June and Decree-Law no. 218/2015 of October 7, considering the detection limits, precision and accuracy specified in the aforementioned diplomas and the quality boundaries of the general physical-chemical parameters applicable in rivers for the North Group for the evaluation of the ecological status assessment (**Table**) (APA, 2021).

Table 2.2. Maximum thresholds of physical-chemical parameters for the Ecological Status in Northern rivers of Portugal (adapted from APA, 2021).

Parameters	Excellent /Good	Good / Moderate
Dissolved Oxygen	**8-12 mg O_2/L**	**6 mg O_2/L**
Saturation rate O_2	**80-115 %O_2**	**70-125 %O_2**
pH	**6.5 – 8.5**	**6 - 9**
Electrical Conductivity	**--**	**250 µS/cm**
Temperature	**--**	**6.5 – 25.5 ºC**

2.3. Hydromorphological elements: channel and riparian quality

River Habitat Survey (RHS)

The hydromorphological characteristics were evaluated using the RHS methodology (Environment Agency, 2003 and update included in the 2022 reprint). The RHS method is applied in the field, filling a sheet with several variables to assess the quality status of aquatic and riparian habitats (Raven et al., 1997). In the RHS technique hydrogeomorphological characteristics are inventoried, complementing 10 *spot-checks* with the riparian corridor evaluation, through the *sweep-up* made along a 500 m long section (**Figure 2.7**). In detail, the observations are recorded at two different scales: in transepts arranged at intervals of approximately 50 m (10 *spot-checks*) and continuous survey throughout the entire sector of approximately 500 m (*sweep-up*). The field inventory is diverse and complete, giving information about different variables, like the dominant substrate, frequency of occurrence of erosion and sedimentation characteristics, types of current and hydrodynamics, vegetation structure, bank morphology and land use in areas adjacent to the riparian corridors.

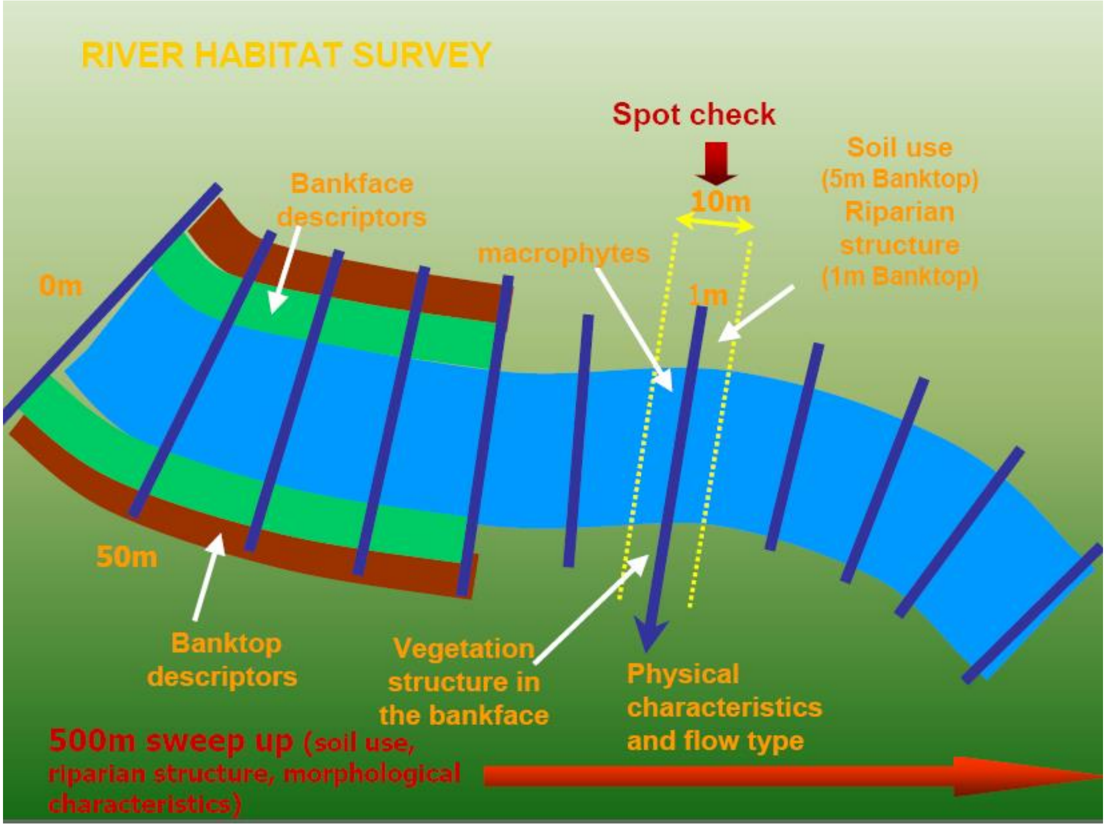


Figure 2.7. Schematic representation, spatial distribution and some of the considered variables (Environment Agency, 2003).

Two RHS indices can be calculated: 1) HQA - Habitat Quality Assessment, related with the measurement of the diversity and degree of naturalness of the physical structure of the sampling site and 2) HMS - Habitat Modification Score, which establishes a rank of the quality and diversity of river habitats, taking into consideration the degree of riparian degradation.

The HQA value can only be used to make comparisons between rivers of the same typology or different sections of the same river, resulting from to the sum of nine sub-indices that evaluate several components of hydromorphological quality, with limits established according to the river typology (**Table 2.3**).

Table 2.3. HQA limit values for the Excellent class (adapted from APA, 2021).

River Type	Acronym	Class Limit Excellent
Alto Douro Rivers of Small Dimension	**N3**	**> 65**
Alto Douro Rivers of Medium-Large Dimension	**N2**	**> 61**
Northern Rivers of Medium-Large Dimension	**N1 > 100**	**> 60**
Northern Rivers of Small Dimension	**N1 ≤ 100**	**> 68**

The assessment of the degree of artificialization of the physical structure of the river corridor is evaluated through the HMS index, similarly to the impact of transverse and longitudinal structures within the 500 m sampling sections. The HMS scores (**Table 2.4**) define different categories of river artificialization and are independent of river type, possible the comparison between rivers.

Table 2.4. HMS Index Score : Artificialization categories (adapted from APA, 2021).

Score	Category	Description	Quality Class
0-16	1	Pristine/Semi-natural	Excellent
17-199	2	Predominantly unmodified	Good or Inferior
200-499	3	Obviously modified	
500-1399	4	Significantly modified	
>1400	5	Severely modified	

Both HQA and HMS indices can be calculated using the RHS Toolbox version 1.56 software by Naura (2021) (http://www.riverhabitatsurvey.org /author/mnaura/).

2.4. Biota: Benthic Macroinvertebrate Communities

2.4.1. Sampling Procedures

The benthic macroinvertebrate communities were captured using the sampling procedures defined by the protocol established by the Environment Portuguese Agency (APA), according to the implementation of the Water Framework Directive (WFD) in Portugal (INAG 2008a). As recommended by the protocol, sampling was made in the spring season of 2023 and 2024. Summarily, the protocol defines a sampling area of 50 m, representative of the detected habitats (e.g., riffle, pool and runs) and microhabitats (e.g., fine and coarse materials, leaves accumulation, aquatic plants). This area must consider the presence of erosion units (turbulent flow) and adjacent sedimentation units (laminar flow). Six subsamples were collected in the sampling selected river zone, using a hand net (25*25 cm dimension and 500 µm of mesh size) 1-meter extension area, removing the river bottom against current, using the movement of the foot (**Figure 2.8** and **Figure 2.9**).

Figure 2.8. Sampling procedures of benthic invertebrates collection (Spring 2024).

Attached invertebrates to substrata (e.g. gastropods, caddisflies) were also collected using tweezers, avoiding to damage the organisms. All invertebrates captured were immediately preserved in ethanol in labelled polyethylene bottles for further processing of the samples in the laboratory.

Figure 2.9. Sampling procedures of benthic invertebrates collection (Spring 2024).

In the laboratory, invertebrates were sorted and subsequently preserved in 70% ethanol. The macroinvertebrates samples were then counted, and identified using a stereomicroscope SMZ10 with 10-132*x* zoom magnification, and dichotomous keys (e.g. TACHET et al. 1981, 2010) (**Figure 2.10**) used to identify until family taxonomic level, except for the subclasses Oligochaeta and Acari.

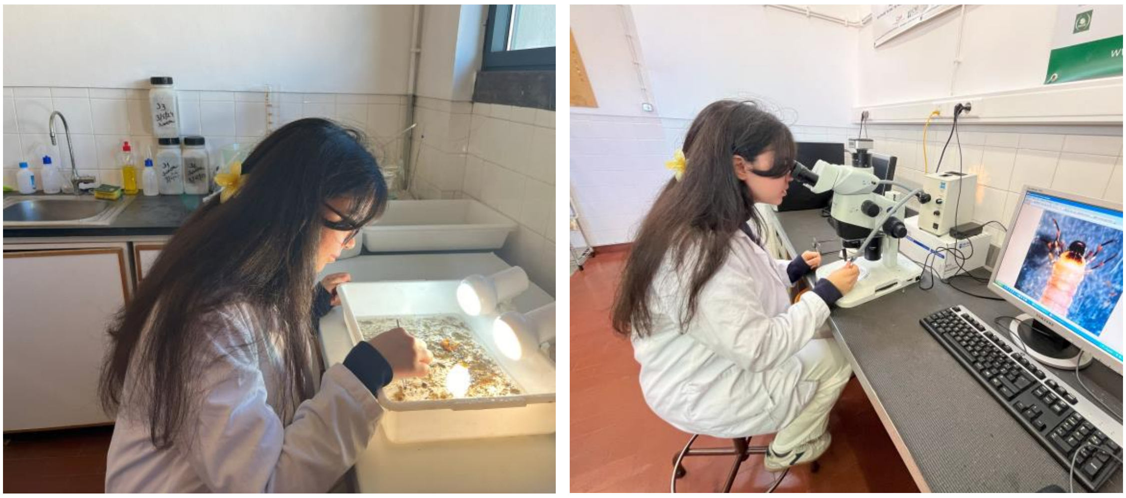


Figure 2.10. Laboratorial procedures : macroinvertebrate sorting and identification.

2.4.2. Biotic indexes and complementary metrics

The biological quality evaluation was made through the calculation of different uni and multimetric variables, some of them calculated using the Software AMIIB@ (***http://dqa.inag.pt/implementacao_invertebrados_AMIIB.html***), such as : 1) Number of individuals (N) and number of *taxa* (S); 2) Diversity (H' Shannon-Wienner index); 3) Evenness (Pielou J' index); 4) Relative abundance of Ephemeroptera, Plecoptera and Trichoptera (% EPT); and 6) Portuguese Northern Invertebrate Index- $IPtI_N$ (INAG, 2009).

The **multimetric Index $IPtI_N$**, developed for Northern rivers of Portugal, includes several metrics such as nº of *taxa*, EPT, Pielou J' evenness, Shannon-Wienner H' diversity, IASPT and Sel. ETD, resulting in the following formula:

$$IPtI_N = Nº\ taxa \times 0.25 + EPT \times 0.15 + Evenness \times 0.1 + (IASPT - 2) \times 0.3 + Log\ (Sel.\ ETD+1) \times 0.2$$

where:

- **EPT**: Nº families belonging to Ephemeroptera, Plecoptera, Trichoptera orders.
- **Evenness**: Defined as Pielou index or Evenness and calculated by the formula:

$$E = H'/Ln\ S$$

where:

H' - diversity of Shannon-Wienner

S - number of present *taxa*

Ln - natural or neper logarithm

The **H' Shannon-Wienner Index** is calculated by the formula:

$$\mathbf{H' = - \sum p_i \, Ln \, p_i} \qquad \text{where:}$$

$\mathbf{p_i = n_i/N}$

$\mathbf{n_i}$- nº of individuals of each *taxon i*

N- total nº of individuals present in sample

- **IASPT**: Iberian ASPT, corresponding to IBMWP (Alba-Tercedor, 2000) divided by the number of families present in the sample.
- **Log (Sel. ETD+1)** - Log_{10} of (1 + abundance of Heptageniidae, Ephemeridae, Brachycentridae, Odontoceridae, Limnephilidae, Goeridae, Polycentropodidae, Athericidae, Dixidae, Dolichopodidae, Empididae, Stratiomyidae).

The reference values and boundary values for the quality classes for the 4 river typologies of Angueira River basin are presented in **Table 2.5**.

Table 2.5. Median reference values and boundaries for river types of the study (APA, 2016).

Typology	Reference value	Excellent	Good	Moderate	Poor	Bad
N3	1.01	≥0.85	[0.69 – 0.85[	[0.40 – 0.69[	[0.20 – 0.40[	[0 – 0.20[
N2	1.01	≥0.83	[0.69 – 0.83[	[0.41 – 0.69[	[0.20 – 0.41[	[0 – 0.20[
N1 > 100	1.00	≥0.88	[0.68 – 0.88[	[0.44 – 0.68[	[0.22 – 0.44[	[0 – 0.22[
N1 ≤ 100	1.01	≥0.85	[0.69 – 0.85[	[0.40 – 0.69[	[0.20 – 0.40[	[0 – 0.20[

2.4.3. Functional Feeding Groups

Another approach to evaluate the biological quality can use the macroinvertebrate functional feeding group (FFG) method, based on their nutritional resource requirements (Merritt & Cummins, 1996). The software ASTERICS developed by the project **AQEM** - The Development and Testing of an Integrated **A**ssessment System for the Ecological **Q**uality of Streams and Rivers throughout **E**urope using Benthic **M**acroinvertebrates was used to calculate the relative abundance of the main functional feeding groups (**Table 2.6**).

Table 2.6. Classification System for invertebrate trophic relations (Merritt & Cummins, 1996).

Functional Group	Dominant Food	Feeding mechanism	Examples of taxa	Particle size of food
Shredders	Living vascular hydrophyte plant tissue	Chewers and miners of live macrophytes	Trichoptera	$> 10^3$
	Decomposing plant tissue and wood- coarse particulate organic matter (CPOM)	Chewers, wood borers, and gougers	Diptera Plecoptera	
Collectors	Decomposing fine particulate organic matter (FPOM)	Gatherers or deposit (sediment) feeders	Ephemeridae Chironomidae	$< 10^3$
Filter-feeders	Decomposing fine particulate organic matter (FPOM)	Filterers or suspension feeders	Hydropsychidae Simuliidae	$< 10^3$
Grazers and Scrapers	Periphyton- attached algae and associated material	Herbivores- grazing scrapers of mineral and organic surfaces	Glossosomatidae Heptageniidae	$< 10^3$
Predators and Parasites	Living animal tissue	Carnivores- attack prey, pierce tissues, cells and suck fluids	Hemiptera	$> 10^3$
	Living animal tissue	Carnivores- ingest whole animals (or parts)	Perlidae	
	Living animal tissue		Plathyelminthes	

2.5. Biota: Fish Communities

2.5.1. Sampling Procedures

The sampling procedures used to capture fish were made based on the Manual for the Biological Assessment of Water Quality in River Systems, according to the WFD - Protocol for sampling and analysis for fish fauna (INAG, 2008b).

The electrofishing was the capture method to sample all representative habitats (i.e., at least, if possible, a riffle/pool sequence) of in each selected site, including the maximum variety of available microhabitats. It was used a capture per unit of effort (CPUE) of 30 minutes and a length of river corresponding to 20 times its width, with a reach length of no less than 100 meters. Sampling was carried out by wading the channel, down to upstream, and the electrofishing equipment adjusted to the water conductivity, to increase fishing efficiency and avoid mortality and fish injuries. It was used a portable electrofishing device with direct current DC to capture fish (Hans Grassl ELT GI ©; 300-600V) (**Figure 2.11**). Fish were subsequently identified to the species level (Collares-Pereira et al., 2021 and Magalhães et al., 2023), counted, measured and weighted and immediately released into the river (**Figure 2.12**).

Figure 2.11. Sampling procedures of fish fauna using electrofishing (Spring 2024).

Figure 2.12. Biometric data collection and fish fauna identification (Spring 2024).

2.5.2. Metrics calculated for fish fauna

To evaluate the biological quality of Angueira River basin, based on fish communities, the Fish Biotic Integrity Index for wadable Rivers of Portugal (F-IBIP), developed by INAG and AFN (2012) for Portugal, was used. This index possesses several metrics (functional and ecological attributes) reflecting basic structural and functional characteristics of the fish communities in freshwater ecosystems (Oliveira et al., 2007, 2010). In summary, these metrics can decrease or increase depending on the anthropogenic disturbance resulting in different responses in terms of richness and specific composition (e.g., number of native species, percentage of exotic individuals) and ecological factors (e.g., food or reproduction characteristics). The F-IBIP index was determined using the software http://www.isa.ulisboa.pt/proj/fibip/.

In Angueira River basin two fish group zones were identified: 1) G3 - Medium-sized Cyprinid rivers of the Northern Region; and 2) G4 - Small Cyprinid streams of the North-Central Interior and South Regions. The F-IBIP score is achieved through the arithmetic mean of the metrics considered in each fish group. The final score of F-IBIP varies between 0 (zero), corresponding to bad quality, and 1 (one) representing excellent quality (**Table 2.7**).

Table 2.7. Variation values of the F-IBIP quality classes (INAG & AFN, 2012)

Score (Ecological Quality Ratio)	Quality Classes
[0.850 – 1.000]	Excellent
[0.675 – 0.850[	Good
[0.450 – 0.675[	Reasonable
[0.225 – 0.450[	Poor
[0 – 0.225[	Bad

2.6. Data treatment

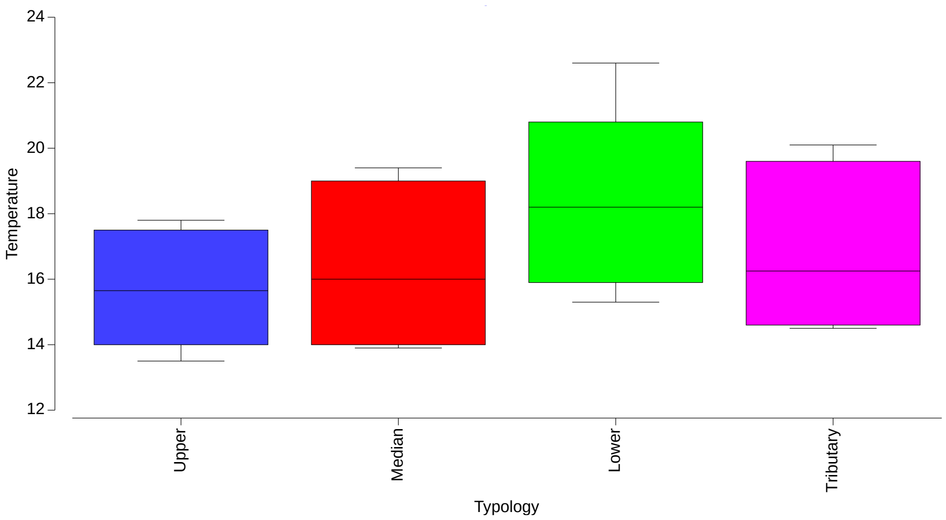
For the water quality elements, several variables were analysed, i.e. temperature, dissolved oxygen, electrical conductivity, total dissolved solids (TDS), pH, total acidity, and oxidability. Box-Whiskers plots and non-parametric analyses, H Kruskal-Wallis tests were made, since data do not fit normal distributions. Multivariate analyses were done after normalisation procedures, and *db*RDA analysis for environment conditions performed (Legendre & Anderson, 1999). The sampling sites, previously grouped in 4 river typologies for River Habitat Survey (RHS) indexes (HMS and HQA) and biota, i.e., macroinvertebrate and fish communities (INAG, 2009; AFN & INAG, 2012) were analysed using non-metric multidimensional scaling (nMDS) and the Bray-Curtis dissimilarity coefficient (Clarke and Gorley, 2015). Ordination was interpreted in ecological terms for stress values <0.2. Prior to analysis, data were Log (x+1) transformed to reduce the influence of abundant taxa and to overcome the unity-sum constraint (Clarke and Gorley, 2015). Analysis of similarity (ANOSIM) was used to assess differences in macroinvertebrate and fish communities among river typology groups. Similarity Percentage (SIMPER) analyses were also performed to identify the macroinvertebrate and fish taxa with the highest contribution to river typology dissimilarity. Richness (S), abundance (N), Shannon-Wiener diversity index (H') and the Pielou's evenness (J') and complementary functional feeding group (FFG) (only for invertebrates), for the invertebrate and fish analyses were performed using nonparametric Kruskall-Wallis multiple comparison tests, since normality or homogeneity of variance were not met, even using several transformations. When the values of the diversity indices were zero, they were removed from the analyses. A two-way PERMANOVA test (999 permutations), was performed to evaluate the influence of sampling years (spring of 2023 and 2024) and the river typologies, for invertebrates and fish communities, both as fixed factors. If the number of permutations was lower than 150 the Monte Carlo test P-value was considered. All the analysis were performed on PRIMER 7 & PERMANOVA+ (Primer-E Ltd, Plymouth) and on STATISTICA (Statsoft, 2004) softwares.

3. RESULTS

The results are discriminated into three different sections, considering the Water Framework Directive elements: 1) water quality; 2) hydromorphological characteristics, and 3) biota, in particular benthic macroinvertebrates and fish communities.

3.1. Physical and chemical water quality

The variation of physical and chemical variables can be observed in **Figure 3.1** to **Figure 3.3**. The water temperature showed a tendency to increase from up to downstream zones, which seems natural, in opposition to the conductivity with lower and higher values obtained in median and in tributary sampling sites, respectively.



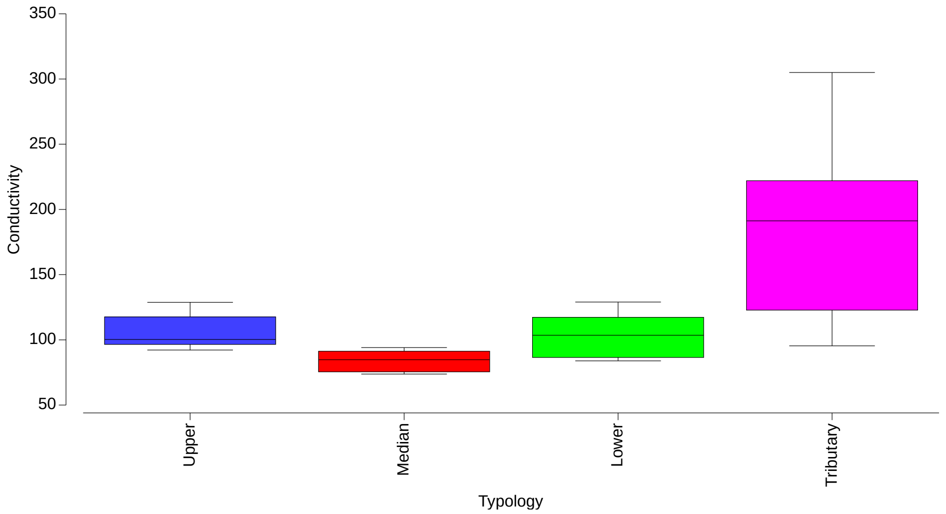


Figure 3.1. Box and whiskers: Water temperature (°C), and conductivity (µS.cm^{-1}) variation for the 4 river typologies. Boxplots show the median values (center line), the range between the 1st and 3rd quartiles (box) and the minimum and maximum values (whiskers). (Spring 2023 and 2024).

The dynamics for the concentration and % of saturation of dissolved oxygen showed higher values in the upperpart of Angueira River. Interestedly, lower values were obtained in the tributary stream. However, the inferior values for both variables are not limiting factors for aquatic life (i.e., 7 mg O_2/L and 75% of saturation) (**Figure 3.2**).

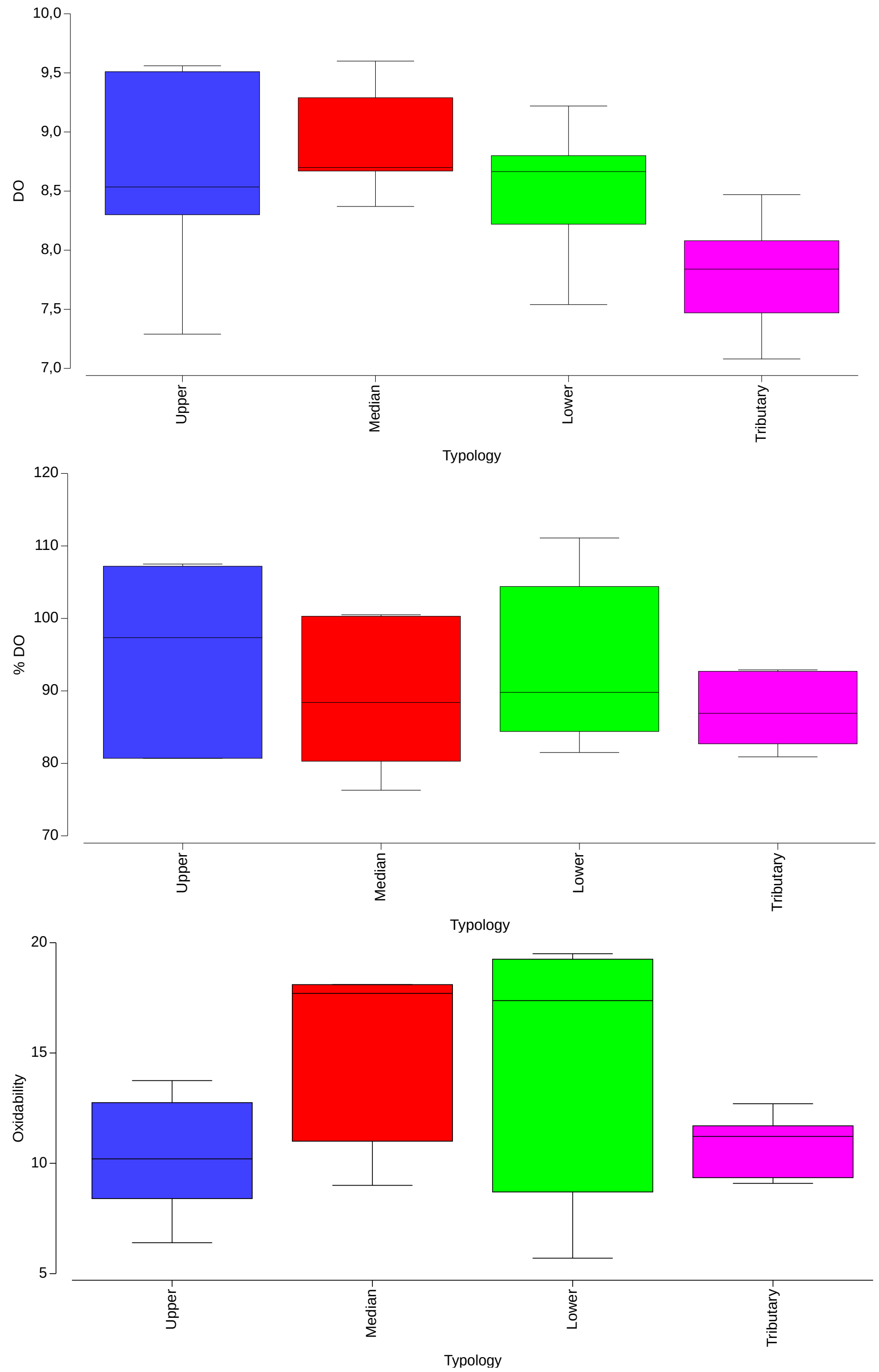


Figure 3.2. Box and whiskers: DO - Dissolved oxygen (mg O_2/L), % saturation DO (% sat.), and Oxidability (mg O_2/L) variation, considering the 4 river typologies. Boxplots show the median values (center line), the range between the 1st and 3rd quartiles (box) and the minimum and maximum values (whiskers). (Spring 2023 and 2024) (Spring 2023 and 2024).

The higher values of oxidability reflected the presence of organic matter and their accumulation along the longitudinal axis of Angueira River (**Figure 3.2**). On the other hand, higher values were also found in the Ferreiros stream for the total of dissolved salts and total acidity (**Figure 3.3**).

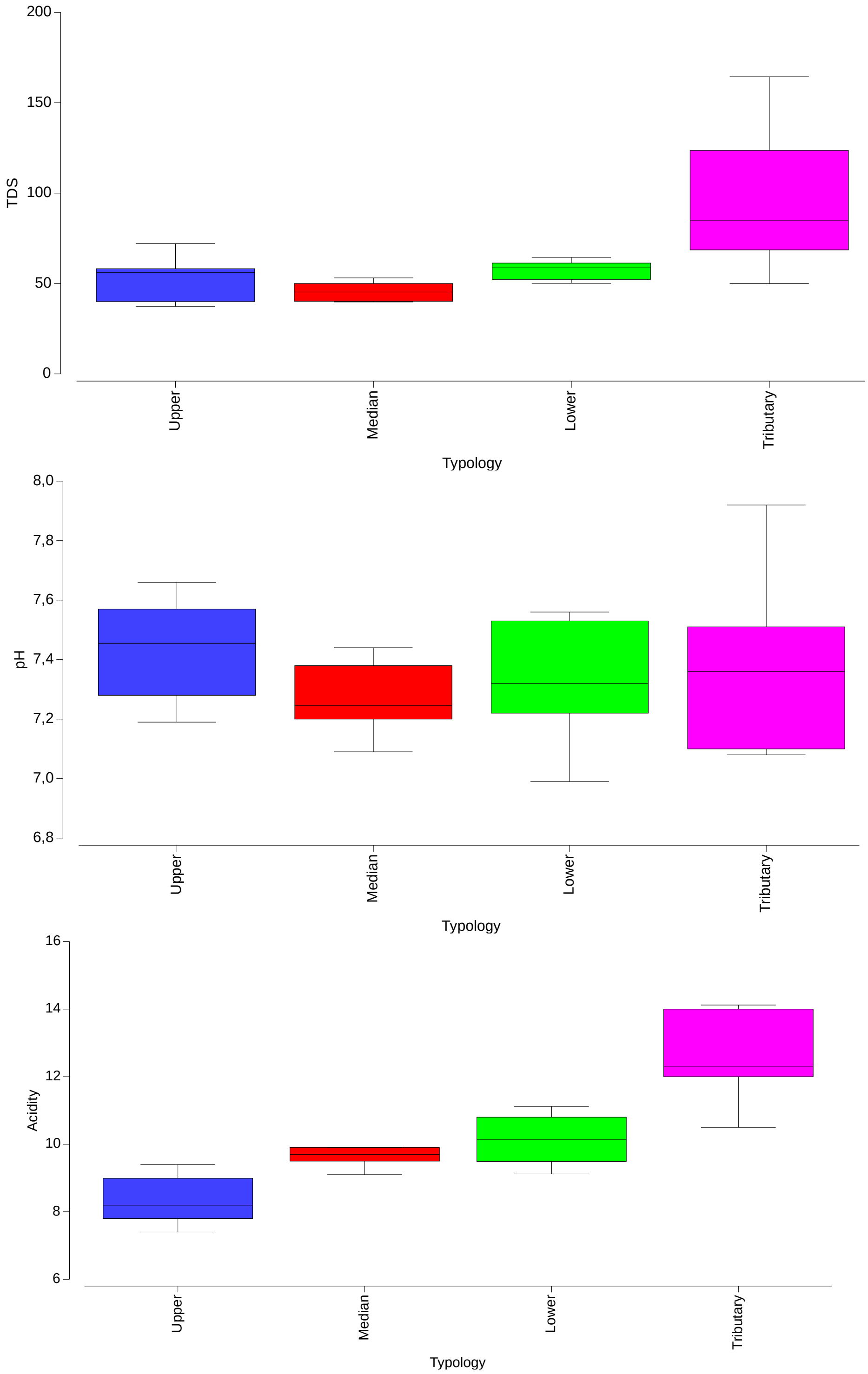


Figure 3.3. Box and whiskers: Total solids dissolved (TDS, mg/L), pH, and Total Acidity (mg HCO_3^-/L) variation, considering the 4 river typologies. Boxplots show the median values (center line), the range between the 1st and 3rd quartiles (box) and the minimum and maximum values (whiskers) (Spring 2023 and 2024).

The results obtained for most of the variables and sampling seasons (i.e., spring 2023 and 2024) for the sampling sites (i.e. considering the 4 typologies) are, in general, within the ranges established by the APA for Good Condition (**see Annex II**). However, the Kruskal-Wallis H tests showed significant differences among the 4 typologies (i.e. upper, median, lower and tributary groups) for dissolved oxygen (H (3, N= 24) =8.824 p =0.032), conductivity (H (3, N= 24) =13.793 p =0.003), Total acidity (H (3, N= 24) =18.316 p =0.0004) and TDS - Total Dissolved Salts H (3, N= 24) =11.347 p =0.010), considering all sampling sites and 2 seasonal periods.

The dbRDA analyses confirmed that conductivity, TDS and DO are among the main variables justifying the discrimination between tributary sites (e.g. F1, F3, F5) and the remain sampling sites (A1 to A9) in the Angueira River, considering a total variance of 60.7% for the two fist axes of the dbRDA analyses (**Figure 3.4**).

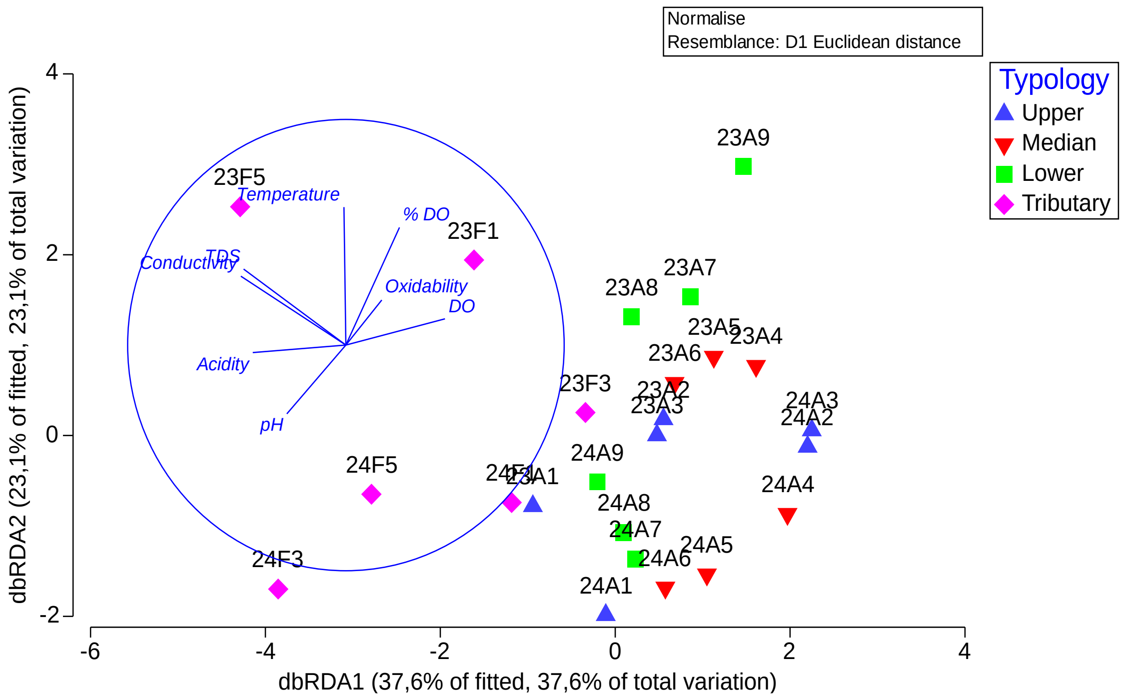


Figure 3.4. Distance-based redundancy analysis (dbRDA) showing the arrangement of the 12 sampling sites on the Angueira and Ferreiros watercourses and 2 seasons, based on the abiotic factors measured and the 4 defined typologies (Spring 2023 and 2024).

In order to understand the variation only in the main watercourse, Angueira River, considering both sampling years, another dbRDA analysis was made (**Figure 3.5**). The results showed the variables of pH, dissolved oxygen and water temperature as the main water quality parameters discriminating among river typologies and both hydrological years.

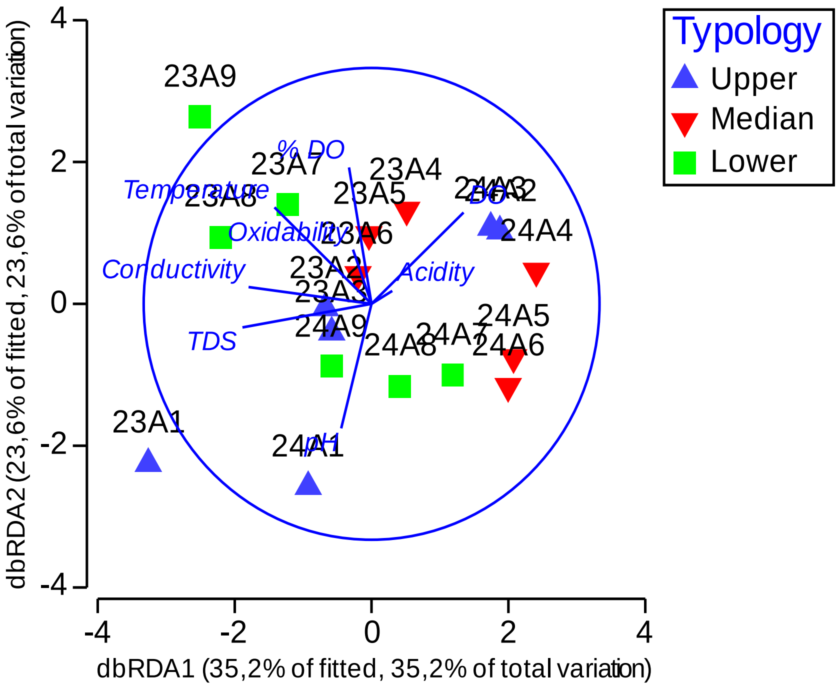


Figure 3.5. Distance-based redundancy analysis (dbRDA) showing the arrangement of the 9 sampling sites on the Angueira River and 2 seasons, based on the abiotic factors measured and the 4 defined typologies (Spring 2023 and 2024).

3.2. Quality of aquatic and riparian habitats (RHS)

The partial and total HQA and HMS scores are presented in **Table 3.1** and **Table 3.2**. The global analysis showed a good/excellent classification for HQA for the 4 different groups. However, for the quality classification according to HMS, most of the sampling sites are affected by hydromorphological modifications. In the lower zone, with low human influence, the natural habitats are more common (**Figure 3.6**).

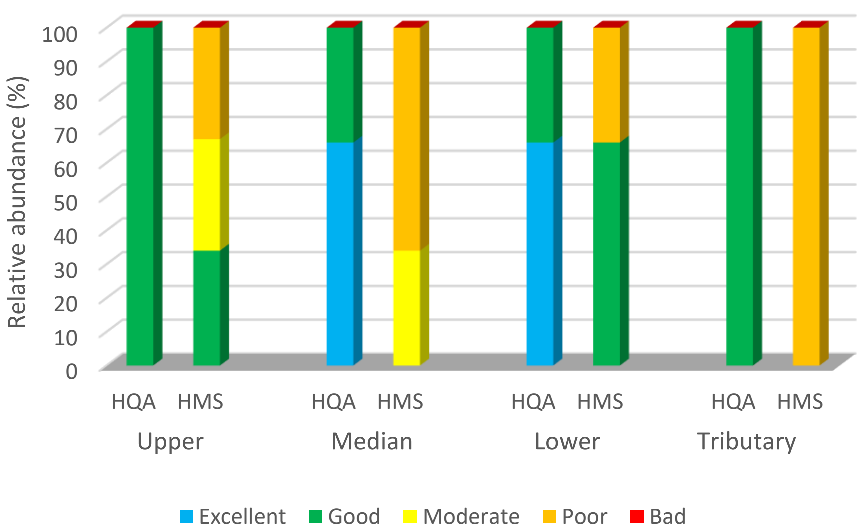


Figure 3.6. Hydromorphological quality: HQA and HMS relative abundance by river typology.

Table 3.1. Partial HQA index values and respective sub-scores for the sites distributed throughout the Angueira River basin. Classification according to APA criteria 2021 (HQA Class: 1 - excellent; ≤ 2 - Good or lower quality).

HQA	A1	A2	A3	A4	A5	A6	A7	A8	A9	F5
Flow type	11	10	9	8	5	9	12	9	10	10
Channel substrate	13	9	8	10	8	9	11	8	8	12
Channel features	5	2	2	7	2	4	6	5	5	4
Bank features	4	5	3	6	0	7	2	6	6	6
Bank veg. structure	6	7	10	12	5	11	12	12	12	10
Point bars	0	0	1	0	0	1	0	0	1	0
Channel vegetation	9	5	7	9	0	7	5	4	6	6
Land use	3	3	3	4	4	4	4	2	4	4
Trees	12	10	8	10	9	11	11	10	10	7
Special features	1	0	0	2	1	4	1	1	1	0
HQA Score	64	51	51	68	34	67	64	57	63	59
HQA Class	≤ 2	≤ 2	≤ 2	1	≤ 2	1	1	≤ 2	1	≤ 2

For the HMS index, weirs, bridges, reinforced banks and poaching were the main negative impacts, responsible for the hydromorphological artificialization (**Table 3.2**).

Table 3.2. Partial HMS index values and respective sub-scores for the sites distributed throughout the Angueira River basin. Classification according to APA 2021 (HMS Class: ≤ 2 - Good or lower quality).

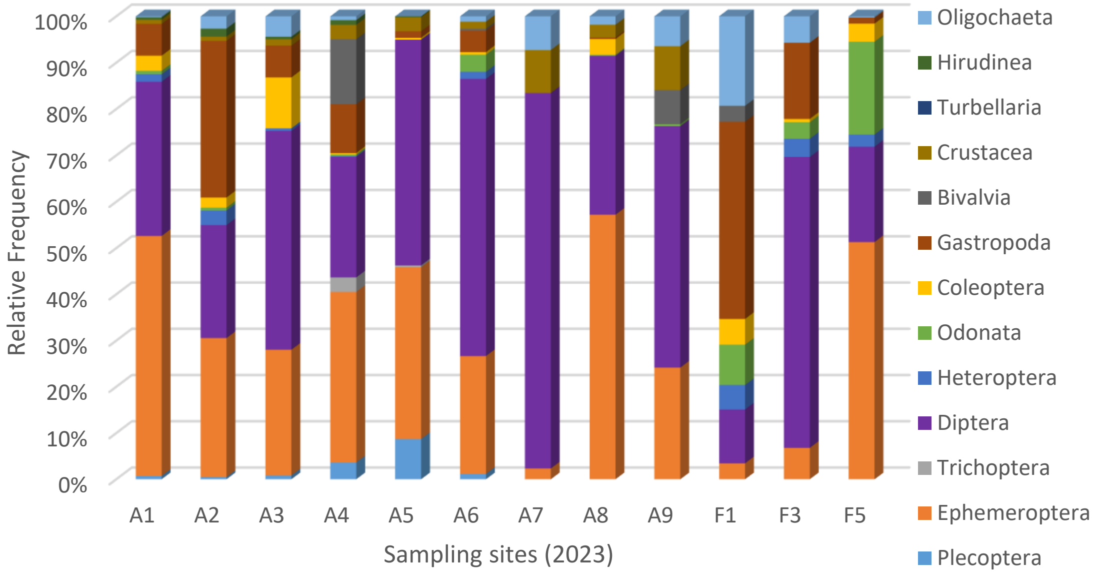
HMS sub-score	A1	A2	A3	A4	A5	A6	A7	A8	A9	F5
Outfall/deflector	0	0	0	0	0	0	0	0	0	0
Berms and embankments	0	0	0	0	0	0	0	0	0	0
Bridges	200	0	0	0	0	200	0	0	100	200
Culverts	0	0	0	0	0	0	0	0	0	0
Fords	0	200	0	0	0	0	0	0	0	0
Poaching	0	0	30	20	10	0	20	20	0	20
Reinforced Bank bed	220	540	0	0	280	80	0	0	90	90
Resectioned Bank bed	0	0	0	0	0	0	0	0	0	0
Weirs, dams, and sluices	900	375	0	900	375	0	375	0	0	300
HMS Score	1320	1115	30	920	665	280	395	20	190	610
HMS Class	4*	3*	2*	4*	4*	3*	3*	2*	2*	4*

(1: Pristine; 2: Predominantly unmodified; 3: Obviously modified; 4: Significantly modified; 5: Severely modified)

3.3. Macroinvertebrate communities – metrics

3.3.1. Faunal composition

The composition of macroinvertebrate communities of all sampling sites, in terms of relative abundance, can be consulted in the **Figure 3.7**, for both years.



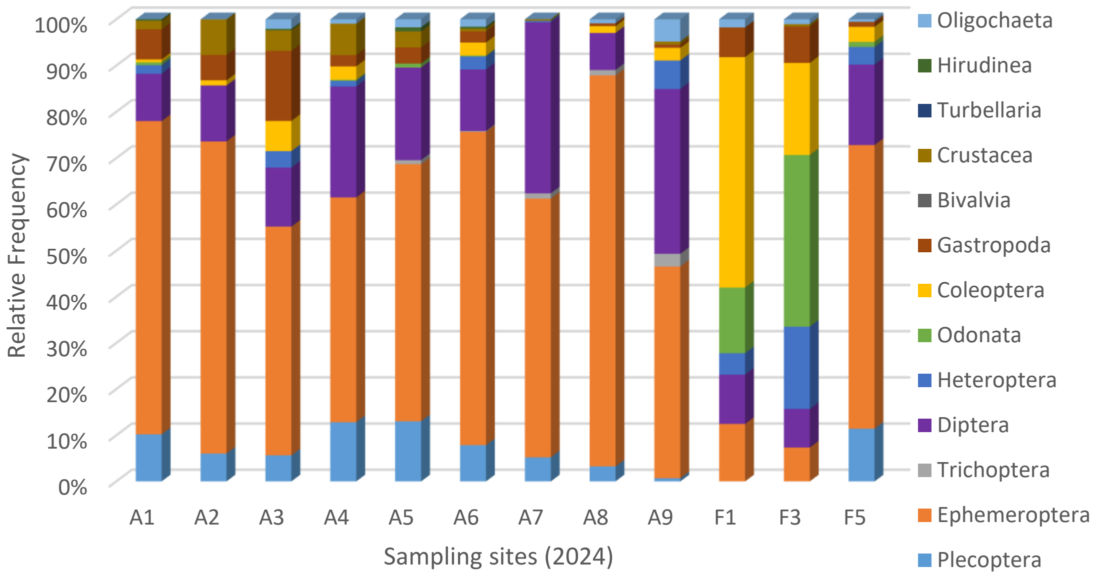


Figure 3.7. Composition of invertebrates in river Angueira basin (Spring 2023 and 2024).

In fact, it was observed a dominance of insects of Diptera (more visible in 2023) and Ephemeroptera (more evident in 2024) orders, along the Angueira sampling sites. In the Ferreiros stream (F1, F3, F5) a different composition was identified, showing other representative groups, like Odonata, Gastropoda, Heteroptera and Coleoptera.

A total of 9497 individuals of invertebrates were identified, belonging to 57 faunistic groups, mainly families. The variation of the total number of individuals (N) captured and distributed by the 4 typology groups can be visualized in **Figure 3.8**.

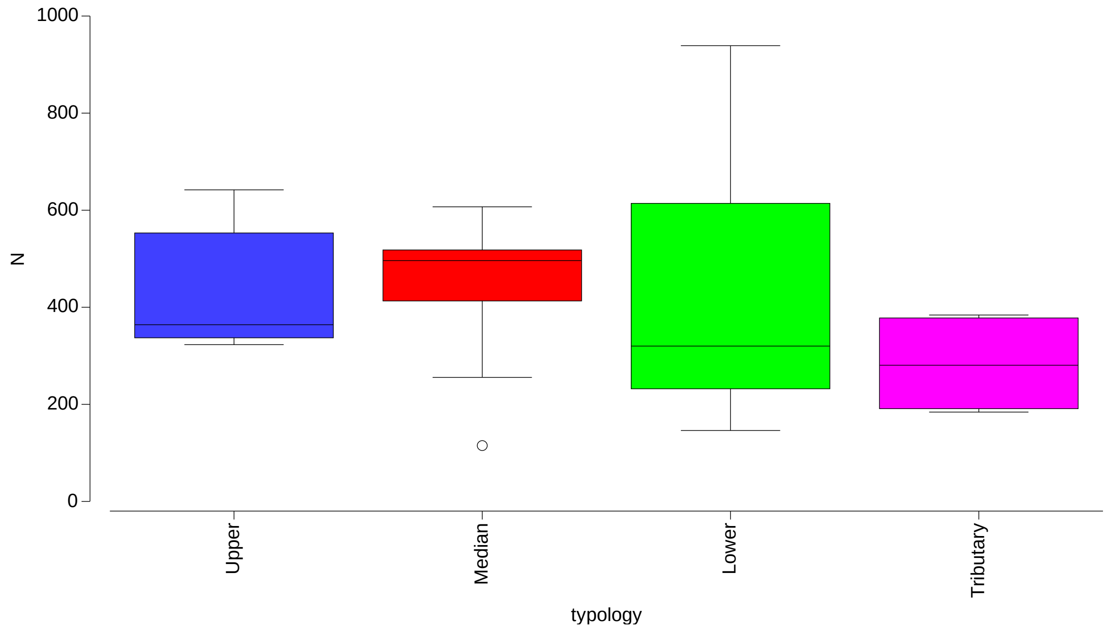


Figure 3.8. Number of individuals present in each typology group (Spring 2023 and 2024).

Relatively to the number of taxa (S) (**Figure 3.9**) it was observed higher values for upstream sampling sites belonging to the Upper (i.e., A1, A2, A3) and Median (A4, A5, A6) Angueira River, reflecting a better ecological condition of the watercourse.

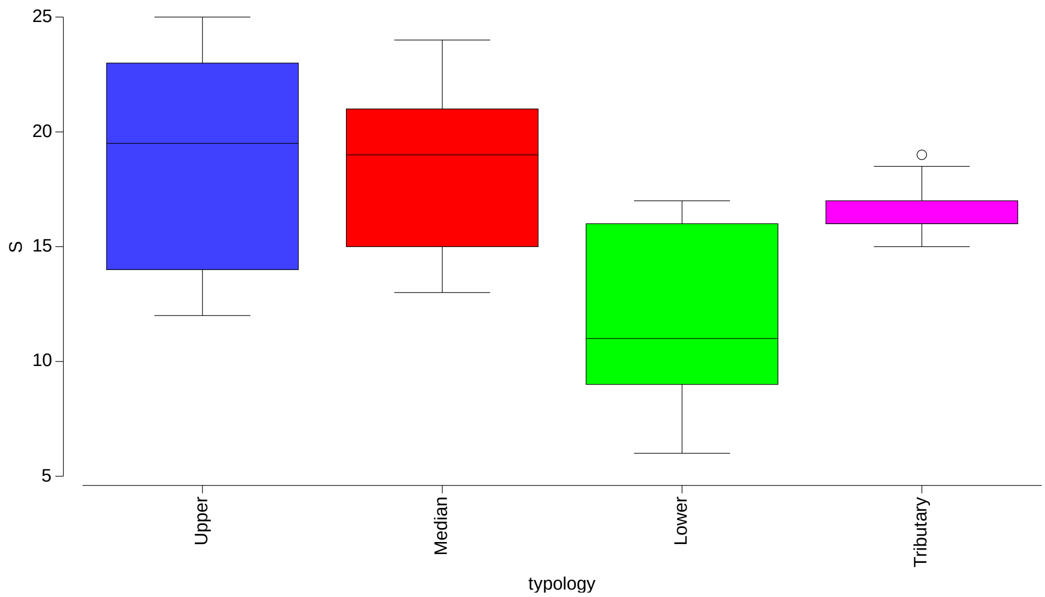


Figure 3.9. Total number of taxa present in each typology group (Spring 2023 and 2024).

Significant differences (Kruskal-Wallis test: H (3, N= 24) =7,407 $p<0.05$) were detected only for the total number of taxa (S) among the 4 typology groups considered.

3.3.2. Diversity (H') and Equitability (J') indexes

The variation of Shannon-Wienner diversity (H') and Pielou Equitability (J') can be observed in **Figure 3.10** and **Figure 3.11**.

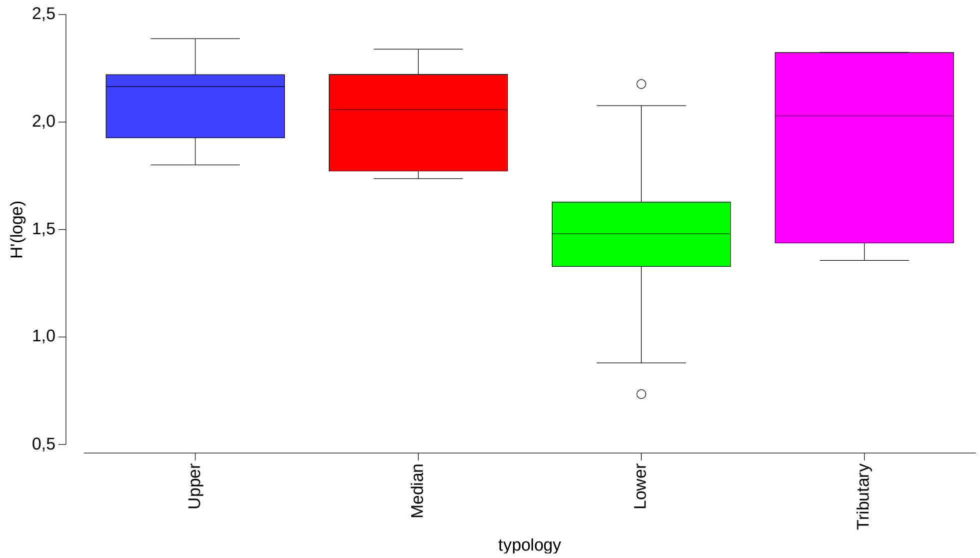


Figure 3.10. Variation of Shannon-Wienner diversity (H') index (Spring 2023 and 2024).

The lowest diversity, i.e., Shannon-Wienner H' index, was obtained the downstream zone (A7, A8, A9) of Angueira River contrasting with similar values found for the remaining typology groups. The same trend was observed for the Pielou J' evenness index among sampling sites, having the lower Angueira the more heterogeneous distribution of individuals in each sampling site.

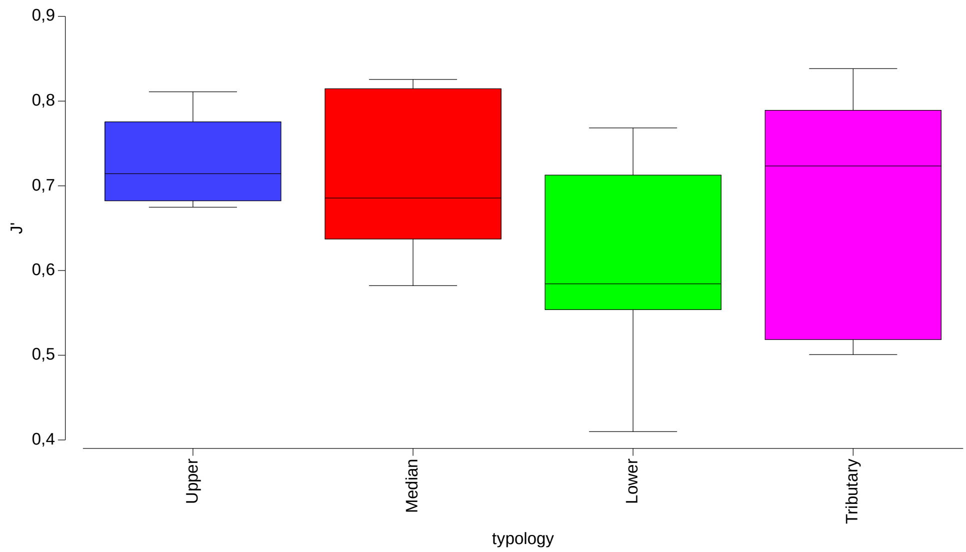


Figure 3.11. Variation of Pielou evenness (J') index (Spring 2023 and 2024).

Significant differences (Kruskal-Wallis test: H (3, N= 24) =6.667 p<0.05) were detected for the the diversity H' of Shannon-Wienner, among the 4 typology groups considered.

3.3.3. Functional Feeding Groups

The analyses of functional feeding groups can be done in the **Figure 3.12** and **Figure 3.13**. The same tendency was observed between years, with a dominance of collectors (mainly gathering collectors), except for the dominance of filter feeders in Lower Angueira, namely A6 and A7 sampling sites, and predators and parasites in the tributary, Ferreiros stream, in the F3 sampling site during the spring of 2024. Shredders were the less representative feeding group, since the input of organic materials and the respective response normally occur in the autumn season. Primary production was present; however the proportion of grazers and scrapers was not very significant.

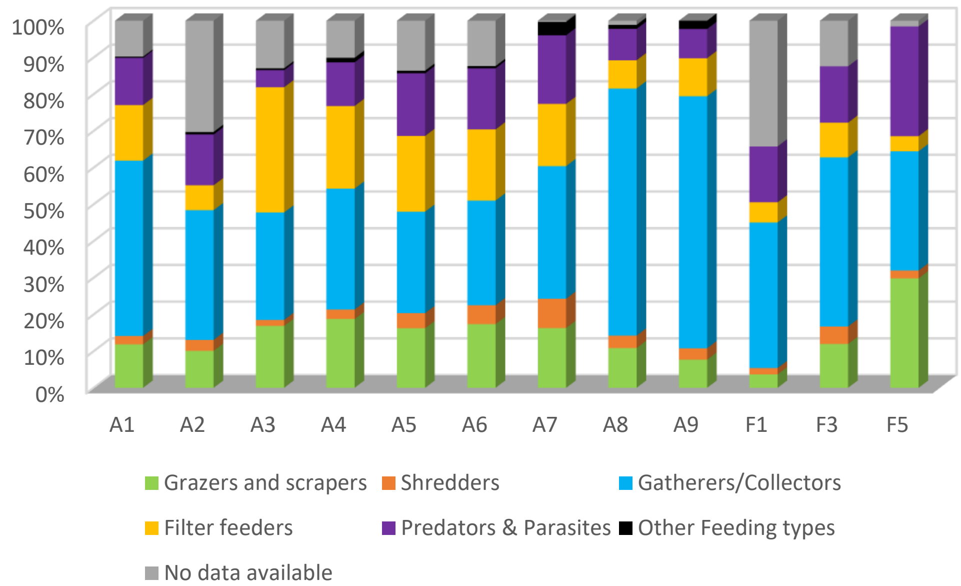


Figure 3.12. Functional feeding analysis in the Angueira River basin (Spring 2023).

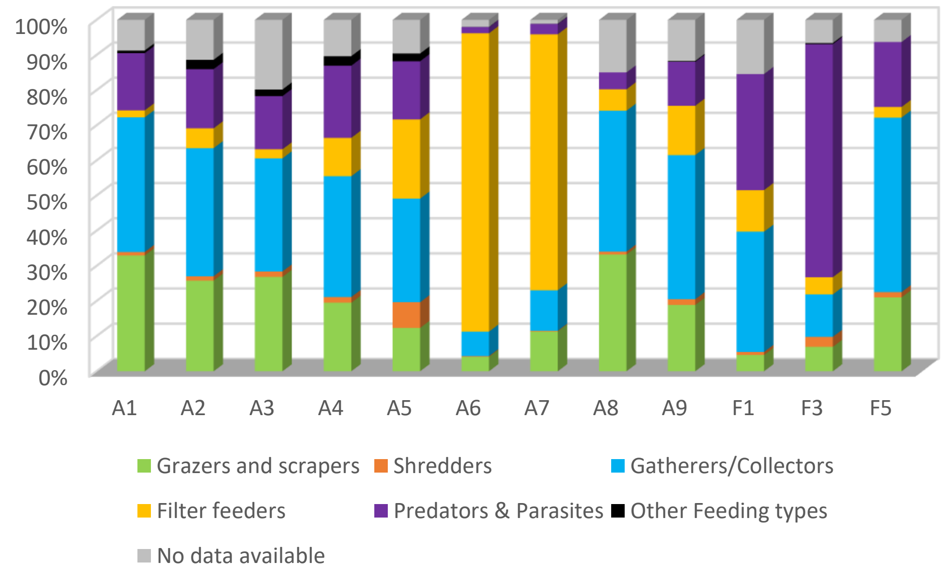


Figure 3.13. Functional feeding analysis in the Angueira River basin (Spring 2024).

3.3.4. IPtIN index

The results obtained for $IPtI_N$ index (**Table 3.3**) showed a better biological condition in the Upper and Median Angueira River. In fact, it was found a good status for both years, except for A1, during spring 2024. In the Lower Angueira and inclusively in the Ferreiros tributary, the environmental conditions (e.g. less flow) contributed to the evident decrease in the biological quality.

Table 3.3. Scores and classification of IPtIN for each sampling site (Spring 2023 and 2024).

Sampling site	$IPtI_N$ 2023		$IPtI_N$ 2024	
	Score	Classification	Score	Classification
A1	0.718	Good	0.590	Moderate
A2	0.737	Good	0.753	Good
A3	0.832	Good	0.723	Good
A4	0.761	Good	0.829	Good
A5	0.875	Good	0.753	Good
A6	0.728	Good	0.726	Good
A7	0.272	Poor	0.685	Moderate
A8	0.442	Moderate	0.822	Good
A9	0.470	Moderate	0.713	Good
F1	0.340	Poor	0.439	Poor
F3	0.383	Poor	0.417	Poor
F5	0.432	Moderate	0.612	Moderate

Other metrics, calculated by AMIIB software (**see Annex III**), corroborated with the ecological condition found in the sampling sites evaluated in the Angueira River basin.

3.3.5. Biotypology of macroinvertebrate communities

The PERMANOVA results, based on the macroinvertebrate communities of the sampling sites, indicated that significant differences were detected among the years (Pseudo-F=7.158, p=0.002), typologies (Pseudo-F=4.424, p=0.001) and inclusively related with the interaction (Pseudo-F=2.167, p=0.003) between both factors (**Table 3.4**), confirming the strong influence of climate interannual variation in a very dynamic ecosystems, from up to downstream zones, subjected to human influences (water pollution, river regulation).

Table 3.4. Results of the PERMANOVA analysis on invertebrate communities along river typologies and sampling years. The asterisks indicate significant values.

Source	dF	SS	MS	Pseudo-F	P (perm)	Unique perms
Year	1	6537	6537	7.158	0.001***	997
Typology	3	12388	4129	4.524	0.001***	998
Year *Typology	3	5938	1979	2.167	0.003**	999
Residual	16	14613	913			
Total	23	39475				

The nMDS analysis (2D, stress value of 0.14) showed a separation in river typologies, mainly between the tributary river, Ferreiros stream (e.g., Alto Douro small rivers, N3), and Angueira River, and between years, more visible between Northern median-large rivers (N1>100) and the two sampling years (**Figure 3.14**).

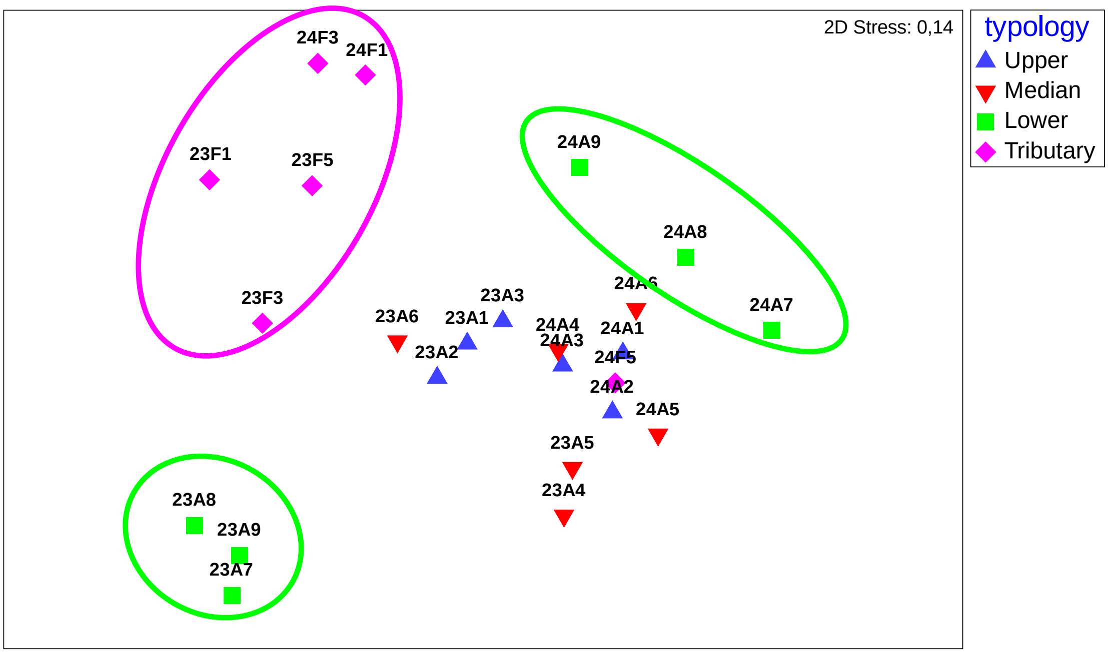


Figure 3.14. Non-metric multidimensional scaling (nMDS) of the 12 sampling sites and 2 sampling seasons, based on macroinvertebrate abundance, according to river typology groups (Spring 2023 and 2024).

The nMDS ordination (**Figure 3.15**) of the macroinvertebrate communities displayed differences in communities from the lower part of Angueira River, represented, for example, by more adapted to harsh environmental conditions, like Corbiculidae, Astacidae, and Planorbidae, in opposition to the Upper Angueira, represented by more sensible taxa to disturbance, like Trichoptera (Hydropsychidae, Rhyacophilidae), Plecoptera (Chloroperlidae), Odonata (Cordulegasteridae, Aeschnidae).

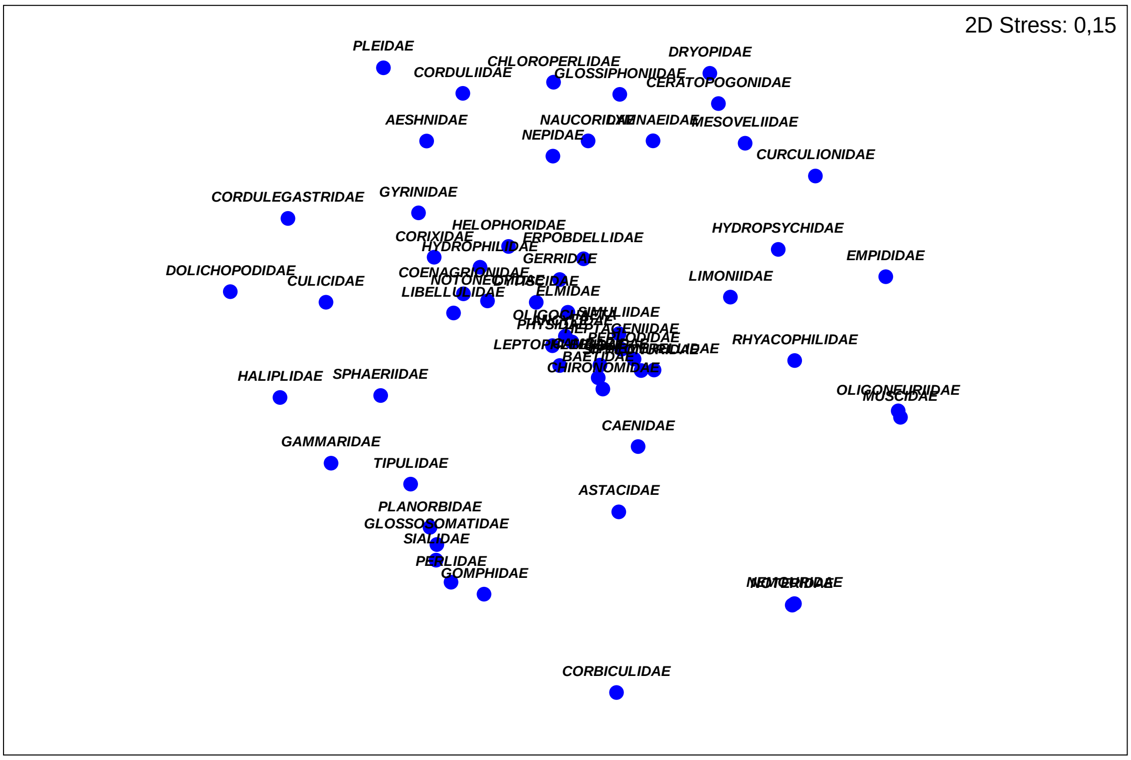


Figure 3.15. nMDS Ordination of macroinvertebrate communities of Angueira River basin (Spring 2023 and 2024).

The pairwise ANOSIM similarity (one-way) tests confirmed the observed tendency in the ordination and significant differences (P <0.01) were detected between sampling years (2023 vs. 2024) and between groups typologies, except between Upper vs. Median reaches of Angueira River.

The SIMPER analysis identified the taxa most contributing to dissimilarity between 1) Upper vs. Median groups (Average dissimilarity = 41.89%) were Chironomidae (8.46%), Ephemerellidae (7.54%), Simuliidae (6.82%), 2) between Upper vs. Lower Groups (60.79) Physidae (8.65%), Siphlonuridae (8.49%), Ephemerellidae (8.10%), 3) between Median vs. Lower groups (60.09%) were Ephemerellidae (9.92%), Chironomidae (8.76%), Siphlonuridae (8.03%), 4) between Upper vs Tributary (58.23%) were Siphlonuridae (7.95%), Baetidae (7.08%), Ephemerellidae (7.00%), 5) between Median vs. Tributary (62.82%) were Ephemerellidae (8.47%), Chironomidae (7.24%), Siphlonuridae (6.90%) and finally 6) between Lower and Tributary groups (69.31%) were Chironomidae (8.24%), Physidae (7.11%), and Caenidae (6.68%).

3.4. Fish Communities - Metrics

3.4.1. Composition and Abundance

Only six fish species were captured in the sampling sites of the Angueira River basin, being three native leuciscids (*Squalius carolitertii*, *Achondrostoma asturicense*, *Pseudochondrostoma duriense)* one cyprinid (*Luciobarbus bocagei*) and two non-native species, a centrarchid (*Lepomis gibbosus)* and a poecilid (*Gambusia holbrooki*) species.

The 4 native leuciscids and cyprinid fishes are endemic species of Iberian Peninsula and *Pseudochondrostoma duriense* and *Achondrostoma asturicense* are classified by Portuguese Red Book (Magalhães et al., 2023) as near threatened (NT) and endandered (EN) species, respectively. Pumpkinseed (*L. gibbosus)* and mosquito fish (*G. holbrooki*) are exotic and invasive species, widely dispersed in Douro basin, showing an ecological plasticity favouring their adaptation to harsh environmental river conditions.

A total of 1253 fishes were captured in the sampling sites and 2 seasons. In the upstream sampling sites (F1 and F3) of Ferreiros stream, fish was not detected. The distribution and abundance, in terms of CPUE (nº fish/30 minutes) of the fish species in the remaining sites of the River Angueira (A1 to A9) and Ferreiros stream (F5) can be observed in following figures, respectively **Figures 3.16** and **3.17.**

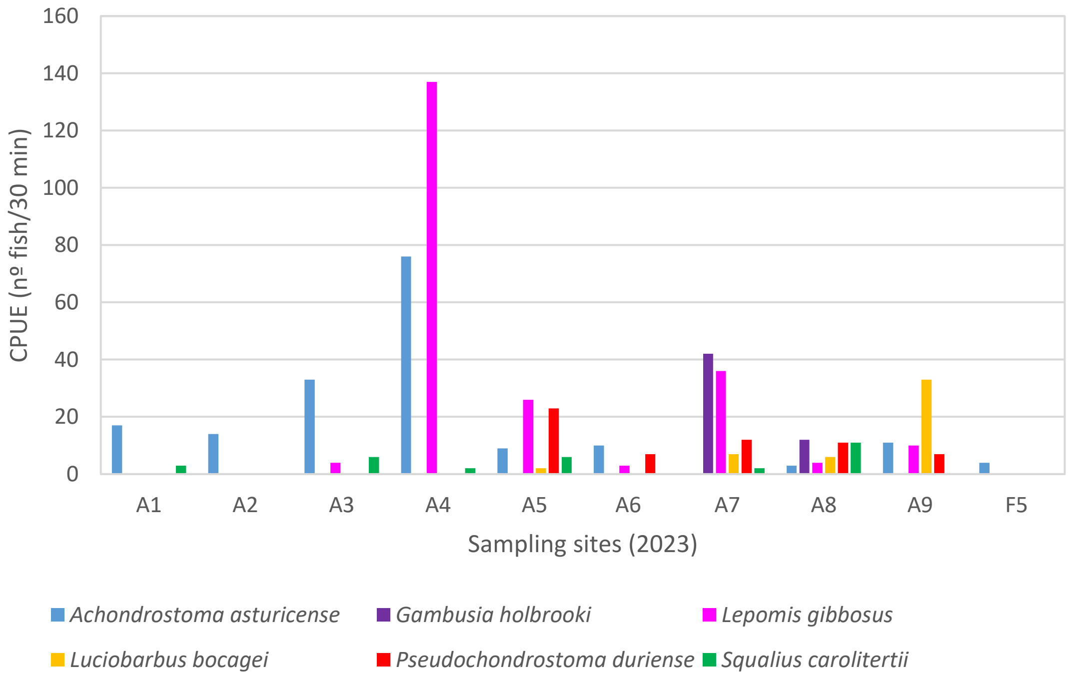


Figure 3.16. (CPUE) and distribution of fish in River Angueira (Spring 2023).

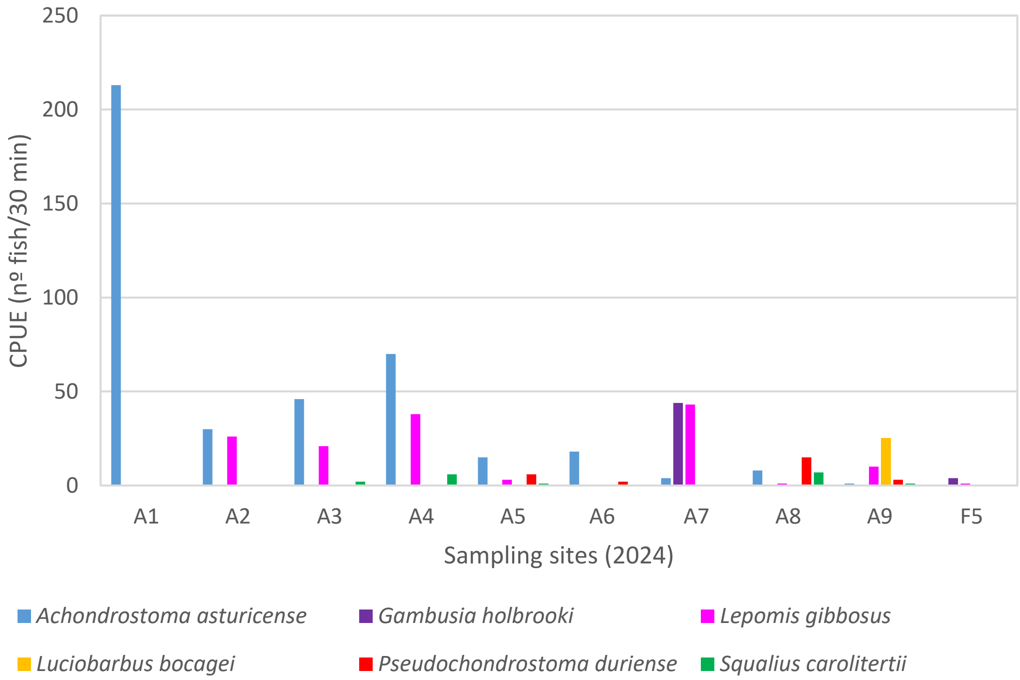


Figure 3.17. (CPUE) and distribution of fish in River Angueira (Spring 2024).

The relative abundance of species can be observed in **Figure 3.18** and **Figure 3.19**. Modified aquatic ecosystems, namely the reservoirs of weirs and the worst water quality in dry periods induced an increasing density and biomass of exotic species, changing drastically the composition and structure of the fish communities and aquatic ecosystems.

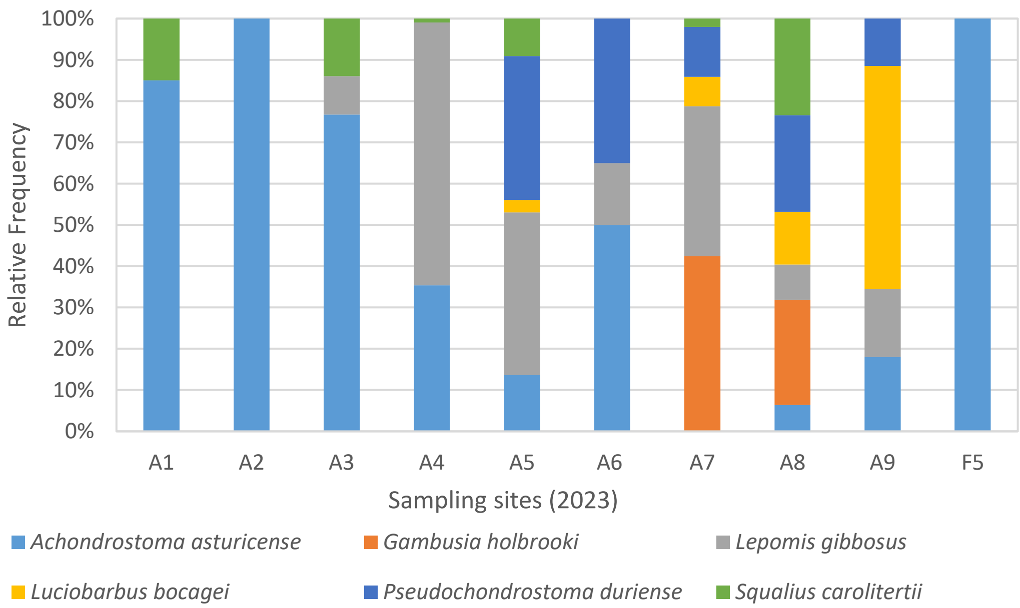


Figure 3.18. Relative abundance and distribution of fish species in River Angueira basin (Spring 2023).

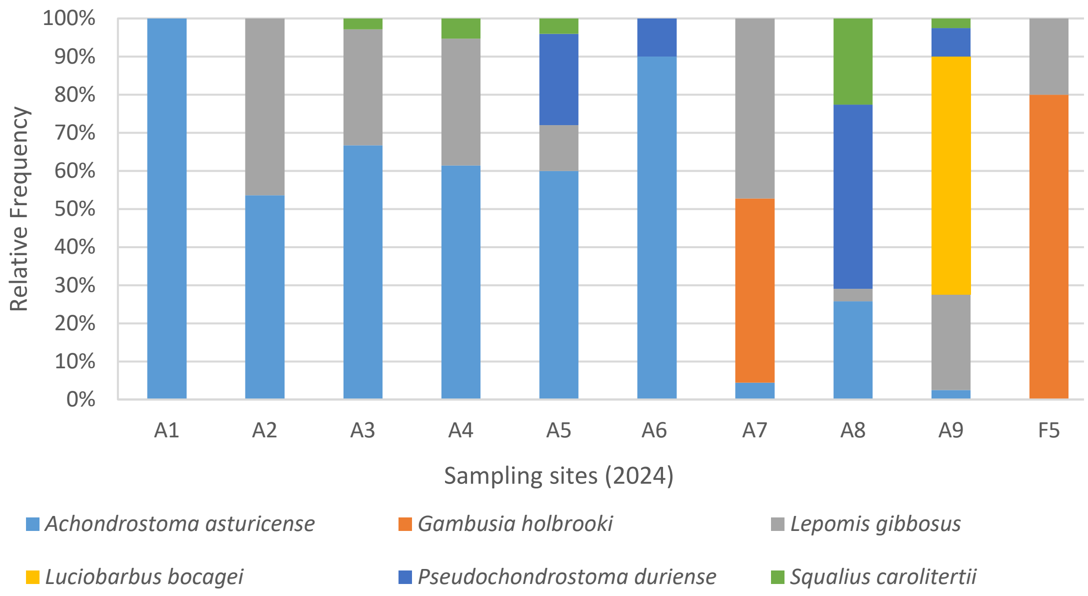


Figure 3.19. Relative abundance and distribution of fish species in River Angueira basin (Spring 2024).

The analysis can also be done in terms of the relative abundance of native vs. non-native species (**Figure 3.20**). Non-native species are increasing, year by year, their abundance and distribution along the longitudinal axis of the Angueira River, being almost exclusively dominant in several sampling sites, such is the case of A7 in more degraded environmental conditions (e.g. dams, pollution and riparian clearcut).

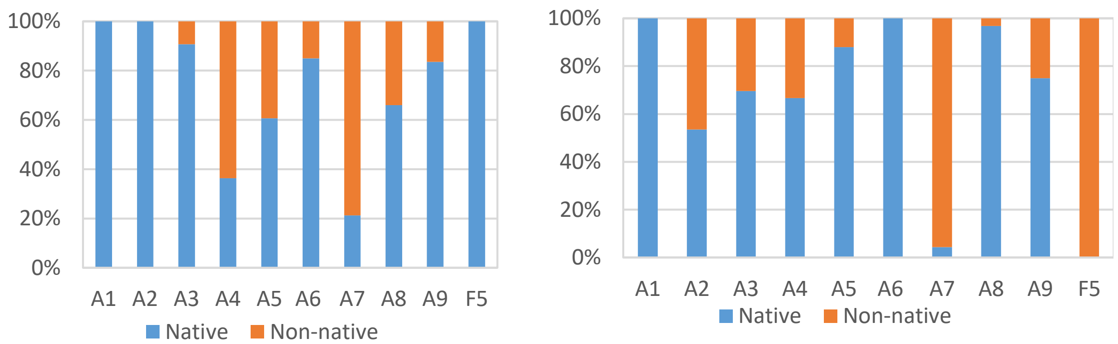


Figure 3.20. Relative abundance and distribution of native vs. non-native fish species in River Angueira basin (left, Spring 2023 ; right Spring 2024).

3.4.2. F-IBIP index

The evaluation of the biological quality of the fish fauna, based on the Biotic Integrity Fish Index for Wadeable Rivers of Portugal (F-IBIP), can be analyzed in **Table 3.5**. All sampling sites did not achieve the requirement of WFD for a good or excellent condition. In fact, the **Moderate to Bad condition** detected, is strongly correlated with the massive abundance of invasive alien species influencing the score and the F-IBIP classification.

Table 3.5. Scores and classification of F-IBIP for the sampling sites (Spring 2023 and 2024).

Sampling site	F-IBIP 2023		F-IBIP 2024	
	Score	Classification	Score	Classification
A1	0.667	Moderate	0.667	Moderate
A2	0.667	Moderate	0.188	Bad
A3	0.563	Moderate	0.262	Poor
A4	0.108	Bad	0.248	Poor
A5	0.220	Bad	0.533	Moderate
A6	0.500	Moderate	0.667	Moderate
A7	0.278	Poor	0.000	Bad
A8	0.389	Poor	0.444	Poor
A9	0.262	Poor	0.444	Poor
F5	0.667	Moderate	0.000	Bad

3.4.3. Biotypology of fish communities

The PERMANOVA results, based on the fish communities of the sampling sites, showed significant differences only among the 4 typologies (Pseudo-F=4.574, p=0.001) (**Table 3.6**).

Table 3.6. Results of the PERMANOVA analysis on fish communities along river typologies and sampling years. The asterisks indicate significant values.

Source	dF	SS	MS	Pseudo-F	P (perm)	Unique perms
Year	**1**	**2292**	**2292**	**2.337**	**0.075**	**999**
Typology	**3**	**13454**	**4485**	**4.574**	**0.001*****	**999**
Year *Typology	**3**	**5991**	**1997**	**2.037**	**0.058**	**998**
Residual	**12**	**11765**	**980**			
Total	**19**	**31809**				

The nMDS analysis (2D, stress value of 0.10) showed a separation in river typologies, mainly between small Alto Douro rivers (N3), represented by the tributary Ferreiros stream, and particularly between the Lower Angueira, representing the Alto Douro Median-Large rivers (N2) and the remaining sampling sites and groups (**Figure 3.21**).

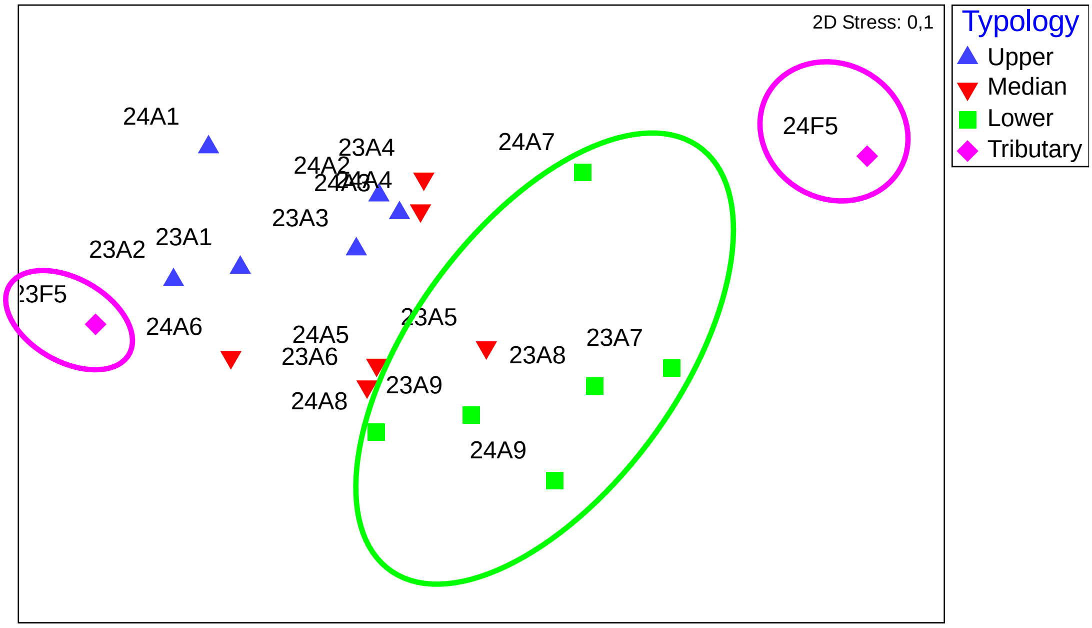


Figure 3.21. Non-metric multidimensional scaling (nMDS) of the 12 sampling sites based on fish abundance, according to river typology groups (Spring 2023 and 2024).

The nMDS ordination (**Figure 3.22**) of the fish communities showed visible differences in typical communities from the lower part of Angueira River, represented by more lentic species such as *Gambusia holbrooki* and *Luciobarbus bocagei*, in opposition to the Upper Angueira, represented by a more rheophilic species like *Achondrostoma asturicense* and *Squalius carolitertii*.

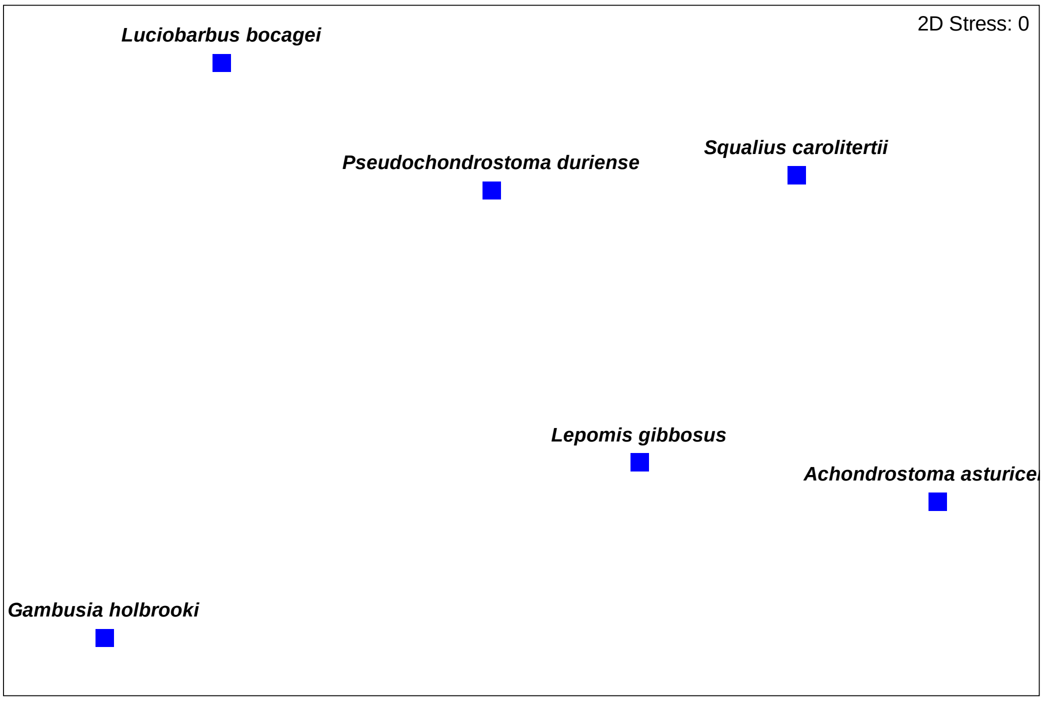


Figure 3.22. nMDS Ordination of fish communities of Angueira River (Spring 2023 and 2024).

Based on the pairwise ANOSIM similarity (one-way) tests no significant differences (P>0.01) were detected between sampling years (2023 vs. 2024). However, significant differences (P<0.01) were observed between all group's typologies, except the pairwise tests of Upper vs. Median and Median vs. Lower groups.

The SIMPER analysis identified the taxa most contributing to dissimilarity between 1) Upper vs. Median groups (Average dissimilarity = 40.44%) were *L. gibbosus* (35.56%), *P. duriense* (26.56%), *A. asturicense* (19.04%), 2) between Upper vs. Lower Groups (65.31%) *A. asturicense* (21.23%), *P. duriense* (18.27%), *L. gibbosus* (17.93%), 3) between Median vs. Lower groups (50.16%) were *L. bocagei* (19.21%), *A. asturicense* (19.15%), *L. gibbosus* (18.83%), 4) between Upper vs Tributary (58.23%) were *A. asturicense* (51.48%), *L. gibbosus* (21.24%), 5) between Median vs. Tributary (76.54%) were *A. asturicense* (31.67%), *L. gibbosus* (25.68%), and finally 6) between Lower and Tributary groups (78.09%) were *L. gibbosus* (21.69%), *P. duriense* (19.84%), and *L. bocagei* (18.58%).

4. DISCUSSION

The ecological evaluation of the water courses of Angueira River basin showed synergic negative impacts on water quality and biota caused mainly by water pollution, habitat fragmentation (e.g. weirs) and degradation (e.g. riparian clearcut) and the introduction of invasive alien species. The impact of these threats has been observed in different studies and sub-basins of Douro River, registering a global tendency to the decrease of biodiversity and ecological integrity, along the time (Escalda, 2004; Silva, 2010; Fonseca, 2011; Portela, 2014; Gomes, 2019; Cunha, 2019; Santos, 2023; Flores, 2023; Zidouh, 2024). All these threats are also well documented, at global scale, leading to the loss of habitats and biodiversity and ecological integrity of freshwater ecosystems and biotic homogenization (Dudgeon et al., 2006; Poff et al., 2007; Woodward et al., 2010; Vörösmarty et al., 2010; Simberloff et al., 2013; Early et al., 2016). Ultimately, these concerns have deserved special attention in the definition of emergency recovery plans and restoration projects (Carvalho et al., 2019; Lynch et al., 2023; ENCNB, 2018)

The pollution and eutrophication are major threats to the freshwater ecosystems, and chemical and microbiological contamination can be correlated with lower biological quality. Agriculture and rural and urban settlements has been responsible for substantial changes in water quality of different watercourses of Sabor River basin, including Angueira River, and substantial increase in dissolved salts (TDS, conductivity, EC > 120 µS/cm), organic matter (BDO_5 > 10 mg $O_2.L^{-1}$) and nutrient contents, such as nitrogen (Total N > 20 $mg.L^{-1}$) and phosphorous (Total P > 5 $mg.L^{-1}$) compounds (Santos et al., 2015, 2017; Zidouh, 2024). This environmental degradation was observed in the Angueira River basin. In fact, in this study, electrical conductivity and total dissolved salts (TDS) were higher in the upper Angueira, probably due to a deficient treatment of the wastewater treatment plant (WWTP) from the Alcañices, located near the border. The same tendency was detected in the different sampling sites of Ferreiros stream, likely due to inputs of dissolved substances, such as pollutants, fertilizers, or rural runoff from surrounding land use. However, the median and lower section showed a slightly lower conductivity and dissolved salts, compared to the Upper section. This could be due to local water dynamics such as dilution from tributaries or less direct impact from anthropogenic sources. Several studies showed that pollution, agricultural runoff, and urbanization, can contribute to the increased levels of dissolved substances and conductivity which leads to eutrophication of aquatic ecosystems (Florescu et al., 2010), threatening biodiversity and interfere with the ecosystem's natural balance (Grizzetti et al., 2017). For these reasons, the European Union's WFD highlights the need to control and maintain the chemical and physical quality of freshwater systems across the river typologies, and the definition of management strategies to achieve the good/excellent ecological status (Directive, 2000; Filipe et al., 2019).

The hydromorphological evaluation allowed to identify other important pressures to the channel and riparian habitats of the watercourses of Angueira River. Both RHS indexes, namely HQA and HMS were sensible and several hydromorphological variables (e.g. bridges, culverts, fords, poaching, weirs, small dams) responsible for the lower aquatic habitat quality. In fact, several weirs are distributed along the longitudinal axis, changing the natural riffle/pool succession and affecting the composition and structure of the flora and fauna. In Portugal, more than 8000 small barriers are referenced (Ordeix et al., 2018) and in the Douro River basin, more than 1000 barriers (Cortes et al., 2017). The loss of river connectivity due to the flow regulation and water retention, and the fragmentation and destruction of habitats leading to the biodiversity loss is focused by several authors (Aarts et al., 2003; Goslan et al., 2019; Reid et al., 2019). On the other hand, the riparian degradation and discontinuity was also observed in Angueira basin due to clearcut and alder tree disease, caused by the pathogen *Phythophotora* x*alni* species complex fungi, detected during 1990s in several European regions (Bjelke et al., 2016). Black alder (*Alnus glutinosa*) is one of the most important species in the Mediterranean rivers, restricted to specific habitat conditions (permanent access to water) (Rodriguez-González et al., 2014). This species is dominant in priority classified European Habitats, (91E0*, 92/43/CEE), and is classified as a target species for conservation (Claessens et al., 2010). In fact, the alder disease and the predicted trend for Mediterranean climate will increase aridity threatening the riparian habitats of Iberia (Lorenzo-Lacruz et al., 2012; González et al., 2012).

The decline of biodiversity and ecological quality was detected in several zones of Angueira River basin. Several metrics calculated for invertebrate (e.g. Shannon-Wienner H' and evenness J' indexes, % EPT, trophic variation) and fish (F-IBIP index, % of exotic species) communities, contributed to highlight differences among sampling sites, subjected to distinct environmental conditions. Taking into consideration the WFD river classification, most of sampling sites did not achieve the good or excellent ecological status. This tendency was markedly confirmed in previous studies, namely based in comparisons along time (e.g. Fidalgo, 1998; Escalda, 2004; Teixeira et al., 2008; Silva, 2010; Fonseca, 2011; Sarmento, 2013; Portela, 2014; Gomes, 2019; Cunha, 2019; Santos, 2023; Flores, 2023; Zidouh, 2014). However, the presence of endangered native fish species (e.g. *A. asturicense*) and priority habitats (91E0*, 92/43/CEE) justify the development of action plans for the conservation or restoration of Angueira River basin.

Portuguese freshwater ecosystems provide a wide variety of ecosystem services including water for human consumption, irrigation, energy production, flood protection and biodiversity (Feio & Ferreira, 2019). Thus, the WFD and other legislation is crucial to protection, sustainable use of water and restoration of rivers in Europe (González del Tánago et al., 2012; Filipe et al., 2019). The biological assessment of inland surface waters under climate change, the effects of invasion by alien species, and the emerging tools and approaches to improve biological assessments (e.g. environmental DNA, eDNA) must be considered to assure the high quality of water and well-being for the society and the conservation of the biodiversity (Postel & Ritcher, 2003; Cardoso & Free, 2008; Filipe et al., 2019).

5. CONCLUSIONS AND FINAL CONSIDERATIONS

The main conclusions of this study can be summarized in the following topics:

- ***Physical and chemical quality of surface water***

The results obtained for **water quality** revealed significant differences between the Angueira and Ribeira de Ferreiros river sectors. In fact, higher dissolved oxygen values were recorded in the upstream (A1 to A3) and middle (A4 to A6) sections of the Angueira River while for conductivity, total acidity and total dissolved solids, higher values were obtained in the downstream section of the Angueira River (A7 to A9) and in the sampling sites of the Ferreiros stream (F1 to F5). These results are clearly illustrated on the Distance-based redundancy analyses (dbRDA). However, they must be analyzed cautiously as it is possible to find more limiting values in the summer season. On the other hand, as indicated by the WFD, at least one annual cycle with seasonal measurements must be carried out to understand, in detail, the critical factors for aquatic and riparian fauna and flora, particularly for stenobiont species, most of them with rheophilic habits. Finally, the determination of nitrogen and phosphorus dynamics will add important information to evaluate the eutrophication phenomena in the Angueira River basin.

- ***Hydromorphological quality: aquatic and riparian habitats***

The **quality of *aquatic and riparian habitats*** of the Angueira River showed, based on River Habitat Survey (RHS) indexes, i.e. HQA and HMS, signs of degradation namely in the dammed river reaches. In fact, the homogenization of environmental conditions in the channel, with strong sedimentation in the river bottom, clogging the substrate interstices, increased depth and decreased water current velocity, contributed to the detected decline in the biodiversity. On the other hand, the riparian clearcut (e.g. clearly visible in A4, near S. Joanico village) and other signs of their degradation (e.g. alder tree disease) contributed to the substantial loss in river shading, nutrient input and habitat for refuge for aquatic and semi-aquatic species.

- ***Biodiversity and biological quality based on the invertebrate community***

Several metrics calculated to the **benthic macroinvertebrate community** showed to have greater biodiversity (e.g. Shannon-Wienner diversity index H', and Pielou evenness

J') and biological quality (e.g. biotic indices IptIN and IBMWP, % EPT) in the upper (i.e. A1 to A3) and medium (A4 to A6) sectors, when compared with the final stretch of the Angueira River (i.e., A7 to A9). Multivariate analysis (i.e. nMDS ordination) allowed to confirm this trend and highlight the differentiation of the invertebrate community present in the Ferreiros stream. The trophic assessment confirmed the dominance of collectors, specializing in the energy use of FPOM (i.e. fine particulate organic material) particularly in the downstream sector of the Angueira River. The different hydrological conditions between years, drier and with lower flow in 2023 and wetter and continuous flow in 2024, led to a dominance of gatherers and filters, respectively. The low percentage of shredders along the river network was noted, related to the degradation of riparian galleries and notable primary productivity with biotic response at the level of grazers and scrapers, because of less shading, greater nutrient content and solar penetration in the aquatic system. Highlights include also the dominance of Diptera (Chironomidae, Simuliidae), Ephemeroptera (Siphlonuridae, Ephemerellidae, Baetidae, Caenidae), Gastropoda (Physidae, Planorbidae) and the high percentage of invasive species, such as *Procambarus clarkii, Pacifastacus leniusculus* and *Corbicula fluminea*.

- ***Biodiversity and biological quality based on the fish community***

Relatively to the **fish community**, a bad biological quality was obtained (e.g. moderate to bad classification of F-IBIP, lower values of Shannon-Wienner diversity H’, higher percentage of exotic species, among others). Successive periods of prolonged drought and the high level of river regulation (i.e. numerous weirs and small dams) are contributing to the massive abundance of exotic species namely *Lepomis gibbosus* and *Gambusia holbrooki* (e.g. Lower Angueira, A7). It is worth highlighting the presence 4 native leuciscids and cyprinid fishes, endemic species of Iberian Peninsula, being two of them, i.e. *Pseudochondrostoma duriense* and *Achondrostoma asturicense*, classified by Portuguese Red Book (Magalhães et al., 2023) as near threatened (NT) and endandered (EN) species, respectively. Multivariate analysis (i.e. nMDS ordination) allowed also to highlight the differentiation lower Angueira (A7 to A9) and Ferreiros stream fish community relatively to the remain communities present in the upper and median sectors of Angueira River.

- ***Main threats to biodiversity and ecological quality in the Angueira River***

Several threats synergistically contributed to the composition and structuring of invertebrate and fish communities and the ecological quality of watercourses in the Angueira River basin. **Water pollution** (e.g., dissolved oxygen, conductivity, dissolved salt content and total acidity), **hydromorphological quality** (e.g., HQA and HMS indexes from the River Habitat Survey methodology, with emphasis on the presence of weirs, typology of vegetation in riparian galleries), are the major detected threats, increased by **interannual hydrological variability** in recent years, due to climate change (e.g. higher frequency of occurrence of extreme periods of drought), particularly in the summer and autumn seasons. Also noteworthy is the presence and increasing **abundance of exotic and invasive species (EEI)**, such as the Louisiana red crayfish *Procambarus clarkii*, the signal crayfish *Pacifastacus leniusculus*, the Asian clam *Corbicula fluminea*, the mosquito fish *Gambusia holbrooki* and other exotic species such as pumpkinseed *Lepomis gibbosus*.

FINAL CONSIDERATIONS

The detected threats in the Angueira River basin justify the implementation of priority measures to safeguard and/or recover biodiversity and ecological quality of aquatic and riparian ecosystems and associated ecosystem services. In this sense, it is important to consider the application of several measures:

- **Ecological restoration and/or rehabilitation:** use of natural engineering techniques, for the restoration or rehabilitation of several stretches of the Angueira River where the ecological integrity has been diminished. In addition to riparian buffer restoration, it is essential to ensure the permeability of physical barriers (e.g. weirs), establishing river connectivity, and allowing the mobility of native species, especially during reproductive migration.

- **Elimination of localized pollution hotspots from urban and rural settlements** and **mitigation of potential diffuse pollution** (e.g. fertilizers and pesticides from agriculture). Promotion of **good agricultural and forestry practices** to reduce the occurrence of rural fires (common in forested areas, as recently happened in São Martinho de Angueira) and avoid soil loss through excessive exposure and mobilization, vulnerable to intense rainfall, increasing in the region with subsequent impacts on river sedimentation.

• **Control/eradication of invasive exotic species:** establishment of an action plan to combat the problem of abundance and dispersion of exotic and invasive species (IAS), such as *Pacifastacus leniusculus, Procambarus clarkii, Corbicula fluminea, Gambusia holbrooki,* detected in this study, but also other species present such as *Neovison vison*. Pay special attention to other abundant exotic species, such as *Lepomis gibbosus* and other non-native species present in neighboring areas (i.e. Maçãs and Sabor Rivers), such as *Alburnus alburnus, Micropterus salmoides, Rutilus rutilus, Sander lucioperca, Esox lucius*, capable of causing even more negative impacts on the biological quality of the Angueira River, particularly in priority habitats with endangered native species.

• **Adequate river management** promotion: through sustainable fish conservation and exploitation plans. **Identification of priority habitats for threatened native species** and establish differentiated management plans aimed at their conservation.

• **Continuous monitoring assessment of aquatic and riparian ecosystems:** Climate change, at least, justifies the need to continue collecting biological and environmental data to obtain in-depth knowledge of biotic variations and responses to such pressures and to understand the dynamics of the invasive alien species (IAS) and changes in the functioning of ecosystems to implement appropriate management measures.

• **Promotion of environmental awareness and public participation:** aiming to contribute to the development of the region, enhancing the associated ecosystem services, such as: 1) support (biodiversity, habitats, soil), provision (water, fish, wood), regulation (insects for pollination, carbon sequestration, air and water purification) and cultural (promotion of associated leisure and educational activities, for example, the creation of River Parks and Recreational and Sport Fishing Zones) services.

• **Establishment of technical and scientific landmarks in the territory:** working towards the conservation of the region's natural values. Establishment of closer interfaces in the specific management of basins shared with Spain, not only between the national/regional authorities (e.g. ICNF, APA, CIM-TTM, Junta Castilla León, Ministries of the Environment and Agriculture), but also with local authorities (e.g. Municipalities of Vimioso, Bragança, Alcañices, Zamora), Academy and research centers (e.g. Polytechnic University of Bragança, University of Valladolid, CIMO/SusTEC), with NGOs installed in the territory (e.g. AEPGA, PALOMBAR) and other entities (e.g. Meseta

Ibérica Transboundary Biosphere Reserve). It is crucial the definition of strategies, considering all stakeholders, and access to financing sources for water management and conservation and restoration of aquatic and riparian ecosystems in the NE region of Portugal.

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ANNEXES

Annex I. Portfolio of images of the main sampling sites of the study (A1 to A12)

Figure A1. Sampling site A1 in River Angueira (near São Martinho de Angueira).

Figure A2. Sampling site A2 in River Angueira (near Angueira).

Figure A3. Sampling site A3 in River Angueira (near Serapicos).

Figure A4. Sampling site A4 in River Angueira (near São Joanico).

Figure A5. Sampling site A5 in River Angueira (Vimioso, Termas da Terronha).

Figure A6. Sampling site A6 in River Angueira (near Vila Chã).

Figure A7. Sampling site A7 in River Angueira (near Uva).

Figure A8. Sampling site A8 in River Angueira (confluence Tortulhas stream, near Mora).

Figure A9. Sampling site A9 in River Angueira (near Algoso).

Figure A10. Sampling site F1 in Ferreiros stream (near Vilar Seco).

Figure A11. Sampling site F3 in Ferreiros stream (near Silva).

Figure A12. Sampling site F5 in Ferreiros stream (near Vila Chã).

Annex II – Physical and chemical water conditions in Angueira basin (spring 2023 and 2024)

Table AII.1 - Water quality of sampling sites in Angueira River and Ferreiros stream (Spring 2023 and 2024), and their classification, considering the maximum threshold for the establishment of Good Ecological Status *(Blue: Excellent; Green: Good; Yellow: Less than or reasonable ecological status*) in Northern rivers of Portugal (APA, 2021)

Site	Year	Temperature (ºC)	Conductivity (µS/cm)	DO (mg/L)	% DO	pH	TDS (mg/L)	Acidity (mg/L)	Oxidability (mg O_2/L)
A1	2023	17.2	128.8	7.29	80.7	7.55	72.1	7.8	11.7
A2	2023	17.8	101.6	8.51	97.5	7.36	57.3	8.99	8.4
A3	2023	17.5	99	8.56	97.2	7.57	55	9.4	13.75
A4	2023	17.0	91.3	9.29	100.5	7.23	53.1	9.8	17.7
A5	2023	19.0	89.9	8.37	96.3	7.2	48.3	9.5	18.1
A6	2023	19.4	94.1	8.67	100.3	7.44	50	9.9	11
A7	2023	20.8	111.3	8.79	104.4	7.22	57.8	9.49	8.7
A8	2023	20.4	117.3	7.54	88.5	6.99	61.3	9.8	19.25
A9	2023	22.6	129.0	9.22	111.1	7.27	64.5	9.12	19.5
F1	2023	17.9	222.0	8.47	92.9	7.08	123.6	10.5	11.7
F3	2023	20.1	95.4	8.08	90.9	7.36	49.9	12	11.09
F5	2023	19.6	305.0	7.98	92.7	7.51	164.4	12.12	11.35
A1	2024	13.5	117.6	8.3	80.7	7.66	58.2	7.4	8.7
A2	2024	14.0	96.5	9.51	107.5	7.19	37.4	7.99	6.4
A3	2024	14.1	92.2	9.56	107.2	7.28	40	8.4	12.75
A4	2024	14	79.6	9.6	80.5	7.09	42.3	9.1	17.7
A5	2024	13.9	75.5	8.7	76.3	7.26	39.8	9.59	18.1
A6	2024	15.0	73.8	8.7	80.3	7.38	40.2	9.91	9.0
A7	2024	15.3	83.9	8.8	84.4	7.37	50.1	10.49	5.7
A8	2024	15.9	86.5	8.54	81.5	7.56	52.3	10.8	18.25
A9	2024	16.0	95.8	8.22	91.1	7.53	60.4	11.12	16.5
F1	2024	14.5	122.8	7.47	82.9	7.1	68.6	12.5	12.7
F3	2024	14.6	191.6	7.08	80.9	7.92	82.5	14	9.09
F5	2024	14.6	190.9	7.7	82.7	7.36	86.9	14.12	9.35

Annex III. Amiib metrics

Table AIII.1 – AMIIB metrics for sampling sites in Angueira River basin (Spring 2023)

Site	A1	A2	A3	A4	A5	A6	A7	A8	A9	F1	F3	F5
Total Taxa	25	23	20	21	15	21	6	11	9	16	15	16
Nº Individuals	553	642	364	607	495	518	302	939	232	378	280	384
ASPT	6.09	5.65	6.16	6.06	6.77	5.58	4.4	5.44	5.71	4.53	5	5.44
IBMWP	125	110	107	117	90	97	19	50	37	58	63	75
IBMWP taxa	24	22	19	20	14	20	5	10	7	16	15	16
IASPT	5.21	5	5.63	5.85	6.43	4.85	3.8	5	5.29	3.63	4.2	4.69
H'	2.17	2.22	2.16	2.22	1.74	1.77	0.73	1.39	1.57	1.89	1.36	1.44
J'	0.67	0.71	0.72	0.73	0.64	0.58	0.41	0.58	0.71	0.68	0.5	0.52
EPT Taxa	8	6	7	7	7	4	2	3	3	1	2	3
Nº taxa - EPT	291	196	102	265	229	138	7	537	56	13	19	197
% Ind. EPT	52.62	30.53	28.02	43.66	46.26	26.64	2.32	57.19	24.14	3.44	6.79	51.3

Table AIII.2 – AMIIB metrics for sampling sites in Angueira River basin (Spring 2024)

Site	A1	A2	A3	A4	A5	A6	A7	A8	A9	F1	F3	F5
Total Taxa	14	12	19	17	13	24	11	16	17	19	16	17
Nº Individuals	323	364	337	413	115	497	614	338	146	281	191	184
ASPT	6.23	6.7	5.41	6.27	6	5.43	6.44	6.31	6.21	5.22	4.87	6.07
IBMWP	74	65	84	92	69	106	57	89	85	81	65	88
IBMWP taxa	13	11	18	16	12	23	10	16	16	19	15	17
IASPT	5.69	5.91	4.67	5.75	5.75	4.61	5.7	5.56	5.31	4.26	4.33	5.18
H'	1.8	1.93	2.39	2.34	2.09	2.02	1.33	1.63	2.18	2.32	2.32	2.17
J'	0.68	0.78	0.81	0.83	0.81	0.64	0.55	0.59	0.77	0.79	0.84	0.77
EPT Taxa	5	6	5	6	6	6	6	9	7	3	2	6
Nº taxa - EPT	252	268	186	254	80	377	383	301	72	35	14	134
% Ind. EPT	78.02	73.63	55.19	61.5	69.57	75.86	62.38	89.05	49.32	12.46	7.33	72.83