Ecological assessment of Sabor River (Douro Basin, NE Portugal): Typology, threats, and implications for the aquatic fauna conservation

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Dedicated to my parents: for their sacrifice, infinite love, and unwavering commitment providing me the education they never had a chance to have. Thank you.

(Ai miei genitori: per il loro sacrificio, amore infinito, e l'importante supporto e sostegno nel darmi l'educazione che non hanno mai avuto modo di avere. Grazie.)

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Resumo

A bacia hidrográfica do Rio Sabor localiza-se no Nordeste de Portugal, na margem direita do Rio Douro, e abrange uma elevada variabilidade ambiental, refletida em diferentes tipologias, onde sobressaem várias espécies endémicas e ameaçadas, habitats singulares e ecossistemas de elevada complexidade e vulnerabilidade. Com efeito, são várias as ameaças, maioritariamente de origem antrópica, que têm vindo a afetar a integridade ecológica na bacia do rio Sabor, com destaque para a poluição e eutrofização da água, a degradação e fragmentação de habitats aquáticos e ribeirinhos, a presença de barreiras de pequena e grande dimensão com regularização de caudais, a introdução de espécies exóticas e ainda as alterações climáticas, nomeadamente a frequência de secas prolongadas e de períodos de curta duração de precipitação intensa, capazes de fomentar a perda de habitats e da biodiversidade.

O principal objetivo do presente estudo foi avaliar a integridade ecológica em 43 locais de amostragem, no verão de 2023, distribuídos pelo rio principal (Rio Sabor) e por diversos afluentes de maior e menor dimensão (e.g. rios Maçãs, Fervença, Angueira, Azibo, Penacal, Vale Moinhos e ribeiras da Vilariça, Veados, Onor, Igrejas, Baçal, Ferreiros, Inferno e Zacarias). Foram consideradas as metodologias e tipologias definidas pela Agência Portuguesa do Ambiente (APA), no âmbito da Diretiva Quadro da Água (DQA), para avaliação dos elementos 1) biológico - macroinvertebrados e peixes; 2) físico-químicos da água; e 3) hidromorfológicos.

Os resultados obtidos permitiram detetar diferenças significativas (testes H Kruskall-Wallis, $P < 0.05$), para alguns parâmetros físico-químicos da água, nomeadamente temperatura, pH e dureza total, considerando as tipologias definidas. Contudo, a variação natural (e.g. longitudinal) aparece condicionada pela perturbação detetada em muitos locais. É o caso do rio Fervença, a jusante da cidade de Bragança (e.g. Fv_04 e Fv_05), onde a qualidade diminuiu drasticamente, tendo sido registados valores elevados de condutividade e de compostos azotados e fosfatados, em contraste com a forte diminuição de oxigénio dissolvido. No que respeita aos habitats aquáticos e ribeirinhos, os índices do River Habitat Survey (RHS) permitiram assinalar alguns locais onde a degradação aquática e ribeirinha (e.g. Sa_01, Sa_07) é evidente. Similarmente, foram encontradas diferenças significativas (PERMANOVA, 2-way testes, $p < 0.05$) para o *biota*, nomeadamente nas comunidades de invertebrados e de peixes, entre as tipologias e perturbações identificadas. A análise multivariada (ordenações nMDS) permitiu diferenciar, as comunidades de invertebrados com predomínio de Gastropoda, Heteroptera e Diptera e espécies exóticas como o lagostim-sinal, o lagostim-vermelho da Louisiana e a ameijoa-asiática. Também na comunidade piscícola esta análise evidenciou um crescente domínio de exóticas (e.g. perca-sol, alburno, achigã, lucioperca) mais comuns em locais mais degradados, particularmente sob efeito da poluição (e.g. Rio Fervença) e regularização de caudais (e.g. barragem do Baixo Sabor), em detrimento das espécies reófilas nativas (e.g. truta-de-rio, boga-do-norte, bordalo, panjorca-do-esla), com maior abundância em locais de boa e excelente integridade ecológica.

A análise dos resultados abióticos e bióticos obtidos para os cursos de água da bacia do rio Sabor, realça a necessidade de continuar a aplicar medidas que, perante um quadro de degradação ambiental e transformação evidente nos ecossistemas aquáticos e ribeirinhos, contemplem ações que integrem a mitigação/minimização dos impactes negativos, a gestão mais eficiente do recurso água e a gestão adaptativa às alterações climáticas, com o intuito de preservar a fauna e flora nativa, integrada num quadro de sustentabilidade ecológica.

Palavras-chave: invertebrados, peixes, métricas, qualidade ecológica, conservação

Abstract

The Sabor basin is located in the Northeast of Portugal on the right bank of the Douro River, encompassing high environmental variability, reflected in different typologies, where endemic species, unique habitats and highly complex and vulnerable ecosystems stand out. In fact, there are several threats, mostly of anthropogenic origin, that have been affecting the ecological integrity of the Sabor basin, with emphasis on water pollution and eutrophication, the degradation and fragmentation of aquatic and riparian habitats, the presence of small and large barriers with flow regulation, the introduction of invasive alien species and more recently the climate change, namely the frequency of prolonged droughts and short-term periods of intense precipitation, with records of loss of habitats and biodiversity.

The main objective of the present study is to evaluate the ecological integrity of 43 sites, sampled in the summer of 2023, distributed along the main river (River Sabor) and several large and small tributaries (e.g. rivers Maçãs, Fervença, Angueira, Azibo, Penacal, Vale Moinhos and streams of Vilariça, Veados, Onor, Churches, Baçal, Ferreiros, Inferno and Zacarias). The methodologies and typologies defined by the Portuguese Environment Agency (APA), within the scope of the Water Framework Directive (WFD), were considered to evaluate several elements 1) biological - fish and macroinvertebrates; 2) physical-chemical water analyses; and 3) hydromorphological. The obtained results made it possible to detect significant differences (Kruskall-Wallis H tests, $P < 0.05$), for physical-chemical parameters, namely temperature, pH and total hardness, considering the defined typologies. However, natural variation (e.g. longitudinal) appears conditioned by the disturbance observed in many sampling sites. This is the case of the Fervença river, downstream from the city of Bragança (e.g. Fv_04 and Fv_05), where the quality decreased drastically, with high values of conductivity, nitrogen and phosphate compounds being recorded, in contrast to the strong decrease in dissolved oxygen. Regarding to aquatic and riparian habitats, it was possible based on the River Habitat Survey (RHS) to identify sampling sites where the aquatic and/or riparian degradation (e.g. Sa_01, Sa_07) was evident. Similarly, significant differences (PERMANOVA, 2-way tests, $p < 0.05$) were found for biota, namely for invertebrate and fish communities, considering both typologies and disturbances identified. Multivariate analysis (nMDS analyses) showed different invertebrate communities, with a predominance of Gastropoda, Heteroptera and Diptera and exotic species such as signal and red crayfish and Asian clam. Also, in the fish community, this analysis highlighted a growing dominance of exotics (e.g. pumpkinseed, common bleak, largemouth bass, zander) to the detriment of native rheophilic species (e.g. brown trout, northern straight-mouth nase, calandino, panjorca-do-esla) which are more common in more degraded sampling sites, particularly under the effect of pollution (e.g. Rio Fervença) and flow regulation (e.g. Baixo Sabor dam).

The analysis of the abiotic and biotic results obtained for the watercourses of the Sabor river basin demonstrates the necessity for the continued implementation of measures that, in the context of environmental degradation and the evident transformation of aquatic and riparian ecosystems, encompass actions that integrate the mitigation and minimization of negative impacts, more efficient management of water resources and adaptive management in response to climate change. These measures are designed with the objective of preserving native fauna and flora, integrated within a framework of ecological sustainability.

Keywords: invertebrates, fish, metrics, ecological quality, conservation

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOSIM	ANALYSIS OF SIMILARITY
APA	AGÊNCIA PORTUGUESA DO AMBIENTE
BDO_5	BIOCHEMICAL OXYGEN DEMAND
CPUE	CAPTURE PER UNIT OF EFFORT
DBRDA	DISTANCE-BASED REDUNDANCY ANALYSIS
DPH	PUBLIC WATER DOMAIN
EQR	ECOLOGICAL QUALITY RATIOS
EN	ENDANGERED
EPT	EPHEMEROPTERA, PLECOPTERA AND TRICHOPTERA
EW	EXTINCT IN THE WILD
EX	EXTINCT
FAO	THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
F-IBIP	FISH INDEX OF BIOTIC INTEGRITY FOR WADEABLE RIVERS IN CONTINENTAL PORTUGAL
FFG	FUNCTIONAL FEEDING GROUPS
H’	SHANNON-WIENER DIVERSITY INDEX
HABS	HARMFUL ALGAL BLOOMS
HQA	HABITAT QUALITY ASSESSMENT
HMS	HABITAT MODIFICATION SCORE
ICNF	INSTITUTE FOR NATURE AND FOREST CONSERVATION
IAS	INVASIVE ALIEN SPECIES
IPBES	INTERGOVERNMENTAL SCIENCE-POLICY PLATFORM ON BIODIVERSITY AND ECOSYSTEM SERVICES
$IPTI_N$	PORTUGUESE NORTHERN INVERTEBRATE INDEX
J’	PIELOU’S EVENNESS INDEX
KBAS	KEY BIODIVERSITY AREAS
MA	MILLENIUM ECOSYSTEM ASSESSMENT
MBBH	THE MEDITERRANEAN BASIN BIODIVERSITY HOTSPOT
MTES	MEDITERRANEAN-TYPE ECOSYSTEMS
N	ABUNDANCE
NE	NORTHEAST
NMDS	NON-METRIC MULTIDIMENSIONAL SCALING
NT	NEAR THREATENED

PCA	PRINCIPAL COMPONENT ANALYSIS
PEABS	SPECIAL PROGRAM FOR THE BAIXO SABOR DAM — UPSTREAM AND DOWNSTREAM RESERVOIRS
PGRH	PLANO DE GESTAO DA REGIAO HIDROGRAFICA
POMS	PROGRAMMES OF MEASURES
RAN	NATIONAL AGRICULTURAL RESERVE
RBMPS	RIVER BASIN MANAGEMENT PLANS
REN	NATIONAL ECOLOGICAL RESERVE
RH3	DOURO RIVER BASIN
RHS	RIVER HABITAT SURVEY
RNA	NATIONAL ROADMAP FOR ADAPTATION
S	RICHNESS
SCI	SITE OF COMMUNITY IMPORTANCE
SCZ	SPECIAL COMMUNITY ZONE
SDGS	SUSTAINABLE DEVELOPMENT GOALS
SIMPER	SIMILARITY PERCENTAGE
TDS	TOTAL DISSOLVED SOLIDS
UN	UNITED NATIONS
WFD	WATER FRAMEWORK DIRECTIVE
WWF	THE WORLDWIDE FUND FOR NATURE
WWTP	WASTEWATER TREATMENT PLANT

1. Introduction

Freshwater ecosystems are among the most threatened in the world, with global declines in their area by 64% from 1997 to 2011, and for Europe by 50% from 1970 to 2008 (Vári et al., 2021). Despite covering less than 1% of the Earth's surface, inland waters are home to a remarkable 10% of all known animal species. Riverine ecosystems around the world host a rich array of biodiversity, including fish, mammals, reptiles, birds, insects, amphibians, invertebrates, fungi, and plants. Notably, aquatic insects comprising a substantial 60% of this biodiversity (Dijkstra et al., 2014; Hanna et al., 2018). This biodiversity of freshwater and inland waters serves as a valuable natural resource, contributing significantly to economic, cultural, aesthetic, scientific and educational domains. However, conservation and management efforts are crucial, as freshwater biodiversity is declining at a rate far exceeding that of the most terrestrial ecosystem (Cazzolla Gatti, 2016).

Water is essential for all living organisms, and its influence extends to humans as well. Globally, the availability of water per person has declined markedly in recent decades therefore many of the existing available water supply sources have become stressed and unpredictable. In some regions of the world, most of them in drylands, water scarcity affects approximately 1–2 billion people today (MA, 2005b). Between 1950 and 2000, the amount of freshwater available per person decreased from 16 800 m^3 to 6800 m^3 (MA, 2005a). Angelakis et al. (2024), attribute the heightened stress and unpredictability of water supply sources to factors such as global population and economic growth, as well as the subsequent intensification of anthropogenic activities across various sectors, including intensified agriculture. Water resources are used and reused for all kinds of economic reasons, such as drinking, food production, flood control, energy, industry, nature, and recreation, with subsequent effects on water quantity and quality (Boelee et al., 2019). Consequently, the significance of water goes beyond human beings, embracing all life forms on Earth, as none would survive without it. For example, infectious disease risks are influenced by changes in the distribution and availability of surface waters (MA, 2005a).

1.1. Freshwater ecosystems and associated services

Freshwater ecosystems include rivers, lakes, ponds, streams, springs, groundwater, and wetlands, stand out for their vital importance to all living things. These ecosystems correspond to a small area of the planet, hosting only 0.01% of the volume of all water on Earth, with lakes, rivers and reservoirs occupying 2.3% of the Earth's surface area (Maes et al., 2018; Rai et al., 2019; Reid et al., 2018). Ecosystems, being multi-functional, provide multiple ecosystem services, which are the benefits they offer (MA, 2005a). According to Bernex (2016), ecosystems services can be divided into four groups: 1) supporting services (or habitat services), which are essential for producing all other ecosystem services; 2) regulating services, which regulate ecosystem processes; 3) provisioning services, which provide material benefits such as food and freshwater; 4) cultural services, which provide many non-material benefits. Freshwater ecosystems provide a wide range of essential services (Figure 1.1), with their condition and functioning tightly connected to natural ecosystems at the water-land interface, such as riparian areas, floodplains and wetlands (Maes et al., 2018). These services include sustaining the global hydrological, carbon and nutrient cycles, supporting water security and regulating flows. They provide natural freshwater storage, water purification, and replenish groundwater. In addition, they provide complementary services like maintaining forest, providing water for crops and fisheries, creating employment opportunities, generating energy, facilitating navigation, and supporting recreation and tourism benefits (Bernex, 2016).

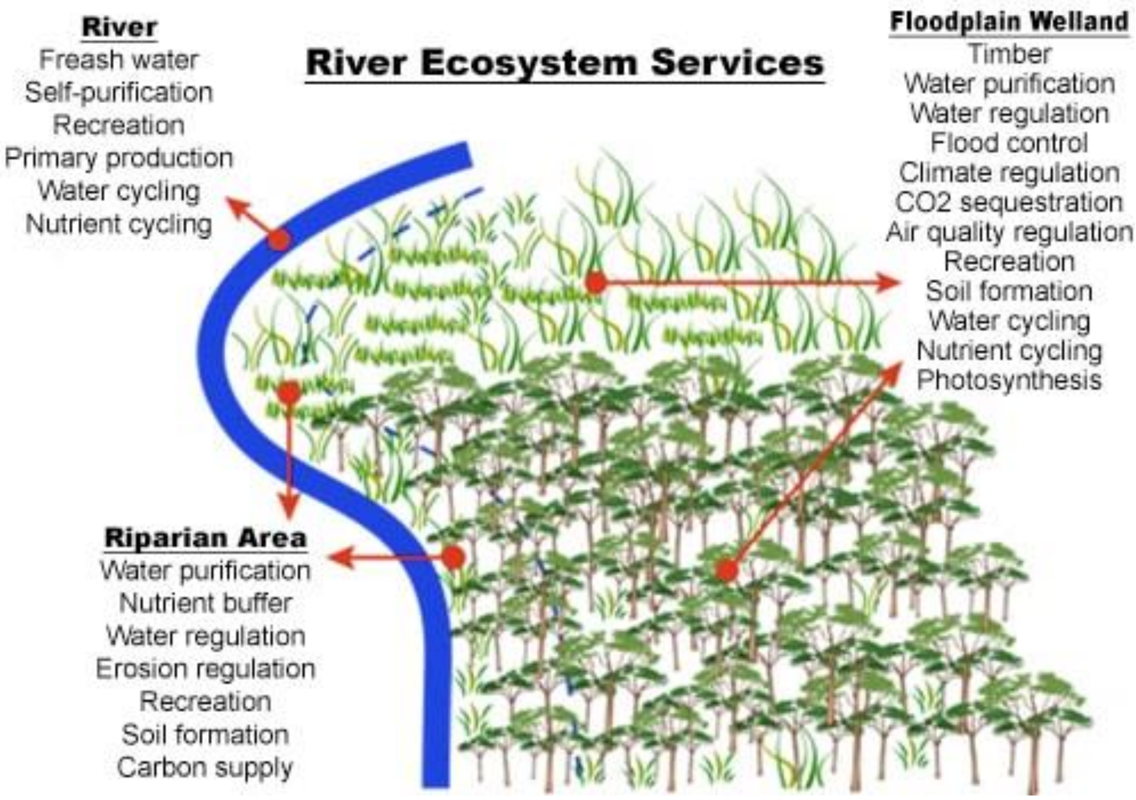

Figure 1.1 River, riparian and floodplain ecosystem services (from https://qsstudy.com/river-ecosystem/#google_vignette).

Freshwater ecosystems play a crucial role in providing essential services, but they are also especially vulnerable to environmental stressors and over exploitation. Water scarcity affects food production, human health, economic development, and geopolitical stability. This is particularly significant in developing countries, where many rural and poor communities rely heavily on natural resources for survival and income. For example, in southern Africa, communities rely on freshwater ecosystems for critically important provisioning services, such as drinking water and food (Jackson et al., 2016; MA, 2005a). Calculating and comparing ecosystem services is difficult and they are not partitioned equally or equitably among stakeholders. For instance, it is easier to value electricity generated from hydroelectric power plants than to measure the spiritual connection of indigenous communities to rivers or the societal importance of iconic species such as salmon, bears, and eagles that depend on freshwaters. Understanding all these values and identifying who benefits from them is necessarily for making informed decisions on river management (Bartley, 2022). Maintaining ecosystems in healthy condition, so that they deliver multiple benefits through ecosystem services, is a key objective of several policies which depend on natural resources, notably agriculture and forestry, fishery, and water policies (Maes et al., 2018).

1.2. Freshwater biodiversity

According to Bernex (2016), biodiversity refers to the total sum of organisms, including their genetic diversity, and the way in which they fit together into communities and ecosystems. The latest consensus definition of biodiversity in the intergovernmental space has been established by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES). It reads: “The variability among living organisms from all sources including terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are a part. This includes variation in genetic, phenotypic, phylogenetic, and functional attributes, as well as changes in abundance and distribution over time and space within and among species, biological communities, and ecosystems” (Díaz & Malhi, 2022). The Food and Agriculture Organization of the United Nations (FAO) and others have stated that there are genuine concerns that biological diversity, ecosystem services, and many fishery resources of inland aquatic ecosystems will be lost as the world strives to accommodate 9 billion people by 2050 (Bartley, 2022).

Aquatic ecosystems are among the world’s most complex and biologically diverse ecosystems, and this adds to the concerns that planners and water managers have in taking them into account (Bernex, 2016). Worryingly, declining populations and an increased risk of extinction have been noted in freshwater species (Collen et al., 2014). Between 1970 and 2018 the Worldwide Fund for Nature (WWF) showed a steeper decline in populations of freshwater species when compared to marine or terrestrial species (Almond et al., 2022). According to the WWF Living Planet Index, freshwater ecosystems and biodiversity are considered among the most threatened on the Earth, with average declines of approximately 83% in the populations of freshwater organisms since 1970 (Cooke et al., 2024). According to Bellard et al. (2022), five main global threats — habitat destruction, over-exploitation, biological invasions, climate change, and pollution — contribute to biodiversity losses. At the global scale, vertebrates are primarily threatened by habitat loss, overexploitation, and then biological invasions. However, within the vertebrates, there are variations: birds and mammals are mainly affected by overexploitation, while amphibians are more susceptible to habitat loss. The Mediterranean Basin Biodiversity Hotspot (MBBH) is among the most biologically diverse regions in the world, renowned for its richness of endemic freshwater species. As the second largest biodiversity hotspot in the world, it spans over 2 million square kilometres, extending eastward from Portugal to Eastern Turkey and southwards from Italy to the Cape Verde archipelago, and including parts of the Iberian Peninsula, France, the Balkan States, Greece, the Middle East, Egypt, and the Maghreb (Freyhof et al., 2020). Off the 5,785 species recorded from the Mediterranean Basin Hotspot and assessed in the IUCN Red List (2016), 1,311 species (23%) are globally threatened. In this case, the proportion of threatened species categorized as Critically Endangered is particularly high for freshwater fishes (26%), reptiles (24%), freshwater molluscs (32%) and plants (34%). Equally important, 32 species from the hotspot are known to have become globally Extinct (EX), or Extinct in the Wild (EW): 11 freshwater fishes; two mammals; one reptile; 14 freshwater molluscs; and four plants (Critical Ecosystem Partnership Fund, 2017). Economic development in the Mediterranean region is dominated by three sectors, all of them having a very large ecological footprint: (i) natural resource sector including agriculture, forestry and fishery, (ii) energy sector based on non-renewable sources, primarily oil and gas, as well as on renewable sources, primarily water but also wind, hydropower and solar energy, and (iii) services sector, primarily tourism and shipping (Critical Ecosystem Partnership Fund, 2017). Mediterranean-type ecosystems (MTEs) are among the most heavily utilized by man and for the longest periods of time. Low rainfall combined with unsustainable agricultural practices has also led to desertification and land degradation in many areas (for example 30% of Greece being declared "threatened" and 60% of Portugal facing a moderate risk of desertification) (Valavanidis & Vlachogianni, 2011).

1.3. Main threats

Since the late nineteenth century, extensive changes to ecosystems, largely driven by population growth and development, have been observed. These alterations, compounded by escalating water abstraction rates, flow modification, wetland habitat conversion, flood protection measures, increased nutrient loads and pollution, result in significant changes to aquatic ecology worldwide (Bernex, 2016; Secretariat of the Convention on Biological Diversity, 2005). Recent losses in freshwater biodiversity are attributed to prevailing anthropogenic pressures, including habitat fragmentation (and destruction), pollution (eutrophication and chemical water pollution), invasive alien species, overfishing, and climate change, which can affect biodiversity at different spatial scales, from local to global (Yoshioka et al., 2014).

Freshwater fish are the most diverse group of vertebrates and have the greatest proportion of threatened species. The main threats to freshwater fish include habitat deterioration or destruction caused by pollution and major modifications like damming and channelization, as well as the introduction of exotic species (Clavero et al., 2004). Additionally, insects play crucial ecological roles as primary consumers, detritivores, predators, and pollinators in both aquatic and riparian ecosystems. However, the biodiversity of freshwater habitats is highly susceptible to environmental change, leading to a disproportionately rapid increase in extinction rates, particularly among less mobile insect groups (Dijkstra et al., 2014). The growth and reproduction of photosynthetic organisms (autotrophs) as well as large-scale ecosystem primary production are frequently limited by the availability of nitrogen (N) or phosphorus (P) in the aquatic ecosystem. However, excessive enrichment of water bodies by nitrogen (N) and phosphorous (P), known as eutrophication (e.g., agriculture), can lead to nutrient imbalance, bloom formation on the water surface, and anoxic conditions inside the water (Elser et al., 2007; Kakade et al., 2021; Wilkinson & Johnson, 2024). These threats not only jeopardize the survival of individual species but also imperil the integrity of entire freshwater ecosystems.

1.3.1. Habitat fragmentation

The fragmentation of river systems is pervasive and growing, posing a substantial threat to freshwater biodiversity. Habitat connectivity is crucial in designing and implementing landscape conservation efforts. Loss of habitat connectivity leads to population fragmentation, posing a significant threat to biodiversity and species persistence. However, dissociating habitat fragmentation from habitat loss is questionable, because the two processes are inherently linked. In river ecosystems, longitudinal connectivity across the branching network structure of streams governs water and sediment transport dynamics and affects the distribution of aquatic habitats and communities (Fuller et al., 2015; Mbora & Mutua, 2023; Sethi et al., 2022). According to Fuller et al. (2015), river networks are naturally fragmented, such as waterfalls, cascades, and beaver dams. However, human actions like damming, road building, water withdrawal, pollution/water quality barriers, and species introductions have further divided these habitats into ever smaller patches. Only a few undammed rivers remain and many of these are affected by culverts and other type of barriers. The amount of water impounded behind dams has quadrupled since 1960; reservoirs now hold three to six times as much water as natural rivers. Most of water use (70% worldwide) is for agriculture (MA, 2005a).

Damming rivers, as noted by Vehanen et al. (2003), has one of the most dramatic impacts on a natural environment. For example, reservoirs substantially affect aquatic biodiversity by impeding the movement of organisms, changing flow regimes, and altering habitat (Vörösmarty et al., 2010). Ecological consequences of hydropower infrastructure include fragmentation of free-flowing rivers, drastic alterations of flow and temperature regimes, diminished water quality, severe reductions in sediment transport, and land degradation. While dams provide benefits such as electricity, flood control, irrigation, and transport, these services often come at the expense of conservation, fisheries and the people that depend on or have strong cultural connections to aquatic ecosystems (Bartley, 2022; Freyhof et al., 2020).

1.3.2. Introduction of invasive alien species

Invasive alien species (IAS) are live specimen of a species or lower taxon of animals, plants, fungi, or microorganisms occurring in a region outside its natural range provoking negative impacts on ecosystems and its services (Magliozzi et al., 2020). Governments are increasingly taking steps to control and eradicate invasive alien species, many of which are aquatic or have significant impacts on water (Secretariat of the Convention on Biological Diversity, 2015). According to Magliozzi et al. (2020), invasive alien species (IAS) cost billions of Euros per year to EU Member States.

Aquatic ecosystems have been subjected to hundreds of introductions of invasive species. These introductions are related to human activities (e.g., trade, aquaculture, construction of canals, aquarium releases, sport fisheries, recreational activities, ballast water) that deliberately or accidentally transported these species worldwide (Sousa et al., 2014). During the 20th century and more acutely in recent decades, freshwater fish introductions into the Iberian Peninsula have significantly increased. It is already common for non-native fish species richness in lakes, reservoirs, and regulated rivers to be greater than that of their native counterparts in Italy, North Africa and the Middle East. Hydroelectric dams and other impoundments are the most important gateway for invasions by non-native fish species, which in the Mediterranean Basin Biodiversity Hotspot (MBBH) mainly occur via the recreational fisheries sector (Freyhof et al., 2020; Leunda, 2010). Introduced species are widespread and expanding their ranges, many of them being piscivorous species, a trophic group almost absent in the original Iberian fauna (Clavero et al., 2004).

Non-native species can successfully establish in natural and particularly in modified environments, impairing their biota. Invasive freshwater fishes can impact native communities and habitats through a variety of processes. They can increase predation pressure and competition, genetic introgression with taxonomically similar species, and disease transmission. Studies on single or multiple IAS in freshwater environments have suggested changes in native communities. These include changes in density, diversity, and the decline of endemic species due to factors such as trophic competition and predation, as well as parasitism and pathogen transfer. Moreover, invasive species can cause major irreversible changes to local physical habitat (i.e. bank stabilization, sedimentation, water quality) (Britton, 2023; Magliozzi et al., 2020; Ruaro et al., 2020). Extinction rates are 100–1000 times higher than prehuman levels in many different taxonomic groups and across a wide range of environments. Despite this alarming trend, habitat degradation and the proliferation of invasive species are frequently identified as the most cited causes driving biodiversity loss. Biotic exchanges, represented by invasive species, are predicted to become the most important driver of biodiversity decrease in lakes and the third most important driver in streams (after land use and climate) for the year 2100 (Hermoso et al., 2011; Thomaz et al., 2015).

1.3.3. Water pollution and eutrophication

Water is fundamental to living organisms, it is important to consider that in a polluted or contaminated ecological state it may pose a health hazard. Many of the pollution sources in the environment include substances or materials for which water can be the main transport or transmission route (Boelee et al., 2019). Water bodies can also be impacted by eutrophication, a phenomenon characterized by the enrichment of aquatic ecosystems with nutrients resulting in high rates of primary production. While eutrophication is a natural process over centuries, human activities have accelerated its rate in many inland and coastal waters (Wilkinson & Johnson, 2024). According to Gilbert (2020), changes in climate and nutrients are contributing to global eutrophication and to the expanding global footprint of harmful algal blooms (HABs). There are more HABs, more often, in new and different places, in both fresh and marine waters, often lasting longer, and which have a range of impacts as well as a range of toxicities. Bloom toxins released in water may cause illnesses, toxicities, and death of aquatic life, including humans via direct contact, inhalation, or ingestion (Kakade et al., 2021). According to Kakade (2021), the main eutrophication causes involve diffuse sources like agricultural areas, wastewater treatment plants (WWTPs) as the point sources, and natural environment, particularly climate change. Freshwater ecosystems play crucial roles in drinking water supply, sanitation, agriculture and industrial. However, these activities lead to the discharge of significant waste, containing N and P, into these water bodies. Eutrophication has been globally recognized since the 20th century and was principally thought to be due to point sources. However, at present, the diffused (non-point) nitrogen (N) and phosphorous (P) sources are considered as the key factors. The long-term use of fertilizer and phosphate mining has doubled phosphorus (P) and tripled nitrogen (N) inputs to the global environment. Also, it should be considered that the rising global food demand and its concomitant fertilizer requirements indicate that eutrophication in freshwater systems is likely to continue increasing soon (Kakade et al., 2021; Zhou et al. 2023). Moreover, the introduction of synthetic organic compounds into these ecosystems can cause physiological changes in aquatic organisms and, at high concentrations, can even be lethal (Cazzolla Gatti, 2016). Overall, rivers actively transform, temporarily store, and permanently remove nutrients within a dynamic environment largely controlled by microbial activity, which is temperature-dependent and therefore sensitive to global warming (Soana et al., 2023).

1.3.4. Overexploitation

Overexploitation, characterized by the unsustainable harvesting or extraction of both renewable and non-renewable natural resources is recognized as one of the primary threats to biodiversity and ecosystem stability, resulting in negative effects on physical and ecological processes and contributing to population declines and biodiversity loss, including the depletion and extinction of non-renewable resources (Cooke et al., 2023). Resource extraction is a global issue affecting all ecosystem types and fresh waters have been particularly impacted. The unequal distribution of freshwater is also a main reason for water overexploitation, given that about 60% of water flowing in all rivers worldwide is shared by two or more countries (Bănăduc et al., 2022; Cooke et al., 2023). The large-scale removal and exploitation of aggregate resources from freshwater systems often exceed the replenishment rate, resulting in significant alterations to the fluvial geomorphology. These alterations can lead to habitat modification and have both direct, such as injuries and mortalities, and indirect impacts, including changes to substrate, increased turbidity, and altered hydraulics at catchment and basin scales, affecting already imperilled freshwater species (Cooke et al., 2023).

According to Dudgeon (2020), overexploitation ranks second in overall importance as a threat to freshwater biodiversity. It primarily affects vertebrates, mainly fishes, as well as reptiles, amphibians and a few mammals and birds and also invertebrates that are also subject to human consumption. Geist (2011) suggests that overexploitation primarily affects fishes and, to a lesser extent, molluscs, crustaceans, and other taxonomic groups. The same author highlight species with high economic value, low fecundity, and late reproduction as particularly prone to overexploitation, with examples such as sturgeon species for caviar production and different species of tuna worldwide.

Organisms that are harvested (e.g., mostly fishes, reptiles, amphibians, invertebrates, and waterbirds) are typically extracted for food, livelihoods, recreation (e.g., angling), or cultural services. Overexploitation can occur due to heavy and/or indiscriminate harvesting, and/or incidental bycatch. Even when fish are released, recreational fishing can result in substantial post release mortality and reduced growth and fitness (Allan et al. 2005; Cooke et al., 2023). Fishing is a crucial source of livelihoods in developing countries, particularly for low-income families in rural areas where job options are limited (Allan et al., 2005). In many parts of the world, a considerable amount of people consumes minimal animal protein, relying primarily on freshwater fish for the dietary needs. Small freshwater fish serve as the main source of lipids and essential micronutrients, including calcium, iron, zinc, vitamin A, in many parts of the developing world (Dudgeon et al., 2020). In developed countries (e.g. USA, Europe, Japan), recreational fishing is the dominant form of the exploitation of fish resources, but also a significant source of income (fishing tourism, sport fishing, etc.) (Bănăduc et al., 2022). Historically, in Europe, freshwater sources were the main providers of fish consumption until the 20th century (Dudgeon et al., 2020).

1.3.5. Climate changes

Mediterranean areas are known as hotspots of biodiversity, highly vulnerable to climate change. The impacts can affect not only humans but also crops, soils, and biodiversity, impacting the overall performance of ecosystems, their provided services, and climate feedback. Furthermore, these impacts can negatively influence the ecosystems (e.g. water availability, air quality, bird phenology, agriculture production). Moreover, they may increase the risk of forest fires, the number and intensity of heatwaves, mortality due to more severe and prolonged droughts, and other extreme weather events (e.g. floods) (Andrade & Contente, 2020; Angelakis et al., 2024; MA, 2005a). Forest fire, as a natural phenomenon, have significant ecological, economic, and sociological repercussions. The effects of fire on aquatic ecosystems, particularly under the influence of climate change, depend on various factors, including fire intensity, pre- and post-fire forest management, and natural climate constraints such as floods leading to soil erosion and run-off into the rivers. Synergically, other anthropogenic pressures like pollution events and the presence of weirs can further constrain aquatic community resilience (Maceda-Veiga, 2012).

Climate modification, closely tied to human population increase, puts enormous pressure on water resources. This global, regional, and local climate change can directly affect water systems by increasing water temperature, decreasing dissolved oxygen content, and increasing pollution toxicity (Bănăduc et al., 2022; Woodward et al., 2010). Rising air temperatures and changing precipitation patterns modify water temperature and flow regimes worldwide, thus affecting two key habitat factors for freshwater species. Fish, being ectotherm, are directly influenced by water temperature, while the hydrologic regime determines the structure and dynamics of the freshwater habitat. The vulnerability of freshwater fish to climatic change results in extinction risk due to altered hydrological regimes and rising temperatures. Recent climate variations have already affected freshwater fish composition (Barbarossa et al., 2021; Manjarrés-Hernández et al., 2021). Predictions indicate increasing climate modification will have widespread consequences on biodiversity across large areas, affecting the dispersion and existence of numerous plants and animal species, and leading to the disappearance of various ecosystem types (Bănăduc et al., 2022). This underscores the need to monitor of ecological integrity and prioritize proactive and or restoration measures to ensure the sustainability of water management practices and the conservation of aquatic and riparian ecosystems.

1.4. Mediterranean rivers as hotspots for conservation

The Mediterranean Basin is one of the most important hotspots of animal and plant diversity worldwide (Cuttelod et al., 2008). However, the loss of freshwater biodiversity in the Mediterranean basin is a major concern (Myers et al., 2000), since terrestrial and aquatic landscapes have been subject to strong anthropogenic pressures during centuries (Cuttelod et al., 2008). For these reasons, protected areas play crucial roles for the protection of biodiversity and national and international conservation polices and legislation have been implemented. Freshwater key biodiversity areas (KBAs) have now been identified, mapped and validated throughout much of the Mediterranean hotspot (Darwall et al., 2014; Nogueira et al., 2021). The EU Biodiversity strategy for 2030 and UN Decade on ecosystem restoration (2021-2030) are recent tools to prevent and reverse the loss of nature. However, mediterranean freshwater ecosystems are particularly vulnerable to water pollution and eutrophication, habitat fragmentation and destruction, biological invasions and homogenization and climate change (Costa et al., 2021; Hermoso et al., 2012; Sáez-Gómez & Prenda, 2022).

The loss of river connectivity, due to flow regulation, and the fragmentation and destruction of habitats resulted, globally, in the proliferation of small (weirs) and large (dams) physical obstacles (Aarts et al., 2003; Gozlan et al., 2019; Reid et al., 2019). These are one of the most serious pressures on the loss of sustainability by native fauna, leading to declines of more than 50% of threatened species in Europe (Northcote, 1998). According to Cortes et al. (2019) the longitudinal dimension of river connectivity in Mediterranean areas has been significantly disrupted by barriers to compensate for water demand in the long periods of water scarcity and increase the hydropower production. In Portugal, more than 8000 small barriers are referenced (Ordeix et al., 2018). Only in the Portuguese Douro River drainage network, more than 1000 barriers are fragmenting and destructing natural habitats, together with intensive agriculture and the introduction of exotic species.

1.5. Monitoring and evaluation of ecological quality

Since 2000, the WFD - Water Framework Directive (2000/60/EC) has been the main law for water protection in Europe. The WFD requires to EU Member States to achieve good status in surface water bodies and groundwater by 2027. According to WFD, the ecological status is defined as “an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters ...’’. This status is assessed using an integrated measure of aquatic ecosystem conditions, defined in five classes based on biological, hydromorphological, and physical and chemical elements (Magliozzi et al., 2020; Nõges et. al., 2007). The WFD requires to the member countries to develop river basin management plans (RBMPs) and Programmes of Measures (PoMs) to protect and, when necessary, restore water bodies to achieve good status and prevent further deterioration. These plans serve as strategic tools that promote an integrated approach to managing water resources, directly impacting activities and water usage within river basin regions (https://environment.ec.europa.eu/topics/water/water-framework-directive_en).

The 2030 Agenda for Sustainable Development, adopted by all United Nations (UN) Member States in 2015, provides 17 Sustainable Development Goals (SDGs), which include specific focus on water. The level of human impact on the planet has been so extreme that it is now widely accepted that we are in a new epoch period called the Anthropocene (Lewis & Maslin, 2015). Although ecosystem degradation and biodiversity loss occur in all regions and ecosystem types, effects are perhaps most extreme in freshwater ecosystems. In response, the UN has declared 2021–2030 the Decade for Ecosystem Restoration, aiming to mitigate ecosystem degradation and biodiversity loss (Cooke et al., 2022). River restoration involves a variety of interventions, including bank stabilization, channel reconfiguration, fish passage, in-stream habitat restoration, floodplain and abandoned channel reconnection, dam and weir removal. These efforts serve multiple purposes, including ecosystem and habitat restoration for biodiversity, flood and sediment management, water quality improvement, provision of ecosystem services, including aesthetic and recreational amenities (Linton, 2021).

1.6. Objectives of the dissertation

The main objectives of the present dissertation were:

1) Evaluate, under the Water Framework Directive and according to the methodology defined by the Portuguese Environmental Agency (APA), from the ecological point of view, 43 sampling sites in the Sabor River basin network, namely:
    - Assess the water quality, based on general physical and chemical parameters.
    - Determine the hydromorphological quality, i.e. aquatic and riparian habitats.
    - Evaluate the biota quality, in particular macroinvertebrates and fish communities.
2) Propose conservation measures mainly oriented to aquatic fauna (invertebrates and fish) present in the Sabor river basin.

2. Methodology

2.1. Study area

The Sabor River is a right-bank tributary of the Douro River, with the origin in the Sierra de la Culebra (province of Zamora, Spain), draining an area of approximately 3,835 km^2, mainly located in northeastern Portugal (86%), subjected to a diverse orographic and climatic conditions (Portela, 2014; Santos et al., 2019) (Figure 2.1).

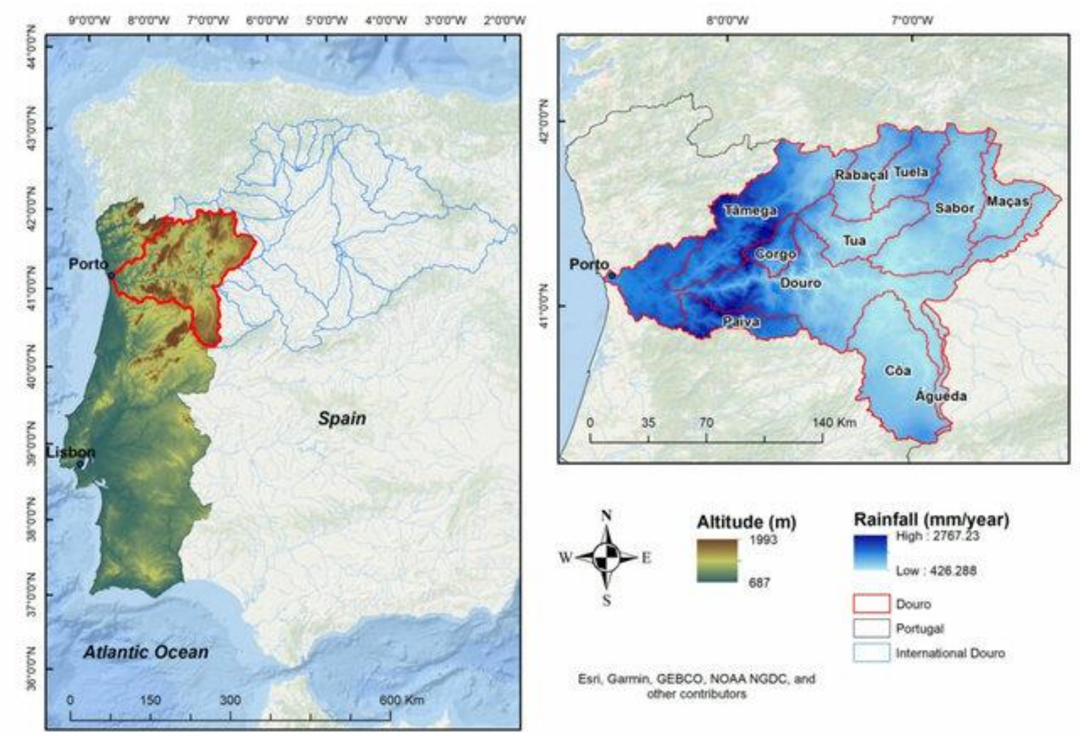

Figure 2.1 Map of Douro Basin: identification of main tributaries and altitude and rainfall distribution in Portugal (from Cortes et al., 2019).

The watershed has an elongated shape, with the main river (120 km long) running in North/South direction and being part of one of the largest geological units of the Iberian Peninsula, the Hesperian Massif, where granitoids, schist, quartzite and metamorphic rocks predominate. The altitude varies between 97 m and 1600 m and is characterized by rainfall gradients, exceeding 1000 mm on annual precipitation in headstream zone (*Terra Fria Transmontana*) contrasting with less than 400 mm near the downstream zone (*Terra Quente Transmontana*) (Gonçalves, 1985, 1991). The climate is predominantly Mediterranean; however, the Continental effect of the Iberian Peninsula and some Atlantic influence can be detected affecting the hydrological regime, markedly pluvial, with strong intra-annual and inter-annual irregularities (Gonçalves, 1985; Nunes, 2008).

Most of the Upper Sabor is in the Montesinho Natural Park, a protected area of wooded natural landscapes and traditional farming, known by their high biodiversity (e.g. 110 bird species and 70% of mammal species detected in Portugal). The headstreams are ecosystems with permanent and turbulent waters, with a marked pool-riffle sequence, coarse substrate (e.g. mainly pebbles and boulders) and stabilized banks supporting extensive riparian galleries dominated by alder (*Alnus lusitanica*), and also ash (*Fraxinus angustifolia*) and willow trees (*Salix salviifolia*) in downstream zones. Among the animal species present in the Upper Sabor, can be highlighted several mammals (e.g. *Galemys pyrenaicus, Lutra lutra*), birds (e.g. *Ciconia nigra, Cinclus cinclus*), reptiles (e.g. *Natrix maura, Lacerta schreiberi*) and amphibians (e.g. *Rana iberica*), and other animals such as *Canis lupus signatus, Felis silvestris,* and *Vipera latastei* regularly frequent the riparian areas (Nogueira et al., 2021; Teixeira et al., 2006).

Downstream of Upper Sabor, almost of the area is classified as SCI (Site of Community Importance) and SCZ (Special Community Zone) and identified as “Rios Sabor e Maçãs site” (PTCON00021) (Figure 2.2).

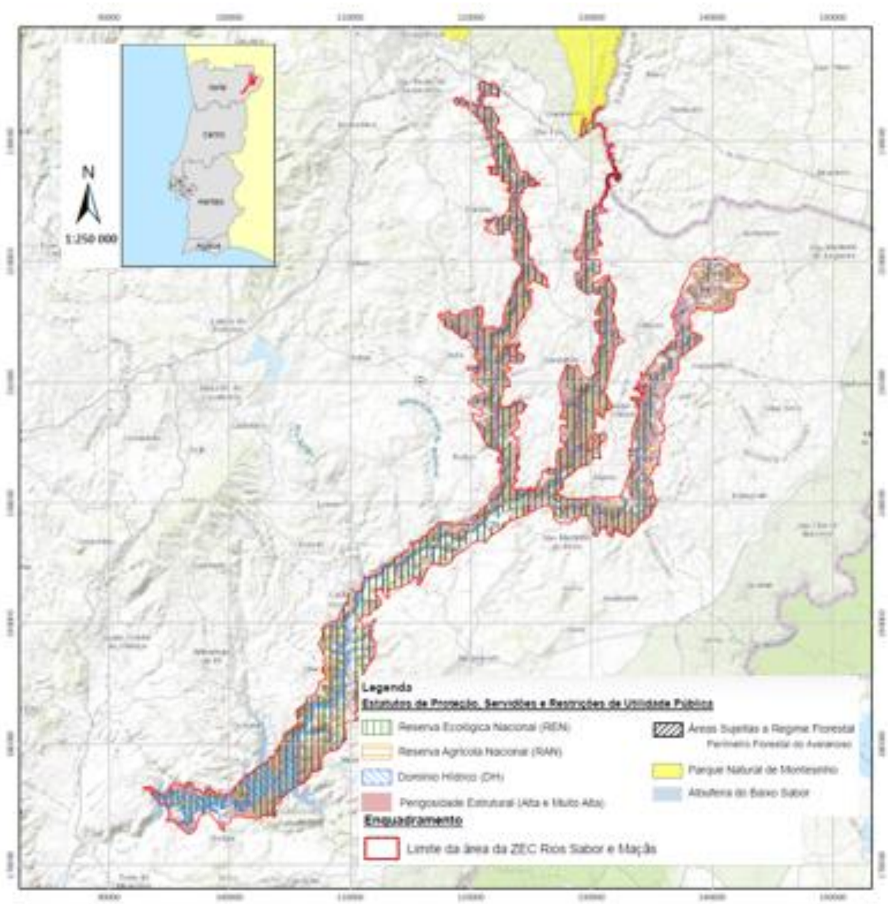

Figure 2.2 Rios Sabor e Maçãs site (PTCON00021): limits and legal figures (from Floradata, 2022).

In this area, other legal figures and conditions contribute to the protection of the target values identified in the Management Plan, namely: Protected Areas, National Ecological Reserve (REN), National Agricultural Reserve (RAN), Public Water Domain (DPH), Public Water Reservoirs, Forestry Regime, and Priority Prevention and Safety Areas (Floradata, 2022). In the SCZ "Rios Sabor and Maçãs" there are 20 types of habitat protected through Annex I of the Habitats Directive, and there are 6 species of flora with a significant presence simultaneously included in Annexes II and IV of the Habitats Directive, along with 2 species listed in Annex IV and 5 in Annex V. Regarding to fauna, can be identified a significant presence of 6 species listed in Annex II of the Habitats Directive, being 1 invertebrate, 4 fish and 2 mammals. Of these, 2 species are also listed in Annex IV. In addition to these, the SCZ is also home to twenty other species of fauna (5 amphibians, 2 reptiles and 13 mammals) also listed in Annex IV and three species (one amphibian, one fish and one mammal) listed in Annex V. In fact, the flora is very diverse (e.g. *Buxus sempervirens, Trigonella polyceratia, Dianthus marizii, Festuca duriotagana* and *Holcus setiglumis* ssp. *duriensis*, among others). Currently, there is a mosaic of agricultural landscape with extensive pastures, extensive olive and almond groves and natural vegetation (mainly composed by xerophilic shrubs of *Cistus* spp. *Cytisus* spp. *Lavandula pedunculata*, and *Juniperus oxycedrus,* and *Quercus suber* and *Q. rotundifolia* trees). The richness of the area is also due to faunal populations and emblematic species with protected status such as invertebrates (e.g. *Coenagrion mercuriale, Gomphus graslinii*), fishes (e.g. *Achondrostoma asturicense, Pseudochondrostoma duriense*), amphibians (e.g. *Triturus marmoratus, Alytes cisternasii, Rana iberica),* reptiles (e.g. *Mauremys leprosa, Chalcides bedriagai*), birds (e.g. *Aquila chrysaetos, Gyps fulvus, Neophron percnopterus*) and mammals (e.g. *Galemys pyrenaicus, Lutra lutra, Canis lupus)* (Floradata, 2022).

The main threats affecting Sabor river and tributaries can be organized in 4 different types of pressures: 1) Qualitative, due to a) point (e.g. domestic and industrial wastewaters) and b) diffuse (e.g. intensive agriculture and livestock) pollution; 2) Quantitative, related with surface and subterranean water abstraction; 3) Hydromorphological, namely significant changes in-channel (e.g. weir, small and big dams) and riparian corridor (e.g. clearcut and tree diseases), and 4) Biological, mainly based on the introduction of exotic species and diseases and species overexploitation (PGRH3, 2022a). More recently, climate change has been contributing to the degradation of aquatic and riparian ecosystems and the habitat loss and biodiversity, since extreme events are nowadays more frequent, prolonged dry alternating with intensive and concentrated rainfall periods, heat waves and forest fires.

The Baixo Sabor hydroelectric power plant system is comprised by two levels. The reservoir created by the upstream dam extends over 60 km. The reservoir created by the downstream dam has a length of about 9.6 km and is comprised between the two dams. Baixo Sabor started operations in 2016 and improved the operational management of the Douro basin. The pump storage system is a combination of an arch gravity concrete dam and a reversible power plant. It has an installed capacity of 144 MW, Average Production: 191 GWh/year. The powerhouse was built underground and comprises two installed Francis reversible turbines of 72 MW each. The concrete dam is 123 meters high and has a crest length of 505 meters. The reservoirs can store in total 1,095 million m^3 of water which makes Baixo Sabor the third largest reservoir of Portugal (Pereira et al., 2017; Movhera, 2022).

2.2. Sampling strategies

Abiotic and biotic variables were sampled in 43 sites, during Summer of 2023, distributed by Sabor (12), Maçãs (5), Angueira (5), Fervença (5), Azibo (3), Vilariça (3), Vale Moinhos (2) and Penacal (2) rivers and one of several small tributaries (e.g. Veados, Onor, Igrejas, Baçal, Ferreiros, Inferno, Zacarias) (Figure 2.3).

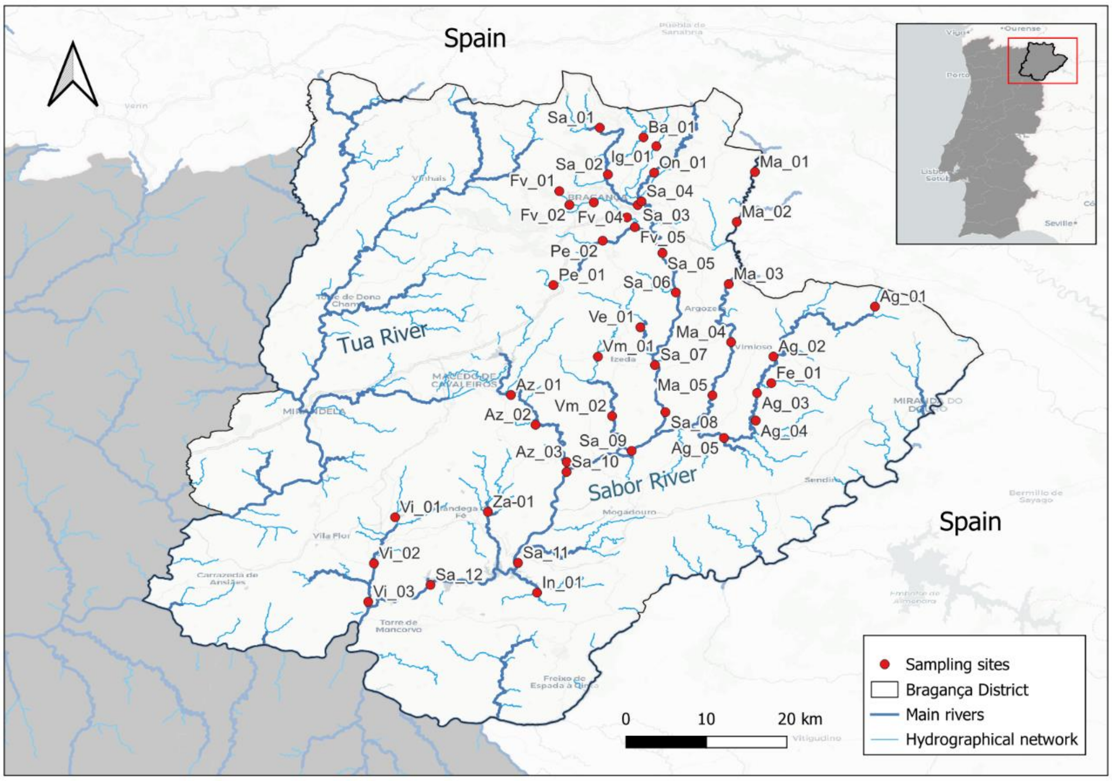

Figure 2.3 Distribution of the forty-three sampling sites in the Sabor basin (NE Portugal).

2.3. Physical and chemical elements: water quality

2.3.1. Water sampling

The water quality evaluation was made based on the measurement of several physical and chemical parameters (Table 2.1). Some parameters were measured ***in situ***, using portable probes, and others determined **in the laboratory**, after the collection of water samples (1.5 L) and their transportation in coolers (to maintain the temperature of 4°C).

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**
	Biochemical Oxygen Demand (BOD_5)	**In laboratory**	**mg O_2/L**
Salinity	**Electric conductivity**	**in situ**	**µS/cm, 20ºC**
Acidification state	**pH**	**in situ**	**Sorensen scale**
	Alkalinity/Acidity	**In laboratory**	**mg HCO_3/L**
	Hardness	**In laboratory**	**mg $CaCO_3$/L**
Nutrient Conditions	**Total Dissolved Solids**	**in situ**	**mg/L**
	Nitrates	**In laboratory**	**mg NO_3/L**
	Ammoniacal Nitrogen	**In laboratory**	**mg NH_4/L**
	Ortophosphates	**In laboratory**	**mg PO_4^{3-}/L**

The procedure for collecting, conservation and water sample transport for general physical-chemical elements followed the 2017 RELACRE guide, as well as the applicable quality standards, established in accordance with Decree-Law No. 42/2016, of August 1st (and complementary documents).

For measurement of the *in-situ* parameters a multiparameter probe (HACH HQ2200 ©) was used, recording it in the datalogger the registered values (Figure 2.4).

Figure 2.4 In situ measurement of water quality in River Sabor (Summer 2023).

The determinations of the physical-chemical parameters at the various sampling stations were carried out in the laboratory of the School of Agriculture of Bragança, using methods following NP EN ISO/IEC 17025 (Figure 2.5).

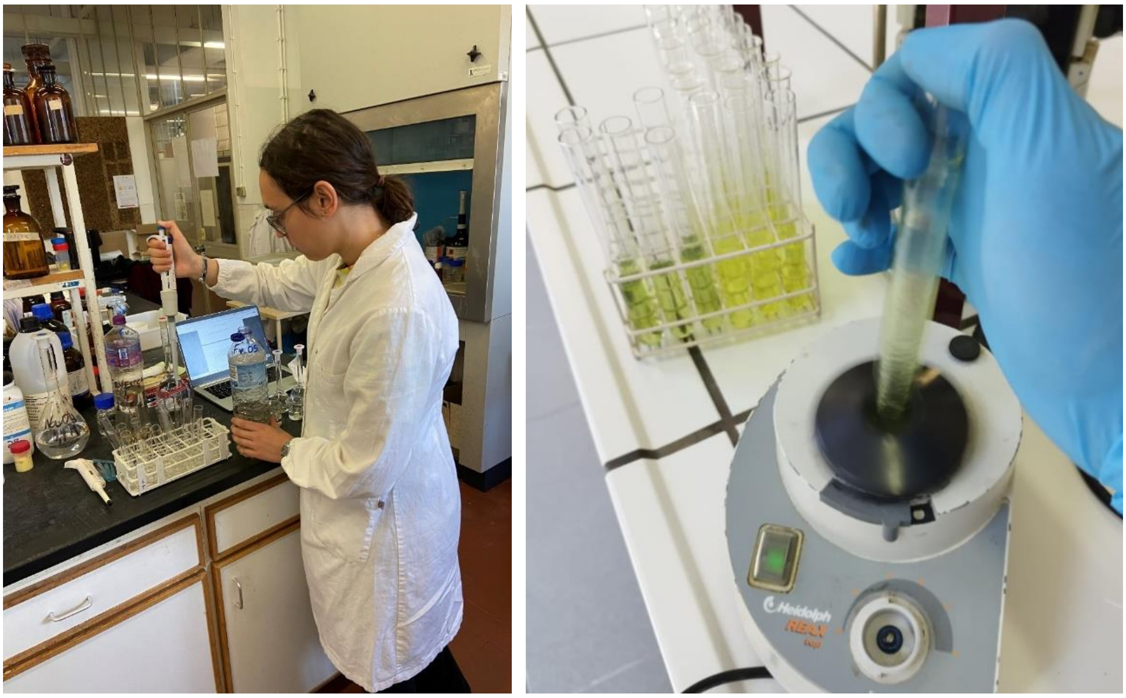

Figure 2.5 Laboratory measurement of water quality of River Sabor (Summer 2023).

2.3.2. Water evaluation criteria

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 used (Table 2.2) (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 / Reasonable
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
Biochemical Oxygen Demand (BOD_5)	3 mg O_2/L	4 mg O_2/L
pH	6,5 – 8,5	6 - 9
Nitrates	5 mg NO_3/L	10 mg NO_3/L
Nitrites	0.01 mg NO_2/L	0.20 mg NO_2/L
Phosphates	0.10 mg PO_4/L	0.20 mg PO_4/L
Conductivity	--	250 µS/cm
Temperature	--	6,5 – 25,5 ºC

2.4. Hydromorphological elements: channel and riparian quality

2.4.1. River Habitat Survey

The inventory of hydromorphological characteristics was carried out through the application of the RHS methodology (Environment Agency, 2003 and update included in the 2022 reprint). The RHS is a method that is easy to apply in the field, for assessing the quality and conservation status of river habitats based on their hydromorphology and structure of riparian and aquatic vegetation (Raven et al., 1997). RHS is a structured technique for inventorying hydrogeomorphological characteristics of the watercourse and riparian corridor along a 500 m long section, covering a 50 m strip on each side of the watercourse (Figure 2.6). Observations are recorded at two different scales: in transepts arranged at intervals of approximately 50 m (10 spot-checks) and continuously throughout the entire sector of approximately 500 m (sweep-up).

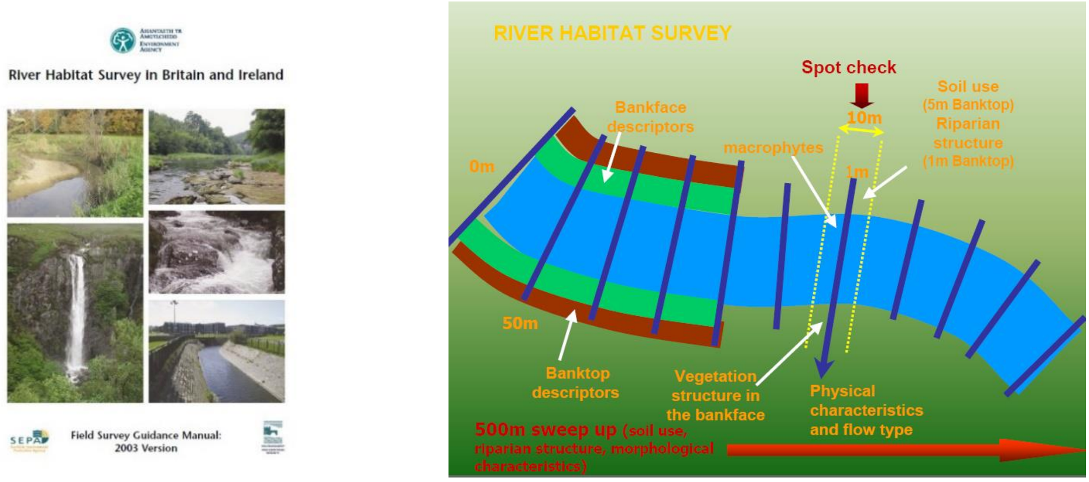

Figure 2.6 RHS Manual and schematic representation of the spatial distribution and variables (adapted from Environment Agency, 2003).

The field inventory includes information on the substrate, frequency of occurrence of erosion and sedimentation characteristics, hydrodynamics, vegetation structure, bank morphology and land use in areas adjacent to the riparian corridors.

2.4.2. Hydromorphological evaluation criteria

In addition to the description of the general features of each monitoring site and its main attributes two RHS indices were also calculated: 1) HQA - Habitat Quality Assessment, corresponding to a measure of the diversity and naturalness of the physical structure of the section and 2) HMS - Habitat Modification Score, which rank the quality and diversity of river habitats and the respective degree of degradation.

The HQA value can only be compared with rivers of the same typology or sections of the same river, corresponding to the sum of nine sub-indices that evaluate specific components of hydromorphological quality (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 HMS allows the assessment of the degree of artificialization of the physical structure of the river corridor and the impact of transverse and longitudinal structures and interventions on river habitats within the 500 m sampling section. This evaluation is independent of the river type, being possible the comparison between results of two river types. Table 2.4 presents the scores for the different categories of river artificialization.

Table 2.4 Artificialization Categories: HMS Index Score (adapted from INAG, 2009).

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**	

The calculation of the HQA and HMS indices was made using the RHS Toolbox version 1.5 software by Naura (2021), available at http://www.riverhabitatsurvey.org /author/mnaura/.

2.5. Biological quality elements

2.5.1. Macroinvertebrate communities

2.5.1.1. Macroinvertebrate sampling

The sampling of benthic macroinvertebrate communities was carried out in accordance with APA criteria, as part of the implementation of Water Framework Directive (WFD) and the Sampling and Analysis Protocol for Benthic Macroinvertebrates (INAG, 2008a). In summary, for the monitoring of the benthic macroinvertebrate community in each sampling site, a 50m long section was selected, representative of the different microhabitats of the watercourse, including in the center an erosion unit (turbulent flow), from which the adjacent sedimentation units (laminar flow) were sampled. Macroinvertebrates were captured using a kick sampling method and a handnet, with a mesh size of 500 µm, to obtain a composite sample of 6 subsamples (corresponding to a sampling area of 1,5 m^2). Benthic macroinvertebrates were captured by placing the handnet against the current and removing the sediment with the feet near the mouth of the net, causing the dislodged organisms to the water column and their movement into the net. Some additional procedures were made, in particularly oriented the capture of the taxa attached to the substrate (e.g. Gastropoda, some caddisflies), washing and scraping of stones or blocks from the substrate, and others that inhabit the surface of the water (e.g., Gerridae, Gyrinidae). Still in the field, the sieved samples were deposited inside 2 L plastic bottles and preserved in alcohol (98%) and labeled (Figure 2.7).

Figure 2.7 Sampling procedures of benthic invertebrates in Sabor River (Spring 2023).

Subsequently, laboratory processing consisted of a prior washing and screening phase, with subsequent sorting and joining of specimens into large groups. The organisms were preserved in 70% alcohol, inside duly labelled vials. Identification was carried out using a stereoscopic microscope OLYMPUS SZX10, with zoom magnification 10-230x, and the aid of dichotomous keys (e.g. Tachet et al., 1981, 2010). The taxonomic level of identification was Family level except for Subclasses Oligochaeta and Acari (Figure 2.8).

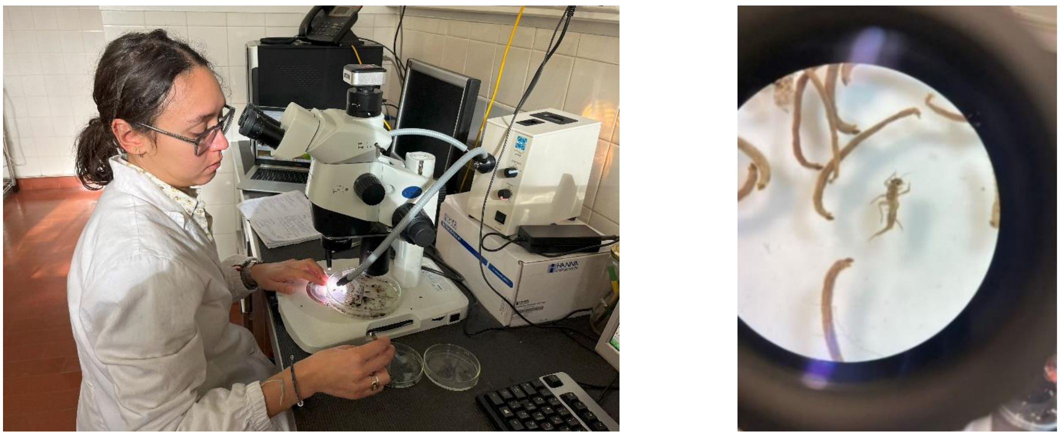

Figure 2.8 Identification of macroinvertebrates samples (Spring 2023)

2.5.1.2. Evaluation criteria for macroinvertebrates

For the evaluation of the biological quality based on macroinvertebrate communities the software AMIIB@ (https://www.apambiente.pt/dqa/amiib%40.html) was used. Some calculated metrics can be highlighted, such as: 1) number of individuals (N) ; 2) number of taxa (S); 3) diversity (e.g. H' Shannon-Wiener index); 4) evenness (e.g. Pielou J' index); 5) relative abundance of Ephemeroptera, Plecoptera and Trichoptera (% EPT); 6) biotic index IBMWP; 7) Portuguese Northern Invertebrate Index $IPtI_N$; and 8) Functional Feeding Group analysis (FFG), using the software ASTERICS v.4.04 developed by the project **AQEM** - **A**ssessment System for the Ecological **Q**uality of Streams and Rivers throughout **E**urope using Benthic **M**acroinvertebrates (http://www.fliessgewaesserbewertung.de/downloads).

The multimetric **Portuguese Northern Invertebrate Index- $IPtI_N$** developed for Northern rivers of Portugal, according to the Water Framework Directive (INAG, 2009), integrate different metrics, resulting in the following formula:

$$IPtI_N = N^o\ taxa \times 0.25 + EPT \times 0.15 + Evenness \times 0.1 + (IASPT - 2) \times 0.3 + Log\ (Sel.\ ETD+1) \times 0.2$$

- **EPT**: Nº families belonging to Ephemeroptera, Plecoptera, Trichoptera;
- **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: $H' = -\sum p_i\ Ln\ p_i$:

$$p_i = n_i/N$$

n_i- nº of individuals of each *taxon i*
N- total nº of individuals present in the 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).

Table 2.5 presents the reference values and boundaries between quality classes in Ecological Quality Ratios (EQR). In Portugal, 15 river types are defined, distributed according to relatively homogeneous morphoclimatic and lithological regions (INAG, 2008b). In the Sabor basin, 4 types were identified: 1) N1 ≤ 100 Km^2 – Small Northern Rivers; 2) N1 > 100 Km^2 - Medium-Large Northern Rivers; 3) N2 - Medium-Large Upper Douro Rivers; and 4) N3 - Small Upper Douro Rivers.

Table 2.5 IPtIN reference values and boundaries for river types of Sabor basin (adapted from APA, 2016).

Typology	Reference value	Excellent	Good	Reasonable	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[

The macroinvertebrate functional feeding groups (FFG) method is based on the association between a set of feeding adaptations found in freshwater invertebrates and their basic nutritional resources categories (Merritt & Cummins, 2006). It was considered the general classification observed in the following table (Table 2.6).

Table 2.6 Classification system for invertebrate functional feeding groups (adapted from Merrit & Cummins, 2006).

Functional Group	Dominant Food	Feeding mechanism	Examples of taxa
Shredders	**Living vascular hydrophyte plant tissue**	**Chewers and miners of live macrophytes**	**Trichoptera**
	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**
Filter-feeders	**Decomposing fine particulate organic matter (FPOM)**	**Filterers or suspension feeders**	**Hydropsychidae Simuliidae**
Grazers and Scrapers	**Periphyton- attached algae and associated material**	**Herbivores- grazing scrapers of mineral and organic surfaces**	**Glossosomatidae Heptageniidae**
Predators and Parasites	**Living animal tissue**	**Carnivores- attack prey, pierce tissues, cells and suck fluids**	**Hemiptera**
	Living animal tissue	**Carnivores- ingest whole animals (or parts)**	**Perlidae**
	Living animal tissue		**Plathyelminthes**

2.5.2. Fish communities

2.5.2.1. Fish sampling

The sampling of fish communities was based on the "Biological Assessment of Water Quality in River Systems protocol for sampling and analysis for fish fauna", according to the Water Framework Directive (INAG, 2008b).

The electrofishing was the capture method used and representative habitats of the sampling site selected, including the riffle/pool sequence and the maximum variety of available microhabitats. The sampled area was related to the width and length of the section. Thus, for watercourses less than 30 meters of width, as in most of the sampled sites, the length of the sampled section corresponded to 20 times its width, with a length of no less than 100 meters, and a capture effort (CPUE) of 30 minutes. Sampling was carried out by wading the channel in a zig-zag way, from down to upstream, with the electrofishing device duly adjusted to the water conductivity, to increase fishing efficiency and simultaneously avoid mortality and fish injuries. A portable electrofishing device with direct current DC was used to sample the 43 sampling sites (Hans Grassl ELT GI ©; 300-600V) (Figure 2.9).

Figure 2.9 Fish capture by electrofishing (spring 2023).

Electrofishing is an efficient and relatively harmless technique based on the creation of an electric field, which can induce changes in the fish behavior, such as involuntary contraction and relaxation muscle movements, allowing their easy capture (Oliveira et al., 2007). After capture, fish were carefully handled and kept, until processing, inside boxes with oxygenated water. The fish species identification was made recurring to Collares-Pereira et al. (2021) and Magalhães et al. (2023). Biometric data were also obtained, namely total length, using an ichthyometer (accuracy of 0.1 cm), and biomass, using a digital balance (accuracy of 0.01 g) (Figure 2.10).

Figure 2.10 Biometric data: measurement and weight of fish fauna (spring 2023).

Native fish were released back to the river and exotic species eliminated, according to the current legislation. All field work carried out by the technical team involved in capturing ichthyofauna was duly accredited by the Institute for Nature and Forest Conservation (ICNF) through the capture and Electrofishing licenses (Licenses 877, 878, 880/2023/CAPT, and credentials 15-B, 19-B and 21-B/2023).

2.5.2.2. Evaluation criteria for fish

The fish data were used to assess the biological quality through the application of the F-IBIP: Fish Index of Biotic Integrity for Wadeable Rivers in Continental Portugal, using the software http://www.isa.ulisboa.pt/proj/fibip/ (INAG and AFN, 2012). The F-IBIP index consists of several metrics trying to reflect basic structural and functional characteristics of the fish communities in freshwater ecosystems of Portugal (Oliveira et al., 2007, 2010). These metrics can decrease or increase depending on the intensity of the anthropogenic disturbance and are included in two major groups: richness and specific composition (e.g. number of native species, percentage of exotic individuals) and ecological factors. In Sabor basin four fish groups were identified: 1) G1 - Salmonid streams of the Northern Region 2) G2 - Salmonid-Cyprinid Transition zone rivers of the Northern Region 3) G3 - Medium-sized Cyprinid rivers of the Northern Region; and 4) G4 - Small Cyprinid streams of the North-Central Interior and South Regions. The F-IBIP score is obtained 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) corresponding to excellent quality (Table 2.7).

Table 2.7 Variation values of the F-IBIP quality classes (adapted from INAG and 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 analysis

For the abiotic characterization several water and habitat variables were analysed, i.e. temperature, dissolved oxygen, electric conductivity, total dissolved solids (TDS), pH, ammoniacal nitrogen, nitrates, total nitrogen, orthophosphates, total phosphorus, alkalinity, hardness, altitude, % shading, maximum depth, water current, substrate composition (%), macrophytes (%), and the River Habitat Survey (RHS) indexes of Habitat Modification Score (HMS) and Habitat Quality Assessment (HQA). The data was previously normalised to perform the *db*RDA analysis for environment conditions, i.e., to sort the different sites according to the abiotic variables measured. The 43 sampling sites were previously distributed by the defined river typology groups (INAG 2009, AFN & INAG 2012) and their macroinvertebrate and fish densities, in terms of CPUE (ind.m^{-1}), were analysed using non-metric multidimensional scaling (nMDS) and the Bray-Curtis dissimilarity index (Clarke and Gorley, 2015). Ordination was interpreted in ecological terms for stress values <0.2. Prior to analysis, data were square root 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 between river typology groups with overall significance level corrected for multiple testing using the Bonferroni sequential correction. Similarity Percentage (SIMPER) analysis was used to identify the macroinvertebrate and fish taxa with the highest contribution to river typology dissimilarity. The Richness (S), abundance (N), Shannon-Wiener diversity index (H') and the Pielou's evenness (J') and complementary functional feeding group (FFG), for the invertebrate 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 the river typologies, for both invertebrates (N1 ≤ 100; N1 > 100; N2; N3) and fish (G1; G2; G3; G4) communities, and river disturbance (i.e., habitat fragmentation, water pollution) 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 (Version 7).

3. Results

3.1. Abiotic Characterization

3.1.1. Water quality: physical and chemical elements

Several physical and chemical parameters were determined for water quality measured in situ and in laboratory. Table 3.1 present partial results corresponding to the longitudinal variation in the main watercourse, the Sabor River (Sa_01 to Sa_11), adding also information regarding to the two sampling sites located in the Fervença River (Fv_04 and Fv_05), downstream of Bragança, where a higher pollution level was detected. The complete abiotic characterization of the 43 sampling sites is presented in **Annex I**.

Table 3.1 Water quality of sampling sites in Sabor and Fervença Rivers (Summer 2023), 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).

Variable	Sa_01	Sa_03	Sa_05	Sa_07	Sa_09	Sa_11	Fv_04	Fv_05
Temperature (ºC)	17.4	21.4	25.3	26.6	27.2	28.8	22.4	23.6
Conductivity (µS/cm)	43.5	168.1	299.0	200.2	208.7	127.1	598.0	494.0
pH (Sorensen scale)	6.77	6.98	7.04	7.16	7.60	7.53	7.36	7.25
Dissolved Oxygen (mg/L)	11.66	8.49	13.52	8.26	8.24	9.33	3.13	5.40
% Saturation O_2	109.2	110.5	162.4	107.0	108.8	117.9	32.9	48.9
BDO_5 (mg/L)	0.63	1.02	1.10	0.70	1.20	1.03	8.20	6.18
Nitrates (mg/L)	0.76	1.27	4.88	1.10	1.28	0.10	30.57	5.99
Nitrites (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01	10.63	9.00
Phosphates (mg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.23	0.34
Hardness (mg/L)	2.4	3.6	2.7	6.9	2.2	2.7	34.9	32.4
Acidity (mg/L)	3.74	9.97	13.64	9.09	4.25	5.97	18.69	34.25
Total Diss. Solids (mg/L)	24.0	78.8	151.5	93.0	98.7	59.3	328.0	295.0

A natural increasing tendency in water temperature and conductivity and decreasing of dissolved oxygen was detected in longitudinal axis, i.e. from headstream (e.g. Sa_01: 17.4 ºC, 43.5 µS/cm, 11.66 mg O_2/L) to downstream (e.g. Sa_11: 28.8 ºC, 127.1 µS/cm, 9.33 mgO_2/L) zones. The obtained results for most of the 43 sampling sites are, in general, within the ranges established by the APA for Good Condition (Table 3.1 and Annex I). In the more disturbed zones of the Fervença River, subjected to nutrient inputs, primarily nitrogen and phosphorus, several parameters (e.g. Conductivity, Dissolved Oxygen, BDO_5, Nitrates, Orthophosphates, among others) identify a bad condition and a low efficiency of the Wastewater Treatment Plant (WWTP) of Bragança (Table 3.1). In

Sabor basin, other detected pressures and their synergic effect negatively influenced the water quality of the watercourses, such as river fragmentation (weir, small and big (Sa_10 to Sa_12) dams), and intensive agriculture (e.g. Vilariça (Vi_01, Vi_02) and Zacarias (Za_01) rivers) (see **Annex I**). The water quality, considering the 4 river typologies defined for macroinvertebrates, confirmed the pattern identified for longitudinal variation of the Sabor river, and significant differences (K-W tests, $p < 0.05$) were identified for the variables of temperature, pH and hardness (Figure 3.1).

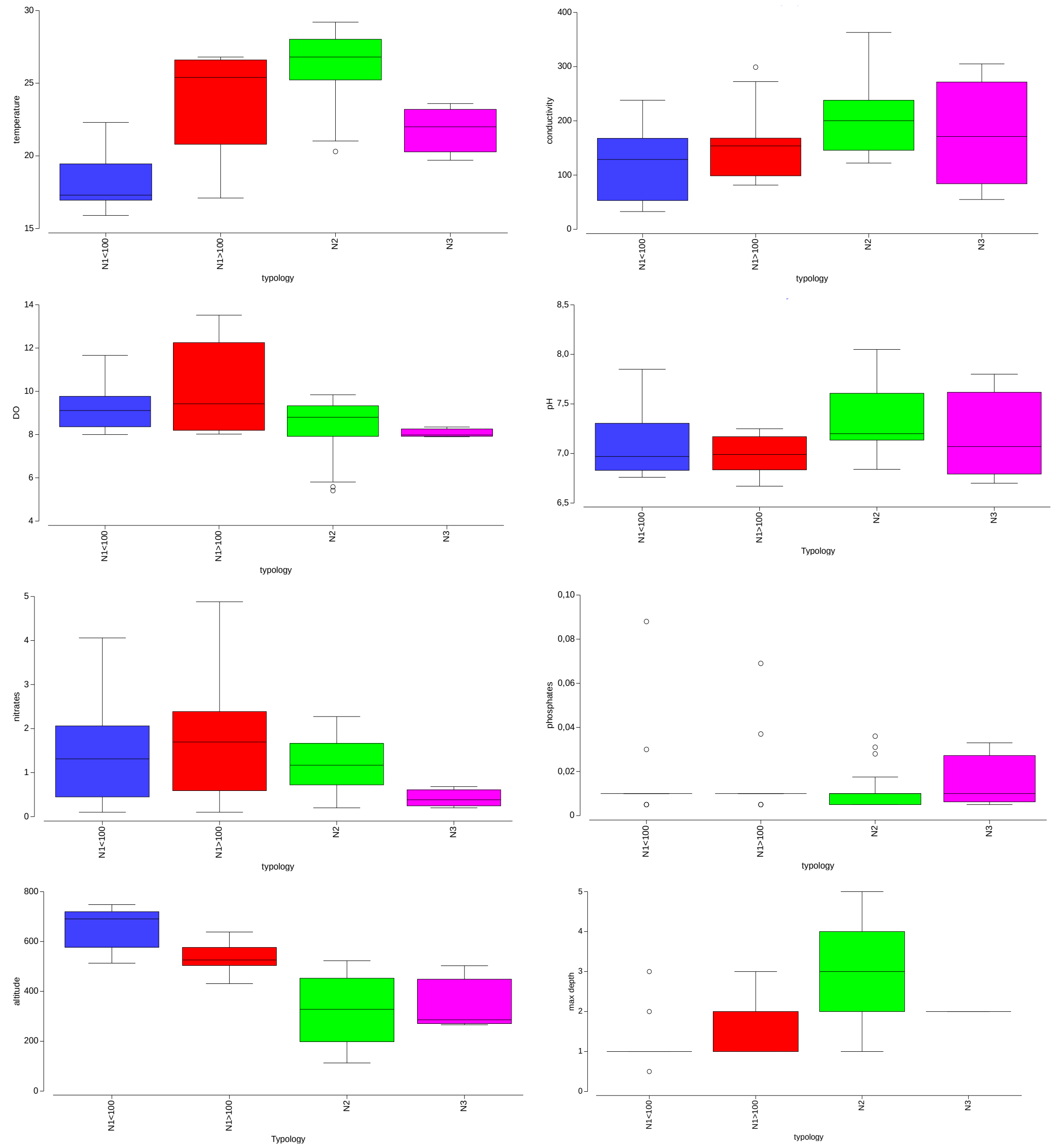

Figure 3.1 Water quality: physical and chemical variation of some parameters, considering the river typologies (Summer 2023).

The dbRDA performed on the abiotic data for the 43 sites (Figure 3.2) showed a marked separation between the polluted sampling sites located in Fervença river (Fv_04 and Fv_05) and the remain sampling sites, basically due to the substantial differences in nutrients (N and P) and dissolved salts (conductivity). However, it is also noticeable the division between the typologies of Small Northern Rivers (N1 ≤100) and Alto-Douro Medium-Large Rivers (N2), mainly due to differences in altitude and hardness which contributed to the separation of sites within the N1 ≤100 typology. Additionally, maximum depth and temperature were responsible for differentiating sites within the N2 typology. The two first axes of dbRDA explained 66.7% of all the variation (Figure 3.2).

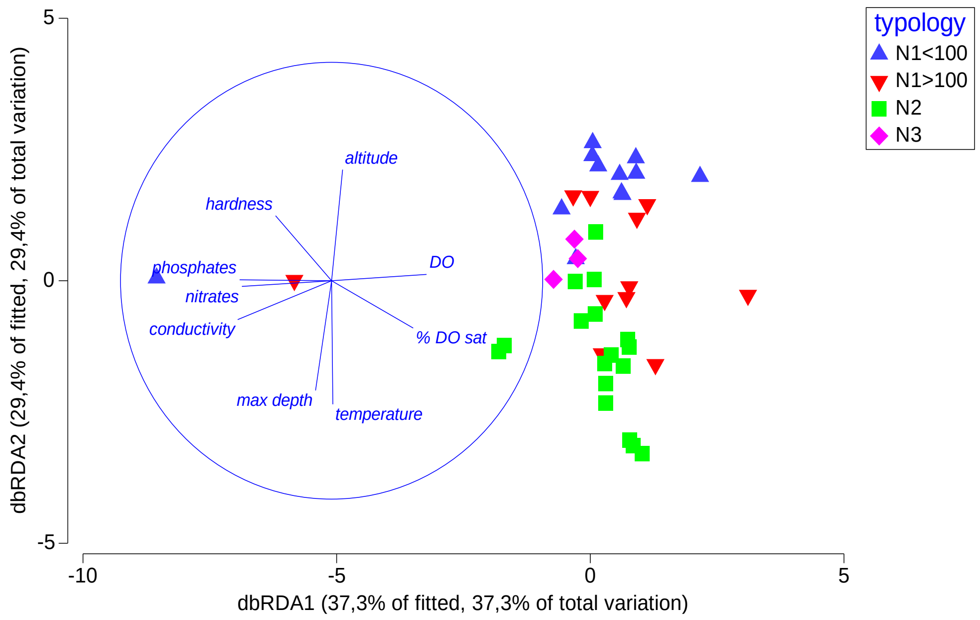

Figure 3.2 Distance-based redundancy analysis (dbRDA) showing the arrangement of the 43 sampling sites based on the abiotic factors measured and the 4 defined typologies.

3.1.2. Riparian and channel quality: hydromorphological elements

The RHS data, collected from the different sampling sites, allowed the obtention of two indexes HQA and HMS for 40 sampling sites (see Annex II, for total scores). Similarly to the water quality, it is presented in Table 3.2 and Table 3.3 the results obtained for the main river, only for the lotic sampling sites (i.e. Sa_01 to Sa_09). The HQA scores did not reach the excellent condition for all the sites in Sabor River, however most of them had a HQA > 50, like in sampling sites located inside the Natural Montesinho Park (e.g. Sa_01) and near the tail of the Baixo Sabor Dam (e.g. Sa_09), where the human influence is not so evident and natural habitats typical from those typologies (i.e. N1 < 100 and N2) can easily be identified, such as different flow types and complex channel and vegetation structures and a low land use by agricultural activities (Table 3.2).

Table 3.2 Partial HQA index values and respective sub-indices for the sites distributed throughout the Sabor River. Classification according to APA criteria 2021.

HQA	Sa_01	Sa_02	Sa_03	Sa_04	Sa_05	Sa_06	Sa_07	Sa_08	Sa_09
Flow type	10	9	9	8	10	9	9	8	11
Channel substrate	8	7	9	6	10	8	9	7	5
Channel features	6	4	7	2	5	5	6	5	6
Bank features	2	5	2	0	2	1	6	6	6
Bank veg. structure	3	3	11	10	5	12	4	5	10
Point bars	0	0	0	0	0	0	0	1	0
Channel vegetation	6	2	4	4	3	4	1	2	1
Land use	3	5	0	6	2	6	4	5	4
Trees	11	11	11	11	15	10	11	6	3
Special features	1	3	2	1	1	1	1	0	1
HQA Score	50	49	55	48	53	56	51	45	47
HQA Class	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2

The HMS index identified weirs as the major negative impact in the fluvial connectivity of this watercourse, responsible for the modification of flow types and riverbed substrate. However, only Sa_07 was classified as a significantly modified sampling site (Table 3.3).

Table 3.3 Partial HMS index values and respective sub-score for the sites distributed throughout the Sabor River. Classification according to APA criteria 2021. * - Good or lower quality

HMS sub-score	Sa_01	Sa_02	Sa_03	Sa_04	Sa_05	Sa_06	Sa_07	Sa_08	Sa_09
Outfall/deflector	0	0	0	0	0	0	0	0	0
Berms and embankments	0	0	0	0	0	0	0	0	0
Bridges	0	0	0	0	0	200	500	0	0
Culverts	0	0	0	0	0	0	0	0	0
Fords	0	0	0	0	0	0	0	0	0
Poaching	0	10	0	0	0	10	10	10	0
Reinforced Bank bed	50	0	20	0	90	0	50	0	0
Resectioned Bank bed	0	0	0	0	0	0	0	0	0
Weirs, dams, and sluices	0	305	375	375	375	0	375	125	0
HMS Score	50	315	395	375	465	210	935	135	0
HMS Class	2*	3*	3*	3*	3*	3*	4*	2*	1

A global analysis of the two RHS indexes, i.e. HQA and HMS, discriminated in terms of relative abundance by the 4 typologies defined for macroinvertebrate communities, confirmed that few sampling sites had good quality. Specifically, fewer than 40% of the sampling sites achieved a good quality classification according to HMS, and only one sampling site (5%) received an excellent classification according to HQA (Figure 3.3). Among the typologies, N3 showed the best results, with higher HMS index values recorded for this typology. In contrast, typology N2 (Sa_07: bridges, weirs, dams and sluices) had the higher number of sampling sites with lower quality.

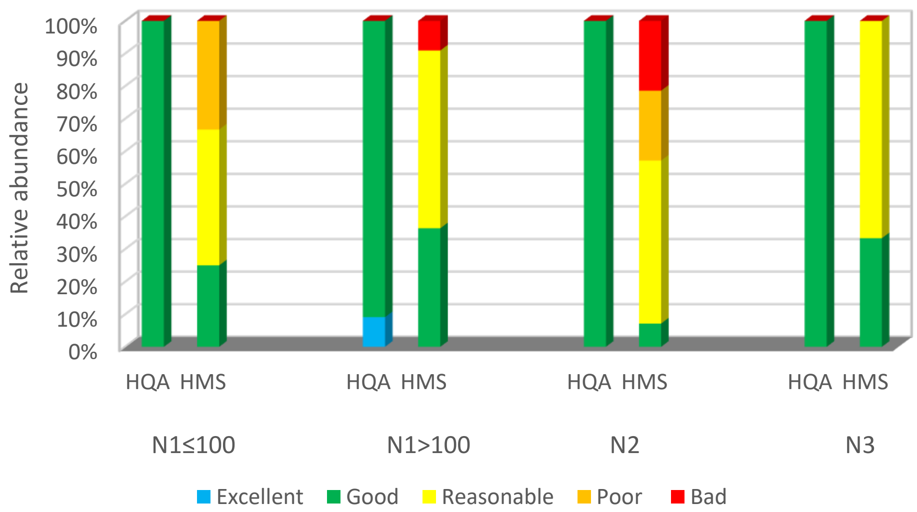

Figure 3.3 Hydromorphological quality: HQA and HMS relative abundance by river typology (Summer 2023).

3.2. Biotic Characterization

3.2.1. Macroinvertebrate communities

A total of 30 021 macroinvertebrates were identified, distributed by 86 different faunistic groups (mainly families) and belonging, the majority, to the phyla Arthropoda, Mollusca and Anellida. Arthropods were the most diverse group, including 69, 1 and 5 taxonomic units respectively distributed by Insecta, Arachnida and Crustacea classes. The most abundant taxa were insects (Diptera: Chironomidae and Simuliidae (11453 individuals), Ephemeroptera: Baetidae, Caenidae, Leptophlebiidae (6412), Plecoptera: Leuctridae (1275), Trichoptera: Hydropsyche (737), Hemiptera: Corixidae (1115)), crustaceans (Asellidae (3990), Atyidae (651)), and anelids (Oligochaeta (601)). The highest number of macroinvertebrates was found in Fervença River (e.g. Fv_02 and Fv_04, with 3274 and 2844 individuals, respectively) and the lowest number was collected in Zacarias stream (Za_01, 132 individuals, 19 different taxa). The presence of invasive alien species must be highlighted, namely the Asian clam (*C. fluminea*), and two decapods, the red-swamp crayfish (*P. clarkii*) and the signal-crayfish (*P. leniusculus*). In addition, it is worth noting the detection of threatened specimens, such as unionid mussels (*U. delphinus, A. anatina, P. littoralis*). The distribution and abundance of organisms and taxa in Sabor basin was naturally influenced by the typology but also by different pressures, such as river habitat (weir and dams), pollution and eutrophication and climate change (drought events), as can be observed by the faunal composition, diversity, and other metrics.

3.2.1.1. Macroinvertebrate community composition

Pronounced differences in the composition of macroinvertebrate communities were observed, namely between up and downstream of the main watercourse (Figure 3.4). In fact, a higher number of EPT taxa was detected (e.g. Plecoptera in Upper Sabor: Sa_01, Sa_02 and Sa_03, respectively with 37.78%, 53.52% and 40.67%), typical from small (N1<100) and median-large (N1>100) northern rivers, with low level of disturbance. In the Medium Sabor (e.g. Sa_06 to Sa_09) it was visible a dominance of Ephemeroptera (Baetidae, Caenidae) which are less sensitive organisms to pollution, comparatively with most of the families of Plecoptera (Leuctridae) and Trichoptera (Brachycentridae, Limnephilidae, Sericostomatidae) order. Due to the environmental changes promoted by the Lower Sabor (e.g. Baixo Sabor Dam: Sa_10, Sa_11, Sa_12) a higher abundance of Heteroptera (Corixidae) and Crustacea (Atyidae) was also observed (Figure 3.4).

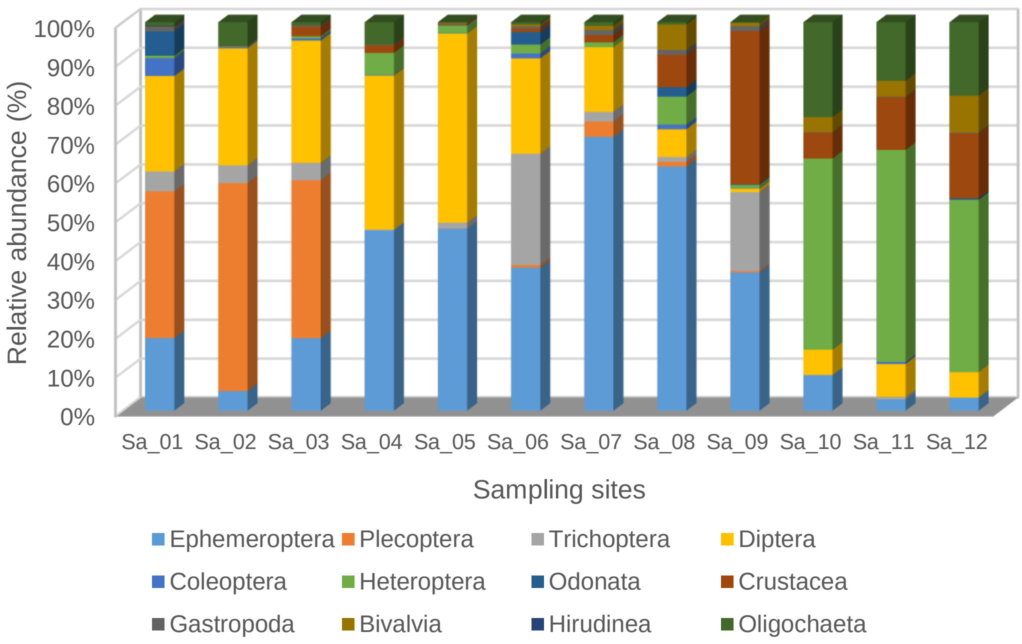

Figure 3.4 Macroinvertebrate relative composition in the Sabor river (Summer 2023).

Other differences were also detected in the macroinvertebrate composition of tributaries. For example, an evident dominance of Diptera and Crustacea was detected in Fervença river, showing the negative influence of domestic and industrial wastewaters of Bragança city, even after their treatment in the wastewater treatment plant (e.g. Fv_04 and Fv_05, with respectively 88.75% and 90.33% with Asellidae individuals) and inclusively in the urban river (Fv_03), and upstream (Fv_01, Fv_02) where Diptera (Chironomidae, Simuliidae) was over 70% (Figure 3.5). Differently, for most of the Maçãs river sampling sites, EPT%, ranged between 54.93% and 81.04%, except for Ma_02 directly influenced by a small dam (e.g. Water Treatment Plant of Vimioso), that clearly showed an important value for Diptera (88.55%) (Figure 3.5). The synergic effect of human activities (e.g. water pollution, fragmentation and loss of channel and riparian habitats, introduction of exotic species) and climate change (e.g. prolonged dry period, heatwaves, and forest fires), namely in Summer 2023, contributed to extensive and unusual variations in the macroinvertebrate composition, decreasing the stenobiont species, particularly in low order streams (Annex III, Figure AIII.1 and AIII.2). Some of these streams, even in mountain areas, acquired temporary conditions during the harsh environmental period of Summer and Autumn seasons of recent decade in the northeastern Portugal.

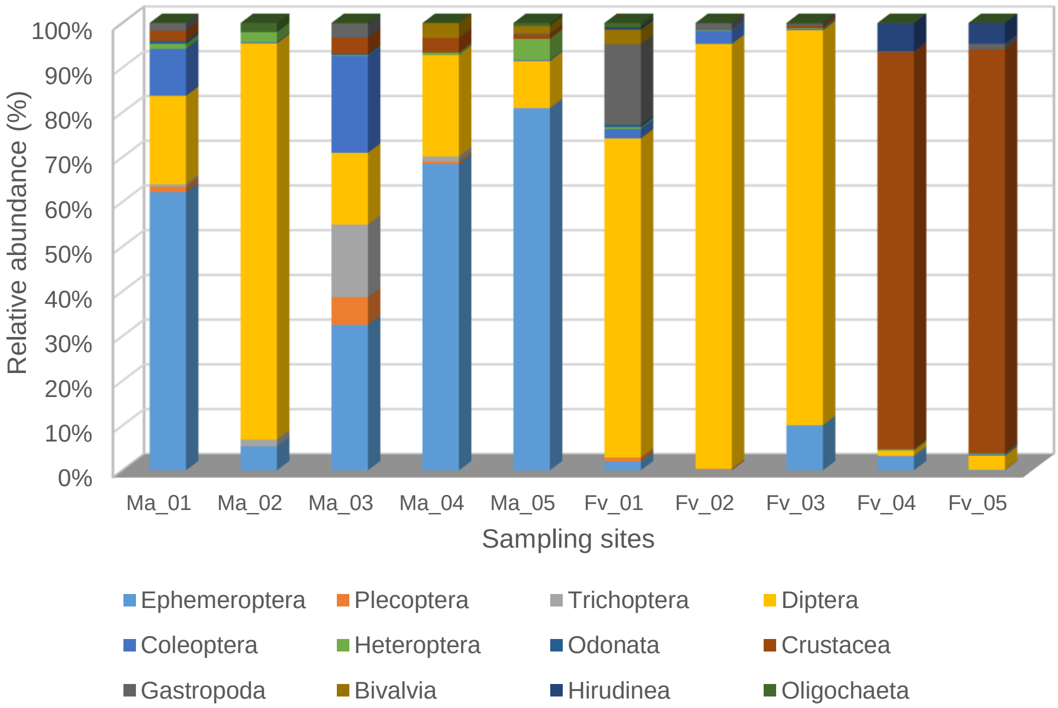

Figure 3.5 Faunal composition of invertebrates in Maçãs and Fervença rivers, tributaries of Sabor river (Summer 2023).

3.2.1.2. Typologies of macroinvertebrate communities

Based on the macroinvertebrate communities of the Sabor basin, according to the 4 defined river typologies (N1≤ 100, N1> 100, N2 and N3), no significant differences (K-W test, p > 0.05) were found for the metrics of richness (S) and abundance (N) (Figure 3.6). Individual scores for different metrics of 43 sampling sites are presented in Annex III (Table AIII.1 to AIII.3).

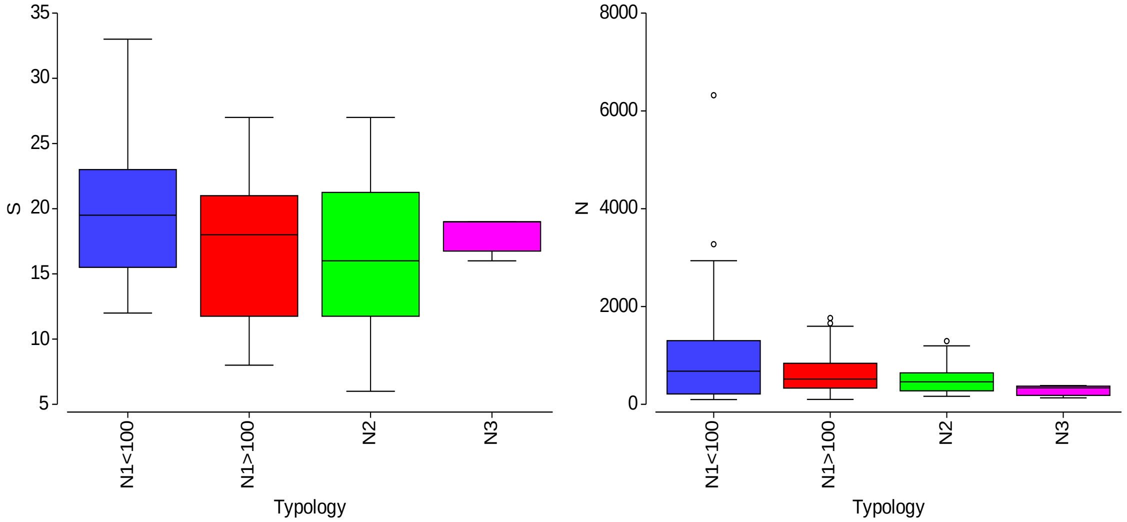

Figure 3.6 Richness (S) and abundance (N) of the macroinvertebrate communities, distributed by river typology groups.

The PERMANOVA results, based on the macroinvertebrate communities of the sampling sites, indicated that there are significant differences were detected among the typologies (Pseudo-F=2.158, p=0.002), disturbance (Pseudo-F=1.819, p=0.041) and the interaction (Pseudo-F=1.480, p=0.028) between both factors (Table 3.4).

Table 3.4 Results of the PERMANOVA analysis on invertebrate communities along river typologies and non-disturbed vs. disturbed. The asterisks indicate significant values.

Source	**dF**	**SS**	**MS**	**Pseudo-F**	**P (perm)**	**Unique perms**
Typology	3	12496	4165	2.158	0.002**	997
Disturbance	1	3511	3511	1.819	0.041*	999
Typology* Disturbance	3	8569	2856	1.4798	0.028*	999
Residual	35	67555	1930			
Total	42	94885				

The nMDS analysis (2D, stress value of 0.19) showed a separation in river typologies, mainly between small northern rivers (N1≤100) and both small (N3) and medium-large rivers and Upper Douro (N2) and for most of the northern rivers (N1≤100) (Figure 3.7).

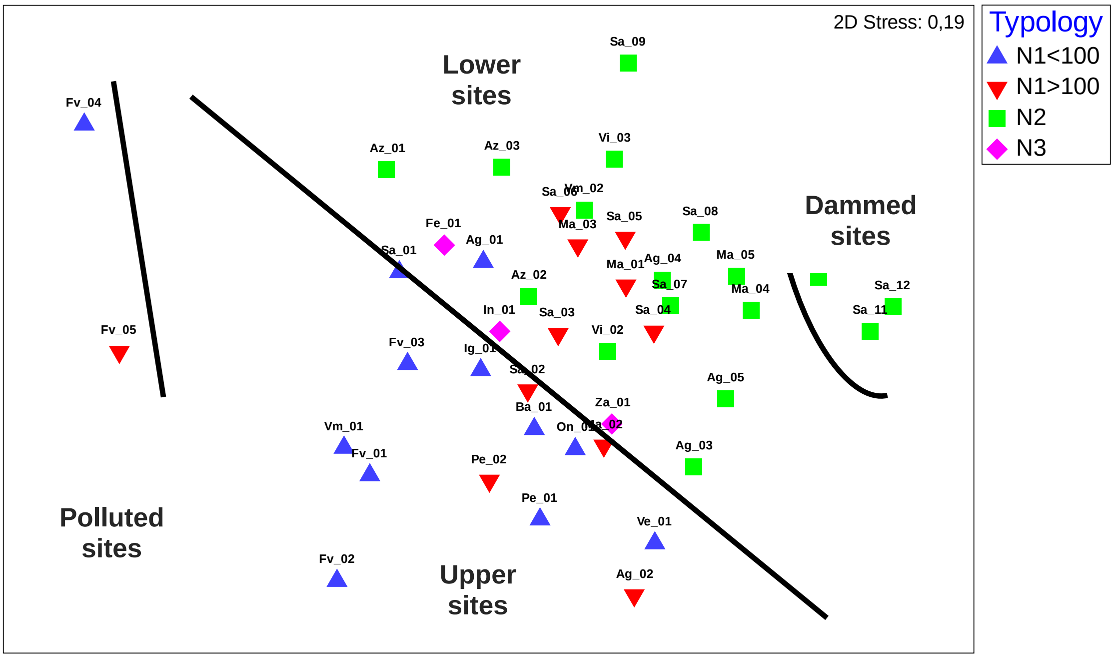

Figure 3.7 Non-metric multidimensional scaling (nMDS) of the 43 sampling sites, based on macroinvertebrate abundance, according to river typology groups and main pressures detected (river regulation and pollution).

Moreover, the main pressures of river regulation and water pollution contributed to an environmental gradient reflected in the dissimilarity among macroinvertebrate communities. In fact, in the Figure 3.8 can also be identified two more groups composed by the 3 sampling sites located in the Baixo Sabor dam (Sa_10, Sa_11, Sa_12) characterized by a higher abundance of Heteroptera, and Fervença River, highly polluted, where euribiont organisms belonging to the faunal groups of Asellidae, Hirudinea and Diptera showed an evident dominance (Figure 3.8).

The ANOSIM tests confirmed significant differences ($p < 0.001$) for the pairwise of Small Northern Rivers (N1≤100) and Alto-Douro Medium Rivers (N2) typologies and between the pairwise of disturbed and non-disturbed sites. The SIMPER analysis identified the taxa most contributing to dissimilarity between Small Northern Rivers (N1≤100) and Alto-Douro Medium Rivers (N2) (72.96%) were Caenidae (8.41%), Simuliidae (8.05%), Chironomidae (8.03%), Baetidae (4.92%), Asellidae (4.82%) and Corixidae (4.60%), while between disturbed and non-disturbed sites (66.73%) were Caenidae (8.17%), Chironomidae (7.32%), Leuctridae (6.30%), Simuliidae (5.00%), Corbiculidae (1.51%), Cambaridae (1.66%) and Astacidae (1.48%).

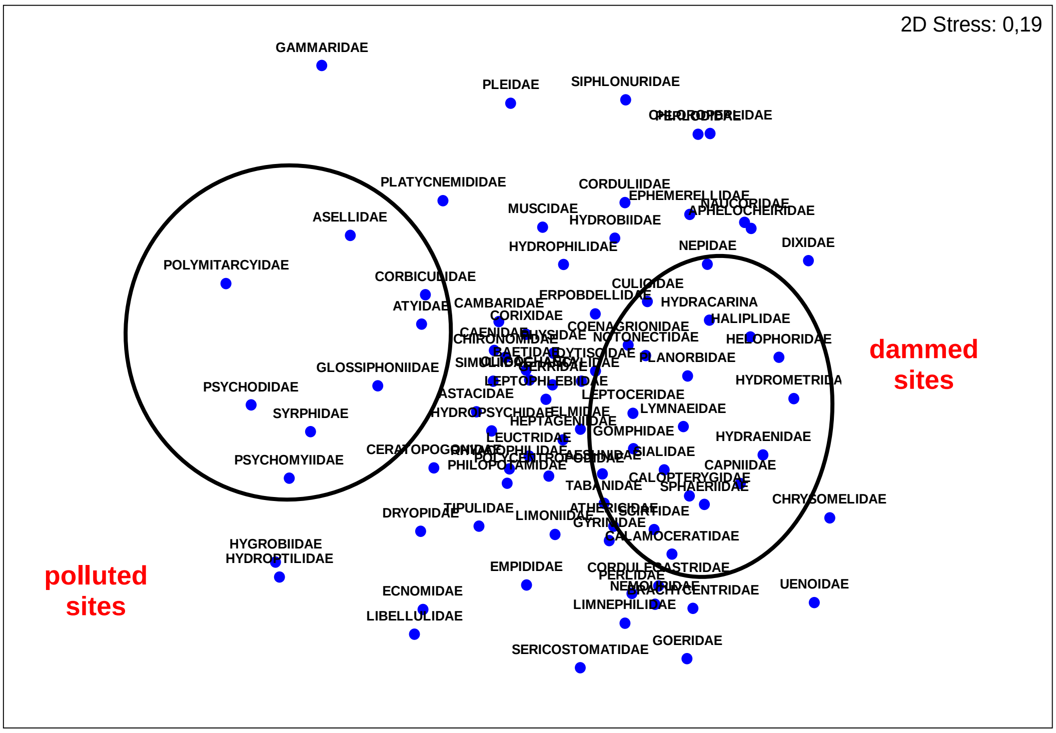

Figure 3.8 Non-metric multidimensional scaling (nMDS) of the invertebrate abundance, according to river typology and main detected pressures (river regulation and pollution).

3.2.1.3. Diversity and trophic indexes

No significant differences (K-W tests, p > 0.05) were found for the metrics of Pielou Evenness index (J') and Shannon-Wienner index (H') considering the 4 river typologies (N1 ≤ 100, N1> 100, N2 and N3) present in the Sabor basin (Figure 3.9).

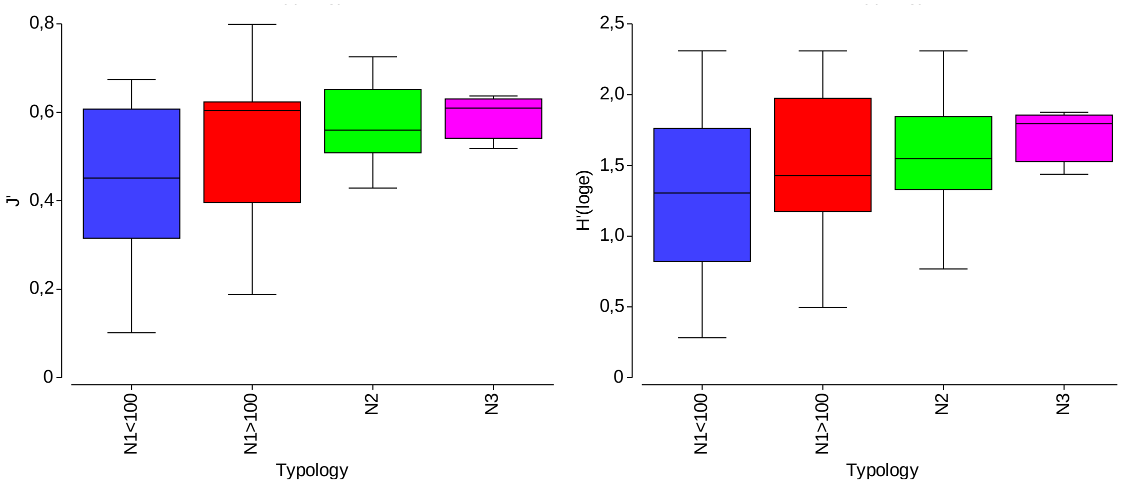

Figure 3.9 Pielou Evenness index (J') and Shannon-Wienner index (H') of the macroinvertebrate communities, distributed by river typology groups.

The biological quality of all sampling site can be observed in the Figure 3.10.

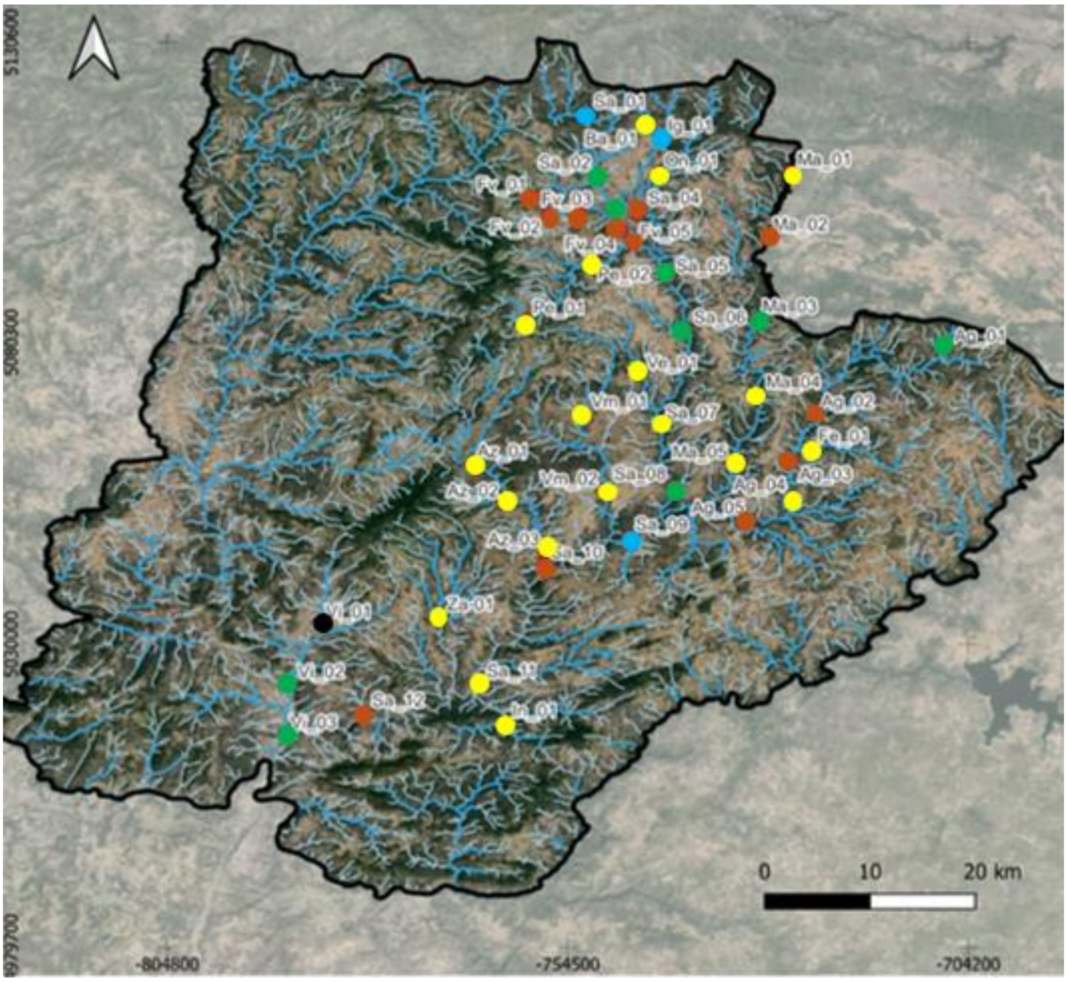

Figure 3.10 Biological quality, based on the invertebrate communities, of the 43 sampling sites surveyed in the Sabor basin. Symbols - Blue: excellent; Green: good; Yellow: reasonable; Orange: poor ecological condition (Summer 2023).

The results for the IPtIN index (see Annex III, Tables AIII.4 to AIII.7), indicated that 72% of 43 sampling sites belong to the reasonable and poor condition (Figure 3.11).

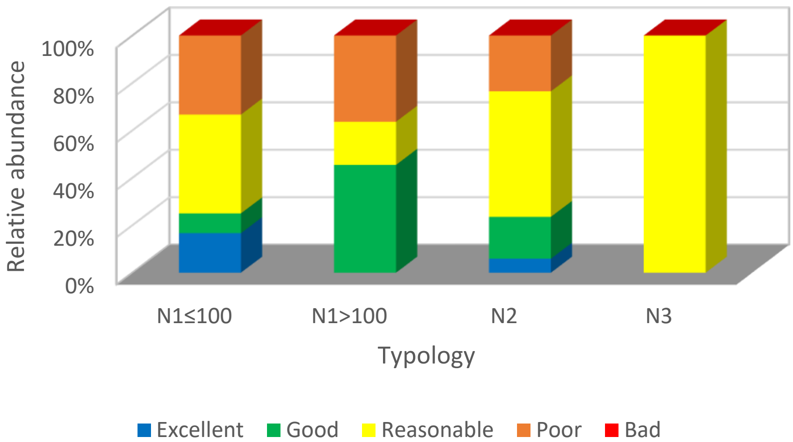

Figure 3.11 IPtIN index of the macroinvertebrate communities, distributed by river typology groups.

The excellent and good classification, i.e. the minimum required by the Water Framework Directive, was observed for only 3 (e.g. 17% - N1≤100 and 6% - N2) and 9 sampling sites (e.g. 8% - N1≤100, 45% - N1>100 and 18% - N2), respectively.

Significant differences (K-W tests, $p < 0.05$) were found for functional feeding groups of shredders, gathering collectors and predator and parasites groups, considering the 4 river typologies (N1 ≤ 100, N1> 100, N2 and N3) present in the Sabor basin. The trophic analyses showed a clear dominance of gatherers/collectors, although the different functional feeding groups (FFG) were represented in almost all the 43 sampling sites. The food resources availability is reflected in the FFG variation along the longitudinal axis (from Sa_01 to Sa_12) of the main watercourse, i.e. Sabor River. In fact, the importance of riparian zones appeared well marked in the Upper Sabor (Sa_01 to Sa_03) by the presence of shredders. The ecological integrity of the headwater streams and particularly the natural heterogeneity of microhabitats allowed, in summer season, to occur both food sources, i.e. allochthonous (e.g. input of leaves, seeds) and autochthonous (e.g. periphyton), used by shredders and grazers and scrapers, respectively. Moreover, the organic matter deposited and transported in the water column is well represented by the presence of gatherers/collectors and filter feeders. Finally, it was found a normal (< 15%) percentage of predators and parasites, which consume directly in available alive preys. A detailed analyses of individual FFG variation can be done in Figure 3.12 and Figure 3.13 e and in the Annex III (Figures AIII.3 and AIII.4).

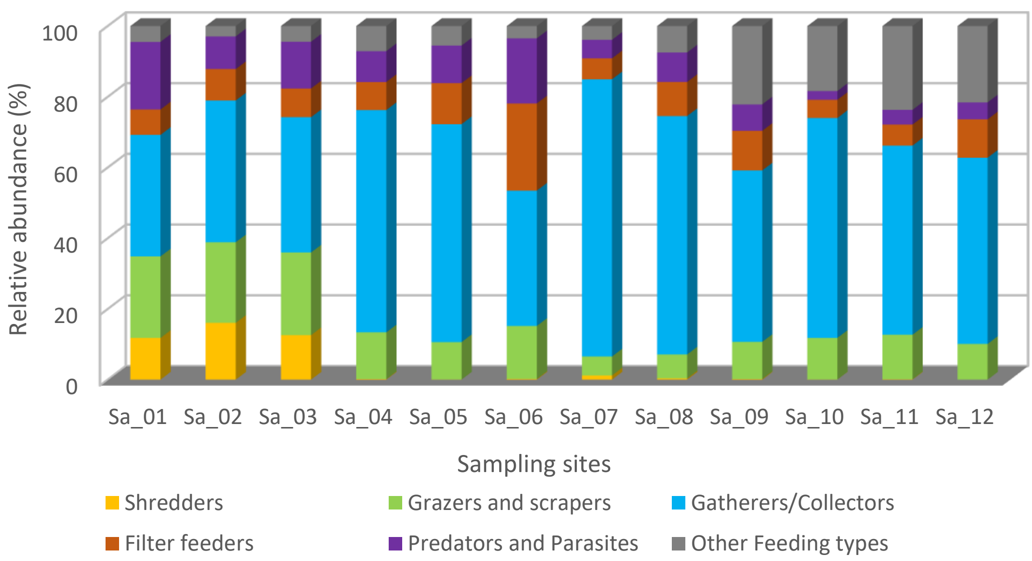

Figure 3.12 Functional feeding groups (FFG) in the Sabor river (Summer 2023).

The trophic analysis demonstrated to be a sensible and representative metric of the effect of the detected disturbance in different sampling sites. For example, it was visible pronounced variations in Fervença River, subjected to water pollution (Figure 3.13).

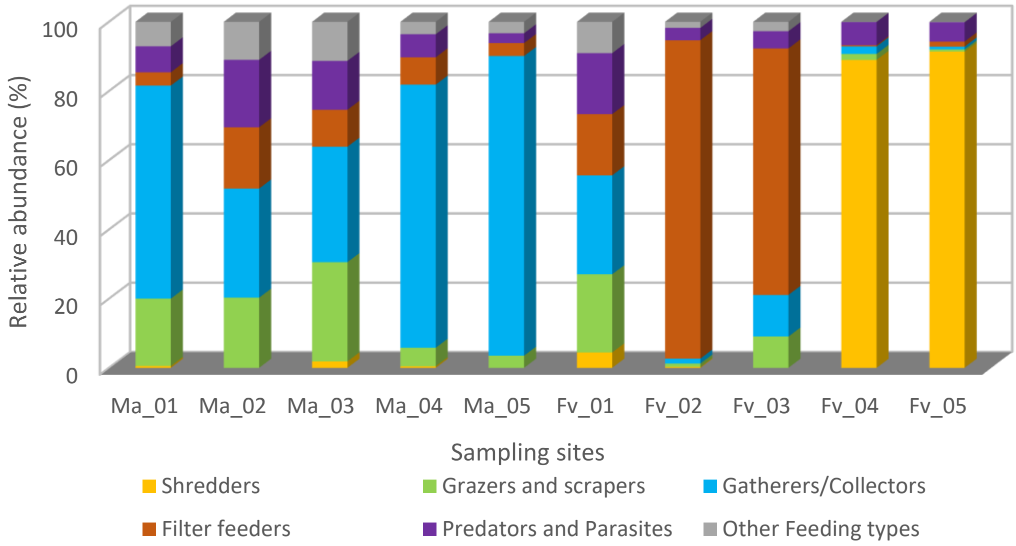

Figure 3.13 Functional feeding groups (FFG) in Maçãs and Fervença rivers (Summer 2023).

3.2.2. Fish communities

A total of 6 086 individuals and 16 species were identified in the Sabor basin. It must be highlighted that 56% are non-native (9 species) and 44% (7 species) are native species. The assemblage of native species belongs to the following 4 families: 1) Salmonidae: *Salmo trutta*; 2) Leuciscidae: *Squalius carolitertii, Squalius alburnoides, Achondrostoma asturicense, Pseudochondrostoma duriense*; 3) Cyprinidae: *Luciobarbus bocagei*; 4) Cobitidae: *Cobitis paludica*. The non-native species are distributed in 4 families of distinct sources: 1) Centrarchidae: *Micropterus salmoides, Lepomis gibbosus* (North America); 2) Leuciscidae: *Alburnus alburnus, Rutilus rutilus* (Europe and Asia); 3) Gobionidae: *Gobio lozanoi* (North Spain and South France); 4) Poecilidae: *Gambusia holbrooki* (North America); 5) Esocidae: *Esox lucius* (North America, Europe, and Asia); 6) Percidae: *Sander lucioperca* (Europe and Asia); and 7) Cyprinidae: *Cyprinus carpio* (Asia). The most abundant species were *Lepomis gibbosus* (18.5%, 1125 individuals), *Squalius alburnoides* (18%, 1115), *Alburnus alburnus,* (12.8%, 781), and *Squalius carolitertii* (11.4%, 692). The highest densities (CPUE, ind.100m^2) were found in Sabor River (e.g. Sa_09 and Sa_03, with 74 and 51 individuals/100 m^2, respectively) and in the tributaries of Vilariça (Vi_03, 92 ind.100 m^2), Vale Moinhos (e.g. Vm_01, 68 ind.100 m^2), Maçãs (e.g. Ma_05, 51 ind.100 m^2) rivers. In opposition, the lowest densities were detected in Azibo (Az_03, 7 ind.100 m^2), Ferreiros (Fe_01, 8 ind.100 m^2), and Zacarias (Za_01, 9 ind.100 m^2) streams (see annex IV, Figure AIV.1 to AIV.3).

3.2.2.1. Fish community composition

Major differences in fish species composition and their variation along the longitudinal axis of the Sabor River were observed (Figure 3.14). Indeed, in the Upper Sabor the natural fish zonation, only with the presence of native species, was detected, ranging from exclusively salmonid (e.g. Sa_01) to transitional salmonid/cyprinid (Sa_02 and Sa_03) and finally to the beginning of the exclusive cyprinid (Sa_04) zone, coinciding with the southern edge of Montesinho influenced by a small hydropower plant. In the medium-sized Sabor (Sa_05 to Sa_09), despite the presence of several weirs, native species cohabit, in running waters, with an increase downstream tendency for higher densities of exotic species. The Lower Sabor is characterized by the presence of a 60 Km extent reservoir (e.g. Baixo Sabor Dam: Sa_10, Sa_11, Sa_12) and this modified environment, typically of lentic conditions lead, with human influence, to the almost disappearance of native fish, substituted by a more adapted exotic fish assemblage. The introduction of invasive alien species, and particularly piscivorous fish, like pike (*Esox lucius*), black-bass (*Micropterus salmoides*) and zander (*Sander lucioperca*), contributed for the decrease of native fish in the Baixo Sabor Dam.

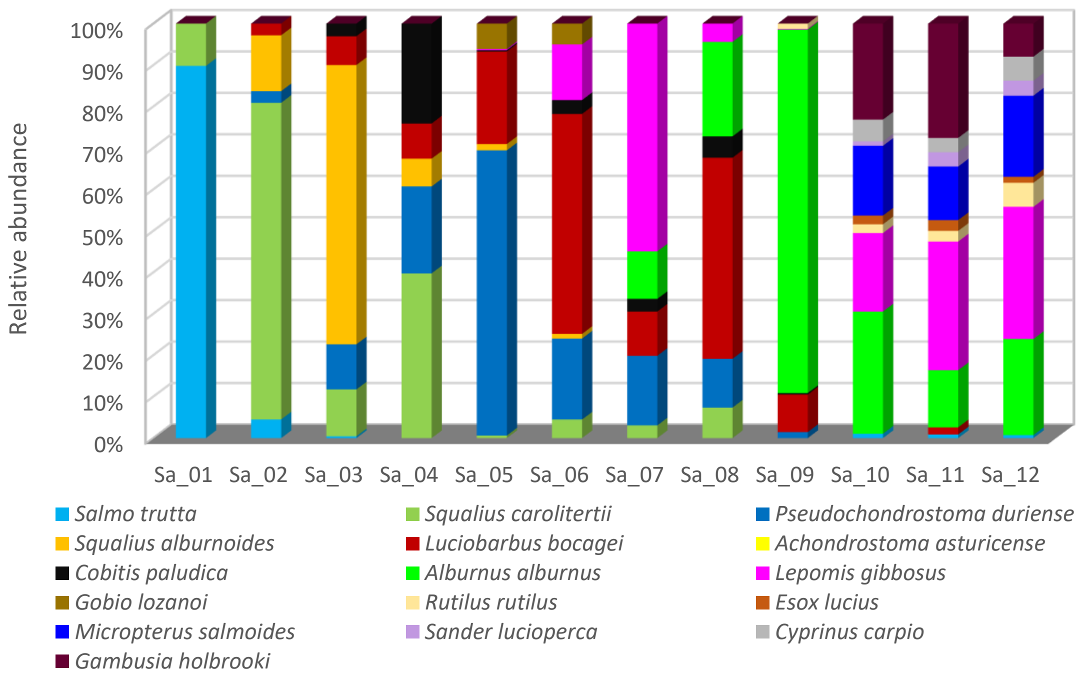

Figure 3.14 Relative composition of fish communities in Sabor river (Summer 2023).

This pattern of substitution of native by exotic species along the longitudinal gradient of Sabor river is clearly observed in Figure 3.15.

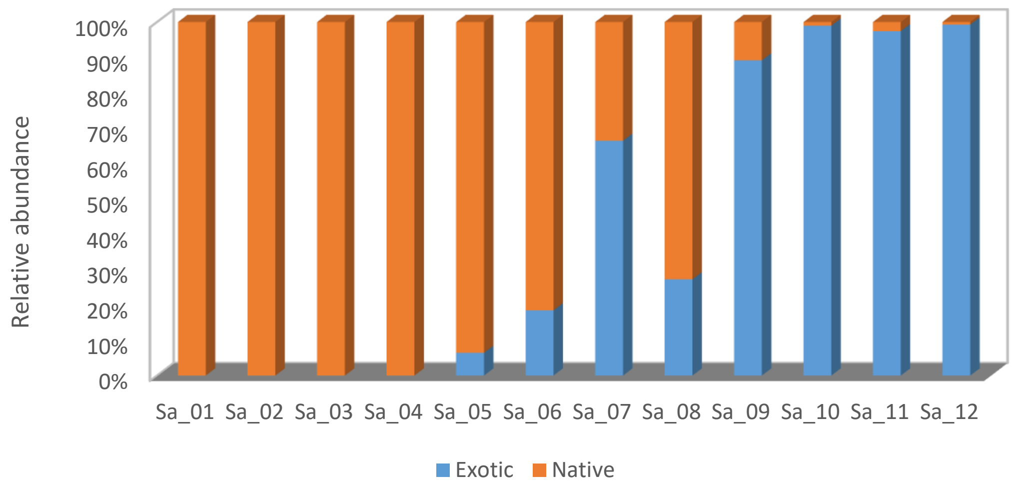

Figure 3.15 Native and non-native distribution of fish in Sabor river (Summer 2023).

The presence of exotic species in tributaries is also evident, inclusively dominating the fish abundance, like in Maçãs and Fervença rivers, for the pumpkinseed (*Lepomis gibbosus*); and Pyrenean gudgeon (*Gobio lozanoi*) species, respectively (Figure 3.16).

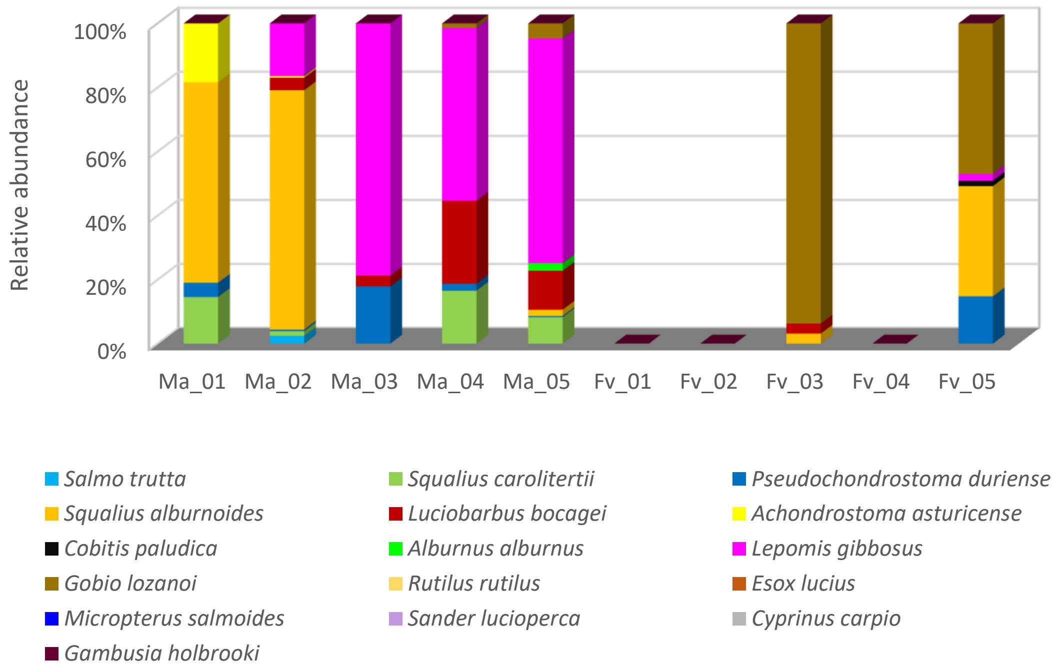

Figure 3.16 Relative composition of fish communities in Maçãs and Fervença rivers (Summer 2023).

In Fervença river, the environmental conditions do not allow the establishment of fish populations. However, most of the streams in the Upper and Medium-sized Sabor basin showed fish communities where native leuciscids, like Northern Iberian chub (*Squalius carolitertii*), Northern straight-mouth nase (*Pseudochondrostoma duriense*), calandino (*S. alburnoides*), and cyprinids, such as common-barbel *(Luciobarbus bocagei*) are dominant species (see Annex IV, Figures AIV.4 and AIV.5). It must be highlighted the presence of *Achondrostoma asturicense*, in Angueira and Maçãs rivers, recently described (Doadrio et al., 2023) and classified as an Endangered (EN) species by the Portuguese Red Data Book for Freshwater and Diadromous Fishes (Magalhães et al., 2023). The regulatory framework and the conservation status of the detected fish in Sabor basin can be observed in Annex IV (Table AIV.1).

3.2.2.2. Typologies of fish communities

Based on the 4 river typologies (G1, G2, G3 and G4) defined for fish communities of Sabor basin, significant differences (K-W tests, $p < 0.05$) were found for the metrics of richness (S) and abundance (N) (Figure 3.17).

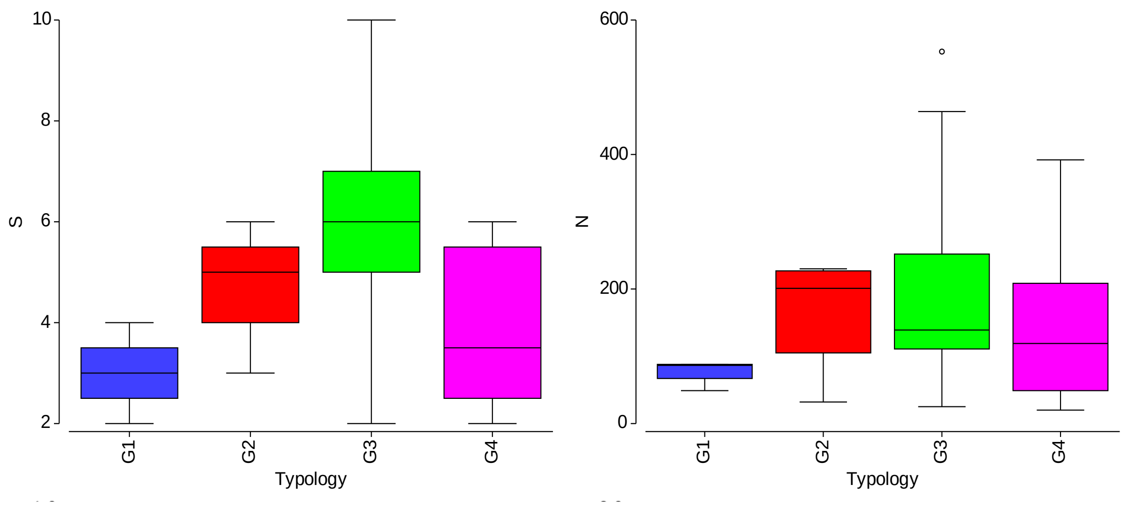

Figure 3.17 Richness (S) and abundance (N) of the fish communities, distributed by river typology groups.

The PERMANOVA results, based on the fish communities of the sampling sites, identified significant differences for both factors, i.e. among the defined typologies (Pseudo-F=2.391, p=0.001) and non-disturbed and disturbed (Pseudo-F=2.411, p=0.018) sites (Table 3.5).

Table 3.5 Results of the PERMANOVA analysis on fish communities along river typologies and non-disturbed vs. disturbed sites. The asterisks indicate significant values.

Source	dF	SS	MS	Pseudo-F	P (perm)	Unique perms
Typology	3	17461	5820	2.391	0.001**	997
Disturbance	1	5870	5870	2.411	0.018*	997
Typology* Disturbance	2	6406	3203	1.315	0.162	998
Residual	33	80342	2435			
Total	39	116320				

The nMDS ordination (2D, stress value of 0.17) showed the best separation in river fish typologies between small streams (i.e. G1 - Salmonid streams of the Northern Region and G4 - Small Cyprinid streams of the North-Central Interior and South Regions) and medium-large rivers (i.e. G2 - Salmonid-Cyprinid Transition zone rivers of the Northern Region and G3 - Medium-sized Cyprinid rivers) (Figure 3.18). However, fish communities colonizing the Baixo Sabor Dam (Sa_10, Sa_11 and Sa_12) can also be differentiated from the dispersion of all sampling sites.

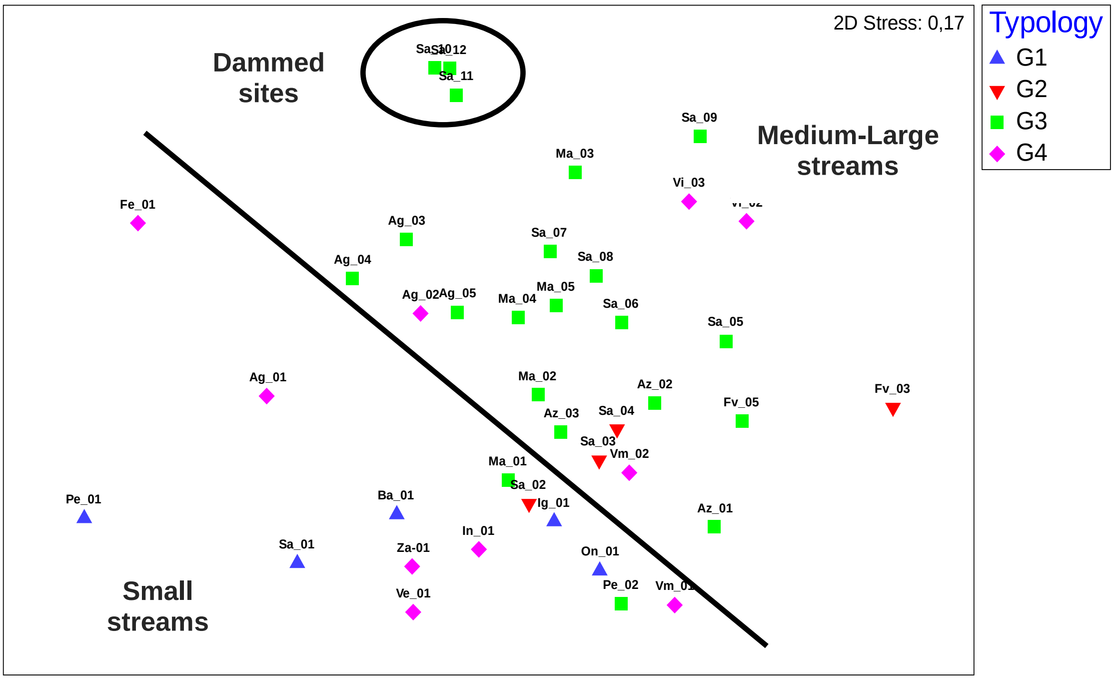

Figure 3.18 Non-metric multidimensional scaling (nMDS) of the 40 sampling sites, based on fish abundance, according to river typology groups and main pressures detected (river regulation and pollution).

Analyzing the nMDS ordination for the fish species communities is possible to identify the species segregation, namely among salmonid zone, represented by *S. trutta*, cyprinid zone, englobing leuciscids and cyprinids and non-native species (*A. alburnus, L. gibbosus, G. holbrooki, G. lozanoi*) and lentic community, composed mainly by piscivorous fish (e.g. *E. Lucius, M. salmoides* and *S. lucioperca*) (Figure 3.19).

The ANOSIM tests confirmed significant differences ($p < 0.05$) between G1 (Salmonid streams of the Northern Region) vs. G3 (Medium-sized Cyprinid rivers), and G3 vs. G4 (Small Cyprinid streams of the North-Central Interior and South Regions) and between disturbed vs. non-disturbed sites ($p = 0.001$). The SIMPER analysis identified the taxa that most contributed to the average dissimilarity between G1 vs. G3 (81.88%) were *L. gibbosus* (16.47%), *S. alburnoides* (14.62%), *L. bocagei* (11.16%), *S. trutta* (10.85%) and *S. carolitertii* (10.04%), and between G3 vs. G4 (78.0%) were the species of *L. gibbosus* (15.58%), *S. alburnoides* (14.46%), *L. bocagei* (10.35%), *A. alburnus* (9.69%) and *S. carolitertii* (9.30%). The fish most contributing to the average dissimilarity between disturbed and non-disturbed sites (78.93%) were *S. alburnoides* (13.61%), *L. gibbosus* (12.80%), *S. carolitertii* (11.59%) and *A. alburnus* (10.34%).

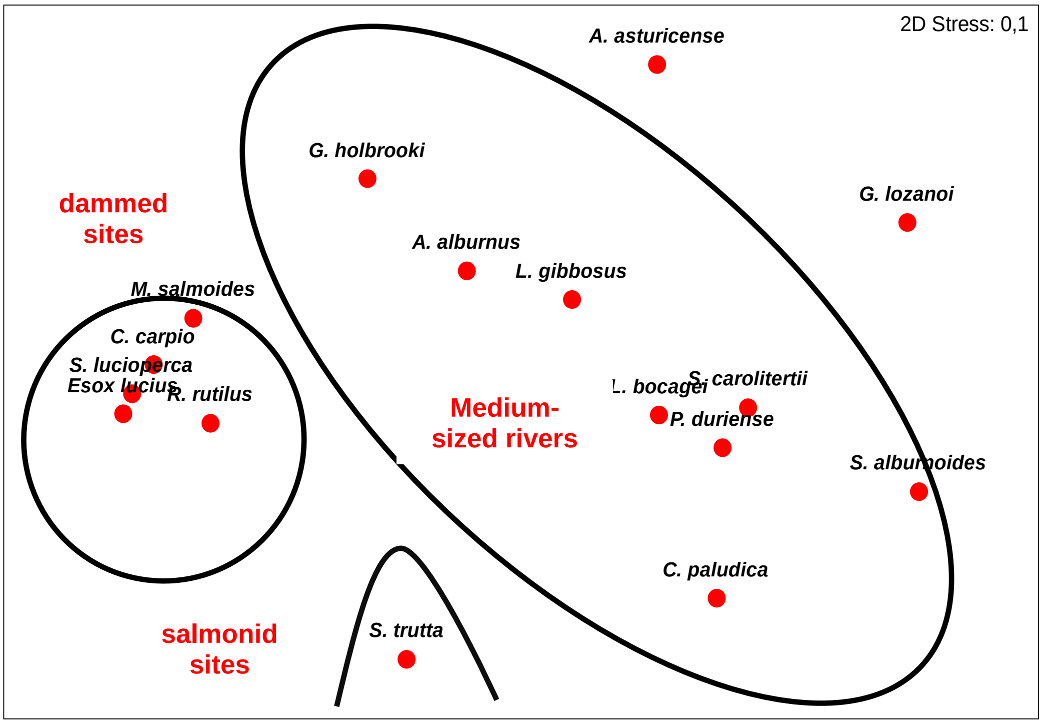

Figure 3.19 Non-metric multidimensional scaling (nMDS) of the fish abundance, according to river typology and main detected pressures (river regulation and pollution).

3.2.2.3. Fish Quality and Diversity indexes

The biological quality of all sampling sites can be observed in the Figure 3.20 (see Annex IV, Tables AIV.2 to AIV.5, for individual scores of sampling sites).

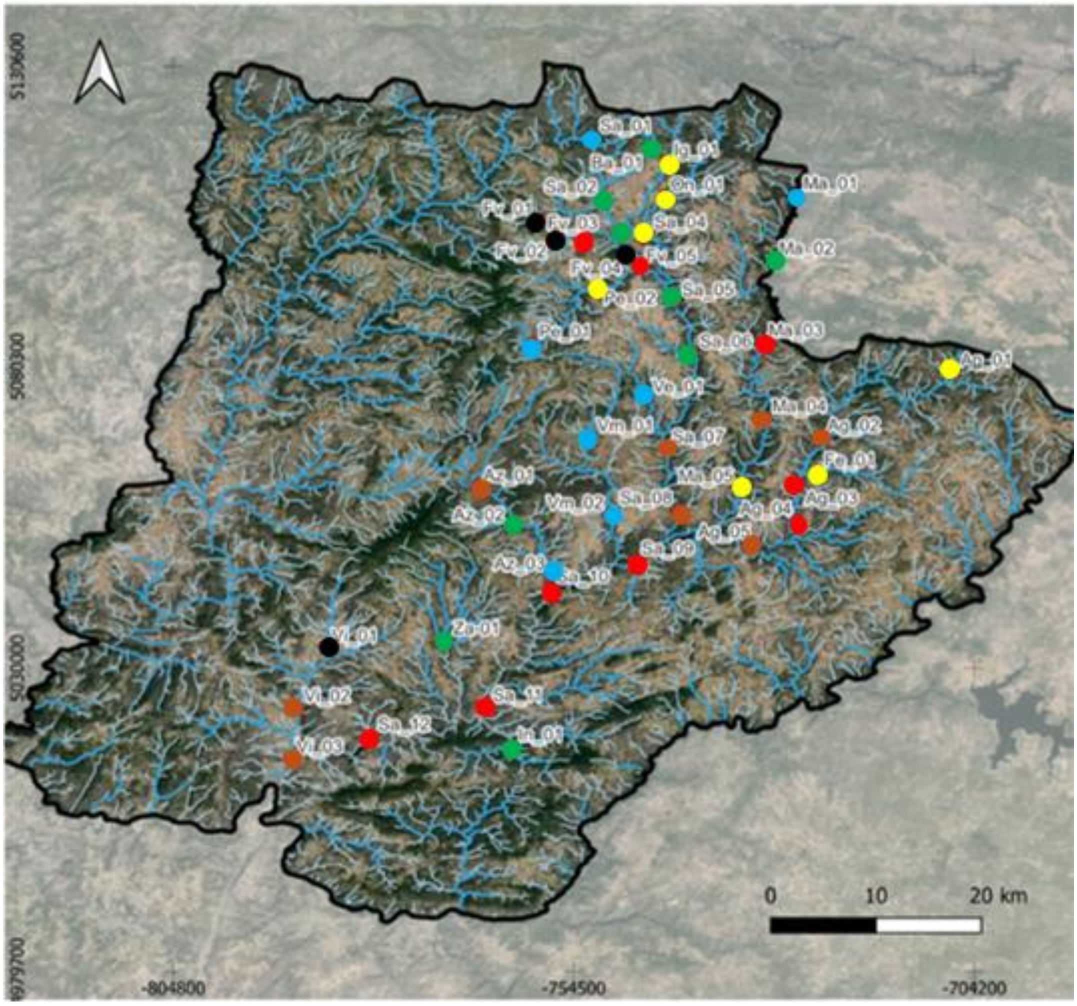

Figure 3.20 Biological quality, based on the fish communities, of sampling sites in Sabor basin (Summer 2023). Symbols - Blue: Excellent; Green: Good; Yellow: Reasonable; Orange: poor; Red: Bad ecological condition (Summer 2023).

The results obtained for the Portuguese fish F-IBIP index indicated that, globally, 40% sampling sites showed good or excellent condition. The best conditions belong to both typologies G1 - Salmonid streams of the Northern Region, G2 - Salmonid-Cyprinid Transition zone rivers of the Northern Region, and G4 - Small Cyprinid streams of the North-Central Interior and South Regions, respectively. The lower condition (less than reasonable) was detected in 15 sampling sites classified as belonging to G3 - Medium-sized Cyprinid rivers of the Northern Region (Figure 3.21).

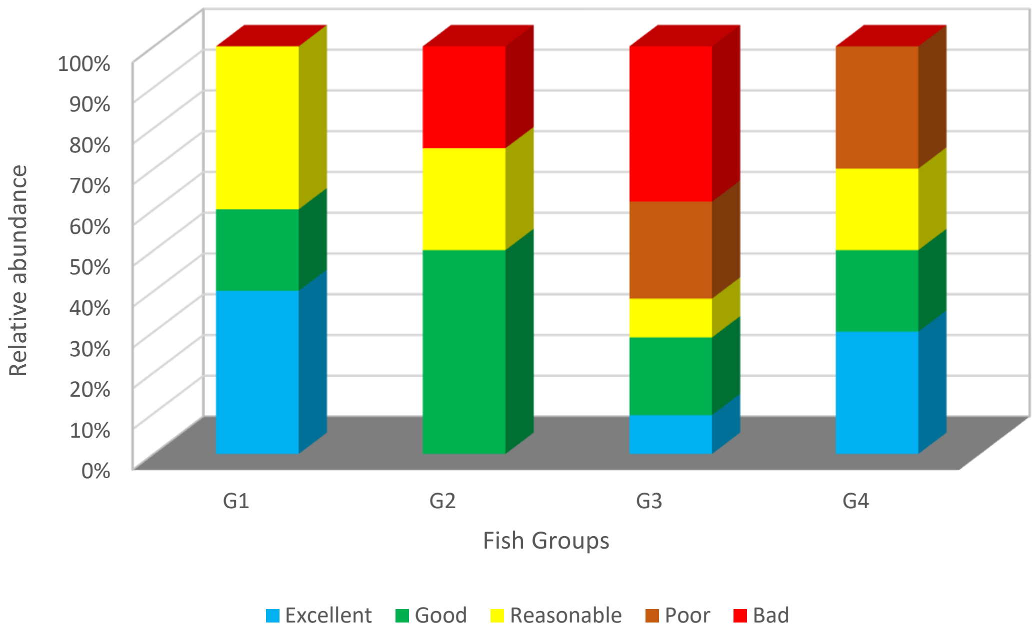

Figure 3.21 F-IBIP of fish communities, distributed by river typology groups.

Significant differences (K-W tests, $p < 0.05$) were also found for the metrics of Pielou Evenness index (J') and Shannon-Wienner index (H') considering the 4 river typologies (G1, G2, G3 and G4) present in the Sabor basin (Figure 3.22).

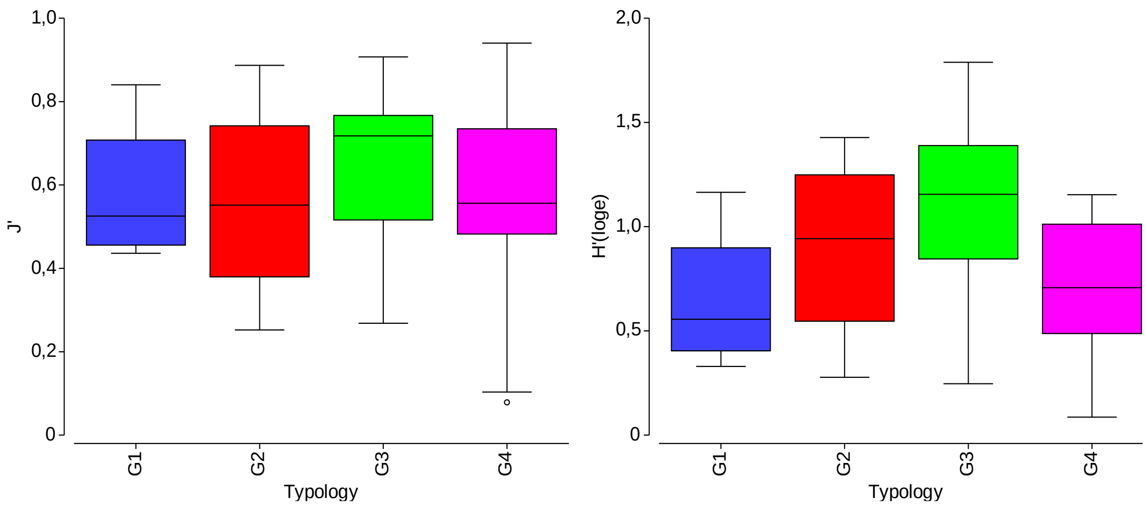

Figure 3.22 Evenness (J') and Shannon-Wienner (H') indexes of fish communities, distributed by river typology groups.

3.3. General ecological assessment status

The general ecological assessment status integrates the ecological status components, based on the assessment criteria of the document "Criteria for the Classification of Water Courses", associated with the 2nd Cycle of PGRH (APA, 2016). Figure 3.23 presents the synthesis of the final classification, considering biological, hydromorphological, and water physical and chemical quality elements.

The results for general ecological assessment status shows that only 8% of Small Northern Rivers of Portugal (N1 ≤ 100) and 18% of Medium-Large Northern Rivers of Portugal (N1 > 100) reached the good ecological status, required by the WFD (Figure 3.23). The final classification for each sampling site can be observed in Annex V.

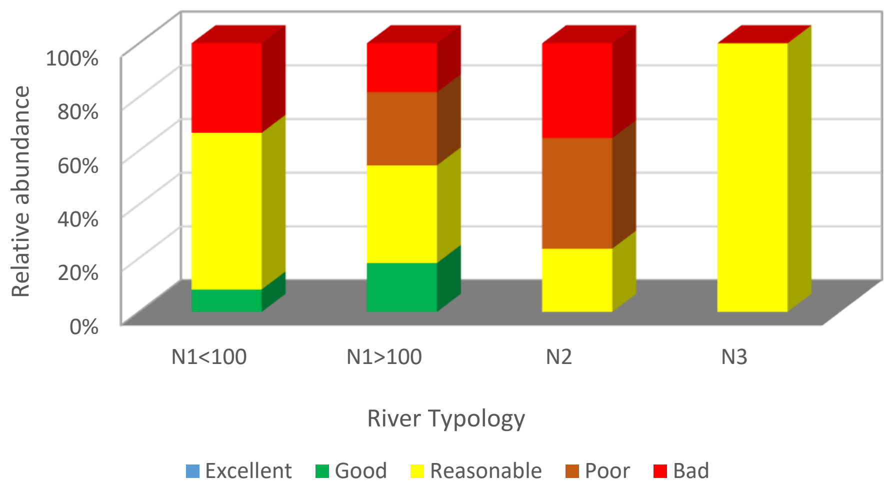

Figure 3.23 General ecological assessment status, based on general physical and chemical water, hydromorphological (RHS, HQA and HMS) and biological (macroinvertebrates and fish), distributed by river typology groups (Summer 2023).

It must be highlighted that the final classification for the general ecological assessment status was based on general physical and chemical water, hydromorphological (RHS, HQA and HMS) and biological (macroinvertebrates and fish). In fact, a complete assessment must also consider the biological evaluation of macrophytes and perifiton (for rivers) and phytoplankton (for reservoirs), and priority hazardous substances.

The classification of physical-chemical elements can only be accurately evaluated after the 4 sampling campaigns, as the evaluation of indicators considered as potentially problematic (e.g., phosphorus and nitrogen compounds) results from averages of the four campaigns. Moreover, it is important to highlight that the element having the most significant impact on the general ecological assessment status was the biological element (macroinvertebrates and fish). The classification index for biological elements is based on five quality classes (for both IptIN and F-IBIP indexes), whereas the HMS index score (hydromorphological elements) define quality using only two classes: “excellent” or “good or inferior”.

4. Discussion

The Water Framework Directive (WFD) was created in 2000 for the protection and sustainable use of freshwater resources in the European Union (EU) (EEA, 2018). However, the requested minimum “good status” was not reached for most of the surface water in EU, considering the initial goals for 2015 deadline (EEA, 2016; Filipe et al., 2019; Reyjol et al., 2014). In Portugal, based on the 2nd River Basin Management Plans (RBMPs) report, only 52.6% of all surface water bodies reached the good ecological status and 25.1% have good chemical status (EU, 2022). In this study, developed in several river typologies along the Sabor river (Douro basin), less than 10% of 43 sampled sites had good classification. It must be referred that not all elements were evaluated, namely primary producers (e.g., macrophytes, periphyton and phytoplankton) and priority hazardous substances for water quality. However, based on the WFD principle “*one out, all out*”, the final ecological scenario is marked by the worst classification. Several factors are contributing to the lower ecological state, namely environmental changes and threats such as habitat fragmentation and degradation (e.g. small and big dams), water pollution and eutrophication, biological invasions and climate change and their hydrological extreme events (Vörösmarty et al., 2010; Voulvoulis et al., 2017).

We hypothesized that along the longitudinal axis of Sabor River, from headstream to downstream, several water physical-chemical parameters tend to increase (e.g. water temperature, dissolved salts and nutrients) in opposition to others (e.g. dissolved oxygen). According to Ilhéu et al. (2014), Portuguese river types reflect two main geographical and climatic gradients. From north to south the gradient is associated with a decrease in altitude, precipitation, annual discharge, and increasing temperature. On the other hand, the west–east gradient is related to a continental effect, with a precipitation decrease and an increase in temperature extremes. In fact, in this study it was detected a natural increase in water temperature and conductivity and a decreasing tendency in dissolved oxygen content along the longitudinal axis, i.e. from headstream to downstream zones. The relation between altitude, water temperature, conductivity and TDS is clearly visible for the following sampling sites: Sa_01 (720 m, 17.4°C, 43.5 µS/cm, 24 mg/L), Pe_01 (748 m, 17.1°C, 32.7 µS/cm, 18.68 mg/L), in contrast with Vi_03 (113 m, 27.8 °C, 363 µS/cm, 100 mg/L) and Sa_12 (143 m, 29.2 °C 126.5 µS/cm, 59.5 mg/L). However, this natural variation can be disrupted or modified by the impact of human activities. For example, the higher conductivity and TDS values can be related with point and diffuse pollution sources, confirmed in Fervença river (e.g. Fv_04: 598.0 µS/cm, 328 mg/L and Fv_05: 494.0 µS/cm, 295.0 mg/L). The lower efficiency of the Wastewater Treatment Plant (WWTP) of Bragança was previously referred by Portela (2014), and this higher organic pollution was, nine years after, detected (e.g. Fv_04: 30.57 mg NO_3^-/L and 0.34 mg PO_4^{3-}/L). According to detections limits from APA (2021), a maximum threshold for excellent/good status is represented by 5 mg NO_3^-/L and 0.10 mg PO_4^{3-}/L. Another major impact is due to the damming effect, observed in different sampling sites and particularly in the Baixo Sabor Dam (Sa_10, Sa_11 and Sa_12) where an increase in water temperature was detected. This pattern was observed in other studies (Santos et al., 2017) and the synergic influence of disturbance events, mainly of anthropic source, affect the abiotic conditions normally found in different river typologies. As highlighted by Gomes (2019) several factors affect the water quality, playing crucial roles, and driving variations, such as the lithology (e.g., regions with sedimentary rocks have waters with more salts and higher conductivity than regions with metamorphic rocks), land use (e.g. vegetation type and cover, urbanization) and particularly negative human pressures (e.g., intensive agriculture, domestic and industrial wastewaters, invasive alien species).

Relatively to the hydromorphological quality, based on the official index of River Habitat Survey, few sampling sites had an excellent condition (e.g., Ma_03, Fv_05 and Pe_02). However, for the Habitat quality index (HQA), most of them had a score HQA > 50. The Habitat modification score (HMS) index identified weirs as the major negative impact in the fluvial connectivity of this watercourse, responsible for the modification of flow types and riverbed substrate. In fact, our results reflect, like other studies (Oliveira, 2023; Portela, 2014), the anthropogenic influence, namely through the presence of bridges, weirs/transverse barriers and the riparian degradation.

The biological data allowed to find responsive patterns and significant differences were found for macroinvertebrate and fish communities considering the different typologies and environmental degradation detected in the Sabor Basin. Several metrics (see Annex III) contributed to highlight the good ecological state, namely in sampling sites of mountain zones (e.g. Upper Sabor: Sa_01, Sa_02, Sa_03), dominated by insects of EPT families (Plecoptera Ephemeroptera, Trichoptera) and native fish species (*Salmo trutta, Squalius carolitertii, Achondrostoma asturicense*) which are particularly sensible to environmental degradation. This study allowed to verify that the presence of river disturbance (i.e., habitat fragmentation, water pollution) in the Sabor basin led to a significant negative impact for macroinvertebrate and fish communities showing significant differences between non-disturbed and disturbed sites, and among river typologies. The statistical analyses (e.g. PERMANOVA and nMDS analyses) confirmed significant differences among river typologies and disturbance, i.e., for water pollution as well as for habitat fragmentation. For example, the highest number and lower diversity of invertebrates was found in the polluted Fervença River (e.g. Fv_02 and Fv_04, with 3274 and 2844 individuals, respectively), according to Cazzolla Gatti (2016) and the concept of indicator species, where the presence and/or absence of dominant taxa depends on the degree of environmental changes. An evident dominance of Diptera and Crustacea was detected in Fervença river, showing the negative influence of domestic and industrial wastewaters of Bragança city, even after their treatment in WWTP (e.g. Fv_04 and Fv_05). Other type of disturbance, related with hydromorphological changes resulting from the barrier effect (weirs, small and big dams), also induced notable variations in the composition, abundance and functioning of macroinvertebrate communities. For example, in most of the Maçãs river sampling sites, EPT%, ranged between 54.93% and 81.04%, except for Ma_02 directly influenced by a small dam (e.g. Water Treatment Plant of Vimioso), where euribiont taxa are dominant (e.g. Diptera: 88.55%). Due to the environmental changes promoted by the Lower Sabor (e.g. Baixo Sabor Dam: Sa_10, Sa_11, Sa_12) a higher abundance of Heteroptera (Corixidae) was observed (around 50%) without the presence of Trichoptera and Plecoptera orders. This macroinvertebrate community changes were also detected in several studies developed in the Sabor basin (Portela, 2014; Rodrigues, 2013; Silva, 2010). This observation aligns with the findings of Santos et al. (2017) study, where the composition and abundance of benthic macroinvertebrate in the areas most impacted by the Baixo Sabor dam were dominated by specimens from Oligochaeta and Heteroptera orders (with predominance of Corixidae family). Moreover, as mentioned by Gomes (2019), the presence of invasive alien species (IAS), namely the Asian clam (*Corbicula fluminea*), and the two decapods, the red-swamp crayfish (*Procambarus clarkii*) and the signal-crayfish (*Pacifastacus leniusculus*), are possibly changing the composition and functioning of invertebrate communities. The same author cited that the impact of human activities (e.g., irrigation, urban use) and the presence of IAS are reflected in the lower percentage of the shredders, as they are characterized by being more sensitive to disturbances occurring in the ecosystem. Additionally, gathering collectors and grazers and herbivores are growing in the headwaters, since riparian degradation (clearcut and alder disease) are decreasing the input of allochthonous organic matter. In addition, it is worth noting the occurrence of threatened specimens, such as the case of odonats (*Coenagrion caerulescens, Sympetrum flaveolum*) and unionid mussels (*Unio delphinus, Anodonta anatina, Potomida littoralis*) (Boieiro et al., 2023; IUCN, 2024).

In the Sabor basin, a total of 16 fish species were identified, 44% (7 species) as native species and 56% (9 species) being non-native species. We hypothesized that the 4 river typologies (G1, G2, G3 and G4) defined for fish communities of Sabor basin would lead to significant differences in some metrics. Consequently, significant differences were found for the metrics richness, abundance, Pielou Evenness index (J') and Shannon-Wienner index (H'). The results for the official F-IBIP index (see Annex IV, Tables AIV.2 to Table AIV.5), indicated that 40% of 40 sampling sites belong to good and excellent biological quality. As in other Mediterranean-type streams, fish communities in the Sabor watershed are highly dynamic, and temporal changes can induce major fluctuations in abundances, while much less variation is found in species composition and richness. Communities tend to show far more marked changes at the local than at the watershed or regional scales (Mota-Ferreira et al., 2020). The present study showed that main river pressures (i.e., habitat fragmentation, water pollution, invasive alien species) led to negative impact for native fish communities. Significant differences were found between non-disturbed and disturbed sites, and among river typologies (G1; G2; G3; G4). In the nMDS ordination (see Figure 3.19) it was possible to observe the segregation of species, namely from salmonid zone (G1), represented by *S. trutta*, to cyprinid zone (G3), represented by leuciscids and cyprinids and non-native species (*A. alburnus, L. gibbosus, G. holbrooki, G. lozanoi*) and a more lentic community (dammed sites Sa_10, Sa_11, Sa_12), composed mainly by piscivorous fish (e.g. *E. lucius, M. salmoides* and *S. lucioperca*). Ilhéu et al. (2014) found that sites experiencing higher human pressure exhibit significantly higher occurrence, abundance, and percentage of non-native species. In fact, species introductions and extinctions is one of the main reasons causing biological homogenization (Hermoso et al., 2011; Olden et al., 2006; Rahel, 2010). The construction of dams creates new environments (reservoirs) that favour biotic homogenization (Clavero et al., 2004; Clavero & Hermoso, 2011) and the dominance of non-native fishes, resulting in higher abundance in regulated rivers, such is the case of Baixo Sabor dam, which unequivocally affected the native fish communities, replaced by various exotic species in a very short temporal period. Notably, the introduction of exotic species by reservoir managers for sport fishing, specifically the black-bass (*Micropterus salmoides*) and pikeperch (*Sander lucioperca*), played a crucial role in this shift (Santos et al., 2017). According to Boavida et al. (2018), the dominant species in Douro River, in the past, were *Luciobarbus bocagei* and the *Pseudochondrostoma duriense*, using the Sabor River to spawn, namely during reproduction migration. However, a significant population decrease of both native species has been detected in recent years, due to the expansion of exotic carnivorous species, such as the black-bass (*Micropterus salmoides*), zander (*S. lucioperca*) and the Northern pike (*Esox lucius*). Cortes et al. (2019) mentioned alternative approaches to dam removal for the conservation of native fish, such as the trap and transport method, currently used to migrate juvenile salmonids in large catchments. This technique has also been tested with cyprinids in the Sabor River, involving the trap and transport of spawners to upstream (or downstream in the case of juveniles). As noted by Boavida et al. (2018), the rehabilitation of impacted tributaries like the Vilariça River is a valid compensatory procedure, during the crucial stage (reproduction) of the dominant native species.

Based on the classification of the overall state of surface water bodies in Douro River Basin (RH3), 66.7% and 86.4% are respectively classified as good and excellent for the Sabor and Maçãs basins (PGRH3, 2022a). However, the recent (Summer 2023) monitoring and assessment showed a decrease in the overall ecological state, justifying the continuous monitoring and management of the different watercourses of Sabor basin.

5. Conclusions and final considerations

The sudden and strong changes in environmental conditions detected in the last decade in Mediterranean streams and rivers justify the need to combat the habitats loss and biodiversity through adequate adaptation strategies to climate change, mitigation and/or minimization of impacts and restoration ecology of degraded areas. This is only possible if continuous monitoring and knowledge of the ecological status evolution of aquatic and riparian ecosystems are a reality. In this context, the present work aimed to contribute to updating the knowledge of the ecological status of several watercourses (43 sampling sites) in the Sabor river basin, one of the main tributaries of the Douro River, in Portugal. The following conclusions can be highlighted:

1. Physical and chemical (general) water elements: Typical variation patterns were found in several physicochemical parameters, i.e., a tendency to increase the water temperature and the content of dissolved salts and nutrients from upstream (lower order watercourses) to downstream (i.e., higher order). However, some of the disturbance phenomena detected (e.g. pollution, regulation, riparian degradation, IAS) in each of the typologies considered (i.e., from small rivers (N1 ≤ 100 and N3) to medium-large rivers in the Northern Portugal and Alto-Douro (N1 > 100 and N2)) induced modifications on the natural variations. However, significant differences were found among typologies for the parameters of water temperature, pH and total hardness. It must be highlighted the detected pollution in the Fervença river and the low efficiency of the WWTP (the same problem for many other smaller ones), removing nitrogen and phosphate compounds, with obvious impacts on water quality and biota. On the other hand, the flow stagnation caused by small and large barriers (e.g., small hydroelectric plants and big dams - Baixo Sabor), namely during summer season, also interfere with water quality, leading to changes in the structure and functioning of aquatic and riparian communities.

2. Hydromorphological elements: Both HQA and HMS indices, resulting from the River Habitat Survey (RHS), showed some concerning signs for many of the sampled sites, since only a minority have an excellent hydromorphological condition. In fact, the degradation of the riparian zone, in part due to the high mortality of alder trees (*Alnus lusitanica*), caused by the fungi *Phythophtora* x*alni* and the presence of physical obstacles (e.g. weirs, small hydroelectric plants and big dams), among other negative pressures, act synergistically and contribute to the reduction of water and biological quality, with the consequent reduction of the biogenic capacity of aquatic ecosystems.

3. Biological elements – macroinvertebrate fauna: 30 021 specimens were identified, distributed across 86 taxonomic groups. This biological quality in the Sabor basin is well expressed in different metrics (e.g. taxonomic richness, abundance, diversity, evenness), with emphasis on the classification obtained by the official IPtIN index (see Figure 3.11), where most of the sampled sites present a quality below the minimum good status, established by the WFD. Usually, the sampling sites with the best biological quality were in the headwater sections of low-order rivers. It is noteworthy that significant differences were found in the communities considering the typologies, but also the disturbances (pollution, regulation, IAS), as main drivers inducing changes in the structure and functioning of invertebrate communities. It is important to highlight, on the one hand, the presence of threatened invertebrate species with conservation status (Portuguese Red Book), such as bivalves (e.g., *Unio delphinus, Anodonta anatina, Potomida littoralis*), obligatory dependent on native cyprinid and leuciscid fishes, and Odonata (*Coenagrion caerulescens, Lestes sponsa, Sympetrum flaveolum*). On the other hand, the increasing impact of widespread dispersion of invasive alien species (IAS), such as decapods (*Pacifastacus leniusculus, Procambarus clarkii*) and bivalves (*Corbicula fluminea*), must also be referred for the Sabor basin.

4. Biological elements – fish fauna: 6,086 specimens were identified, distributed across 16 species, being 7 native (*Salmo trutta, Squalius carolitertii, Squalius alburnoides, Achondrostoma asturicense, Pseudochondrostoma duriense, Luciobarbus bocagei* and *Cobitis paludica*, in this case probably translocated) and 9 non-native species (*Micropterus salmoides, Lepomis gibbosus, Alburnus alburnus, Rutilus rutilus, Gobio lozanoi, Gambusia holbrooki, Esox lucius, Sander lucioperca* and *Cyprinus carpio*). Significant differences were detected between typologies and disturbed vs. non-disturbed sites. In fact, the dispersion pattern of non-native species is very clear and occurs from downstream to upstream with the particularity of being also dominant in environmentally modified sites, mainly in reservoirs of small and especially large dams (e.g. Baixo Sabor Dam). Among other metrics (e.g. taxonomic richness, abundance, diversity, evenness) the classification of the official F-IBIP index (see Figure 3.21) allows to highlight the decrease in biological quality of middle and final section of the Sabor river, and the “opportunity” for tributaries to be refuges for native fauna with rheophilic habits, as long as the water flow conditions are maintained, and the sequence of riffles and pools preserved.

5. General ecological assessment status: The application of the official APA criteria for measuring the Ecological State revealed, based on just one sampling season (summer 2023), that only 7% of sampling sites reached the good ecological state. This analysis must be confirmed and or reinforced with other assessments, considering other seasons and complementary surveys for primary producers (e.g. periphyton, phytoplankton and macrophytes) and a more detailed water quality evaluation, measuring both general and specific pollutants (priority hazardous substances). However, harsh environmental conditions, ultimately observed in NE Portugal, namely linked to prolonged droughts and a more temporary character of several tributaries are leading to stressful conditions to the aquatic fauna survival, in particular sensible and threatened taxa, more prone to diminish their abundance and inclusively to become extinct in some watercourses.

6. Implications for aquatic fauna (native invertebrate and fish communities): The results presented a very relevant bioecological information e.g., i) presence of native ichthyofauna typical of rivers in this region (i.e., Cyprinidae and Leuscicidae, particularly *Achondrostoma asturicense* (EN, endangered status) and *Pseudochondrostoma duriense* (NT - Near threatened); ii) presence of several invasive alien species of fish (e.g., *Alburnus alburnus, Lepomis gibbosus, Gobio lozanoi, Micropterus salmoides*), invertebrates (*Pacifastacus leniusculus, Procambarus clarkii, Corbicula fluminea*) and also plants (e.g., *Arundo donax, Acacia* sp.); iii) high decrease of some relevant taxa in terms of conservation, namely bivalves (*Anodonta anatina, Unio delphinus* and *Potomida littoralis*). The observed patterns in terms of distribution and abundance of native invertebrate and fish species imply the development of mitigation actions oriented to ongoing pressures, and habitat restoration and adaptation to climate change.

Final considerations: Conservation measures and future research

Conservation measures must be considered and implemented to promote the good/excellent ecological condition of aquatic and riparian ecosystems, namely:

- Amplify the regular monitoring assessment made by APA and define specific measures to adjust ongoing pressures and adaptation to climate change, including in the National Roadmap for Adaptation 2100 – Assessment of the vulnerability of Portuguese territory to climate change in the 21st century (RNA 2100).
- Increase and integrate the monitoring of other faunistic groups, and particularly of threatened species (e.g. mammals: *Galemys pyrenaicus, Lutra lutra, Neomys anomalus*; birds: *Cinclus cinclus; Ciconia nigra;* Amphibians: *Rana iberica*, Reptiles: *Lacerta schreiberi*) to apply oriented measures aimed at their conservation.

- Establish measures aimed to control non-native species (e.g. *Pacifastacus leniusculus, Procambarus clarkii, Corbicula fluminea, Lepomis gibbosus, Alburnus alburnus, Neovison vison*), at least in the priority habitats of threatened species.
- Promote habitat improvement and barrier permeability in small obstacles (e.g. weirs) to guarantee river connectivity and species mobility, allowing their reproductive migratory pulses.
- Conclude the Special Program for the Baixo Sabor Dam — upstream and downstream reservoirs (PEABS), according with the legislation (Dispatch nº 2230 from 29th February 2024).
- Develop the regulation and management of recreational fishing in Sabor basin, namely in protected areas, to balance the conservation of species (priority) with the exploitation (use) of resources.
- Consider the creation of a Natural Regional Park in the Sabor Valley (like the PNRVT - Natural Regional Park of Tua Valley), including municipalities in the effort of the conservation of "Rios Sabor e Maçãs site" (PTCON00021) highlighting the SCI (Site of Community Importance) and SCZ (Special Community Zone) of the NE Portugal.
- Development of education, and awareness measures for species and habitat conservation and the implementation of appropriate agriculture and forest management practices in the Sabor basin.

Several measures are, nowadays, programmed to be implemented in Douro basin region (RH3) - 3rd cycle of River Basin Management Plans (RBMPs) (PGRH3, 2022b). Most of the measures are oriented to:

- Development of the national action plan for river continuity restoration.
- Development of drought and water scarcity management plans.
- Implementation of the national strategy for river and stream rehabilitation.
- Development of the national action plan for the control, containment, and eradication of invasive alien species (IAS).
- Expansion/Remodelling/Improvement of domestic and industrial WWTP.
- Improvement of drainage networks, avoiding substantial losses of water.
- Adopting regulations for the discharge of industrial wastewater.
- Implementation of post-intervention measures for mine exploitation zones.
- Application of environmental constraints in the assessment of projects for the agricultural valorisation of livestock effluents and wastewater sludges, based on the mapping of restricted or prohibited areas.

Further studies are recommended to be undertaken, as the situation can be worst in the future. Mediterranean-type ecosystems (MTEs) are among the most heavily impacted by human activities and more intense catastrophic events are expected due to climate change, leading to a loss in biodiversity. It is emphasized that monitoring the aquatic fauna is essential for maintaining aquatic ecosystems in healthy condition, given the multiple benefits they provide.

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Attachments

Annex I – Physical and chemical water characterization of sampling sites in Sabor basin (Summer 2023)

Table AI.1 - Water quality of sampling sites in Sabor (Sa) and Fervença (Fv) Rivers (Summer 2023), 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).

Variable	Sa_01	Sa_02	Sa_03	Sa_04	Sa_05	Sa_06	Sa_07	Sa_08	Sa_09	Sa_10	Sa_11	Sa_12	Fv_01	Fv_02	Fv_03	Fv_04	Fv_05
Temperature (ºC)	17.4	20.8	21.4	25.5	25.3	26.6	26.6	26.7	27.2	28.7	28.8	29.2	16.5	17.3	22.3	22.4	23.6
Conductivity (µS/cm)	43.5	98.6	168.1	158.6	299	190.65	200.2	168.7	208.7	130.5	127.1	126.5	166.2	168.3	238	598	494
pH (Sorensen scale)	6.77	6.79	6.98	6.77	7.04	7.19	7.16	7.06	7.6	8.05	7.53	7.8	6.95	7.28	7.85	7.36	7.25
Diss. Oxygen (mg/L)	11.66	10.12	8.49	8.02	13.52	9.27	8.26	8.8	8.24	9.08	9.33	9.78	9.4	9.1	9.11	3.13	5.4
% Saturation O_2	109.2	73	110.5	97.1	162.4	112.2	107	90.5	108.8	114.8	117.9	122.4	90.2	99	98.7	32.9	48.9
Nitrates (mg/L)	0.755	0.745	1.271	2.386	4.88	4.713	1.096	1.01	1.281	1.662	0.1	1.168	2.304	2.066	4.059	30.573	5.989
Nitrites (mg/L)	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	10.626	8.995
Phosphates (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.229	0.342
Hardness (mg/L)	2.4	4.4	3.6	5.3	5.7	3.8	6.9	3.9	2.2	4.1	5.7	5.8	7.8	32.3	20.4	34.9	32.4
Acidity (mg/L)	3.74	9.2	9.97	21.31	13.64	9.22	9.09	9.05	4.25	3.25	5.97	3.4	9.1	7.13	4.57	18.69	34.25
Total Diss. Solids (mg/L)	24	59.9	78.8	82.7	151.5	133	93	77.2	98.7	60.7	59.3	59.5	90.7	95.9	140.9	328	295

Table AI.2 - Water quality of sampling sites in Maçãs (Ma), Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) Rivers (Summer 2023), 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).

Variable	Ma_01	Ma_02	Ma_03	Ma_04	Ma_05	Ag_01	Ag_02	Ag_03	Ag_04	Ag_05	Pe_01	Pe_02	Vm_01	Vm_02	Az_01	Az_02	Az_03
Temperature (ºC)	23.8	26.6	26.8	26.1	25.7	17.2	17.1	23.8	26.8	27.4	17.1	17.4	18.7	29	20.3	23.3	23.5
Conductivity (µS/cm)	82.6	81.5	152.6	122.1	150.8	128.8	154.8	229	181.6	231.8	32.7	100.4	173.6	256	161.5	300	213.1
pH (Sorensen scale)	7.11	6.67	6.97	7.15	6.84	7.33	6.99	7.2	7.05	7.33	6.76	7.19	6.99	7.57	7.19	7.63	7.9
Diss. Oxygen (mg/L)	12.25	13.11	9.57	7.68	9.33	8.29	8.2	8.95	8	9.73	8.22	8.17	8	9.84	7.61	8.28	9.05
% Saturation O_2	63.2	174.3	126.4	93.7	118.9	80.7	80.8	111.7	105.5	129	91.8	91	86	134.7	89.1	95.7	109.6
Nitrates (mg/L)	0.1	0.59	2.118	0.755	1.973	1.705	2.128	1.694	1.075	2.273	0.2	0.476	2.045	0.621	1.663	0.6	0.2
Nitrites (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	0.02	0.02	<0.01	<0.01	<0.01
Phosphates (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Hardness (mg/L)	18.4	10.9	22.8	17.6	12.5	15	24.4	22.3	21	13.8	24.6	31	26.4	11.4	9.6	16.4	2

Acidity (mg/L)	5.62	14.24	7.88	4.15	9.1	8.14	17.6	17.49	15.2	13.53	6.5	6.06	17.78	9.68	3.88	7.45	4.04
Total Dis. Solids (mg/L)	40	37.2	69.1	64.7	69.8	72.1	87.2	111.8	84.6	106.3	18.68	56	94.7	112.3	84.5	149.5	105.2

Table AI.3 - Water quality of sampling sites in Vilariça (Vi), Veados (V2), Onor (On), Igrejas (Ig) Baçal (Ba), Ferreiros (Fe), Inferno (In) and Zacarias (Za) streams (Summer 2023), 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).

Variable	Vi_02	Vi_03	Ve_01	On_01	Ig_01	Ba_01	Fe_01	In_01	Za_01
Temperature (ºC)	27.5	27.8	19.7	19.7	15.9	16.9	23.6	22	19.7
Conductivity (µS/cm)	363	291.2	42.2	81.9	129	112.8	305	54.9	171.2
pH (Sorensen scale)	7.15	7.09	6.85	6.93	7.15	6.81	7.8	7.07	6.7
Diss. Oxygen (mg/L)	5.41	5.58	8.57	9.25	9.89	10.5	7.98	7.9	8.35
% Saturation O_2	58.5	62.7	100.4	90.8	94.7	100.2	92.7	90.3	92.6
Nitrates (mg/L)	0.2	1.633	0.425	0.518	0.1	1.312	0.683	0.2	0.383
Nitrites (mg/L)	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phosphates (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Hardness (mg/L)	10	22.2	28	21.7	13.8	22.5	18.9	46.5	31
Acidity (mg/L)	12.22	6.51	5.95	12.87	9.17	13.13	14.42	6.91	26.38
Total Dis. Solids (mg/L)	192	100	22.1	43.2	75	63.9	164.4	28.7	93.5

Annex II – River Habitat Survey (RHS): Total scores for HQA and HMS indexes, considering the 40 sampling sites in Sabor basin (Summer 2023)

Table AII.1 – Total scores HQA (Habitat Quality Assessment) and HMS (Habitat Modification Score) indexes (Summer 2023), for sampling sites in Small Northern Rivers (N1 ≤ 100 Km^2) in Sabor Basin (Summer 2023).

Sampling site	HQA	HMS Class	HMS Score
Sa_01	50	2	50
Fv_01	56	3	260
Fv_02	51	3	330
Fv_03	42	4	770
Fv_04	58	3	350
Ag_01	64	4	1320
Pe_01	48	3	200
Vm_01	53	4	1075
Ve_01	45	3	405
On_01	42	2	40
Ig_01	51	2	40
Ba_01	47	2	60

Table AII.2 – Total scores HQA (Habitat Quality Assessment) and HMS (Habitat Modification Score) indexes (Summer 2023), for sampling sites in Medium-Large Northern Rivers (N1 > 100 Km^2) in Sabor Basin (Summer 2023).

Sampling site	HQA	HMS Class	HMS Score
Sa_02	49	3	315
Sa_03	55	3	395
Sa_04	48	3	375
Sa_05	53	3	465
Sa_06	56	3	210
Ma_01	54	4	690
Ma_02	52	4	555
Ma_03	62	4	1145
Fv_05	70	3	375
Ag_02	34	4	665
Pe_02	66	1	0

Table AII.3 – Total scores HQA (Habitat Quality Assessment) and HMS (Habitat Modification Score) indexes (Summer 2023), for sampling sites in Medium-Large Alto Douro Rivers (N2) in Sabor Basin (Summer 2023).

Sampling site	HQA	HMS Class	HMS Score
Sa_07	51	4	935
Sa_08	44	2	135
Sa_09	47	1	0
Ma_04	47	3	375
Ma_05	41	1	0
Ag_03	44	3	375
Ag_04	35	3	400
Ag_05	44	2	40
Vm_02	37	1	10
Az_01	36	2	40
Az_02	39	3	270
Az_03	58	3	220
Vi_02	53	3	330
Vi_03	60	3	415

Table AII.4 – Total scores HQA (Habitat Quality Assessment) and HMS (Habitat Modification Score) indexes (Summer 2023), for sampling sites in Small Alto Douro Rivers (N3) in Sabor Basin (Summer 2023).

Sampling site	HQA	HMS Class	HMS Score
Fe_01	60	4	620
In_01	52	3	475
Za_01	30	3	320

Annex III – Ecological metrics (AMIIB; APA, 2021) for the 43 sampling sites in Sabor basin (Summer 2023)

Table AIII.1 – Ecological (AMIIB, APA 2021) metrics for the sampling sites in Sabor (Sa) and Fervença (Fv) Rivers (Summer 2023).

Metrics	Sa_01	Sa_02	Sa_03	Sa_04	Sa_05	Sa_06	Sa_07	Sa_08	Sa_09	Sa_10	Sa_11	Sa_12	Fv_01	Fv_02	Fv_03	Fv_04	Fv_05
Total nº taxa	33	19	21	11	23	29	17	22	28	12	16	12	20	20	12	16	14
Total nº individuals	802	654	359	605	1767	904	637	167	1299	675	590	503	1197	3274	1360	2844	1614
BMWP	186	84	112	51	109	152	89	111	131	35	51	40	82	86	52	70	52
Nº Taxa BMWP	29	14	18	11	18	24	15	18	21	8	11	9	18	18	12	14	13
ASPT	6.41	6	6.22	4.64	6.06	6.33	5.93	6.17	6.24	4.38	4.64	4.44	4.56	4.78	4.33	5	4
IBMWP	209	101	121	42	118	159	82	112	150	38	57	39	87	86	45	71	48
Nº of Taxa - IBMWP	33	19	21	11	22	28	15	20	26	10	14	10	20	20	12	16	14
IASPT	6.33	5.32	5.76	3.82	5.36	5.68	5.47	5.6	5.77	3.8	4.07	3.9	4.35	4.3	3.75	4.44	3.43
Shannon-Wienner H'	2.31	1.43	1.88	1.41	1.14	2.09	1.2	2.25	1.68	1.53	1.55	1.71	1.12	0.44	0.95	0.53	0.51
Evenness J'	0.66	0.48	0.62	0.59	0.36	0.62	0.42	0.73	0.51	0.62	0.56	0.69	0.37	0.15	0.38	0.19	0.19
EPT Taxa	14	8	10	3	8	9	7	7	8	2	3	1	2	4	2	5	1
Nº Individuals - EPT	494	413	229	282	855	598	490	109	731	62	21	17	34	12	137	93	2
% of Individuals - EPT	61.6	63.15	63.79	46.61	48.39	66.15	76.92	65.27	56.27	9.19	3.56	3.38	2.84	0.37	10.07	3.27	0.12

Table AIII.2 – Ecological (AMIIB, APA 2021) metrics for the sampling sites in Maçãs (Ma), Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) Rivers (Summer 2023),

Metrics	Ma_01	Ma_02	Ma_03	Ma_04	Ma_05	Ag_01	Ag_02	Ag_03	Ag_04	Ag_05	Pe_01	Pe_02	Vm_01	Vm_02	Az_01	Az_02	Az_03
Total nº taxa	17	10	18	11	12	26	8	6	11	7	19	21	15	21	25	24	22
Total nº individuals	172	393	517	181	422	550	182	282	923	178	180	321	1242	1089	599	308	459
BMWP	90	53	101	60	62	150	33	22	49	28	92	110	76	99	119	113	99
Nº Taxa BMWP	14	10	17	9	11	24	8	5	9	5	16	20	15	16	22	19	19
ASPT	6.43	5.3	5.94	6.67	5.64	6.25	4.13	4.4	5.44	5.6	5.75	5.5	5.07	6.19	5.41	5.95	5.21
IBMWP	88	46	94	55	57	135	29	19	50	25	111	106	68	111	124	113	98

Nº of Taxa - IBMWP	**16**	**10**	**17**	**9**	**11**	**25**	**8**	**5**	**10**	**5**	**19**	**21**	**15**	**20**	**25**	**23**	**22**
IASPT	**5.5**	**4.6**	**5.53**	**6.11**	**5.18**	**5.4**	**3.63**	**3.8**	**5**	**5**	**5.84**	**5.05**	**4.53**	**5.55**	**4.96**	**4.91**	**4.45**
Shannon-Wienner H'	**2**	**0.57**	**2.31**	**1.4**	**1.3**	**2.16**	**1**	**0.54**	**1.34**	**0.99**	**1.38**	**1.84**	**0.7**	**1.7**	**2.31**	**1.8**	**1.98**
Evenness J'	**0.71**	**0.25**	**0.8**	**0.58**	**0.52**	**0.66**	**0.48**	**0.3**	**0.56**	**0.51**	**0.47**	**0.6**	**0.26**	**0.56**	**0.72**	**0.57**	**0.64**
EPT Taxa	**5**	**4**	**7**	**6**	**4**	**8**	**1**	**2**	**3**	**2**	**6**	**7**	**3**	**6**	**7**	**6**	**6**
Nº Individuals - EPT	**110**	**27**	**284**	**127**	**342**	**291**	**1**	**7**	**537**	**44**	**28**	**35**	**45**	**770**	**141**	**94**	**285**
% of Individuals - EPT	**63.95**	**6.87**	**54.93**	**70.17**	**81.04**	**52.91**	**0.55**	**2.48**	**58.18**	**24.72**	**15.56**	**10.9**	**3.62**	**70.71**	**23.54**	**30.52**	**62.09**

Table AIII.3 - Ecological (AMIIB, APA 2021) metrics for the sampling sites in Vilariça (Vi). Veados (V2). Onor (On). Igrejas (Ig) Baçal (Ba). Ferreiros (Fe). Inferno (In) and Zacarias (Za) streams (Summer 2023).

Metrics	**Vi_02**	**Vi_03**	**Ve_01**	**On_01**	**Ig_01**	**Ba_01**	**Fe_01**	**In_01**	**Za_01**
Total nº taxa	**18**	**21**	**13**	**17**	**28**	**20**	**16**	**19**	**19**
Total nº individuals	**381**	**398**	**167**	**204**	**553**	**218**	**384**	**335**	**132**
BMWP	**98**	**95**	**60**	**71**	**157**	**97**	**87**	**118**	**93**
Nº Taxa BMWP	**16**	**16**	**11**	**13**	**23**	**16**	**16**	**19**	**18**
ASPT	**6.13**	**5.94**	**5.45**	**5.46**	**6.83**	**6.06**	**5.44**	**6.21**	**5.17**
IBMWP	**97**	**97**	**62**	**87**	**180**	**117**	**75**	**109**	**78**
Nº of Taxa - IBMWP	**17**	**19**	**13**	**17**	**28**	**20**	**16**	**19**	**18**
IASPT	**5.71**	**5.11**	**4.77**	**5.12**	**6.43**	**5.85**	**4.69**	**5.74**	**4.33**
Shannon-Wienner H'	**1.42**	**2.17**	**1.92**	**1.23**	**1.8**	**1.66**	**1.44**	**1.79**	**1.88**
Evenness J'	**0.49**	**0.71**	**0.75**	**0.44**	**0.54**	**0.55**	**0.52**	**0.61**	**0.64**
EPT Taxa	**11**	**9**	**4**	**5**	**13**	**6**	**3**	**7**	**4**
Nº Individuals - EPT	**84**	**236**	**59**	**34**	**394**	**71**	**197**	**196**	**12**
% of Individuals - EPT	**22.05**	**59.3**	**35.33**	**16.67**	**71.25**	**32.57**	**51.3**	**58.51**	**9.09**

Annex III – Macroinvertebrate data: Composition, IPTIN scores and classification, for the 43 sampling sites in Sabor basin (Summer 2023)

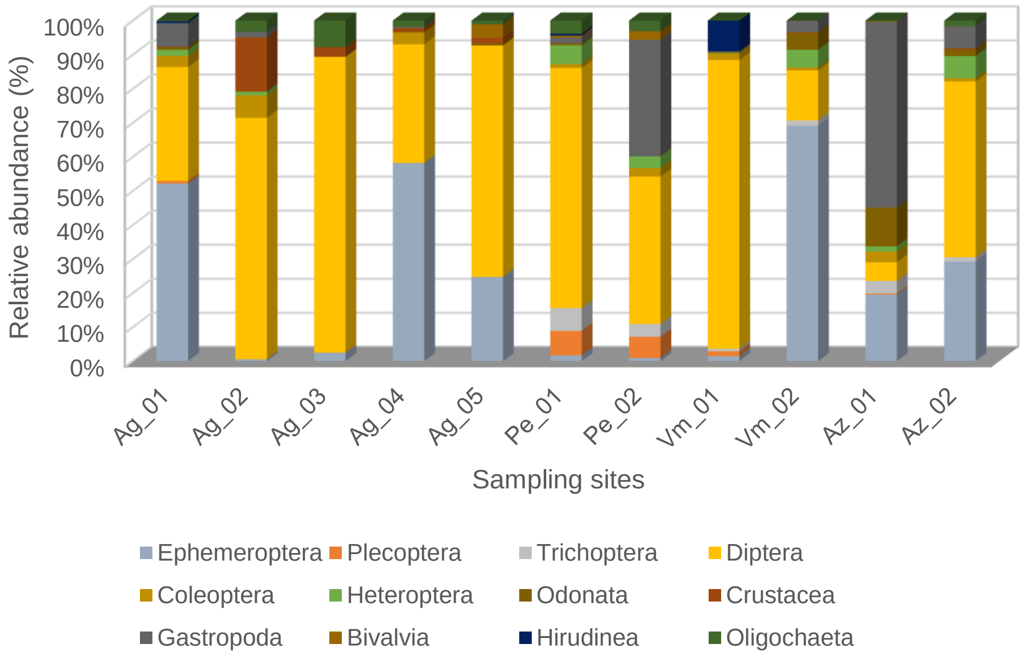

Figure AIII.1 - Faunal composition of invertebrates in Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) rivers of Sabor basin (Summer 2023).

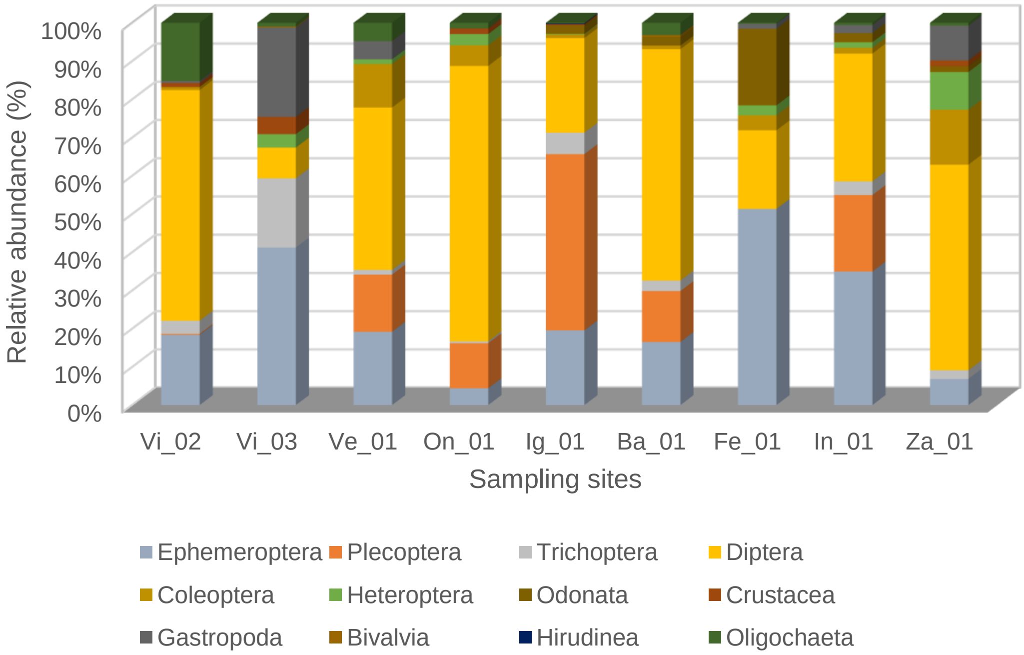

Figure AIII.2 - Faunal composition of invertebrates in Vilariça (Vi), Veados (Ve), Onor (On), Igrejas (Ig), Baçal (Ba), Ferreiros (Fe), Inferno (In) and Zacarias (Za) streams of Sabor basin (Summer 2023).

Table AIII.4 – Total scores and ecological classification, based on the Northern Portuguese Invertebrate index (IPTIN) for the sampling sites located in Small Northern Rivers (N1 ≤ 100 Km^2) in Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Sa_01	N1 ≤ 100 Km^2	0.926	Excellent
Fv_01	N1 ≤ 100 Km^2	0.434	Poor
Fv_02	N1 ≤ 100 Km^2	0.401	Poor
Fv_03	N1 ≤ 100 Km^2	0.283	Poor
Fv_04	N1 ≤ 100 Km^2	0.362	Poor
Ag_01	N1 ≤ 100 Km^2	0.737	Good
Pe_01	N1 ≤ 100 Km^2	0.595	Reasonable
Vm_01	N1 ≤ 100 Km^2	0.451	Reasonable
Ve_01	N1 ≤ 100 Km^2	0.475	Reasonable
On_01	N1 ≤ 100 Km^2	0.497	Reasonable
Ig_01	N1 ≤ 100 Km^2	0.876	Excellent
Ba_01	N1 ≤ 100 Km^2	0.654	Reasonable

Table AIII.5 – Total scores and ecological classification, based on the Northern Portuguese Invertebrate index (IPTIN) for the sampling sites located in Medium-Large Northern Rivers (N1 > 100 Km^2) in Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Sa_02	N1 > 100 Km^2	0.759	Good
Sa_03	N1 > 100 Km^2	0.843	Good
Sa_04	N1 > 100 Km^2	0.372	Poor
Sa_05	N1 > 100 Km^2	0.708	Good
Sa_06	N1 > 100 Km^2	0.795	Good
Ma_01	N1 > 100 Km^2	0.598	Reasonable
Ma_02	N1 > 100 Km^2	0.378	Poor
Ma_03	N1 > 100 Km^2	0.822	Good
Fv_05	N1 > 100 Km^2	0.284	Poor
Ag_02	N1 > 100 Km^2	0.288	Poor
Pe_02	N1 > 100 Km^2	0.608	Reasonable

Table AIII.6 – Total scores and ecological classification, based on the Northern Portuguese Invertebrate index (IPTIN) for the sampling sites located in Medium-Large Alto Douro Rivers (N2) in Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Sa_07	N2	0.608	Reasonable
Sa_08	N2	0.705	Good
Sa_09	N2	0.843	Excellent
Sa_10	N2	0.352	Poor
Sa_11	N2	0.500	Reasonable
Sa_12	N2	0.360	Poor
Ma_04	N2	0.625	Reasonable
Ma_05	N2	0.466	Reasonable
Ag_03	N2	0.255	Poor
Ag_04	N2	0.439	Reasonable
Ag_05	N2	0.390	Poor
Vm_02	N2	0.673	Reasonable
Az_01	N2	0.677	Reasonable
Az_02	N2	0.608	Reasonable
Az_03	N2	0.527	Reasonable
Vi_02	N2	0.717	Good
Vi_03	N2	0.718	Good

Table AIII.7 – Total scores and ecological classification, based on the Northern Portuguese Invertebrate index (IPTIN) for the sampling sites located in Small Alto Douro Rivers (N3) in Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Fe_01	N3	0.432	Reasonable
In_01	N3	0.651	Reasonable
Za_01	N3	0.423	Reasonable

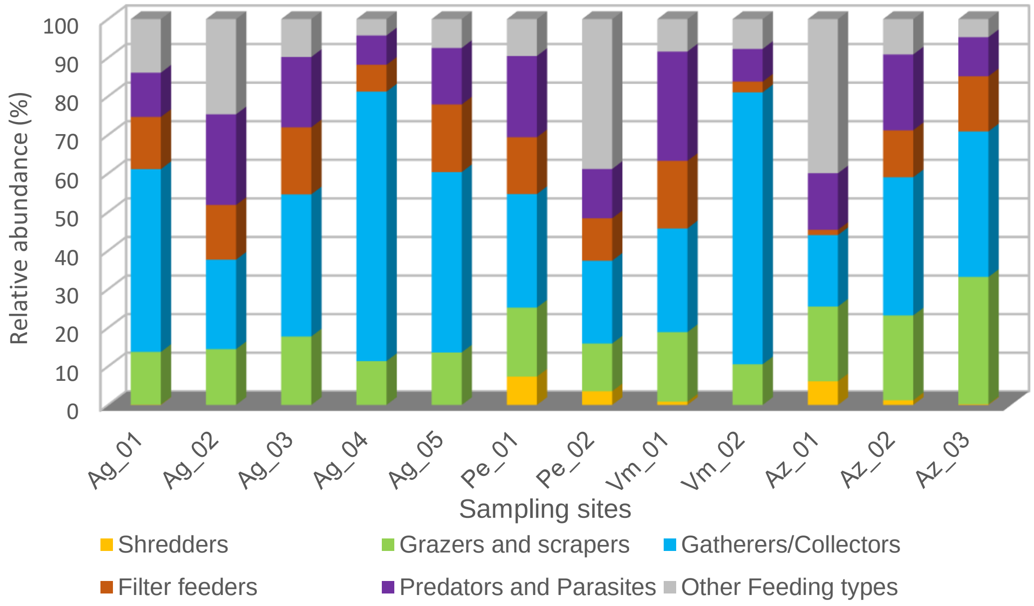

Figure AIII.3 – Relative composition of functional feeding groups of macroinvertebrate communities in Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) rivers of Sabor basin (Summer 2023).

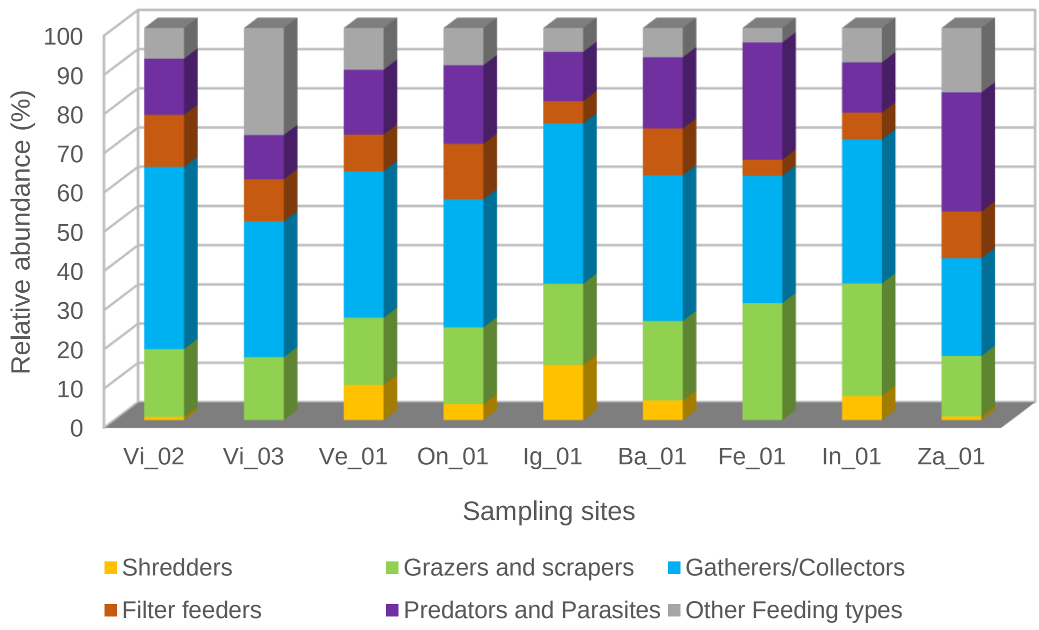

Figure AIII.4 - Relative composition of functional feeding groups of macroinvertebrate communities in Vilariça (Vi), Veados (Ve), Onor (On), Igrejas (Ig), Baçal (Ba), Ferreiros (Fe), Inferno (In) and Zacarias (Za) streams of Sabor basin (Summer 2023).

Annex IV – Fish data: Captures per Unit of Effort (CPUE), relative composition, quality index: F-IBIP scores and classification for the 43 sampling sites in Sabor basin (Summer 2023)

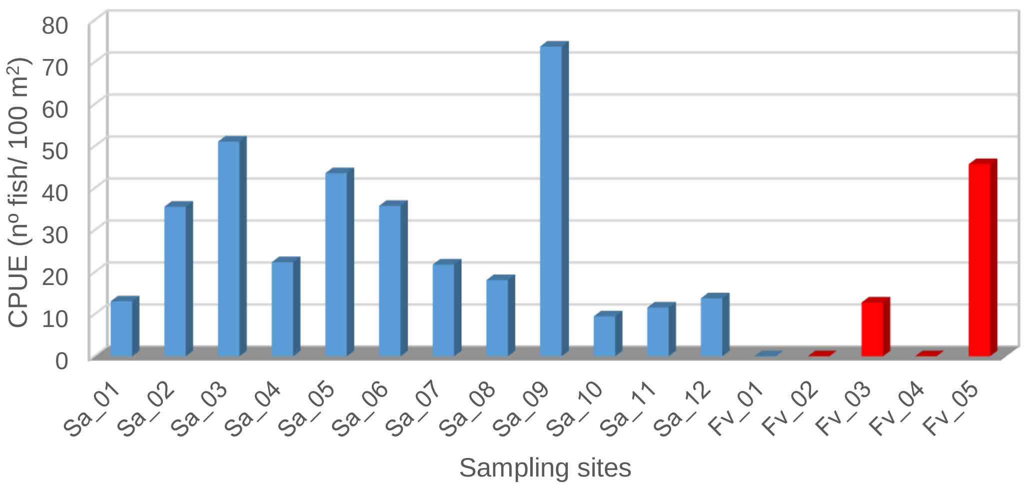

Figure AIV.1 – Captures per Unit of Effort (CPUE, nº fish/ 100 m^2) in sampling sites of Sabor and Fervença rivers (Summer 2023).

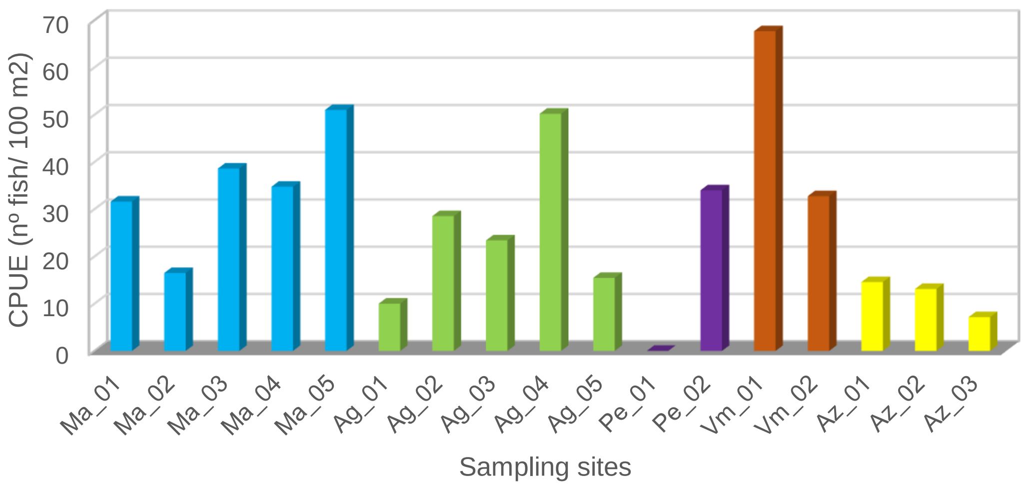

Figure AIV.2 – Captures per unit of effort (CPUE, nº fish/ 100 m^2) in sampling sites of Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) rivers of Sabor basin (Summer 2023).

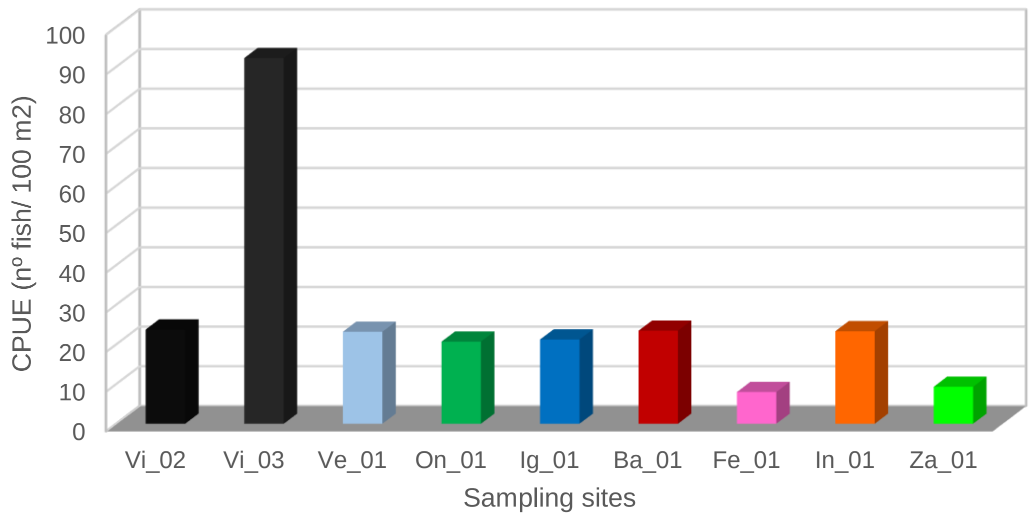

Figure AIV.3 - Captures per unit of effort (CPUE, nº fish/ 100 m^2) in sampling sites of Vilariça (Vi), Veados (Ve), Onor (On), Igrejas (Ig), Baçal (Ba), Ferreiros (Fe), Inferno (In) and Zacarias (Za) streams of Sabor basin (Summer 2023).

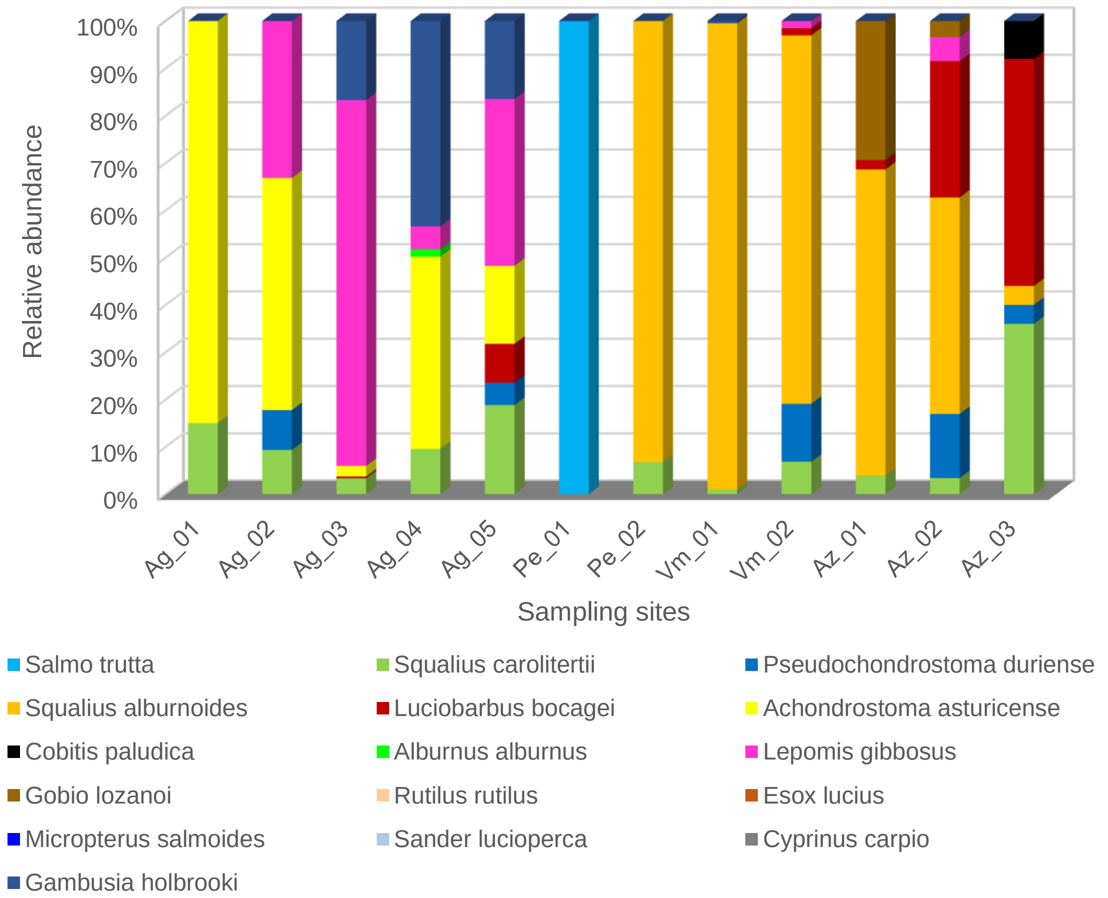

Figure AIV.4 - Relative composition of fish communities in Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) rivers of Sabor basin (Summer 2023).

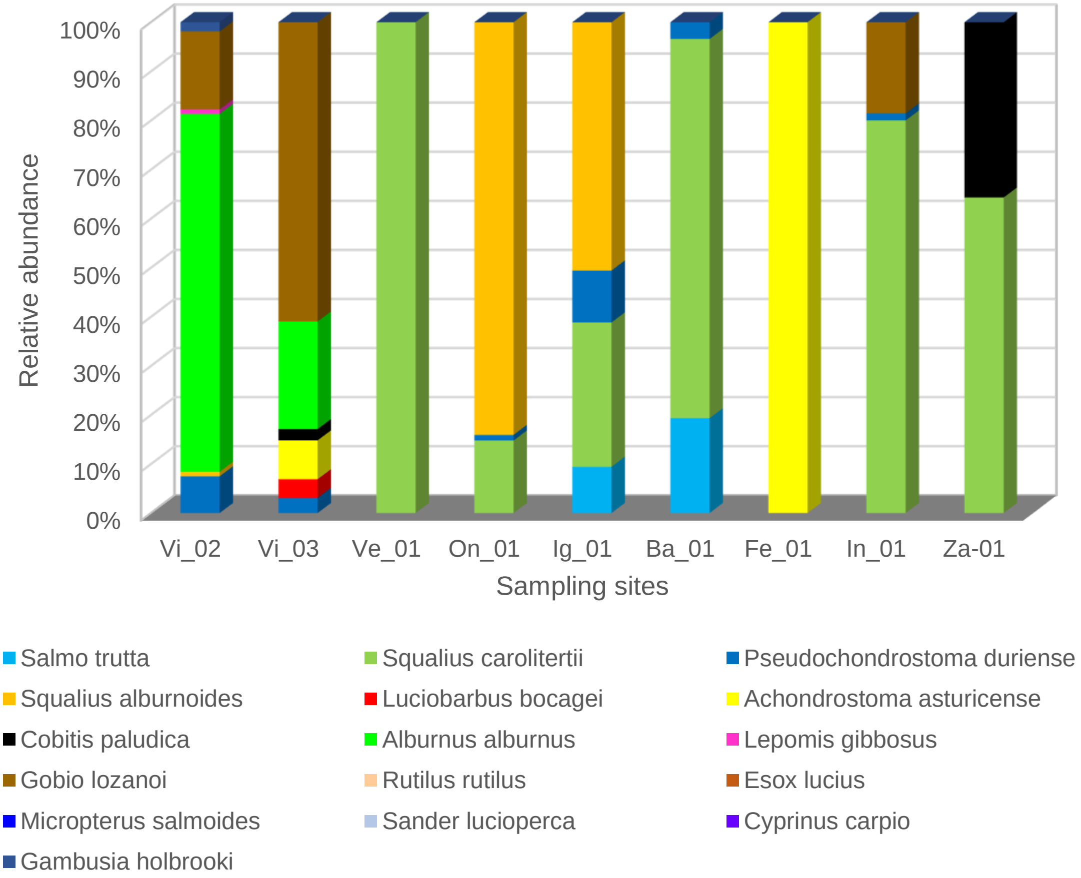

Figure AIV.5 - Relative composition of fish communities in Vilariça (Vi), Veados (Ve), Onor (On), Igrejas (Ig), Baçal (Ba), Ferreiros (Fe), Inferno (In) and Zacarias (Za) streams of Sabor basin (Summer 2023).

Table AIV.1 – Regulatory framework for referred species (native species are identified in bold). Symbols: PRB: Portuguese Red Book Data; IUCN: International Union for the Conservation of Nature

Species	Common name	Threaten category		Bern Convention	Habitat Directive
		PRB	IUCN		
Cyprinidae					
Luciobarbus bocagei	**Iberian Barbel**	**LC**	**LC**	**III**	**V**
Cyprinus carpio	**Carp**	**NA**			
Centrarchidae					
Lepomis gibbosus	Pumpkimseed	NA			
Micropterus salmoides	Black-bass	NA			
Cobitidae					
Cobitis paludica	Southern Iberian spined-loach	LC			
Esocidae					
Esox lucius	Northern Pike	Na			
Gobionidae					
Gobio lozanoi	Pyrenean gudgeon	NA			
Leuscicidae					
Achondrostoma asturicense	**Panjorca-do-esla**	**EN**		**III**	**II**
Alburnus alburnus	Common-bleak	NA			
Pseudochondrostoma duriense	**Northern straight-mouth nase**	**NT**	**VU**	**III**	**II**
Rutilus rutilus	Roach	NA			
Squalius alburnoides	**Calandino**	**LC**	**VU**	**III**	
Squalius carolitertii	**Northern Iberian Chub**	**LC**	**LC**	**III**	
Percidae					
Sander lucioperca	Zander	NA			
Poecilidae					
Gambusia holbrooki	Mosquito fish	NA			
Salmonidae					
Salmo trutta	**Brown trout**	**NT**			

Table AIV.2 – Total scores and ecological classification, based on the Fish Index of Biotic Integrity for Wadeable Rivers in Continental Portugal (F-IBIP) for the sampling sites located in Salmonid Streams of Northern Region (G1) of Sabor Basin (Summer 2023).

Sampling site	Typology	F-IBIP score	F-IBIP Class
Sa_01	**G1**	**0.966**	**Excellent**
Pe_01	**G1**	**1.000**	**Excellent**
On_01	**G1**	**0.661**	**Reasonable**
Ig_01	**G1**	**0.649**	**Reasonable**
Ba_01	**G1**	**0.715**	**Good**

Table AIV.3 – Total scores and ecological classification, based on the Fish Index of Biotic Integrity for Wadeable Rivers in Continental Portugal (F-IBIP) for the sampling sites located in Salmonid-Cyprinid Transition zone rivers of the Northern Region (G2) of Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Sa_02	G2	0.767	Good
Sa_03	G2	0.735	Good
Sa_04	G2	0.616	Reasonable
Fv_03	G2	0.010	Bad

Table AIV.4 – Total scores and ecological classification, based on the Fish Index of Biotic Integrity for Wadeable Rivers in Continental Portugal (F-IBIP) for the sampling sites located in Medium-sized Cyprinid rivers of the Northern Region (G3) of Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Sa_05	G3	0.818	Good
Sa_06	G3	0.684	Good
Sa_07	G3	0.278	Poor
Sa_08	G3	0.309	Poor
Sa_09	G3	0.000	Bad
Sa_10	G3	0.000	Bad
Sa_11	G3	0.000	Bad
Sa_12	G3	0.000	Bad
Ma_01	G3	0.889	Excellent
Ma_02	G3	0.817	Good
Ma_03	G3	0.000	Bad
Ma_04	G3	0.278	Poor
Ma_05	G3	0.556	Reasonable
Fv_05	G3	0.167	Bad
Ag_03	G3	0.111	Bad
Ag_04	G3	0.000	Bad
Ag_05	G3	0.389	Poor
Pe_02	G3	0.500	Reasonable
Az_01	G3	0.284	Poor
Az_02	G3	0.795	Good
Az_03	G3	0.889	Excellent

Table AIV.5 – Total scores and ecological classification, based on the Fish Index of Biotic Integrity for Wadeable Rivers in Continental Portugal (F-IBIP) for the sampling sites located in Small Cyprinid streams of the North-Central Interior and South Regions (G4) of Sabor Basin (Summer 2023).

Sampling site	Typology	IPTIN score	IPTIN Class
Ag_01	G4	0.6670	Reasonable
Ag_02	G4	0.249	Poor
Vm_01	G4	0.95	Excellent
Vm_02	G4	0.942	Excellent
Vi_02	G4	0.333	Poor
Vi_03	G4	0.333	Poor
Ve_01	G4	0.964	Excellent
Fe_01	G4	0.667	Reasonable
In_01	G4	0.704	Good
Za_01	G4	0.720	Good

Annex V – General quality assessment of sampling sites in Sabor basin, based on the measured elements (Summer 2023)

Table AV.1 - Water quality of sampling sites in Sabor (Sa) and Fervença (Fv) Rivers (Summer 2023), and their classification, considering the maximum threshold for the establishment of Good Ecological Status *(Blue: Excellent; Green: Good; Yellow: Reasonable; Orange: Poor; Red: Bad; White: no sampled; Black: no fish*) (APA, 2021). Water analyses – Yellow: *Less than or reasonable status; Hydromorphological analises: Green: Equal or Less than or good status.*

Elements	Sa_01	Sa_02	Sa_03	Sa_04	Sa_05	Sa_06	Sa_07	Sa_08	Sa_09	Sa_10	Sa_11	Sa_12	Fv_01	Fv_02	Fv_03	Fv_04	Fv_05
Physical and Chemical																	
General																	
Hydromorphological																	
HQA	*	*	*	*	*	*	*	*	*				*	*	*	*	
HMS	*	*	*	*	*	*	*	*					*	*	*	*	*
Biological																	
Macroinvertebrates																	
Fish																	
Final Classification																	

Table AV.2 - Water quality of sampling sites in Maçãs (Ma), Angueira (Ag), Penacal (Pe), Vale Moinhos (Vm) and Azibo (Az) Rivers (Summer 2023), and their classification, considering the maximum threshold for the establishment of Good Ecological Status *(Blue: Excellent; Green: Good; Yellow: Reasonable; Orange: Poor; Red: Bad; White: no sampled; Black: no fish*) (APA, 2021). Water analyses – Yellow: *Less than or reasonable status; Hydromorphological analises: Green: Equal or Less than or good status*

Elements	Ma_01	Ma_02	Ma_03	Ma_04	Ma_05	Ag_01	Ag_02	Ag_03	Ag_04	Ag_05	Pe_01	Pe_02	Vm_01	Vm_02	Az_01	Az_02	Az_03
Physical and Chemical																	
General																	
Hydromorphological																	
HQA	*	*		*	*	*	*	*	*	*	*		*	*	*	*	*
HMS	*	*	*	*		*	*	*	*	*	*		*		*	*	
Biological																	
Macroinvertebrates																	
Fish																	
Final Classification																	

Table AV.3 - Water quality of sampling sites in Vilariça (Vi), Veados (V2), Onor (On), Igrejas (Ig) Baçal (Ba), Ferreiros (Fe), Inferno (In) and Zacarias (Za) streams (Summer 2023), and their classification, considering the maximum threshold for the establishment of Good Ecological Status *(Blue: Excellent; Green: Good; Yellow: Reasonable; Orange: Poor; Red: Bad; White: no sampled; Black: no fish*) (APA, 2021). Water analyses – Yellow: *Less than or reasonable status; Hydromorphological analises: Green: Equal or Less than or good status.*

Elements	Vi_02	Vi_03	Ve_01	On_01	Ig_01	Ba_01	Fe_01	In_01	Za_01
Physical and Chemical									
General									
Hydromorphological									
HQA	*	*	*	*	*	*	*	*	*
HMS	*	*	*	*	*	*	*	*	*
Biological									
Macroinvertebrates									
Fish									
Final Classification									

Presidente do juri

Maria da Natividade Ribeiro Vieira

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