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River sickness: Impacts of alder decline on river ecological quality and brown trout populations in Montesinho Natural Park (northern Portugal)

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Resumo e palavras-chave

Os ecossistemas dulçaquícolas estão sujeitos a inúmeras pressões, sendo muito vulneráveis aos efeitos das doenças infeciosas. Atualmente, a doença provocada pelo oomiceto *Phytophthora,* daqui em diante denominada doença do amieiro, está amplamente distribuída a nível mundial tendo em Portugal especial incidência sobre o amieiro (*Alnus lusitanica*), que desempenha um papel essencial no funcionamento dos sistemas lóticos. Esta doença produz inúmeros sintomas e, em última análise, conduz à morte das árvores infetadas. Em Portugal, esta doença foi oficialmente registada no centro do país, mas afeta também já a região norte, nomeadamente o Parque Natural de Montesinho, onde o amieiro é uma espécie arbórea dominante nas galerias ripárias.

O Parque Natural de Montesinho é uma das maiores áreas protegidas portuguesas, cujos cursos de água albergam populações residentes de truta-de-rio (*Salmo trutta),* com elevado valor socioeconómico, genético e ecológico, e altamente sensíveis a alterações ambientais. A deterioração das galerias ripárias associada e a perda de amieiros devido à doença do amieiro poderá ter impactos significativos na qualidade dos sistemas lóticos e em particular nas populações de truta-de-rio.

Neste estudo, realizado no verão de 2019, procurou-se analisar os efeitos da doença do amieiro, na qualidade biológica e morfológica dos rios Sabor e Baceiro, e na ecologia das populações de truta-de-rio aí residentes.

Devido às suas características particulares, o amieiro influencia múltiplos aspetos do funcionamento e integridade ecológica dos rios, os quais podem ser alterados devido ao declínio e perda das árvores infetadas. Durante o período inicial da doença, o deterioramento e queda de folhas dos amieiros conduz à redução da qualidade de matéria alóctone nos rios e consequente diminuição dos níveis de azoto, o que vai influenciar as toda a cadeia trófica, incluindo macroinvertebrados e peixes. Adicionalmente, a diminuição da copa aumenta a exposição solar, com subida da temperatura e produção de algas, podendo afetar espécies estenotérmicas, como a truta-de-rio. Por outro lado, o deterioramento das raízes e a morte dos amieiros tem consequências ao nível da hidromorfologia, alterando o dinamismo do rio, diminuindo a estabilidade das margens e aumentando a turbidez da água.

Tendo em conta estes possíveis efeitos, neste trabalho foram analisadas as seguintes hipóteses: o deterioramento dos amieiros provoca alterações ao nível da qualidade físico-química da água (H_A), hidromorfologia dos rios (H_B), comunidades de macroinvertebrados (H_C), comunidades de peixes (H_D), e condição física da truta-de-rio (H_E).

Assim, foram selecionados 6 locais nos rios Sabor e Baceiro, de acordo com a degradação dos amieiros, distribuição da truta-de-rio e acessibilidade. Em cada rio, foram selecionados dois locais em cada uma das seguintes categorias de condição dos amieiros: 1) boa integridade ecológica sem desfolhamento, 2) sinais de doença com desfolhamento moderado e 3) desfolhamento crítico e amieiros mortos.

Em cada local, foram medidas 6 variáveis físico-quimicas seguindo as normas da Agência Portuguesa Ambiental (APA): temperatura, oxigénio dissolvido, condutividade elétrica, pH e azoto e fósforo total. Adicionalmente, as características hidromorfológicas foram obtidas através da aplicação do *River Habitat Survey* (RHS) e determinação dos índices *Habitat Quality Assessment* (HQA) e *Habitat Modification Score* (HMS). As amostragens das comunidades de macroinvertebrados bentónicos e peixes foram realizadas de acordo com protocolos de aplicação da Diretiva Quadro da Água. Os macroinvertebrados foram identificados ao nível da família e classificados em termos de grupo funcional de alimentação. Os peixes foram identificados ao nível da espécie e, no caso da truta-de-rio, foram também determinados o seu comprimento e peso. As comunidades de macroinvertebrados foram avaliadas, através dos índices IBMWP e IPtIN, e as comunidades piscícolas através do Índice Piscícola

de Integridade Biótica para Rios Vadeáveis de Portugal Continental (F-IBP). A condição das populações de truta-de-rio foi determinada com base no índice K de condição corporal.

A qualidade da água variou entre boa e excelente, tendo sido registadas apenas ligeiras variações nas condições de temperatura, condutividade, azoto e fósforo. Contudo, estas não ocorreram em associação com o deterioramento do amieiro, estando possivelmente relacionadas com outros fatores. Pelo contrário, foram detetadas diferenças significativas nas características hidromorfológicas tendo os locais com amieiros mortos apresentado menores valores de HQA.

As comunidades de macroinvertebrados apresentaram qualidade boa a excelente, incluindo principalmente taxa sensíveis, e existindo reduzida variação entre zonas com amieiros em diferentes estados. Nas comunidades piscícolas, verificou-se uma diminuição da qualidade consistente com o deterioramento dos amieiros. No entanto, estas comunidades mantiveram níveis de qualidade boa a excelente, incluindo apenas espécies nativas, e sendo dominadas por truta-de-rio. A condição física desta espécie variou seguindo um padrão semelhante ao deterioramento do amieiro, apresentando menores valores em locais com amieiros mais degradados.

Este primeiro conjunto de resultados indica que o deterioramento dos amieiros poderá ter conduzido a uma diminuição da integridade ecológica dos rios e condição física da truta-de-rio, encorajando em particular a realização de estudos adicionais mais detalhados sobre a ecologia das espécies e comunidades de peixes.

Nesse sentido, a segunda parte deste estudo centrou-se na análise dos efeitos da doença do amieiro na dieta e uso de microhabitat da truta-de-rio, espécie bioindicadora da qualidade de rios de montanha, muito sensível a fatores de degradação ambiental.

A truta-de-rio apresenta variações de dieta ao longo da ontogenia. As trutas jovens, de menores dimensões, consomem principalmente pequenos macroinvertebrados bentónicos de primeiros instares. Contrariamente, as trutas adultas, de maiores dimensões, consumem principalmente presas maiores como pupas, insetos adultos ou peixes.

Do mesmo modo o uso de microhabitat também varia ao longo da ontogenia. As trutas mais pequenas preferem água rasas com corrente (riffles), enquanto as maiores preferem águas mais profundas (pools), com pouca corrente e presença de blocos de grande dimensão e rocha.

Uma vez que a disponibilidade das presas de grandes dimensões é altamente dependente da vegetação ripária, e que o amieiro influencia o dinamismo do rio, analisaram-se as seguintes hipóteses: o deterioramento dos amieiros causa alterações na composição da dieta da truta-de-rio (H_F), promove um aumento da sobreposição da dieta entre as classes de tamanho (H_G), conduz a alterações na disponibilidade de microhabitat (H_H) e influencia o seu uso pela truta-de-rio (H_I)

Para avaliar estas hipóteses, foram considerados os 6 locais definidos nos rios Sabor e Baceiro, e classificados em termos de estado dos amieiros. Cada local foi amostrado através de pesca elétrica, segundo o protocolo definido para aplicação da Diretiva Quadro da Água. Para além de dados biométricos, foram também recolhidos conteúdos estomacais dos indivíduos de truta-de-rio capturados. Os conteúdos estomacais foram triados e as presas identificadas ao nível da família.

A disponibilidade de microhabitat em cada local foi determinada com base na realização de transtos, em que foram registados a profundidade, presença de corrente, tipo de substrato dominante e subdominante e cobertura. O uso de habitat pela truta-de-rio foi avaliado através de observação subaquática (*snorkelling).*

As análises de dieta e uso de habitat foram realizadas considerando três classe de tamanho, nomeadamente: A) ≤13.0; B)]13.0 – 20.0[; C) ≥ 20.0 cm.

A dieta da truta-de-rio variou com o deterioramento dos amieiros, tendo o consumo de presas de origem terrestre diminuído como esperado. Simultaneamente, registou-se uma maior percentagem de insetos das ordens Ephemeroptera e Trichoptera na dieta. Contudo, não foi detetado qualquer aumento significativo na sobreposição de dieta entre as diferentes classes de tamanho .

Quer a disponibilidade quer o uso de microhabitat variaram entre locais, mas de forma independente. A disponibilidade de habitat não apresentou padrões definidos com o estado da vegetação ripária, sendo possivelmente explicada por outros fatores. No caso do uso de habitat, as alterações no tipo de cobertura foram claras, estando provavelmente associadas ao deterioramento dos amieiros. Em locais com deterioramento mais pronunciado, a truta-de-rio aumentou o uso de cobertura, em especial de pedras e blocos. Como o consumo de presas de origem terrestre diminuiu, a predação por trutas maiores sobre as mais pequenas possivelmente aumentou, encorajando o uso de estruturas de cobertura. Por outro lado, o aumento do consumo de Trichoptera poderá também estar relacionado com o aumento do uso de pedras e blocos, pois estes macroinvertebrados são tipicamente encontrados junto destas estruturas.

Em conclusão, a doença do amieiro é recente no norte de Portugal e ainda não apresenta consequências ecológicas substanciais. No entanto, algumas alterações na qualidade dos rios e ecologia da truta-de-rio seguiram padrões semelhantes ao deterioramento do amieiro. Este estudo destaca a importância de compreender as relações ecológicas entre comunidades e doenças infeciosas, fornecendo indicações para pesquisas futuras. Uma vez que as alterações observadas ainda são pequenas, prevê-se que seja possível evitar consequências futuras sobre as comunidades do Parque Natural do Montesinho se forem tomadas medidas adequadas. Como tal, recomenda-se a poda ou remoção das árvores infetadas, utilizando técnicas adequadas, e substituindo-os por espécies que ocorrem naturalmente com o amieiro, como o salgueiro e o freixo. A identificação de amieiros resistentes aos efeitos da doença do amieiro também é recomendada, permitindo o mapeamento de áreas mais suscetíveis à degradação e uma gestão mais eficiente dos recursos disponíveis. Adicionalmente, em rios regulados, deve ser garantido um regime de fluxo natural, evitando períodos de cheias que contribuem para o aumento da vulnerabilidade dos amieiros à infeção por *Phytophthora*. Por último, a monitorização das populações locais de truta-de-rio é essencial, servindo de indicador da eficácia da gestão em prática.

***Palavras-chave**: amieiro, truta-de-rio, doença infeciosa, qualidade ecológica, rios de montanha*

Abstract and Keywords

One of the most contemporary threats to freshwater ecosystems is the spread and effects of infectious diseases. Specifically, plant diseases can be harmful for the host and can translate into changes at the ecosystem level. Although these repercussions may be severe, available information is mostly focused on virus/host interactions and virus characteristics while wild plant virus effects on communities remain poorly understood.

The alder (*Alnus spp.*) disease caused by *Phytophthora* is a widely spread contemporary plant disease with special incidence in freshwater ecosystems. In Europe, the disease was first reported in the U.K, and followed by several other countries, including in central Portugal. Unofficially, the disease has already been detected in Montesinho Natural Park, northern Portugal, where alder (*Alnus lusitanica*) is the dominant riparian tree.

Mountain rivers in the Montesinho Natural Park are some of the few Portuguese rivers harboring resident populations of brown trout (*Salmo trutta),* a species with high genetic, ecological, and socio-economic value. Because brown trout is highly sensitive to environmental degradation, it may be highly vulnerable to ecological changes caused by alder disease.

As such, this study explored the effects of riparian deterioration resulting from the alder disease on the ecosystem, using Montesinho Natural Park as setting. The first chapter focused on river ecological integrity of two mountain rivers (Sabor and Baceiro, River Douro basin) currently affected by a decrease in riparian condition, mainly provoked by the recently detected alder disease, and the second chapter explored the indirect effects of the disease on the ecology of resident brown trout populations.

Physical and chemical characteristics, hydromorphology, and benthic macroinvertebrate and fish communities were assessed in 6 sites in each river, identified as: 1) excellent riparian condition (health trees) 2) signs of disease (dieback and lower canopy) and 3) extensive dead alder tree areas. It would be expected that ecological conditions were better where the riparian integrity is high. Physical and chemical conditions were typical of salmonid rivers and showed little change with alder decay. Significant decrease in hydromorphological quality was detected with alder decay. Macroinvertebrate communities had a good/excellent quality, mainly including sensitive taxa. Fish communities showed changes in structure between the highly degraded riparian areas and the zones with high integrity and with signs of alder disease. Nevertheless, communities still were in good/excellent quality, only including native species. Brown trout dominated the communities at all sites, but its condition significantly decreased with alder decay. Overall, these results highlight and suggest some decrease in ecological integrity of rivers associated with alder disease, encouraging further studies and the definition of adequate measures to rehabilitate Montesinho Natural Park riparian zones.

To better comprehend alder disease repercussions on the ecology of sensitive species brown trout was used as study subject for diet and microhabitat use analyses. Sabor and Baceiro rivers were again used as setting, and populations were analyzed in the same sites previously categorized according to riparian conditions. Diet was characterized through stomach contents analysis. Microhabitat use and availability were determined through snorkeling observations and transects randomly distributed within each site. Fish were divided into 3 size classes (small, medium, large) and evaluated independently. Diet of brown trout changed between zones, reflecting variations in the use of terrestrial origin prey, Ephemeroptera, Plecoptera and Trichoptera. Terrestrial origin prey, which is typically highly influenced by riparian condition, decreased with alder decay, while consumption of other prey types increased. However, no significant increase in diet overlap between brown trout size classes was found with alder decay. Availability and use of microhabitat were not dependent but both changed between sampled sites. Specifically, changes in the use of cover were noticeable likely reflecting indirect effects of the riparian condition. Although further studies are needed, current results suggest that degradation of alder may have a significant impact on brown trout diet and microhabitat use.

In conclusion, the alder disease is recent in northern Portugal and does not yet imply substantial ecological consequences. However, the decay of alder has already led to some changes in river quality and brown trout habits. Disease consequences may grow in the future and imply further considerable risk to sensitive species. This study highlights the importance of understanding ecological relationships between communities and infectious diseases, providing guidelines for future research. Furthermore, perceived changes are small and there is still possible to avoid future consequences in this region if action is taken. As such, it is recommended pruning or removing of infected trees, using appropriate techniques, and replacing them with species that naturally co-occur with alder, such as willow and ash trees. The identification of *Phytophthora* resistant alders is also recommended, allowing the mapping of areas that are most susceptible to degradation and a more efficient management of available resources. Additionally, in regulated rivers, a natural flow regime must be ensured, avoiding periods of flood that contribute to increased vulnerability of alders to infection by *Phytophthora*. Finally, monitoring of local brown trout populations is essential, serving as an indicator of the effectiveness of current management strategies.

***Keywords**: mountain rivers, Phytophthora, infectious diseases, ecological impacts, sensitive species*

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1. Introduction

Freshwater ecosystems provide multiple ecosystem services, including provisioning (e.g. supply of fresh water, source of energy), regulating (e.g. water regulation and purification), cultural (e.g. recreation and ecotourism) and supporting (e.g. water and nutrient cycling) services (Millenium Ecosystem Assessment, 2005; Grêt-Regamey et al., 2012). However, ecosystem condition is increasingly affected by several human pressures such as water pollution and eutrophication, habitat loss and degradation, overexploitation, flow modification and the introduction of alien species (Malmqvist and Rundle, 2002; Dudgeon et al., 2006). Furthermore, the interaction of human pressures with climate change is damaging freshwater biodiversity and modifying irreversibly ecosystem condition (Gozlan et al., 2019; Lake et al., 2000; Sala et al., 2000) and consequently compromising ecosystem services (**Figure 1.1**).

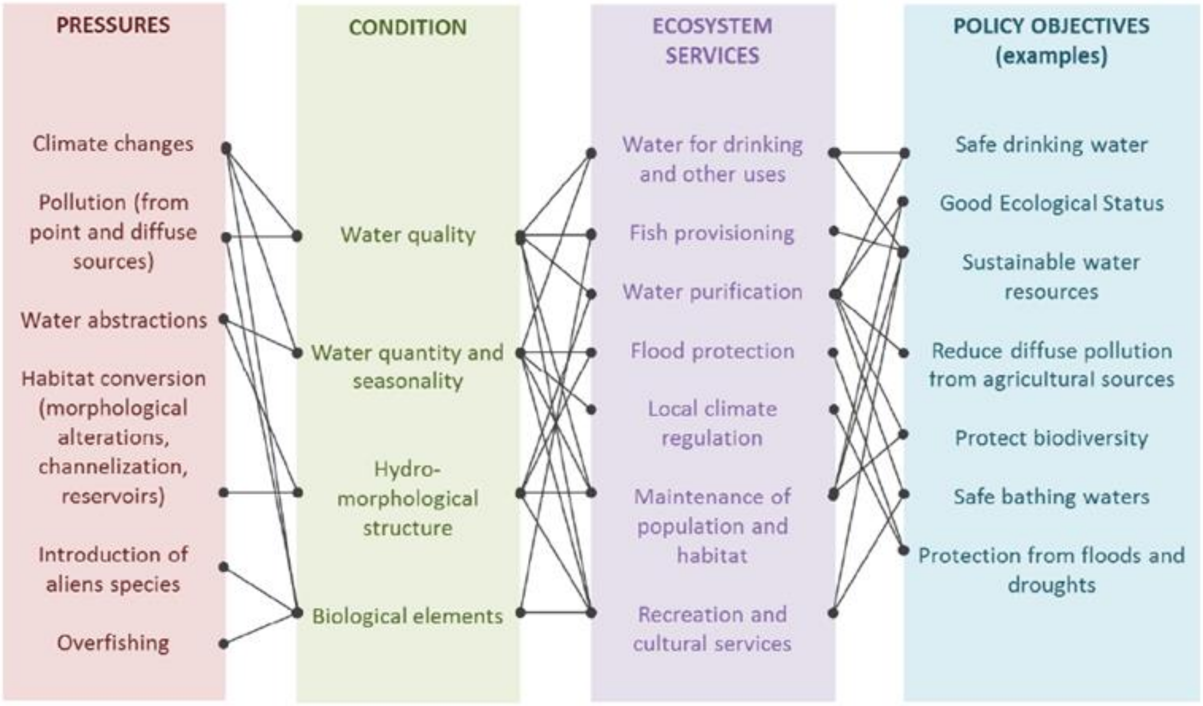

Figure 1.1 Links between pressures, condition and ecosystem services in freshwater ecosystems (adapted from Grizzetti et al. (2016)).

One of the most contemporary threats to freshwater ecosystems is the spread of infectious diseases. Infectious diseases are dependent on a multitude of environmental conditions and access to specific and adequate host or hosts. These conditions can be more easily met in the proximity of freshwater sources, making aquatic ecosystems not only highly susceptible but also efficient platforms for disease spread. Although their importance is yet to be fully understood, evidence on the significant impacts of infections on several species is already worrying (Reid et al., 2019).

Specifically, plant diseases can be harmful for the host and can translate into changes at the ecosystem level. Among other, repercussions of plant diseases include changes in resource availability, plant cover and community structure, soil and geomorphological characteristics, climate and water regulation, land use, nutrient cycling, increased pollution through pesticide use and vulnerability to invasion by exotic species (Cheatham et al. 2009). Although these repercussions may be severe, available information is mostly focused on virus/host interactions and virus characteristics while wild plant virus effects on natural communities remain sparsely studied (Alexander et al., 2014).

The alder disease is a widely spread contemporary plant disease with special incidence in freshwater ecosystems, mainly due to transplantations from plant nurseries combined with other sources (Jung et al., 2007; Rodríguez González et al., 2021). This disease is caused by *Phytophthora.* These are unicellular organisms called oomycetes that closely resemble fungi (Cooke et al., 2000; Fry and Grünwald, 2010). Hydrochore dissemination allows oomycete to contaminate a vast portion of rivers and riverine host species like alder (Gibbs et al., 2003). Furthermore, its spread is highly effective given that fallen dead trees also contribute to the downstream sickness's spread (Bjelke et al., 2016). In Europe, the disease was first reported in the U.K (Brasier et al., 1995), and followed by several other countries (Bjelke et al., 2016), including Portugal. Here, two species of *Phytophthora* were identified, *Phytophthora lacustris* and *Phytophthora x alni*, which due to its hybrid origin is even more damaging than the parental species. Currently, very few studies have focused on the distribution of the disease in Portugal, having only been recorded in the central region (Kanoun-Boulé et al., 2016). However, unofficially, the disease as already been detected in Montesinho Natural Park, northern Portugal, where alder (*Alnus lusitanica* Vít, Douda & Mandák, 2017) is the dominant riparian tree species and the target of alder disease (Castro et al., 2010).

Montesinho Natural Park is one of the largest protected areas in Portugal with high faunal and floral diversity. The riverine network is extensive, including the headwaters of the Sabor and the Tua rivers, two tributaries of the Douro, the river with the largest drainage basin in Portugal. Given the mountainous characteristics of these rivers, they are some of the few where it is possible to find resident populations of brown trout (*Salmo trutta* Linnaeus, 1758) species with high genetic, ecological, and regional socio-economical value. As sensitive species requiring specific environmental conditions, its vulnerability to the consequences of ecological changes caused by alder disease may be significant (Castro et al., 2010; Collares-Pereira et al., 2021).

Symptoms of the disease include usually leaf size reduction and color change, rotting of roots and bark, external stem lesions and ultimately death of the infected individual. Even though infected alder can live up to 10 years, loss of vitality may cause significant damages to riverine systems and communities (Bjelke et al., 2016). However, these effects and consequences of the disease are poorly studied.

As such, in the next chapters, the effects of riparian deterioration resulting from the alder disease will be explored. Using as setting the Montesinho Natural Park, the first chapter focuses on river ecological integrity. In that sense, chemical and physical, hydromorphological and biological characteristics were evaluated, and changes highlighted. Furthermore, in the second chapter, brown trout diet and microhabitat use was evaluated to better comprehend alder disease repercussions on sensitive species. Finally, the overall results are highlighted and discussed in the fourth chapter, presenting guidelines and perspectives for future studies.

2. Is the ecological integrity of mountain rivers in Montesinho Natural Park (Northern Portugal) affected by the alder (*Alnus lusitanica*) tree disease?

2.1. Abstract

Mountain rivers provide a multitude of ecosystem services essential for the conservation of biodiversity and human well-being. However, climate change and human impacts are threatening these regions and in particular freshwater ecosystems, very sensitive to disturbance, that can function as sentinels for long term effects. The objective of the present study was to evaluate the ecological integrity of two mountain rivers (Sabor and Baceiro, River Douro basin, North-eastern Portugal), currently affected by a decrease in riparian condition, mainly provoked by the recently detected alder disease. Physical and chemical characteristics, hydromorphology, and benthic macroinvertebrate and fish communities were assessed in 6 sites in each river, according with the Water Framework Directive procedures. Several metrics were calculated and compared among three zones identified as: 1) excellent riparian condition (health trees) 2) signs of disease (dieback and lower canopy) and 3) extensive dead alder areas. It would be expected that there were better conditions in zones where the riparian integrity is high. Physical and chemical conditions were typical of salmonid rivers and showed little change with alder decay. Significant differences were obtained for hydromorphological characteristics. Macroinvertebrate communities had a good/excellent quality, mainly including sensitive taxa. Variation between riparian condition zones were less evident than for fish communities which showed changes in structure between the high degraded riparian areas and both reference and zones with signs of alder disease. Nevertheless, communities had good/excellent quality, only including native species. The dominant species was the brown trout (*Salmo trutta*) and its physical condition significantly changed between disturbed riparian zones and good integrity zones. Overall, results highlight a link between alder disease and the decrease in ecological integrity of rivers, encouraging further studies and definition of adequate measures to rehabilitate Montesinho Natural Park riparian zones.

Keywords*: water quality, hydromorphology condition, macroinvertebrates, fish, Salmo trutta*

2.2. Introduction

Ecosystem condition can be assessed using indicators. According with the Water Framework Directive (WFD) the ecological status of fresh waters in Europe is defined by the quality of: 1) biological 2) physicochemical, and 3) hydromorphological elements (European Comission, 2000). In the case of rivers, biological indicators include, among other, benthic macroinvertebrates and fish (Schaumburg et al., 2004; Delgado et al., 2010). Macroinvertebrates and fish communities are responsive to multiple local and basin-scale pressures including organic pollution and hydromorphological and land use modifications. As such, information on the composition and structure of both communities is critical for the evaluation of the ecological status of rivers (Birk et al., 2012; Breine et al., 2007; Hering et al., 2006, 2003; Morais et al., 2004; Pardo et al., 2014).

Mountain rivers generally have low biodiversity and functional complexity due to the harshness of environmental conditions. They are highly vulnerable to climate events, hydrological extremes and in particular temperature increases, and to the spread of alien species, including plants, animals, and microorganisms. For these reasons, mountain headwater rivers can play a crucial role as sentinels of ecosystem change (**Figure 2.1**) making its monitoring key in supporting policy and decision making (Céréghino et al., 2008; Schmeller et al., 2018).

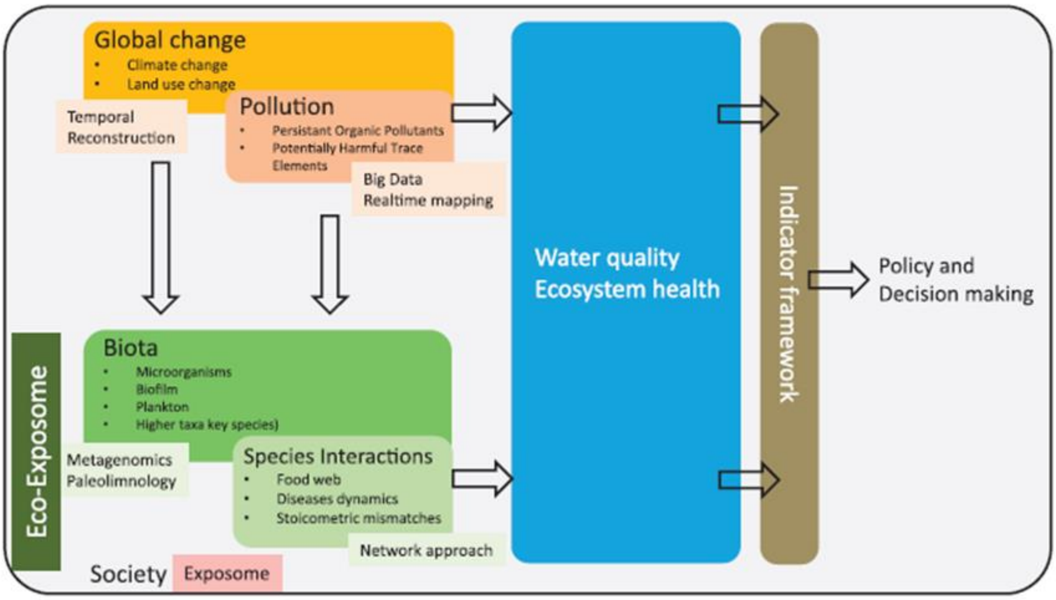

Figure 2.1 A research approach to establish mountain freshwater ecosystems as sentinels of change. Drivers and pressures (orange) of biodiversity change need to be reconstructed (adapted from Schmeller et al., 2018).

Given their fragility, mountain rivers are highly susceptible to the negative impacts by infectious riparian diseases. This is particularly the case of the alder (*Alnus spp.*) disease which affects the ecological integrity of the aquatic/riparian ecotone (Bjelke et al., 2016).

In the early stages of the disease, physical and chemical parameters (e.g. water temperature, nutrients, dissolved salts) can be impacted due to precocious leaf fall. Leaf litter decrease would be expected in subsequent 2-3 years, leading to a reduction of the allochthonous organic matter input and nutrient (mainly nitrogen) concentrations in rivers (Bjelke et al., 2016). Given the major role of exogenous source of organic materials in ecosystem productivity (Wang and Wen, 2018), significant changes in ecological state are also expected.

For instance, nitrogen dynamics shifts lead to macroinvertebrate diversity changes both in species composition and functional feeding groups, possibly having a strong influence on shredders (Irons et al., 1988; Feio and Ferreira, 2019). Therefore, changes on species diversity and on the ratio between sensitive (Ephemeroptera, Plecoptera and Trichoptera) versus resilient macroinvertebrates (Diptera, Annelida) are expected.

The cascade effects, beginning on changed nutrient levels, can influence directly or indirectly the food webs, including fish (Bjelke et al., 2016). For instance, crown deterioration will result in overexposure to direct sunlight and rise in water temperature (Garner et al., 2017), which would increase algal productivity (Béchet et al., 2014).

Finally, changes in the river dynamics due to root decay and alder dieback would likely decrease riverbank stability and possibly water clarity, changing the hydromorphological characteristics of the river (Bjelke et al., 2016). Some of these negative impacts can be mitigated by the reinforcement of riparian galleries with ash (*Fraxinus spp.*) and willow (*Salix spp.*) trees, while resistant strains of alder species can be found. However, ash and willow trees are less resilient than *Alnus* species and produce lower quality leaf litter, with lower percentage of nitrogen, being incapable of fully preventing ecological state deterioration (Bjelke et al., 2016).

Given the overall importance of riparian vegetation for the functioning and maintenance of ecosystem condition in mountain rivers, it is crucial to understand how alder disease may affect the ecological status and in particular the local biota. This information can be used by authorities, managers, and stakeholders to define the best measures to promote the conservation and balance the exploitation of aquatic resources.

The objective of the present study was to assess the ecological integrity of two mountain freshwater ecosystems, Sabor and Baceiro rivers, encompassing distinct riparian condition zones, mainly determined by the alder disease. Located in Montesinho Natural Park in northern Portugal, these rivers are home to sensitive and both ecologically and economically important species including *Salmo trutta*. In detail, the specific objectives were to:

1) **Assess the water quality**, based on physical and chemical parameters.

    H_A: Physical and chemical parameters change with alder decay. More specifically, temperature increases with alder decay while nutrients decrease.

2) **Identify modifications in the hydromorphology**, i.e., aquatic and riparian habitats.

    H_B: Quality based on hydromorphological conditions decreases with increase in alder decay.

3) **Evaluate changes in biota**, specifically in macroinvertebrates and fish communities.

    H_C: Macroinvertebrates communities change with alder decay.

    H_D: Fish communities change with alder decay.

    H_E: *Salmo trutta* condition decreases with alder decay.

2.3. Study area

The Sabor and Baceiro rivers are two tributaries of the Douro river that drain the eastern part of Montesinho Natural Park. Dominated by alder (*Alnus lusitanica), ash (Frax*inus *angustifolia* Vahl, 1804), poplar (*Populus nigra* L., 1753) and willow (*Salix* spp), these rivers are surrounded by natural landscape in the upper regions, while agricultural and livestock activities increases downstream.

As mountain rivers, they exhibit cold and highly oxygenated waters with low content of dissolved salts and nutrients and flow throughout deep valleys with narrow channels and highly shaded by riparian vegetation. These aquatic and riparian habitats sustain several threatened species and contribute to preserve high ecological integrity in this protected area (Castro et al., 2010).

Both the Sabor and Baceiro rivers support a variety of aquatic habitats including riffle-erosion (boulders and high-water current) and pool-sedimentation (sands and low water current) zones. The functioning of these low productive systems is highly dependent on the input of allochthonous organic materials, displaying strong relationships with the marginal areas and in particular with the riparian vegetation (Teixeira and Cortes, 2006). Because aquatic primary production is very low, ecological integrity is extremely linked to the management of the riparian ecotones. Human influence is nowadays very low and only a subsistence agriculture is practiced mainly in the downstream sections of each river. However some small and rustic weirs limit the river longitudinal connectivity and the upstream movement of fish, namely during the reproduction period (Castro et al., 2010).

Fish assemblages are dominated by brown trout. However, other species may also be found, including Northern Iberian chub (*Squalius carolitertii* Doadrio, 1988), Northern straight-mouth nase (*Pseudochondrostoma duriense* Coelho, 1985), Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864), calandino (*Squalius alburnoides* Steindachner, 1866) and Northern Iberian spined-loach (*Cobitis calderoni* Băcescu, 1962) (Collares-Pereira et al., 2021).

2.4. Methods

Six sampling sites were selected in each river based on the recent degradation of riparian vegetation (mainly alder), on brown trout distribution, and accessibility. Specifically, alder crown condition and damaging agents were determined based on Eichhorn et al. (2010), leading to the identification of three riparian zones: 1) good integrity condition, with no defoliation (< 10%) (GI), 2) signs of disease, with slight to moderate defoliation (10-50%) (SD) and 3) the presence of severe defoliation (> 50%) and dead vegetation, mainly alder (DR) (**Figure 2.2 to Figure 2.4)**. Two sites were selected in each zone, in both the Sabor (S1-S6) and the Baceiro (B1-B6) rivers (**Figure 2.5**). Site S4 was near a village.

The sampling sites are included in salmonid waters, with a longitudinal/altitudinal difference between the upstream (S1/B1) and downstream (S6/B6) sampling sites of approximately 16,5 Km/200 m for the Baceiro river and 20,1 Km/270m for the Sabor river.

Water, hydromorphology, macroinvertebrate and fish sampling were conducted in the summer season of 2019.

Figure 2.2 Sampling site with riparian vegetation in good integrity condition (GI).

Figure 2.3 Sampling site with riparian vegetation with signs of disease (SD).

Figure 2.4 Sampling site with presence of dead riparian vegetation, mainly alder (DR).

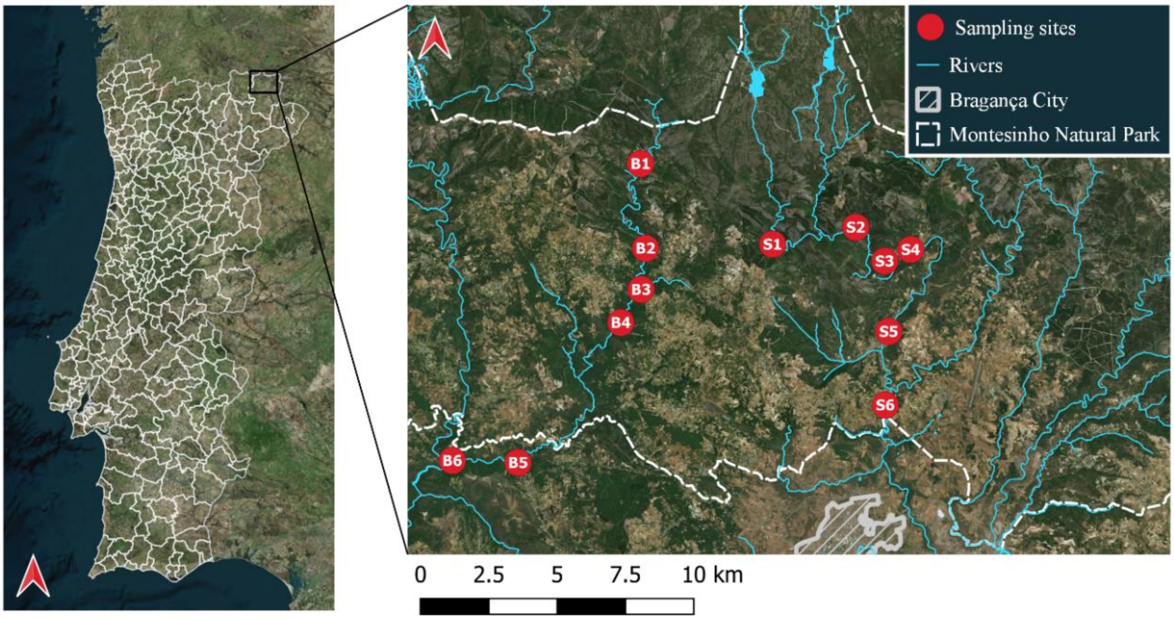

Figure 2.5 Location of sampling sites, in the Baceiro river (B) and in the Sabor river (S).

2.4.1. *Water quality*

The water quality was evaluated based on physical and chemical parameters. Four variables were measured *in-situ*, using multiparametric portable probes (**Figure 2.6**) namely: temperature (T, °C), dissolved oxygen (DO, mg O_2/L and % saturation), electrical conductivity (EC25, µS/cm), and pH. In the laboratory, water samples were analysed for total nitrogen (mg N_{total}/L) and total phosphorus (mg P_{total}/L) contents, according to the official Portuguese criteria for water quality assessment (APA, 2014).

Figure 2.6 In situ measurement of water quality parameters.

2.4.2. *Hydromorphology*

The hydromorphological characteristics were obtained through the application of the River Habitat Survey (RHS) methodology (version 2003) (Environment Agency, 2003). The RHS is a semi-objective method commonly used to evaluate the quality and conservation status of aquatic and riparian habitats (Raven et al., 1997, 1998). The RHS allows the inventory of the channel and the riparian corridor along a 500 m stretch, covering a 50 m strip on each side of the watercourse (**Figure 2.7**). Observations are made in transects defined every 50 m (spot-checks), and in continuous over the entire 500 m sector (sweep-up).

The RHS data were used to determine two indexes: 1) Habitat Quality Assessment - HQA and 2) Habitat Modification Score – HMS, using the software “River Habitat Survey Toolbox - http://www.riverhabitatsurvey.org/author/mnaura/” (Naura, 2016).

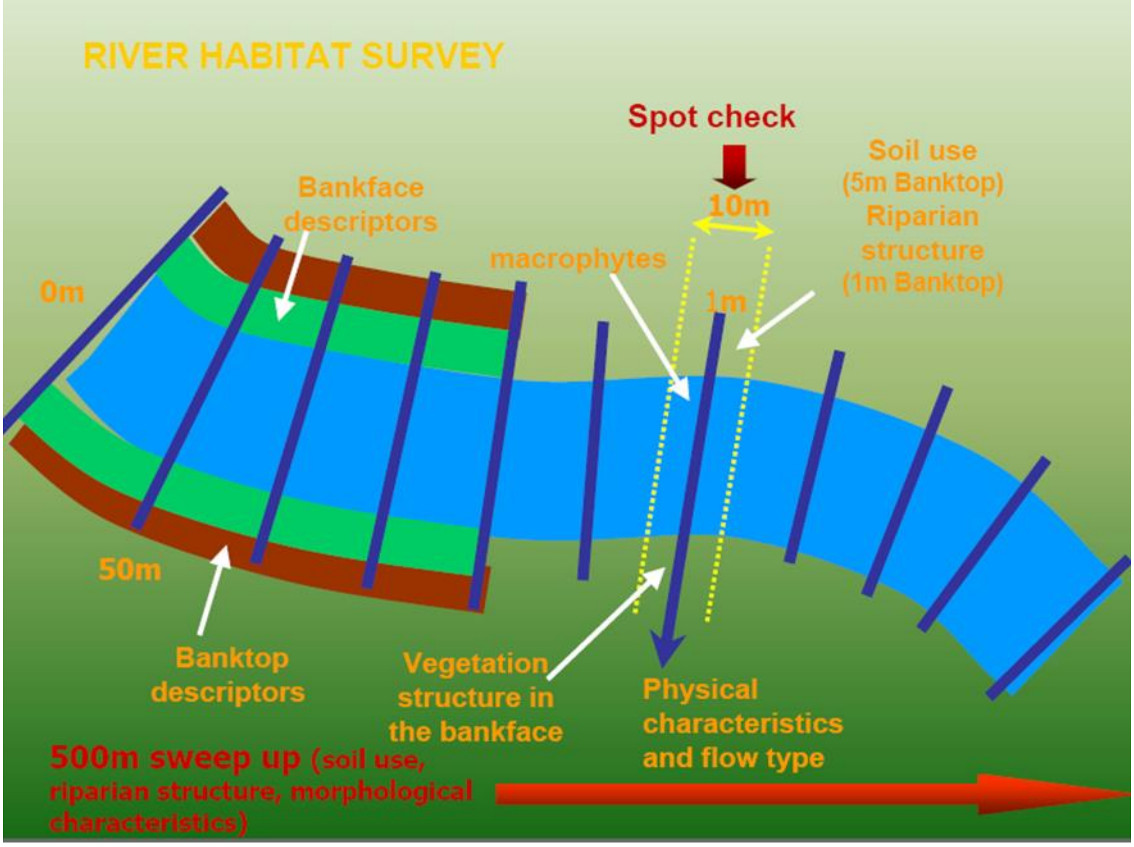

Figure 2.7 Scheme of RHS sampling (adapted from Environment Agency, 2003).

2.4.3. *Benthic macroinvertebrates*

2.4.3.1. Sampling

Benthic macroinvertebrates were sampled using the protocol established for WFD application in Portugal (INAG I.P., 2008a). A stretch with 50 m was selected at each site, considering erosion (turbulent flow) and adjacent sedimentation (laminar flow) units and the representativeness of habitats (e.g. riffle, pool) and microhabitats (e.g. inorganic and organic materials). Samples were collected in 6, 1-m long sectors, using a hand net (25*25 cm mouth dimension, 500 µm mesh size) removing the substrata, preferably against current, using foot movements (). Samples were preserved in 70% alcohol, for further analysis and identification. In the laboratory, the macroinvertebrates were sorted, identified and counted under a stereomicroscope (OLYMPUS SMZ10, 10-132x zoom magnification), using dichotomous keys (Tachet et al., 1981, 2010) (**Figure 2.9**). The identification was made to the family level, except for Oligochaeta and Acari which were identified to subclass.

Figure 2.8 Benthic macroinvertebrates kick sampling.

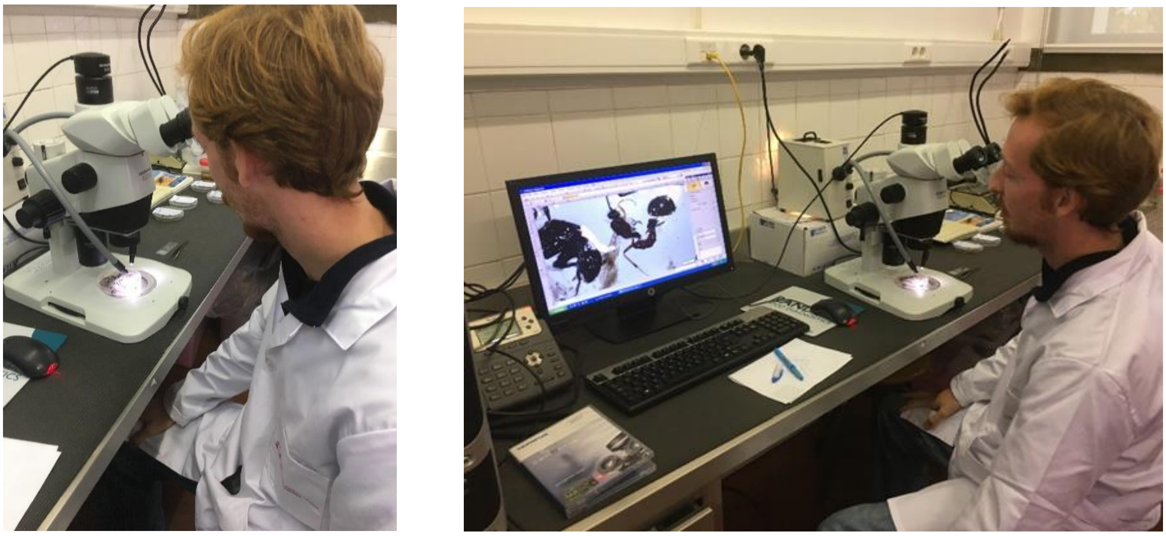

Figure 2.9 Laboratorial identification of benthic macroinvertebrates.

2.4.3.2. Community metrics

Community metrics included the: number of individuals (N), number of *taxa* (S), diversity (H' Shannon-Wienner index), evenness (Pielou J' index), relative abundance of Ephemeroptera, Plecoptera and Trichoptera (% EPT), biotic Index IBMWP (Alba-Tercedor, 1996; Alba-Tercedor et al., 2002) and the Portuguese Northern Invertebrate Index- $IPtI_N$ (INAG I.P., 2009).

The multimetric Index $IPtI_N$, is defined as:

$$\mathbf{IPtIN = N^{o}taxa*0.25+EPT*0.15+Evenness*0.1+(IASPT-2)*0.3+Log\,(Sel.\,ETD+1)*0.2,} \tag{2.1}$$

where, **EPT**: number families belonging to Ephemeroptera, Plecoptera, Trichoptera orders; **Evenness**: Pielou index or Evenness and calculated by the formula: $\mathbf{E = H'/Ln\ S}$**,** where: **H'** - diversity of Shannon-Wienner; **S** - number of *taxa* and **Ln** - natural logarithm. The **H' Shannon-Wienner Index** is calculated as: $\mathbf{H' = -\sum p_i\ Ln\ p_i}$, where: $\mathbf{p_i = n_i/N}$, where $\mathbf{n_i}$- nº of individuals of each *taxon I*; and **N**-

total nº of individuals present in sample. The **IASPT** corresponds to the ratio IBMWP/number of taxa present in the sample and finally **Log (Sel. ETD+1)** - Log$_{10}$ of (1 + abundance of Heptageniidae, Ephemeridae, Brachycentridae, Odontoceridae, Limnephilidae, Goeridae, Polycentropodidae, Athericidae, Dixidae, Dolichopodidae, Empididae, Stratiomyidae). All metrics were calculated using the Software AMIIB@ (http://dqa.inag.pt/implementacao_invertebrados_AMIIB.html).

Sampling sites were categorized into 2 river typologies, according to INAG, I.P. (2008b): Northern rivers of small dimension (NRSD; Sites S1, S2, S3, S4, S5, B1, B2, B3) and Northern rivers of medium dimension (NRMD; S6, B4, B5 and B6)). In **Table 2.1** are indicated the reference and boundary values for the quality classes of the IPtI$_N$ for each river typology.

Table 2.1 IPtI$_N$ reference values and boundaries for river types of the study (INAG I.P., 2009)

River Typology	Reference Values	Excellent	Good	Fair	Poor	Bad
Northern rivers small dimension $\leq$ 100 km^2	**1.02**	**≥0.87**	**[0.68-0.87[**	**[0.44-0.68[**	**[0.22-0.44[**	**[0-0.22[**
Northern rivers medium dimension > 100 km^2	**1.00**	**≥0.88**	**[0.68-0.88[**	**[0.44-0.68[**	**[0.22-0.44[**	**[0-0.22[**

Additionally, macroinvertebrates were classified into functional feeding groups (FFG) based on its feeding adaptations and basic nutritional resources (Merritt and Cummins, 2007). The software ASTERICS developed by the project **AQEM** (Hering et al., 2003) was used, and its classification system for aquatic invertebrate trophic relations is shown in **Table 2.2**.

Table 2.2 Classification System for invertebrate trophic relations (adapted from Merritt and Cummins, 2007)

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

2.4.4. Fish

2.4.4.1. Sampling

Fish sampling was conducted according to the protocol for the application of the WFD in Portugal (INAG I.P., 2008c). Fish were sampled by single pass electrofishing, in at least 100-m stretches (Hans Grassl ELT IIGI, 300–600 V, 2–3 A, DC) and collected with hand-nets (**Figure 2.10**). Fish were identified to species level. Captured brown trout individuals were measured for total length (in mm) and weight (in 0.1 g) (**Figure 2.11**), and additional data were also gathered for habitat and diet analyses (further described in chapter 3), after which fish were released into the same place in the river where they had been captured.

Figure 2.10 Sampling of fish using electrofishing.

Figure 2.11 Fish identification and biometric data collection.

2.4.4.2. Community metrics

The quality of fish communities was determined using the Biotic Integrity Index for wadable Rivers of Portugal (F-IBIP; http://www.isa.ulisboa.pt/proj/fibip/; INAG I.P. and AFN (2012). Sampling sites were categorized in the Northern Salmonid Region (NRS) and in the Northern Salmonid-Cyprinid Transition Region (NRSC). Metrics in the F-IBIP for NRS sites (S1, S2, S3, S4, S5, B1, B2 and B3) included the percentages of intolerant individuals (%), exotic individuals (%) and omnivorous individuals (%), and metrics in the F-IBP for NRSC sites (S6, B4, B5 and B6) included the percentages of exotic individuals (%), intolerant + intermediate individuals (%), invertivores individuals (%) (excluding tolerant species) and potamodromous individuals (%). The F-IBIP scores were obtained through the arithmetic mean of the metrics, with standardized final scores ranging between 0 (zero), corresponding to bad quality, and 1 (one) corresponding to excellent quality (**Table 2.3**).

Table 2.3 F-IBIP scores for each quality class (INAG I.P. 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**

2.4.4.3. Population parameters for brown trout

To estimate length-weight relationship, data were transformed logarithmically, and the following equation was used:

$$W = a\,L^{b} \tag{2.2}$$

where, W - weight (g); L - Fork length (cm); a,b – equation coefficients. The parameters **a** and **b** were calculated as the intercept and slope, respectively, of the linear regression:

$$\mathrm{Ln}\ W = \mathrm{Ln}\ a + b * \mathrm{Ln}\ L. \tag{2.3}$$

The standard value for b is 3 indicating an isometric growth (Ricker, 1958). Significant deviations from this value represent allometric growth and were assessed through a Student’s T test.

Fish condition was calculated using the Fulton index, as:

$$K = (100 \times W) / L^{b} \tag{2.4}$$

where: K - Condition factor.

For salmonids, K ranges between 0.8 and 2 (Baxter, 1998). Based on the K values, general fish appearance and fat reserves, condition was categorized from excellent to extremely poor, as in Table 2.4. For analysis, individuals were sorted in 3 size-classes (total length in cm): A) $\leq$13.0; B)]13.0 – 20.0[; C) $\geq$ 20.0 cm, to account for ontogenetic variation in condition (Grey, 2001).

Table 2.4 Condition Factor K for salmonid fish (adapted from Baxter, 1998).

Range	Quality
> 1.4	EXCELLENT
[1.2 – 1.4 [	GOOD
[1.0 – 1.2 [	FAIR
[0.8 – 1.0 [	POOR
< 0.8	EXTREMELY POOR

2.4.5. *Data analysis*

Non-metric multidimensional scaling (nMDS) was used for summarizing variation in invertebrate and fish communities among sites, using the Bray-Curtis similarity coefficient. Prior to analysis, abundance data were transformed [Log (x + 1)]. Ordination was interpreted in ecological terms for stress values <0.20, an indicator of reasonable two-dimensional data representation (Clarke and Warwick, 2001). Furthermore, ANOSIM tests were used to assess dissimilarities in communities between sites (Clarke and Gorley, 2006).

Fish condition data were assessed for normality and homocedasticy using Shapiro and Levene tests, respectively. When these conditions were met, ANOVA was used to compare data among sites while Kruskall-Wallis H test was used on non-normal or non-homocedastic data. The Tukey test was used for post-hoc comparisons between sites.

Data were analysed using PRIMER 7 and PERMANOVA + software (Clarke and Gorley, 2006) and R software (R Development Core Team, 2014).

2.5. Results

2.5.1. *Water quality*

The water quality in the Sabor and Baceiro rivers was generally good (**Table 2.5** and **Table 2**.6), with low water temperature (T < 20.0 ºC), and nutrient content (Total-N < 1 mg/L; Total- P < 0.05 mg/L) and high dissolved oxygen concentration (OD > 7.7 mg/L).

Table 2.5 Physical and chemical characteristics of the water in the Sabor river. Riparian condition is defined as: GI – Good integrity; SD – Signs of disease; DR –Dead riparian vegetation, mainly alder.

	GI		SD		DR	
Variable	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Temperature (ºC)	14.7	14.5	14.6	10.6	11.4	11.1
DO (mg/L)	8.5	9.7	9.1	9.8	9.7	9.7
DO (% sat.)	90.9	99.6	97.6	95.9	94.8	93.9
pH	7.3	7.3	7.2	7.2	7.1	7.1
Conductivity (µS.cm^{-1})	44.4	50.3	53.7	68.2	43.2	49.5
Total – N (mg/L)	<0.1	<0.1	<0.1	0.5	0.45	0.75
Total – P (mg/L)	<0.01	<0.01	<0.01	0.01	0.02	0.05

Table 2.6 Physical and chemical characteristics of the water in the Baceiro river. Riparian condition is defined as GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation, mainly alder.

	GI		SD		DR	
Variable	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Temperature (ºC)	**16.8**	**13.5**	**12.5**	**13.6**	**13.9**	**16.5**
DO (mg/L)	**8.3**	**8.3**	**7.7**	**8.6**	**8.9**	**8.9**
DO (% sat.)	**95.1**	**87.6**	**77,5**	**89.1**	**92.8**	**96.5**
pH	**6.9**	**6.9**	**7.0**	**7.1**	**7.0**	**7.2**
Conductivity (µS.cm^{-1})	**18.1**	**44.7**	**91.1**	**77.8**	**158.2**	**167.0**
Total – N (mg/L)	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.15**	**0.25**
Total – P (mg/L)	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**0.01**	**0.01**

2.5.2. *Hydromorphology*

The classification of HQA and HMS indices is presented in **Figure 2.12** andFigure **2.13**, respectively. The HQA scores ranged between Excellent and Reasonable, showing a slight decrease in sites with more disturbed riparian habitats (e.g. S4, near França village) and those with recent mortality of alder (S5, S6 and B5, B6). With respect to HMS, in the Sabor river, S6 categorized as modified while S1 and S4 were significantly modified. In the Baceiro river, no sites were categorized as significantly modified, but B1, B2 and B4 were considered modified.

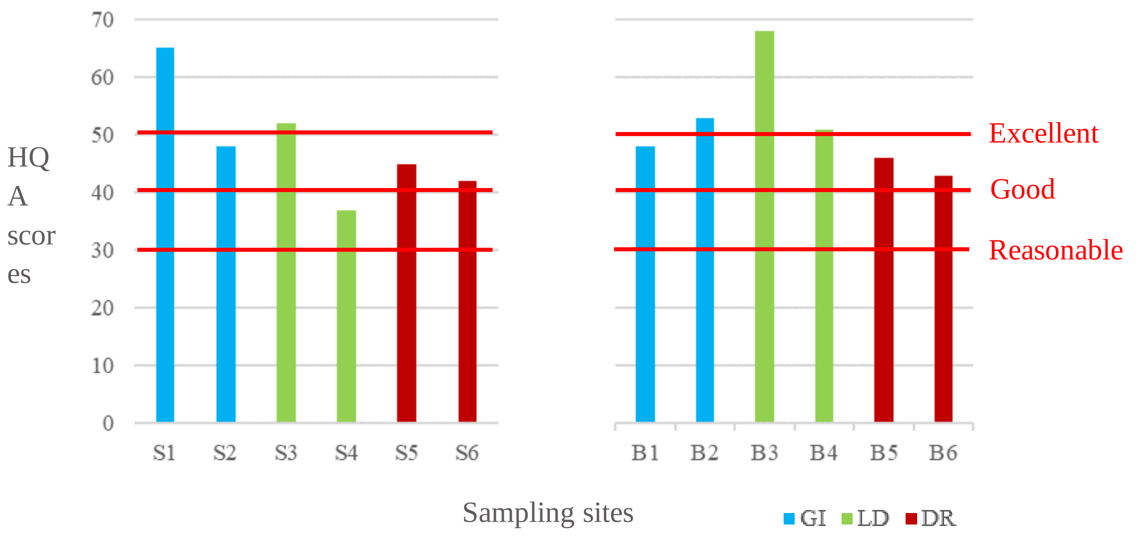

Figure 2.12 Scores of HQA index for the Sabor and Baceiro rivers.

Figure 2.13 Scores of HMS index for the Sabor and Baceiro rivers.

2.5.3. *Macroinvertebrate communities*

2.5.3.1. Faunal composition

A total of 15 201 invertebrates were identified for both the Sabor and Baceiro rivers, belonging to 57 taxa (Annexes, **Table 6.4**). The global composition of the communities in each river is presented in **Figure 2.14**.

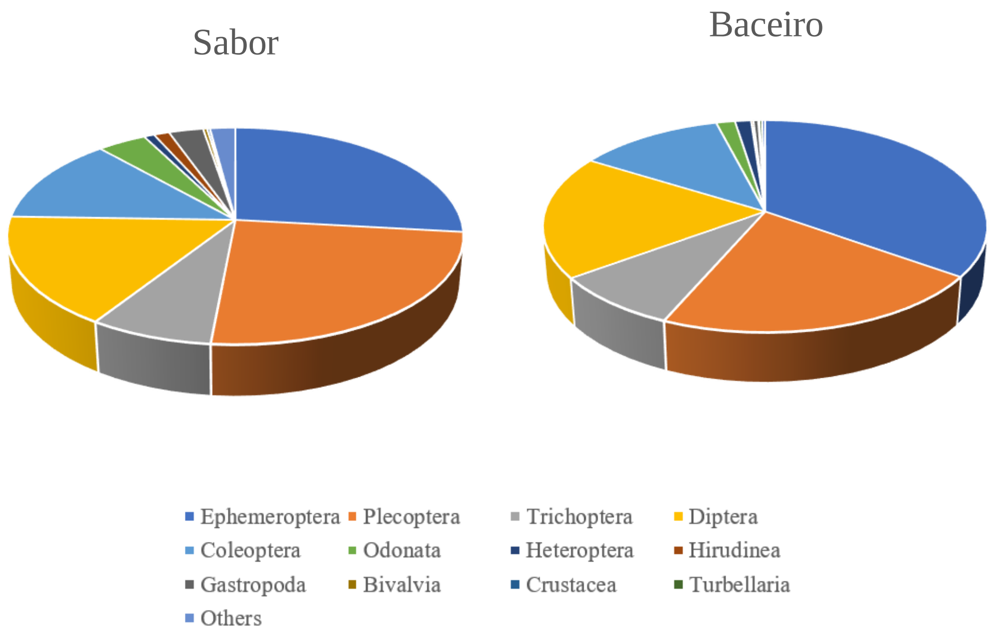

Figure 2.14 Overall composition of macroinvertebrate communities in the Sabor and Baceiro rivers.

Macroinvertebrate communities were dominated by EPTs, representing 59.1% and 65.0% of total numbers in the Sabor and Baceiro rivers, respectively. The relative abundance of macroinvertebrate groups in each sampling site in the Sabor and Baceiro rivers is shown in **Figure 2.15** andFigure **2.16**, respectively. In Sabor river, S4, S5 and S6 had more resistant organisms, such as leeches (Hirudinea) and gastropods (Mollusca).

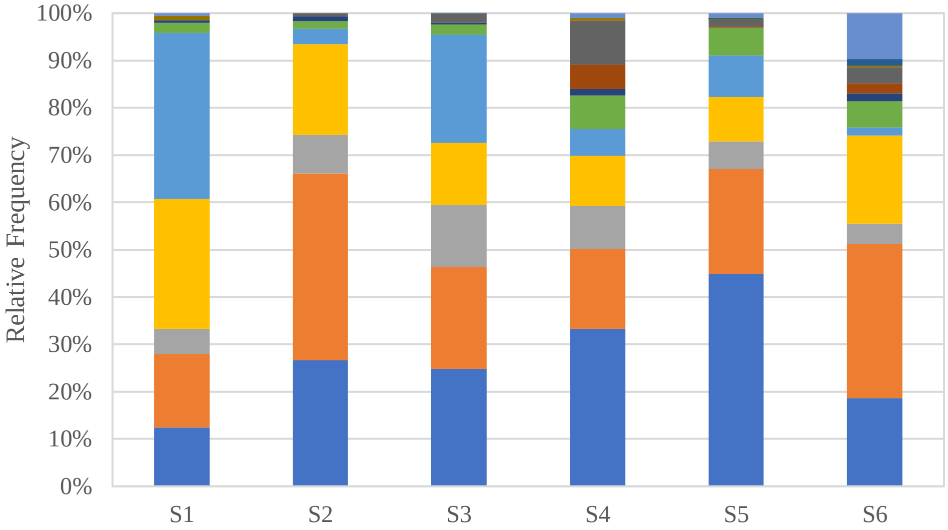

Figure 2.15 Composition of macroinvertebrate communities in the Sabor river.

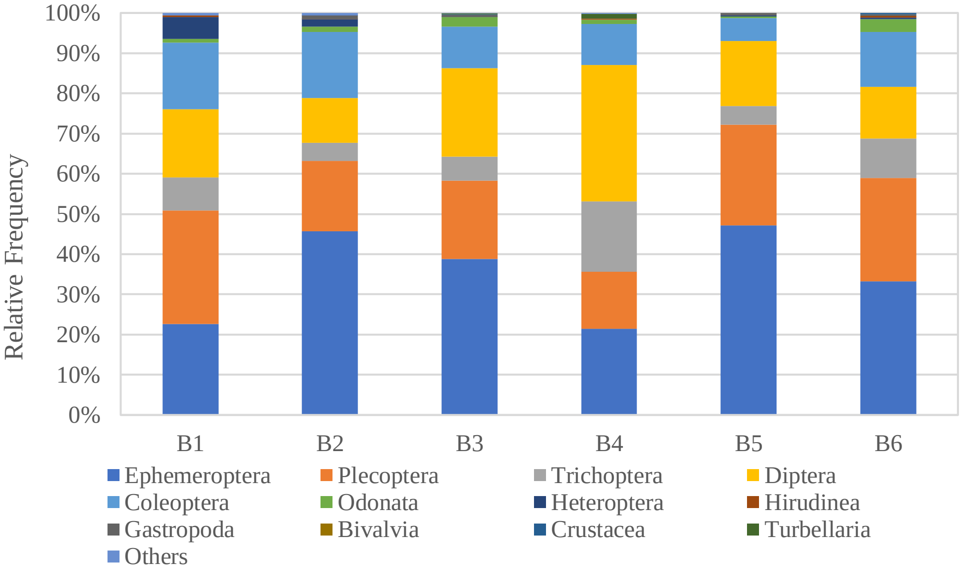

Figure 2.16 Composition of macroinvertebrate communities in the Baceiro river.

Community metrics are shown in **Table 2.7** and **Table 2.8.** For the Sabor river, SD macroinvertebrate communities had the highest total number of individuals and diversity (H’ Shannon-Wienner index). All sites had high EPT% with the lowest value being 33.3% (S1). For Baceiro river, B1 and B6 had the lowest total of individuals, maintaining however high EPT% as all other sites. Through the IBMWP all sites in both rivers were considered of excellent quality. However, using the IPtIN, in the Sabor river, the community in S1 was considered to have a good quality while communities in all other sites were considered excellent. The same pattern was observed for Baceiro river, where only the community in B1 was assessed as good quality.

Table 2.7 Macroinvertebrate community metrics and quality scores for the Sabor river. Riparian condition is defined as: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation, mainly alder.

	GI		SD		DR	
Metric	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Total Number of taxa	**23.00**	**24.00**	**34.00**	**35.00**	**25.00**	**25.00**
Total nº Individuals	**339.00**	**458.00**	**2618.00**	**1103.00**	**597.00**	**236.00**
H’ Shannon-Wienner	**2.4**	**2.3**	**2.53**	**2.72**	**2.43**	**2.36**
J’ Pielou Evenness	**0.77**	**0.72**	**0.72**	**0.76**	**0.75**	**0.73**
EPT Taxa	**7.00**	**10.00**	**15.00**	**10.00**	**11.00**	**8.00**
EPT - Nº Individuals	**113.00**	**340.00**	**1557.00**	**654.00**	**435.00**	**131.00**
EPT - % Individuals	**33.33**	**74.24**	**59.47**	**59.29**	**72.86**	**55.51**
IBMWP Score	**135.00**	**165.00**	**218.00**	**198.00**	**166.00**	**142.00**
IBMWP Quality Class	**Excellent**	**Excellent**	**Excellent**	**Excellent**	**Excellent**	**Excellent**
IPtIn Score	**0.73**	**0.87**	**1.05**	**0.89**	**0.91**	**0.88**
IPtIn Quality Class	**Good**	**Excellent**	**Excellent**	**Excellent**	**Excellent**	**Excellent**

Table 2.8 Macroinvertebrate metrics and quality scores for the Baceiro river. Riparian condition is defined as GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation, mainly alder.

	GI		SD		DR	
Metric	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Total Number of taxa	**24.00**	**30.00**	**30.00**	**36.00**	**28.00**	**20.00**
Total nº Individuals	**525.00**	**1872.00**	**1370.00**	**3777.00**	**1684.00**	**256.00**
H’ Shannon-Wienner	**2.24**	**2.35**	**2.56**	**2.26**	**2.33**	**2.28**
J’ Pielou Evenness	**0.7**	**0.69**	**0.75**	**0.63**	**0.7**	**0.76**
EPT Taxa	**10.00**	**14.00**	**12.00**	**15.00**	**11.00**	**9.00**
EPT - Nº Individuals	**310.00**	**1267.00**	**881.00**	**2007.00**	**1295.00**	**176.00**
EPT - % Individuals	**59.05**	**67.68**	**64.31**	**53.14**	**76.9**	**68.75**
IBMWP Score	**159.00**	**187.00**	**187.00**	**223.00**	**171.00**	**124.00**
IBMWP Quality Class	**Excellent**	**Excellent**	**Excellent**	**Excellent**	**Excellent**	**Excellent**
IPtIn Score	**0.77**	**1.00**	**0.98**	**1.02**	**1.08**	**0.94**
IPtIn Quality Class	**Good**	**Excellent**	**Excellent**	**Excellent**	**Excellent**	**Excellent**

2.5.3.2. Functional feeding groups

The variation of functional feeding groups is shown in **Figure 2.17**. There was a considerable abundance of grazers and collectors in all the sites, while the proportion of shredders was very low.

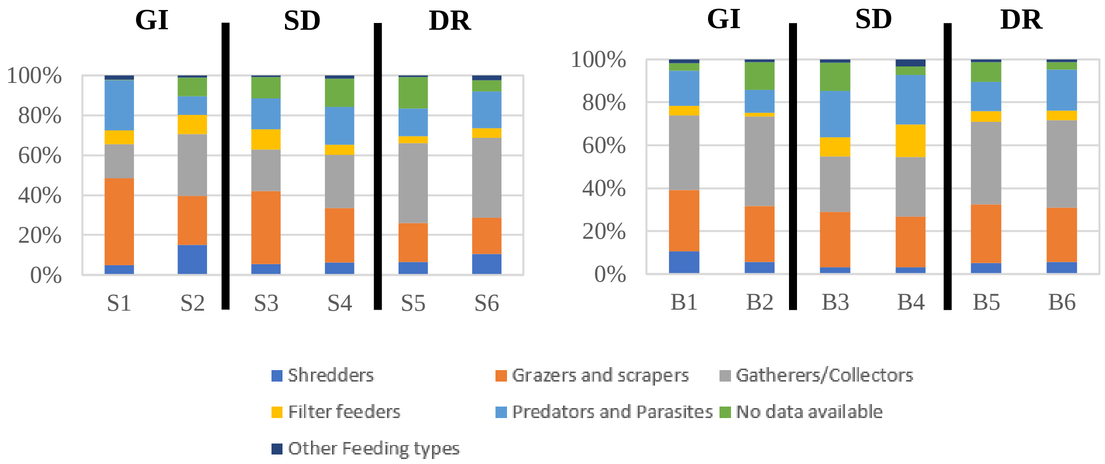

Figure 2.17 Functional feeding groups in the Sabor (left) and Baceiro (right) rivers. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

2.5.3.3. Community structure

The nMDS analysis (stress value of 0.09) showed a separation between GI in the Sabor (S1, S2) and Baceiro (B1) rivers, and DR (S5, S6 and B6) along with S4, from the remaining sites (**Figure 2.18**). Furthermore, the ordination showed a separation between macroinvertebrate families, mainly between more sensitive taxa including Trichoptera (Uenoidae, Rhyacophilidae, Goeridae, Calamoceratidae), and more resilient taxa including Heteroptera (Corixidae, Notonectidae), Hirudinea (Erpobdellidae), Diptera (Dolichopodidae) and Crustacea (Astacidae, Cambaridae) (**Figure 2.19**).

Based on the ANOSIM similarity (one-way) pairwise tests, no significant differences (P> 0.05) were detected among the macroinvertebrate communities of the 3 alder condition zones (i.e. GI, SD and DR).

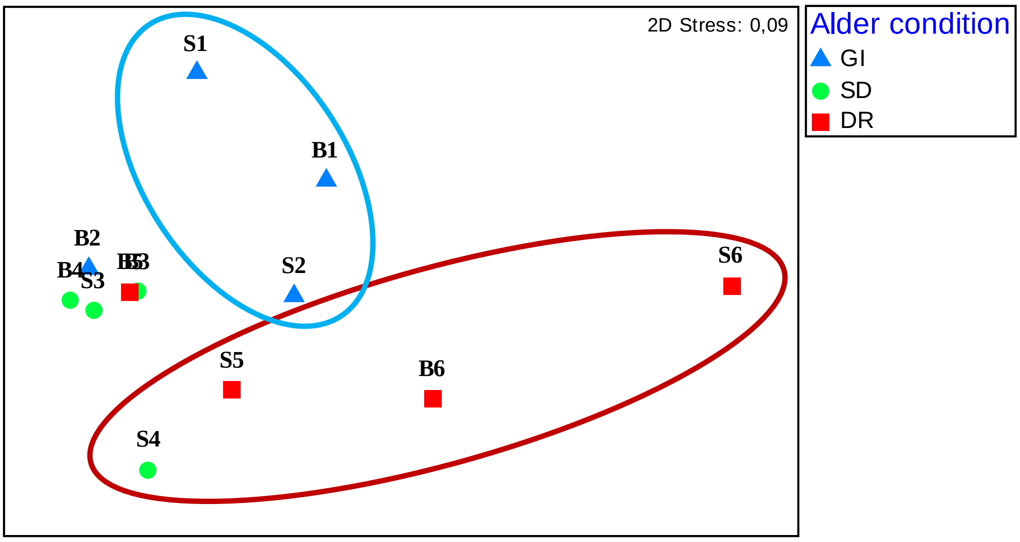

Figure 2.18 nMDS ordination of macroinvertebrate communities in the Sabor (S) and Baceiro (B) rivers by sites. Riparian condition is defined: good riparian integrity (GI); Signs of disease (SD), and Dead riparian trees (DR).

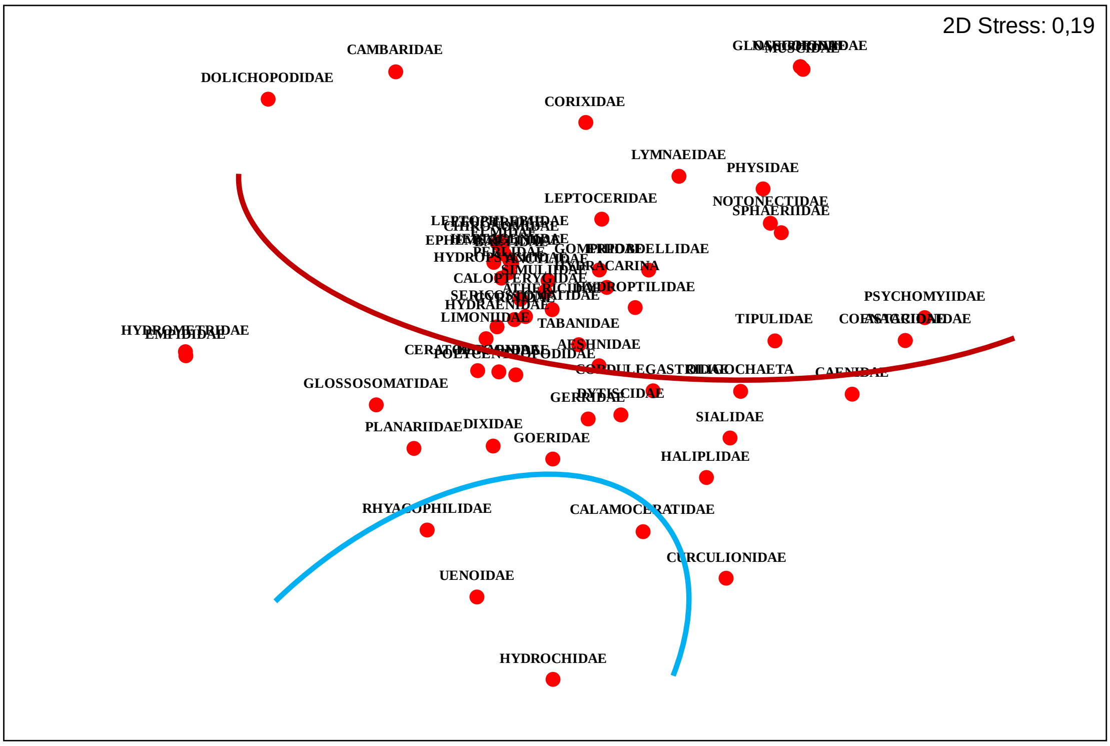

Figure 2.19 nMDS ordination of macroinvertebrate communities in the Sabor (S) and Baceiro (B) rivers by macroinvertebrate taxa.

2.5.4. *Fish communities*

2.5.4.1. Composition and abundance

A total of 1584 fishes and 5 species were captured in Sabor river (**Figure 2.20**). S1 and S2 were exclusively salmonid, with brown trout being the only species detected. The remaining sites were in the transition salmonid/cyprinid zone where brown trout coexists with native cyprinids, including *P. duriense*, *S. carolitertii*, *S. alburnoides* and *L. bocagei*.

In total, 508 fishes and 4 species were captured in the Baceiro river **(Figure 2.21).** Brown trout was the only species at all sites but in B5 and B6 where a few *P. duriense*, *S. carolitertii* and *S. alburnoides* were found.

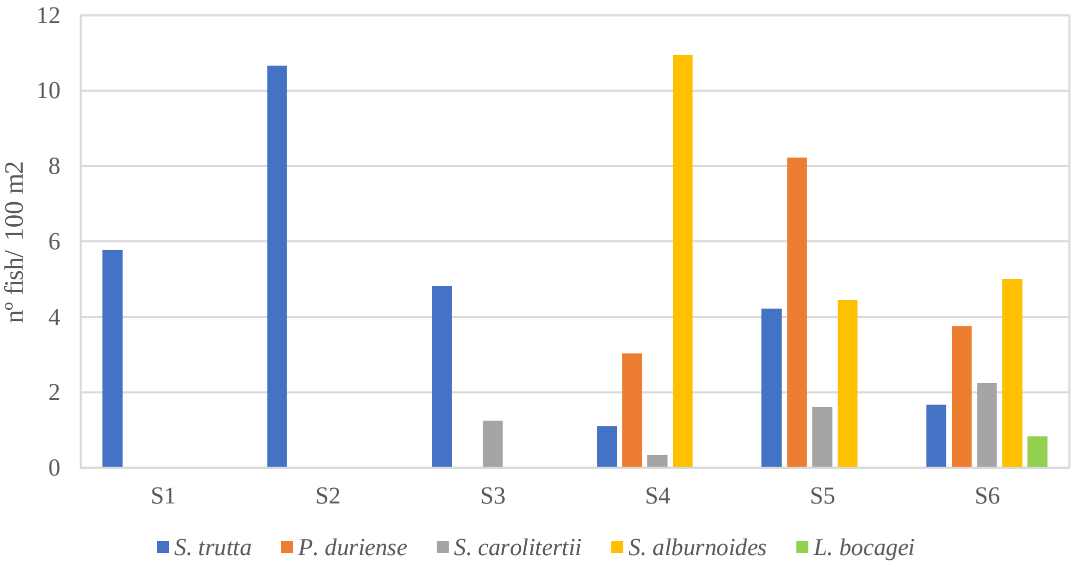

Figure 2.20 Density (nº fish/100 m2) in the Sabor river.

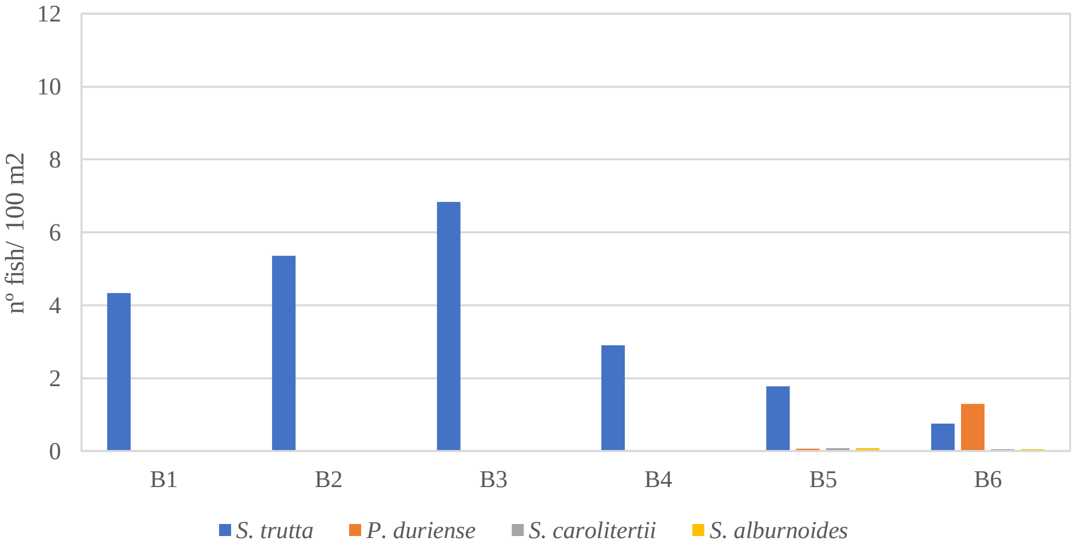

Figure 2.21 Density (nº fish/100 m2) of fish in the Baceiro river.

2.5.4.2. F-IBIP index

F-IBIP metrics, scores, and quality classification for each site in the Sabor and Baceiro rivers are shown in **Table 2.9** and **Table 2.10,** respectively. Scores revealed that fish communities in the Sabor river, were in excellent condition only in 3 sampling sites (S1, S2 and S3) while in the remaining sites (S4, S5, S6) the classification was good. In the Baceiro river, fish communities were in excellent condition at all sites except B6, where the classification was good.

Table 2.9 F-IBIP metrics and scores for fish communities in the Sabor river.

Sampling site	Alder condition	Exotic (%)	Intolerant (%)	Omnivorous (%)	Potamodromous (native) %	Potamodromous (%)	Score	Classification
S1	**GI**	**0**	**100**	**0**	**-**	**-**	**1.000**	**Excellent**
S2	**GI**	**0**	**100**	**0**	**-**	**-**	**1.000**	**Excellent**
S3	**SD**	**0**	**79.4**	**0**	**-**	**-**	**0.931**	**Excellent**
S4	**SD**	**0**	**100**	**-**	**80.3**	**26.9**	**0.781**	**Good**
S5	**DR**	**0**	**100**	**-**	**55.6**	**67.3**	**0.845**	**Good**
S6	**DR**	**0**	**93.9**	**-**	**66.1**	**46.3**	**0.792**	**Good**

Table 2.10 F-IBIP metrics and scores of fish communities in the Baceiro river.

Sampling site	Alder condition	Exotic (%)	Intolerant (%)	Omnivorous (%)	Potamodromous (native) %	Potamodromous (%)	Score	Classification
B1	**GI**	**0**	**100**	**0**	**-**	**-**	**1.000**	**Excellent**
B2	**GI**	**0**	**100**	**0**	**-**	**-**	**1.000**	**Excellent**
B3	**SD**	**0**	**100**	**0**	**-**	**-**	**1.000**	**Excellent**
B4	**SD**	**0**	**100**	**0**	**-**	**-**	**1.000**	**Excellent**
B5	**DR**	**0**	**92**	**0**	**-**	**-**	**0.937**	**Excellent**
B6	**DR**	**0**	**100**	**-**	**40**	**95.2**	**0.832**	**Good**

2.5.4.3. Community structure

The nMDS analysis of fish communities showed that GI (S1, S2, B1 and B2) and SD (S3, B3 and B4) were separated from DR (S5, S6, B5 and B6) and S4 (**Figure 2.22**). Furthermore, the ordination by fish species highlighted a discrimination between brown trout and other cyprinid species, and specially *L. bocagei* (**Figure 2.23)**.

Significant differences ($P < 0.05$) were obtained between the upper (i.e., GI – Good integrity) and lower (DR – Dead riparian trees) zones in each river, based on the ANOSIM similarity (one-way) pairwise tests.

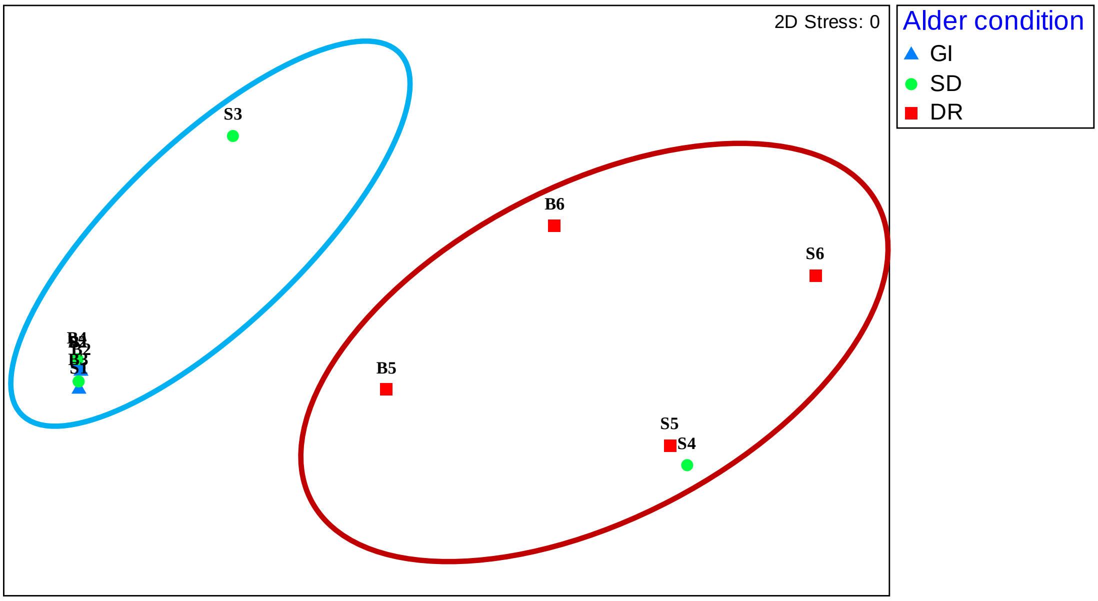

Figure 2.22 nMDS ordination of fish communities in Sabor (S) and Baceiro (B) rivers by sites. Riparian condition is defined , good riparian integrity (GI); Signs of disease (SD), and Dead riparian trees (DR).

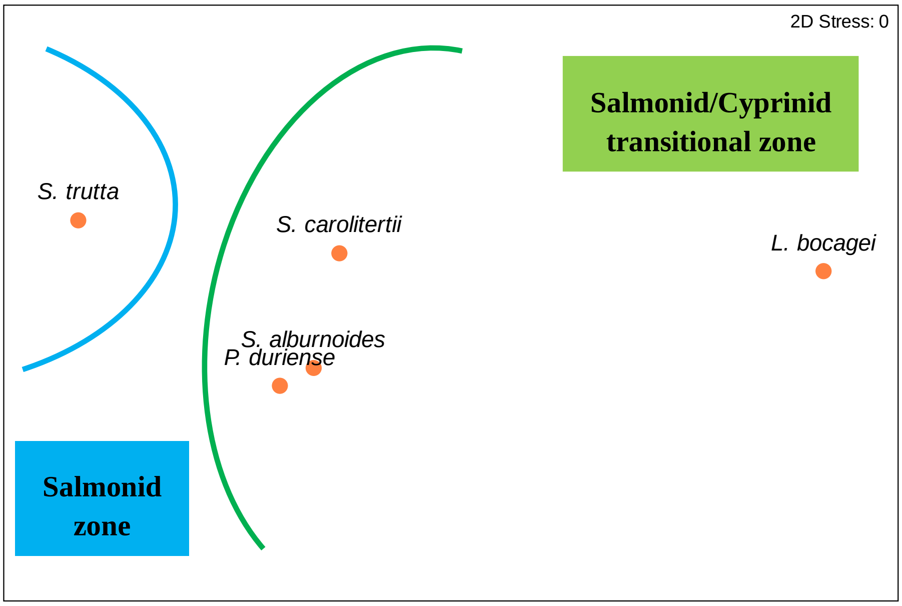

Figure 2.23 nMDS ordination of fish communities in Sabor (S) and Baceiro (B) rivers by fish species.

2.5.4.4. Brown trout populations

Population parameters for brown trout in the Sabor and Baceiro rivers are in **Table 2.11** and **Table 2.12,** respectively. Brown trout showed allometric growth in all sites in the Sabor and Baceiro rivers. In the Sabor river, only brown trout from S3 and S4 were in a good condition overall while brown trout from S1, S2, S5 and S6 were assessed as fair. In the Baceiro river, brown trout from all sites were in good condition overall.

Table 2.11 Biometric data and population parameters for brown trout in the Sabor river.

Site	N	Length (cm)	Body weight (g)	Growth coefficient					Condition	
				α	b	r2	t test	Growth Type	K	
S1	92	15.87±4.89	50.28±55.31	0.183	2.79±0.02	0.993	0.005	Allo (-)	1.18±0.14	Fair
S2	62	14.24±2.43	29.79±18.01	0.193	2.74±0.04	0.987	0.010	Allo (-)	1.18±0.08	Fair
S3	28	12.31±6.15	34.50±54.50	0.173	2.85±0.05	0.992	0.007	Allo (-)	1.26±0.17	Good
S4	27	18.27±7.71	94.12±105.7	0.201	2.74±0.06	0.986	0.017	Allo (-)	1.27±0.36	Good
S5	76	15.51±4.92	46.58±54.25	0.174	2.83±0.03	0.992	0.005	Allo (-)	1.15±0.13	Fair
S6	100	14.28±4.99	38.17±39.74	0.170	2.85±0.01	0.995	0.003	Allo (-)	1.18±0.12	Fair

Table 2.12 Biometric data and population parameters for brown trout in the Baceiro river.

Site	N	Length (cm)	Body weight (g)	Growth coefficient					Condition	
				α	b	r2	t test	Growth Type	K	
B1	30	13.93±5.24	38.98±36.41	0.168	2.89±0.03	0.996	0.004	Allo (-)	1.26±0.12	Good
B2	73	11.60±5.51	28.6±42.41	0.167	2.89±0.03	0.989	0.004	Allo (-)	1.29±0.21	Good
B3	81	14.48±4.86	39.99±35.37	0.190	2.77±0.02	0.992	0.006	Allo (-)	1.25±0.18	Good
B4	58	8.88±3.10	9.36±20.16	0.177	2.80±0.05	0.977	0.011	Allo (-)	1.26±0.17	Good
B5	91	13.30±6.50	42.23±58.49	0.183	2.82±0.01	0.997	0.003	Allo (-)	1.31±0.16	Good
B6	33	12.69±5.45	33.77±47.07	0.181	2.82±0.04	0.991	0.008	Allo (-)	1.30±0.17	Good

Figure 2.24 and **Figure 2.25** show the total number and condition factor of brown trout discriminated by size classes A, B and C in relation to riparian condition. In Sabor river, condition factor was significantly higher ($P < 0.01$) in GI (S1 and S2) than DR (S5 and S6). In Baceiro river, class A condition factor was significantly lower in GI than SD ($P < 0.01$) and DR ($P < 0.05$).

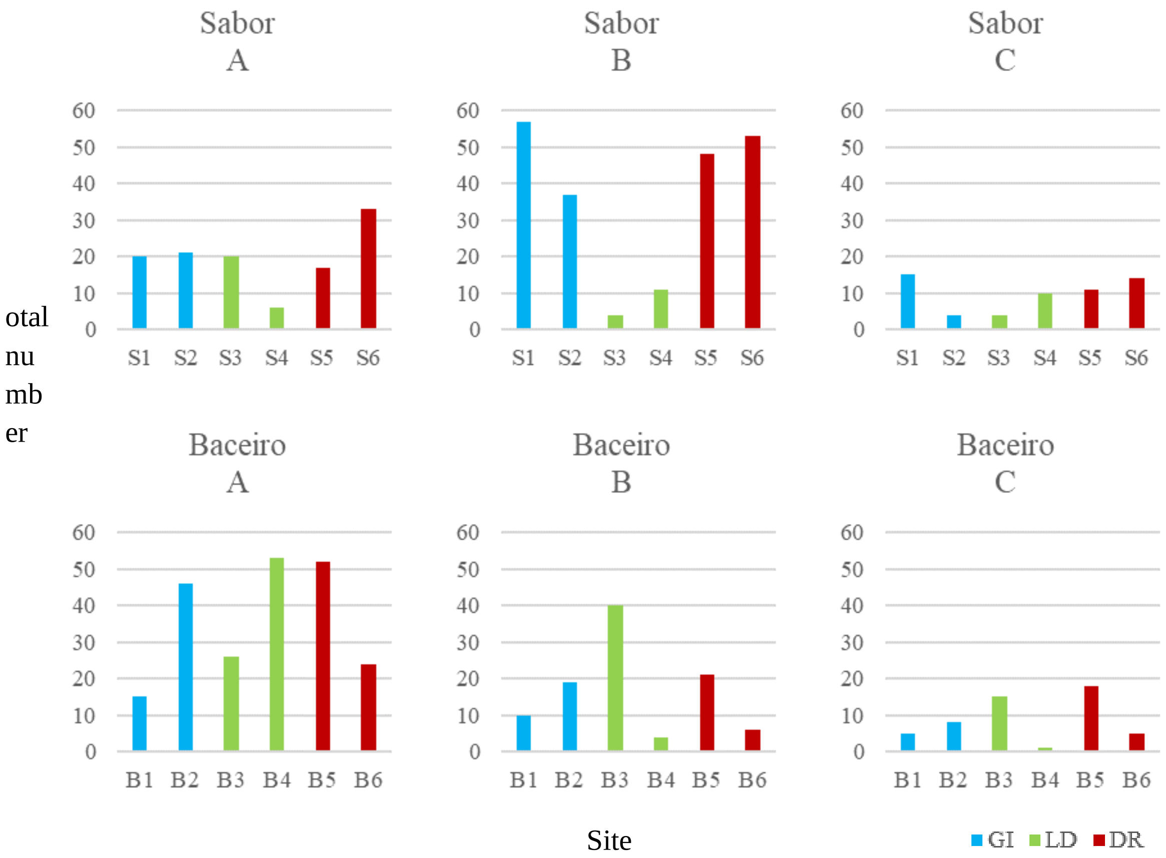

Figure 2.24 Total number of brown trout of each size class (A, B and C) in each riparian vegetation zone in Sabor and Baceiro rivers. GI corresponds to good integrity condition zones, SD to zones with signs of disease and DR to zones with presence of dead riparian vegetation, mainly alder.

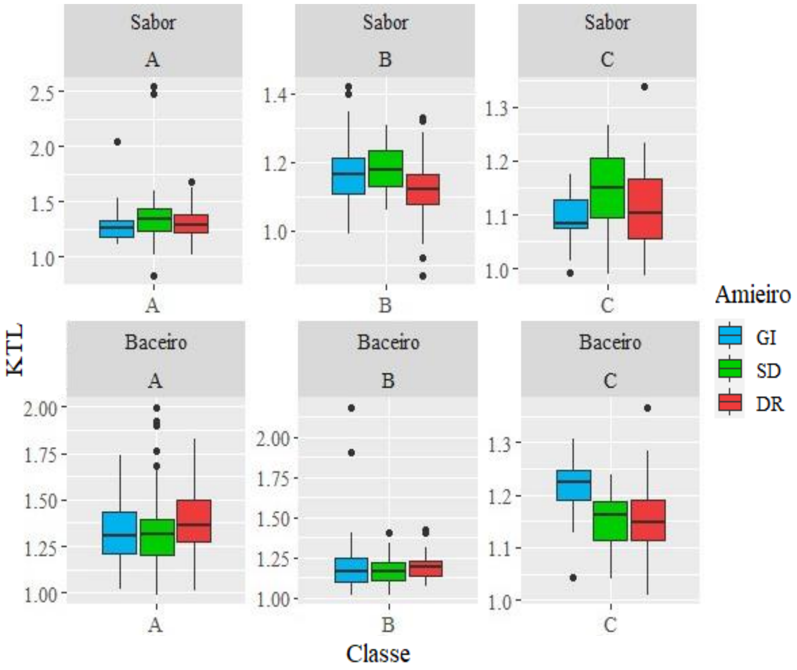

Figure 2.25 Condition Factor (K) of each brown trout size class (A, B and C) in each riparian vegetation zone in Sabor and Baceiro rivers. GI corresponds to good integrity condition zones, SD to zones with signs of disease and DR to zones with presence of dead riparian vegetation, mainly alder.

2.6. Discussion

Alder state gradient was consistent with changes in ecological conditions across the two headwater salmonid rivers, located in the protected area of the Montesinho Natural Park. However, these changes were small and their connection with alder decay was partially unclear.

Physical and chemical conditions were sufficiently stable to maintain good levels of water quality, even though slight variations were observed. These variations, mainly in terms of temperature, conductivity, and nutrients, did not occur as expected with alder decay and were most likely associated with other unmeasured factors.

Hydromorphological assessment revealed a decrease in quality with increase in alder decay.

Macroinvertebrates and fish communities showed similar responses but different sensitivity levels to changes in riparian condition. Macroinvertebrates were little sensitive while fish showed signs of diminished quality with longitudinal changes consistent with alder decay. More specifically brown trout condition was lower in highly disturbed sites.

Even though time constrains and synergy between factors limit conclusions, these results highlight the need for further studies, not only focused on the direct effects of infectious diseases on its hosts but also on the effects that it may have on related communities.

2.6.1. Physical/chemical conditions and hydromorphology

Overall, physical and chemical conditions reflected the maintenance of good quality water. However, some variations are worth considering. In Sabor river, temperature and nutrients were the main agents of change along the longitudinal river path. The higher temperature in upper sections of Sabor river was atypical and possibly explained by the presence of two upstream dams. Retention and exposure to solar radiation of water likely contributed for the increase of temperature, influencing downstream sections (Lessard and Hayes, 2003). Inversely, the accumulation of detritus due to the dam presence would’ve decreased nutrient levels on the adjacent downstream river sections (Maavara et al., 2020). Any organic detritus originated from these river sections would rapidly be transported downstream resulting in relatively higher concentrations of nitrogen and phosphorus.

The Habitat Modification Index was sensitive to the presence of rustic small weirs, constructed in the past to supply mills resulting in high scores in Baceiro River. However, since this river has no nearby dams, it should retain a more natural pattern when compared to Sabor river. However, both nitrogen and phosphorous showed a slight increase, not consistent with predictions. Furthermore, an increase in conductivity was also observed along the river path. Nutrient levels and conductivity can be affected by numerous factors including water levels and rainfall, pollution, geology, among others (Fondriest Environmental Inc., 2014). In this case, the most likely cause is agricultural runoff. Alongside Baceiro river and mainly in the Sabor river downstream zones, there is an increase of agriculture and livestock activity which may promote the increase in nutrient levels and downstream accumulation.

It is worth mentioning that the spike in conductivity in one of Sabor river sampling sites can be possibly explained by its closeness to the França village. Besides having the lowest HQA score of all 6 sampling sites, it also was the most significantly modified location of both rivers.

2.6.2. Biological communities

Regarding to macroinvertebrate community composition, the proportion of EPT (e.g. sensitive insects belonging to Ephemeroptera, Plecoptera and Trichoptera orders) was high for all sampling sites in both rivers. As such, and given its focus on sensitive taxa, using the IBMWP index, all sites were classified as excellent. However, the $IPtI_N$ index is a more rigorous approach, considering the combination of several metrics related with the community abundance, diversity, and evenness. Using this index, it was possible to determine that upper limits of our sampling range in both Sabor and Baceiro rivers had the lowest quality. For Sabor river, this condition may reflect physicochemical and hydromorphological characteristics. However, in Baceiro river, reasons for this decrease are unclear.

No significant differences were detected on functional feeding groups for the different riparian condition zones (e.g. GI, SD and DR). Given this study was conducted in the summer, shredders proportions may be naturally low due to the seasonal decrease of detritus input (Haapala and Muotka, 1998), which might explain the lack of differences between zones.

The nMDS results showed 3 groups of communities consistent with the 3 previously determined zones given alder condition. However, sites B2 and B5 resembled the sites with signs of disease while S4 resembled sites with dead alder (S5, S6 and B6). This probably contributed for the lack of significance in pairwise site comparisons obtained through ANOSIM tests. Nevertheless, B2 and B5 could be transition zones where the alder effects on macroinvertebrates are not yet well established and S4 is a highly disturbed site, as observed through the physical and chemical characteristics and hydromorphological analysis. This suggests that, even though it may yet not be significant, variation in macroinvertebrates communities may be related to alder decay.

For fish communities, the F-IBIP classified the upstream sampling sites in GI and SD zones as excellent and downstream zones as good quality. The high ecological integrity of these rivers obtained through this index is due to the exclusive presence of native fish, including brown trout and Iberian cyprinids (e.g. northern straight-mouth nase, northern Iberian chub, calandino, and, residually, Iberian barbel in the Sabor river). Nevertheless, this pattern reflects a longitudinal variation that is consistent with alder decay patterns. The nMDS analysis and ANOSIM tests confirmed this tendency while again including S4 with sites with dead alder.

These results must be carefully interpreted, because other factors could have synergically contributed for the detected differences, in particular the longitudinal distribution of species and some human pressures, such as agriculture, pollution, angling activities and brown trout restocking programmes. Furthermore, river fragmentation may limit fish migration when river flow is scarce during summer season.

Physical condition of brown trout, the dominant species in both studied rivers, significantly differed between disturbed riparian zones and good integrity zones. In the Sabor river, brown trout with fair condition were mainly found at the upper and lower limits of the sampling zone (S1 , S2, S5 and S6) while brown trout from the remaining sites were mainly in good condition. These may reflect the influences of the dams on the upper region and the decrease in alder condition on the lower region. In Baceiro river, overall changes in condition were residual. This is likely explained with the increase in the condition of small size individuals and the decrease in that of large size individuals, which are possibly linked with differences in diet between size classes analysed in the following chapter.

Nevertheless, very few brown trout in excellent condition were found in both rivers and all showing negative allometric growth rates.This may due to the fact that this region is a salmonid/cyprinid transition zone and the lower limit of brown trout distribution.

2.6.3. Conservation implications and future directions

Previous studies, developed in Montesinho Natural Park rivers in recent years, corroborate our results highlighting the good biological quality of rivers (Gomes, 2019; Halkhoums, 2017; Nogueira, 2019; Ronchesel, 2016; Santos, 2014). However, recent natural and anthropic pressures including riparian degradation mainly due to the alder disease are threatening the ecological integrity of mountains rivers of Montesinho Natural Park. No studies have focused on the impacts of this disease on the functioning of salmonid rivers of Portugal, despite this has been increasingly recognized as important.

We believe that, in this initial phase of the alder disease, the local decrease of organic matter input (leaves, seeds, branches) can be compensated by the downstream transport of materials from upstream zones. Furthermore, river connectivity is high in these mountain rivers allowing the circulation of materials, although two small dams (only in upstream zone of Sabor river) and several rustic weirs, most of them permeable, are present along the sampled area. Unfortunately, the upstream dispersion of the black alder disease will promote, in a very near future (3-4 years), a substantial decrease in riparian condition of ecosystems where alder is the dominant species. The initial phase of dieback and the reduction on the density of the canopy will increase opening extended areas contributing to potential modifications of mountain rivers. This condition can be, over time, mitigated by the natural (or human induced) dispersion of willow and ash trees, present in both rivers. However, *Salmo trutta* is highly vulnerable to changes in Iberian ecosystems since it is at the southwest limit of its natural biogeographical distribution. As such, further ecological studies and development of proper management measures are essential to sustain local populations.

2.7. References

Alba-Tercedor, J., 1996. Macroinvertebrados acuáticos y calidad de las aguas de los ríos. IV Simposio del agua en Andalucía (SIAGA). Almer. 2, 203–213.

Alba-Tercedor, J., Jaímez-Cuéllar, P., Aĺvarez, M., Avileś, J., Bonada, N., Casas, J., Mellado, A., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Robles, S., Elisa Saínz-Cantero, C., Sańchez-Ortega, A., Suaŕez, M.L., Toro, M., Vidal-Abarca, M.R., Vivas, S., Zamora-Munõz, C., 2002. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP’). Limnetica 21, 175–185.

APA, 2014. Relatórios de Caracterização das Regiões Hidrográficas (Art.o 5o da DQA). Agência Port. do Ambient., Lisboa.

Baxter, A., 1998. Condition Factor K for Salmonid fish. Fish. notes.

Béchet, Q., Shilton, A., Guieysse, B., 2014. Full-scale validation of a model of algal productivity. Environ. Sci. Technol. 48, 13826–13833. https://doi.org/10.1021/es503204e

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., Van De Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe’s surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Indic. 18, 31–41. https://doi.org/10.1016/j.ecolind.2011.10.009

Bjelke, U., Boberg, J., Oliva, J., Tattersdill, K., Mckie, B.G., 2016. Dieback of riparian alder caused by the Phytophthora alni complex: Projected consequences for stream ecosystems. Freshw. Biol. 61, 565–579. https://doi.org/10.1111/fwb.12729

Breine, J.J., Maes, J., Quataert, P., Van den Bergh, E., Simoens, I., Van Thuyne, G., Belpaire, C., 2007. A fish-based assessment tool for the ecological quality of the brackish Schelde estuary in Flanders (Belgium). Hydrobiologia 575, 141-159.

Castro, J., de Figueiredo, T., Fonseca, F., Castro, J.P., Nobre, S., Pires, L.C., 2010. Montesinho Natural Park: General Description and Natural Values, in: Evelpidou, N., Figueiredo, T., Mauro, F., Tecim, V., Vassilopoulos, A. (Eds.), Natural Heritage from East to West: Case Studies from 6 EU Countries. Springer, New York, pp. 119–132. https://doi.org/10.1007/978-3-642-01577-9_15

Céréghino, R., Biggs, J., Oertli, B., Declerck, S., 2008. The ecology of European ponds: Defining the characteristics of a neglected freshwater habitat. Hydrobiologia 597, 1–6. https://doi.org/10.1007/s10750-007-9225-8

Clarke, K., Gorley, R., 2006. “PRIMER v6: User Manual/Tutorial.” Primer-E Ltd Plymouth.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation., 2nd ed. Primer-E Ltd, Plymouth.

Coimbra, C.N., Graça, M.A.S., Cortes, R.M., 1996. The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. Environ. Pollut. 94, 301–307. https://doi.org/10.1016/S0269-7491(96)00091-7

Collares-Pereira, M.J. (coord.), Alves, M.J., Ribeiro, F., Domingos, I., Almeida, P.R., da Costa, P., Gante, H., Filipe, A.F., Aboim, M.A., Rodrigues, P.M., Magalhães, M.F., 2021. Guia dos Peixes de Água Doce e Migradores de Portugal Continental. Edições Afrontamento, Porto.

Delgado, C., Pardo, I., García, L., 2010. A multimetric diatom index to assess the ecological status of coastal Galician rivers (NW Spain). Hydrobiologia 644, 371-384.

Eichhorn, J., Roskams, P., Potocic, N., Timmermann, V., Ferretti, M., Mues, V., Szepesi, A., Durrant, D., Seletkovic, I., Schroeck, H.-W., Nevalainen, S., Bussotti, F., Garcia, P., Wulff, S., 2010. Visual assessment of Crown condition and damaging agents, in: Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests. UNECE ICO Forests Programme Co-ordinating Centre, Hamburg.

Environment Agency, 2003. River Habitat Survey in Britain and Ireland: Field Survey Guidance Manual: 2003 Version. Forest Research, Bristol.

European Comission, 2000. Directive 2000/60/EC of the European Parlament and of the Council of 23 October 2000, establishing a framework for Community action in the field of water policy. Official Journal of the European Parliament.

Feio, M.J., Ferreira, V., 2019. Rios de Portugal: comunidades, processos e alterações. Coimbra University Press, Coimbra. https://doi.org/https://doi.org/10.14195/978-989-26-1624-7

Fondriest Environmental Inc., 2014. “Conductivity, Salinity and Total Dissolved Solids.” Fundamentals of Environmental Measurements [WWW Document]. URL https://www.fondriest.com/environmental-measurements/parameters/water-quality/conductivity-salinity-tds/ (accessed 1.10.21).

Garner, G., Malcolm, I.A., Sadler, J.P., Hannah, D.M., 2017. The role of riparian vegetation density, channel orientation and water velocity in determining river temperature dynamics. J. Hydrol. 553, 471–485. https://doi.org/10.1016/j.jhydrol.2017.03.024

Gomes, G., 2019. Avaliação da integridade ecológica de rios da bacia hidrográfica do Rio Douro. Escola Superior Agrária do Instituto Politécnico de Bragança, Bragança.

Grey, J., 2001. Ontogeny and dietary specialization in brown trout (Salmo trutta L.) from Loch Ness, Scotland, examined using stable isotopes of carbon and nitrogen. Ecol. Freshw. Fish 10, 168–176. https://doi.org/10.1034/j.1600-0633.2001.100306.x

Haapala, A., Muotka, T., 1998. Seasonal dynamics of detritus and associated macroinvertebrates in a channelized boreal stream. Arch. für Hydrobiol. 142, 171-189.

Halkhoums, W., 2017. Management of brown trout populations (Salmo trutta L.) in northeastern Portugal (Douro basin): Analysis of habitat use and feeding strategies. Escola Superior Agrária do Instituto Politécnico de Bragança, Bragança.

Hering, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., Feld, C., Johnson, R., Pinto, P., Skoulikidis, N., Verdonschot, P., Zahrádková, S., 2003. The development of a system to assess the ecological quality of streams based on macroinvertebrates - Design of the sampling programme within the AQEM project. Int. Rev. Hydrobiol. 88, 345–361. https://doi.org/10.1002/iroh.200390030

Hering, D., Johnson, R.K., Kramm, S., Schmutz, S., Szoszkiewicz, K., Verdonschot, P.F.M., 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: A comparative metric-based analysis of organism response to stress. Freshw. Biol. 51, 1757–1785. https://doi.org/10.1111/j.1365-2427.2006.01610.x

Hermoso, V., Clavero, M., Blanco-Garrido, F., Prenda, J., 2010. Assessing the ecological status in species-poor systems: a fish-based index for Mediterranean Rivers (Guadiana River, SW Spain). Ecol. Indic. 1152–1161.

INAG I.P., 2009. Critérios para a Classificação do Estado das Massas de Água Superficiais – Rios e Albufeiras. Ministério do Ambient. do Ordenam. do Territ. e do Desenvolv. Reg. Inst. da Água, Lisboa.

INAG I.P., 2008a. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segunda a Directiva Quadro da Água Protocolo de amostragem e análise para os macroinvertebrados bentónicos. Ministério do Ambient. Ordenam. do Territ. e Desenvolv. Reg. Inst. da água, Lisboa.

INAG I.P., 2008b. Tipologia de rios em Portugal Continental. Directiva Quadro da Água I - Caracterização abiótica. Instituto da Água, Lisboa.

INAG I.P., 2008c. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para a fauna piscícola. Ministério do Ambient. do Ordenam. do Territ. e do Desenvolv. Reg. Inst. da Água, Lisboa.

Irons, J.G., Oswood, M.W., Bryant, J.P., 1988. Consumption of leaf detritus by a stream shredder: Influence of tree species and nutrient status. Hydrobiologia 160, 53–61. https://doi.org/10.1007/BF00014278

Lessard, J.L., Hayes, D.B., 2003. Effects of elevated water temperature on fish and macroinvertebrate communities below small dams. River Res. Appl. 19, 721–732. https://doi.org/10.1002/rra.713

Maavara, T., Chen, Q., Van Meter, K., Brown, L.E., Zhang, J., Ni, J., Zarfl, C., 2020. River dam impacts on biogeochemical cycling. Nat. Rev. Earth Environ. 1, 103–116. https://doi.org/10.1038/s43017-019-0019-0

Merritt, R.W., Cummins, K.W., 2007. Trophic Relationships of Macroinvertebrates, in: Hauer, F.R., Lamberti, G.A. (Eds.), Methods in Stream Ecology: Second Edition. Academic Press, pp. 413–433. https://doi.org/10.1016/B978-0-12-416558-8.00020-2

Morais, M., Pinto, P., Guilherme, P., Rosado, J., Antunes, I., 2004. Assessment of tempo-rary streams: the robustness of metric and multimetric indices under different hydrological conditions. Hydrobiologia 516, 229–249.

Naura, M., 2016. River Habitat Survey (RHS) Toolbox software. http://www.riverhabitatsurvey.org/author/mnaura/

Nogueira, J.G., 2019. Biodiversidade em água doce: comparação entre áreas com e sem estatuto de proteção. Escola de Ciências. Universidade do Minho, Braga.

Pardo, I., Gómez-Rodríguez, C., Abraín, R., García-Roselló, E., Reynoldson, T.B., 2014. An invertebrate predictive model (NORTI) for streams and rivers: Sensitivity of the model in detecting stress gradients. Ecol. Indic. 45, 51–62. https://doi.org/10.1016/j.ecolind.2014.03.019

R Development Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria. http://www.r-project.org

Raven, P.J., Fox, P.J.A., Everard, M., Holmes, N.T.H., Dawson, F.H., 1997. River Habitat Survey: a new system for classifying rivers according to their habitat quality, in: Boon, PJ and Howell, D.L. (Ed.), Freshwater Quality: Defining the Indefinable? The Stationery Office, Edinburgh, pp. 215–234.

Raven, P.J., Holmes, N.T.H., Dawson, F.H., Fox, P.J.A., Everard, M., I.R., F., Rouen, K.J., 1998. River Habitat Survey, the physical character of rivers and streams in the UK and Isle of Man. River Habitat Survey Repport. 2, 84.

Ricker, W.E., 1958. Handbook of Computations for Biological Statistics of Fish Populations. Fish. Res. Board Canada 119, 1-300. https://doi.org/10.2307/3796760

Ronchesel, M., 2016. Efeitos de pequenas obras de regularização nas comunidades de peixes e invertebrados do NE de Portugal. Escola Superior Agrária, Instituto Politécnico de Bragança, Bragança.

Santos, M.H., 2014. Bioecologia e conservação das populações de Salmo trutta (L.) na bacia hidrográfica do rio Tua (NE Portugal). Instituto Politécnico de Bragança, Bragança.

Schaumburg, J., Schranz, C., Hofmann, G., Stelzer, D., Schneider, S., Schmedtje, U., 2004. Macrophytes and phytobenthos as indicators of ecological status in German lakes – a contribution to the implementation of the Water Framework Directive. Limnologica 34, 302–314.

Schmeller, D.S., Loyau, A., Bao, K., Brack, W., Chatzinotas, A., De Vleeschouwer, F., Friesen, J., Gandois, L., Hansson, S. V., Haver, M., Le Roux, G., Shen, J., Teisserenc, R., Vredenburg, V.T., 2018. People, pollution and pathogens – Global change impacts in mountain freshwater ecosystems. Sci. Total Environ. 622–623, 756–763. https://doi.org/10.1016/j.scitotenv.2017.12.006

Tachet, H., Bournaud, M., Richoux, P., 1981. Introduction à l'étude des macroinvertebrés d'eaux douces. Université Lyon I - Association Française de Limnologie., Lyon.

Tachet, H., Richoux, P., Bournard, M., Usseglio-Polatera, P., 2010a. Invertébrés d'eaux douces. Systématique, biologie, écologie. CNRS Éditions, Paris.

Teixeira, A., Cortes, R.M.V., 2006. Diet of stocked and wild trout, Salmo trutta: Is there competition for resources? Folia Zool. 55, 61–73.

Wang, D., Wen, Y., 2018. Analysis on influential factors of water quality in key sections of Yangtze River basin in China. IOP Conf. Ser. Mater. Sci. Eng. 394. https://doi.org/10.1088/1757-899X/394/5/052049

3. Is the feeding ecology and microhabitat use of brown trout (*Salmo trutta*, L.) affected by the alder (*Alnus lusitanica)* tree disease in the Montesinho Natural Park (Northern Portugal)?

3.1. Abstract

Populations of brown trout in Portugal have a high ecological, genetic, and socioeconomic value, but currently face numerous threats, some of which are yet to be fully assessed. This study aimed to evaluate the consequences of the alder disease on the diet and habitat use of brown trout in two mountain streams, Sabor and Baceiro rivers (River Douro basin, North-eastern Portugal), covering 1) excellent riparian condition (health trees) 2) signs of disease (dieback and lower canopy) and 3) extensive dead alder areas. Brown trout's diet was assessed in 6 sampling sites in each river through stomach contents analysis. Microhabitat use and availability were determined through snorkeling observations and transects randomly distributed within each sampling site, respectively. Individuals were categorized as small, medium, large size and evaluated independently. Diet of brown trout changed between zones, showing variations mainly in the use of terrestrial origin prey, and of aquatic insects belonging to Ephemeroptera, Plecoptera and Trichoptera orders. Terrestrial origin prey, which is typically strongly influenced by riparian condition, decreased with alder decay, while consumption of other prey types increased. However, there was no significant increase in diet overlap between size classes. Furthermore, availability and use of microhabitat were not dependent but both changed with alder decay. Specifically, changes in the use of cover were noticeable likely reflecting indirect effects of the riparian condition. Although further studies are needed, current results suggest that the alder tree disease may have significant impacts on brown trout diet and microhabitat use, affecting the conservation of this species in the Montesinho Natural Park.

***Keywords**: Salmo trutta, diet, habitat, riparian, threats, conservation*

3.2. Introduction

Iberian populations of brown trout (*Salmo trutta*) in the southern edge of the species native distribution range are naturally small in abundance. However, these populations present unique characteristics, exhibiting high genetic diversity, high degree of isolation between and within basins, and an extensive spawning period (Larios-López et al., 2015). This makes them highly vulnerable to local extinction due not only to future climate change scenarios but also to many other threats, such as habitat loss, alteration and fragmentation, introduction of exotic species, genetic introgression due to restocking programs, overfishing, and diseases (Almodóvar et al., 2011; De Silva, 2012; Macedo-Veiga and De Sostoa, 2011). Currently, the spread of the alder disease is considered to be one of these threats, leading to environmental constrains that can possibly impact brown trout populations (Bjelke et al., 2016).

In Portugal, brown trout is restricted to the northern and central mountainous rivers (Collares-Pereira et al., 2021). Mountainous rivers present typical environmental conditions, namely a limited range of water temperatures, high saturation of dissolved oxygen, low nutrient content, high habitat diversity and dense riparian galleries (Teixeira and Cortes, 2007). Brown trout has a complex life cycle, exhibiting anadromous and resident forms, both with high genetic, ecological, and socioeconomic value (Collares-Pereira et al., 2021). In anadromous forms, adult individuals move into fresh waters to spawn. Younglings spend their first years here and occasionally remain for further years or even their entire lives, establishing resident populations with a wide range of ages and sizes (Nelson et al., 2016).

The feeding ecology of brow trout has been addressed in many studies, most of which report an opportunistic and generalist behavior and high trophic diversity, with little productive and small sized prey being typically consumed in larger quantities (Sánchez-Hernández and Cobo, 2015, 2013). In fresh waters, aquatic macroinvertebrates, mainly insects, but also, mollusks, crustaceans and annelids, are preferred prey for brown trout (Halkhoums, 2017).

Even though trophic diversity is relatively similar among size/age classes, brown trout often exhibits ontogenetic and size-specific diets and feeding strategies. YOY (young of the year) and juveniles typically consume a higher proportion of benthic macroinvertebrates. For example, small sized insects and early instars of the orders Diptera, Ephemerotera, Trichoptera and Plecoptera comprised between 50 to 80% of the prey total for small brown trout in the Baceiro river (Portugal); by contrast, larger brown trout, typically aged 3 or more years, usually fed on larger prey, such as pupae, adult insects, or even small fish (Halkhoums, 2017; Santos, 2014; Teixeira and Cortes, 2006). When prey are abundant, the activity pattern, feeding behavior, and selection of feeding habitat by brown trout are density independent. As such, even though there may be a high diet overlap, temporal and spatial segregation between age/size classes can avoid competition (Sánchez-Hernández and Cobo, 2012).

Abundance and composition of macroinvertebrates communities in mountainous rivers are highly influenced by riparian vegetation (Teixeira and Cortes, 2007). Consequently, the current decline of alder raises some concerns about the feeding ecology of brown trout. Specifically, larger prey items consumed by larger brown trout have a strong connection with riparian vegetation (Wipfli and Musslewhite, 2004), mainly including terrestrial and aerial insects that fall from the crowns of riparian trees. Given the dominance of alder trees in mountainous rivers of Portugal, decline of larger prey could be an indirect result of alder disease (Bjelke et al., 2016; Grey, 2001). Furthermore, in these circumstances larger brown trout can be expected to explore other prey leading to diet shifts and possibly increasing overlap between age/size classes.

Flow velocity is an important feature in brown trout habitat selection since it relates to foraging and prey capture rates and energy costs (Mouton et al., 2011). Typically, brown trout prefers shallow and fast waters, with brown trout density being also associated with cover and refuge (Gosselin et al., 2012). In sites with woody debris and vegetation, brown trout appears to aggregate around such structures, while in sites with less cover is usually more disperse (Enefalk et al., 2019). Substrate preferences are less clear. Some studies suggest a preference for coarse substrate (Teixeira et al., 2006) possibly related with cover and refuge features, while others suggest that lower caliber substrate are favored (Gosselin et al., 2012). Gravel beds are typically used for spawning (Collares-Pereira et al., 2021).

Microhabitat use is size dependent, and possibly related to diet ontogenetic variation, resulting in spatial segregation among individuals (Jonsson, 1989). Since their prey are typically benthic, young brown trout tend to be present in riffle zones characterized by shallow and running waters while older and larger brown trout explore pools, with deeper waters and high percentage of boulders and bedrock, and display a more pelagic behavior (Grey, 2001; Jonsson, 1989; Santos, 2014).

The patterns of habitat use are dependent on microhabitat diversity, which is commonly high in mountainous rivers in northern Portugal (Feio and Ferreira, 2019). However, alder decline may limit habitat availability and diversity (Bjelke et al., 2016). Root decay and changes in river dynamics decrease riverbank stability and refuge availability which may force younger brown trout to be more exposed or to select other types of refuge.

Overall, alder decline can lead to changes in diet and microhabitat use and intraspecific overlap increasing competition and predation risks for brown trout in low productive headwater rivers. However, no studies have provided evidence of such interactions so far. This study aims to fill this knowledge gap which is important to better understand the consequences of degradation of riparian ecotones on freshwater ecosystems. Brown trout populations of two mountain rivers in the Montesinho Natural Park in northern Portugal, the Sabor and Baceiro rivers, were assessed for diet and habitat use in three distinct riparian condition zones, mainly determined by the alder disease. In detail, the specific objectives were to:

1) **Evaluate changes in diet.**

   H_A: Diet composition of each size classes changes with riparian condition.

   H_B: Diet overlap between size classes increases with riparian condition decrease.

2) **Evaluate changes in microhabitat use.**

   H_C: Microhabitat availability changes with riparian condition.

   H_D: Microhabitat use by each size class is dependent on microhabitat availability.

3.3. Study area

The Sabor and Baceiro rivers are two tributaries of the Douro river that drain the eastern part of the Montesinho Natural Park. Although, these mountain rivers have high physical and chemical, hydromorphological and biological quality, and little anthropogenic modification, the recent mortality of alder (*Alnus lusitanica)*, one the most common riparian tree in the region, lead to a small decrease in ecological quality (see chapter 2). Macroinvertebrates are still abundant and diverse, mainly including Ephemeroptera, Plecoptera, Diptera, Coleoptera and Trichoptera. Available microhabitats include fast flowing areas with coarse substrate and typically shallow and low water current areas with small calibre substrate and deeper.

Brown trout populations in the Sabor and Baceiro rivers are resident and complete their life cycle in these rivers, partially sharing habitat with native cyprinids. Brown trout has a significant regional socioeconomic value specially in recreational fisheries (Miranda, 2012). Given their importance, brown trout populations have been targeted by restocking programs in the last few years, which however, have proven to be inefficient (Teixeira et al., 2013, 2006).

3.4. Methodology

For each studied river, three riparian condition zones were identified based on Eichhorn et al. (2010): 1) good integrity condition, with no defoliation (< 10%) (GI), 2) signs of disease, with slight to moderate defoliation (10-50%) (SD) and 3) severe defoliation (> 50%) and dead vegetation, mainly alder (DR). From each defined zone, two sampling sites were selected making a total of 6 per river. Further details on each site are presented in chapter 2, section 4.

3.4.1. Diet

3.4.1.1. Fish sampling and stomach content collection

Fish sampling followed the protocol for the application of the WFD in Portugal (INAG I.P., 2008c). Fish were sampled by single pass electrofishing, in at least 100-m stretches (Hans Grassl ELT IIGI, 300–600 V, 2–3 A, DC), and collected with hand-nets. Brown trout were measured for total length (in mm). For individuals over 5 cm in total length, stomach contents were collected using a non-destructive stomach flushing method (Hyslop, 1980) and immediately frozen, *in situ*, for further laboratory analysis. After recovery from collection and handling, fish were released into the same place in the river where they had been captured.

3.4.1.2. Stomach content analysis

In the laboratory, stomach contents were analysed under a stereoscope microscope (Olympus SMZ10 with 10-132x zoom magnification). Prey items were counted and identified to family level, except for Oligochaeta and Acari subclasses, using dichotomous keys (Tachet et al., 2010, 1981).

3.4.1.3. Diet analysis

Individuals were sorted in 3 size-classes (total length in cm): A) ≤13.0; B)]13.0 – 20.0[; C) ≥ 20.0 cm, to account for ontogenetic variation in diet (Grey, 2001). Fish with less than 3 prey items in their stomachs were excluded from analysis. Only prey categories corresponding to at least 1% of total prey for at least one size class and one sampling site were individually considered. The remaining prey items were included in the "Other" category.

Diet composition for each size class was calculated in terms of percent abundance (A_i), and frequency of occurrence (F_i), following Amundsen (1996):

$$A_i = (\Sigma P_i/\Sigma P_t) \times 100, \tag{3.1}$$

$$F_i = (N_i/N) \times 100, \tag{3.2}$$

where P_i is the contribution of prey i to total prey, P_t is the total prey of all the fish, N_i is the number of fish with prey i in their stomach and N is the total numbers of fish. Pearson correlation coefficient was used to assess redundancy between A_i and F_i

Non-metric multidimensional scaling (nMDS) with Bray-Curtis similarity coefficient was used to assess variation in diet composition and niche similarity among riparian zones (GI, SD, DR). Ordination was interpreted for stress values <0.2 (Clarke and Warwick, 2001). Prior to analysis, data were square root transformed [$\sqrt{(x + 0.5)}$] to reduce the influence of abundant prey and to overcome the unity-sum constraint and submitted to Wisconsin double standardization to improve the detection of gradients in the Bray-Curtis dissimilarity index (Clarke and Gorley, 2006).

Analysis of similarity (ANOSIM) was used to assess differences in diet between riparian zones, with the significance value calculated using a procedure including 5000 permutations of the dataset and corrected for multiple testing using the Bonferroni sequential method (Creque and Czesny, 2012). Similarity Percentage (SIMPER) analysis was used to identify prey categories with the highest contribution to diet dissimilarity. Prey categories were listed in decreasing order by their mean contribution to the total average dissimilarity, with a cut-off at 50% of cumulative average dissimilarity (Clarke and Gorley, 2006).

To assess diet specialization, the evenness index was calculated using the following equation:

$$E = H'/H'_{max}, \tag{3.3}$$

where H' corresponds to the Shannon-Wiener diversity index value and H'_{max} is the maximum value of H' if every prey category was equally eaten. Evenness E values close to zero indicate a stenophagous diet (i.e. individuals feed on preferred type or limited range of prey) and values close to one a euryphagous diet (i.e. individuals show no specific preference or consume a wide variety of prey) (Oscoz et al., 2005).

H' and H'_{max} were calculated using the following formulas, respectively:

$$H' = -\sum P_i \log_2 P_i \tag{3.4}$$

$$H'_{max} = \log_2 N_S \tag{3.5}$$

where P_i is the contribution of prey i to total prey and N_S is the total number of prey categories (Pielou, 1966).

The Schoener index (1970) was used to assess diet overlap between size classes through the following formula:

$$S = 100\,(1-0.5\sum\left|A_{x,i} - A_{y,i}\right|) \tag{3.6}$$

where $A_{x,i}$ is the percent abundance of item i for size class x and $A_{y,i}$ is the percent abundance of item i for size class y. According to Wallace (1981), overlap is considered high when values exceeded 60%.

The Ivlev Electivity Index (D) modified by Jacobs (1974) was used to quantify diet selectivity, that is the degree to which a fish selects a particular prey relative to the range of available prey. The index was determined by the following formula:

$$D = (P_i-R_i)/(P_i+R_i-2P_iR_i) \tag{3.7}$$

where Pi is the contribution of prey i to total consumed prey and R_i is the contribution of prey i to total available prey. The index ranges from -1 to +1 indicating that a prey is avoided or preferred by fish, respectively. Prey availability was approached using macroinvertebrate abundance data collected in chapter 2 (see section 2.4.3.1). Kruskal-Wallis tests were used to assess variation in diet selectivity between riparian condition zones.

Analyses were performed using the PRIMER 7 & Permanova (Clarke and Gorley, 2015) and R (R Development Core Team, 2014) software, and the significance of statistical testing was assessed at $P < 0.05$.

3.4.2. Microhabitat

3.4.2.1. Microhabitat availability

Microhabitat availability was surveyed following the procedures described by Teixeira et al. (2006). In brief, available microhabitat was evaluated along 11 to 16 transects randomly distributed within each sampling site. In each transect, at intervals of 50 cm, the following variables were measured:

Total depth (cm) – Measures made with a graduated rod.

Water current ($m.s^{-1}$) – Measures taken using a Water current Valeport® electronic flowmeter over a 30 second period at 0.6 of total depth when water was less than 75 cm deep, and at 0.2 and 0.8 of total depth and averaged when water depth exceeded 75 cm, velocity measures were taken (Bovee et al., 1978).

Substrate – Sorted in categories based on measurements of particle medium size made with a graduated rod, and classified into dominant and subdominant, corresponding to the first and second most noticeable particles, by visual estimation.

Cover – Determined as features in or near the channel which could promote shelter for at least 50% of the fish's body. "No cover" was noted when no features were present.

Categories for each variable are described in **Table 3.1**.

Table 3.1 Total depth, water column velocity, substrate, and cover categories. Categories for substrate and cover were adapted from Bovee (1982)

Total depth

Code	Description (cm)
D25	< 25
D2550	$25 \leq x < 50$
D5075	$50 \leq x < 75$
D75100	$75 \leq x < 100$
D100	≥ 100

Water current

Code	Description ($m.s^{-1}$)
Nc	No current
Wc	With current (> 0.001)

Substrate

Code	Description (cm)
Fs	Fine sediment (sand, silt, clay) < 0.2
G	Gravel ($0.2 \leq x < 6.0$)
Sc	Small cobble ($6.0 \leq x < 20.0$)
Bc	Big cobble ($20.0 \leq x < 30.0$)
Bo	Boulders (≥ 30.0)
Be	Bedrock

Cover

Code	Description
Nc	No cover
C	Cobbles
B	Boulders
Rus	Aquatic roots, undercut banks and submerged logs
Ov	Overhanging vegetation (riparian tree)
Av	Aquatic vegetation

3.4.2.2. Microhabitat use

The microhabitat used by brown trout was evaluated through underwater observation via snorkelling. Two snorkellers moved in zig-zag preferably in upstream direction, while an operator in the riverbank registered the data.

Whenever an undisturbed fish (i.e.maintaining its activity) was observed, the snorkeler communicated the values of habitat variables at its exact location (i.e. total depth, water current, dominant and subdominant substrate and cover as in **Table 3.1**) to the operator in the river bank. Total length of fish was determined by comparison with objects in the river bottom (e.g. cobble) measured using a graduated rod. This method allows for more accurate measurements, avoiding possible errors due to light's refraction in water (Teixeira A., personal communication).

Snorkelling was performed during the day, between 09:00 a.m and 6:00 p.m., and at maximum depth of 5 m in shaded areas and 6 m in non-shaded areas, to guarantee good visibility.

3.4.2.3. Data Analysis

Individuals were sorted in 3 size-classes (cm): A) ≤13.0; B)]13.0 – 20.0[; C) ≥ 20.0 cm TL, to account for ontogenetic variation in habitat use (Grey, 2001).

Chi-square goodness-of-fit tests were used to determine if variation in availability of each microhabitat variable was significant between riparian zones and to assess variation between habitat availability and use.

To easy interpretation, use of microhabitat variables was graphically represented and a third-degree polynomial regression line was fitted to the data. Analyses were performed using R (R Development Core Team, 2014) software, and the significance of statistical testing was assessed at $P < 0.05$.

3.5. Results

3.5.1. Overall diet composition

In total 542 brown trout were analysed for stomachs contents, 296 from Sabor river with 4778 prey items, and 246 from Baceiro river with 2921 prey items. **Figure 3.1** shows the number of stomachs analsysed by each brown trout size class considered.

Percent abundance and frequency of occurrence were positively correlated for all prey categories, as shown in **Figure 3.2**. Therefore, to avoid redundancy, further analysis focused on prey percent abundance.

Diet composition differed between rivers (**Figure 3.3)**. The main observable difference was in the proportion of terrestrial origin prey (TOP), which was higher in the Sabor (40.2%) than in the Baceiro river (15.7%). Furthermore, in the Sabor river, this prey category was highly consumed by brown trout of all size classes (A: 29.3 %; B: 41.3 %; C: 59.0 %) while in Baceiro river it was mainly consumed by larger individuals only (A: 1.1 %; B: 15.6%; C: 35.4 %).

Besides TOP, in Sabor river, classes A and B mainly consumed Ephemeroptera (A: 34.9 %: B: 23.8 %), Diptera (A: 16.7 %; B: 8.4 %) and Trichoptera (A: 8.7 %; B: 12.2 %). In Baceiro river, class A individuals consumed more Diptera (34.6 %), Ephemeroptera (32.9 %), Plecoptera (12.1 %) and Trichoptera (12.0 %) while class B individuals add a more equitable diet. Class C mainly consumed TOP in both rivers, having also a high proportion of Ephemeroptera in the diet in Baceiro river (33.5 %).

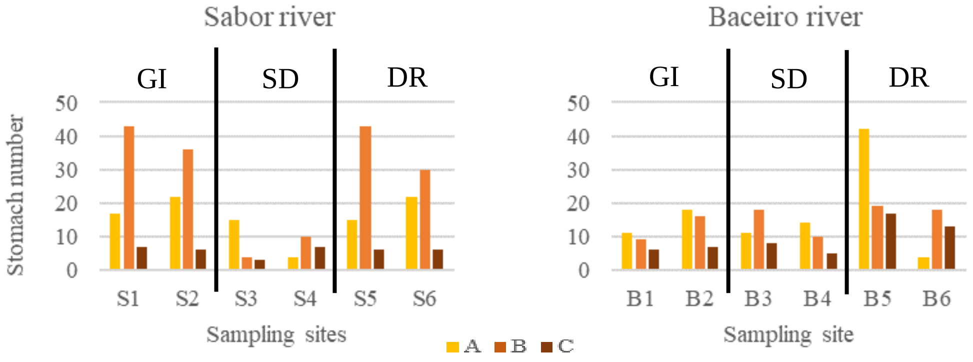

Figure 3.1 Number of stomachs of brown trout of each size class (A, B and C) considered in the analysis from Sabor and Baceiro rivers. Riparian condition is defined in 3 categories: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

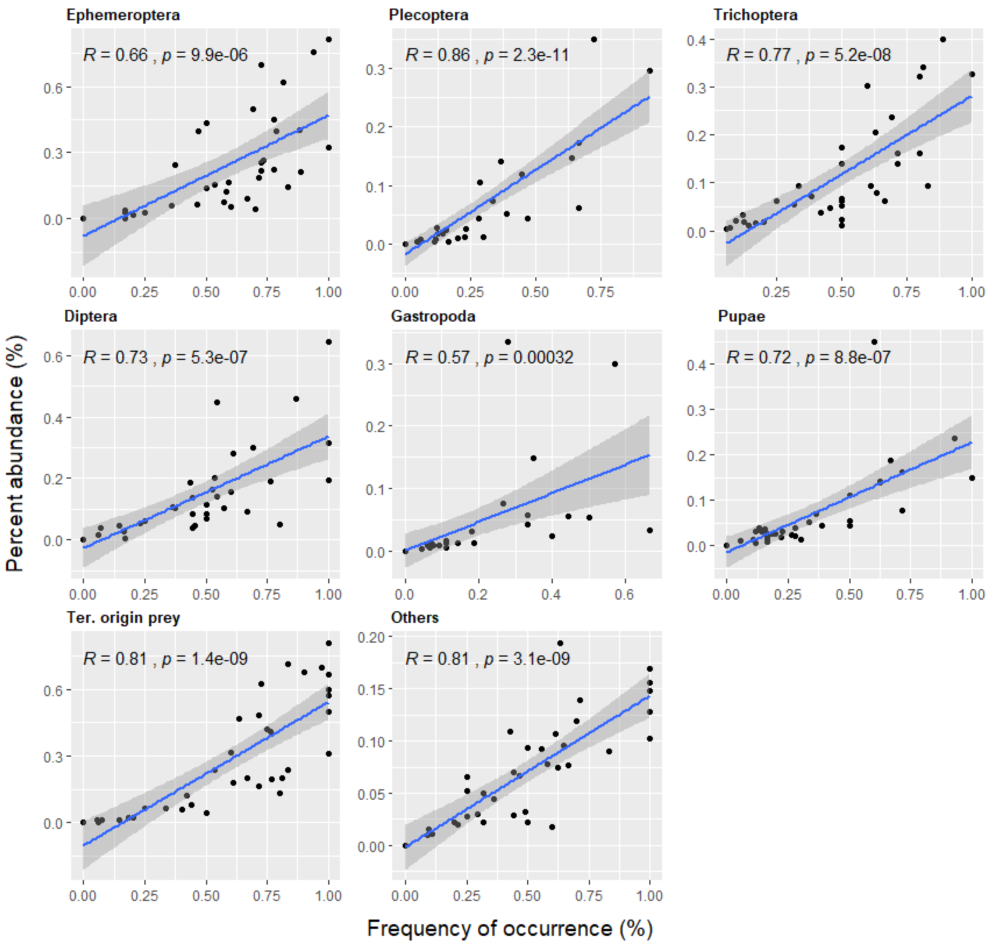

Figure 3.2 Relation between frequency of occurrence (%) and percent abundance (%) for each prey category. Regression lines, and Pearson correlations (R) and p-values (p) are shown.

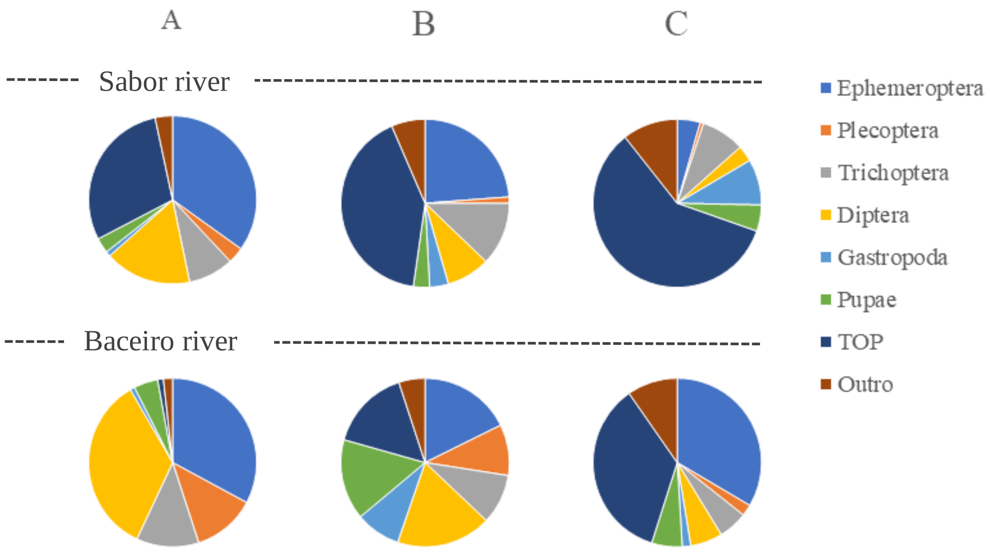

Figure 3.3 Proportions of total prey consumed by brown trout of each size class (A, B and C) in the Sabor and Baceiro rivers.

3.5.2. Variation in diet among riparian zones

Results of the nMDS are presented in **Figure 3.4**. In both rivers and for all size classes, the diet was more variable in GI than in DR. Diet variability was similar in SD and DR in the Sabor river. In the Baceiro river, diet was more variable in SD than in both GI and DR for classes A and B.

In the Sabor river (**Table 3.2**), ANOSIM results indicated a significant difference in the diets of class A individuals between GI and DR (P < 0.01), with 56.7% of the dissimilarity being explained by Ephemeroptera, TOP and Trichoptera. For class B, significant differences in the diets were obtained for both GI and SD with DR (P < 0.01). Trichoptera, Ephemeroptera and TOP explained 57.3% of dissimilarity between GI and DR, while Trichoptera, TOP and Other explained 64.6% of the dissimilarity between SD and DR. No significant differences in the diets among zones were found for class C individuals. For both classes A and B percent abundances of Ephemeroptera and TOP decreased from GI to DR while that of Trichoptera increased (**Table 3.4**).

For the Baceiro river (

Table 3.3), diets of classes A and B showed significant differences between all sites (P < 0.01). The main contributors for diet dissimilarities were Plecoptera, Trichoptera, Diptera and Ephemeroptera. Conversely, the diet of class C was only significantly dissimilar between GI and DR (P < 0.01), with Ephemeroptera, TOP and Trichoptera explaining up to 52.6% of dissimilarity. For class A, percent abundance of Plecoptera decreased and that of Trichoptera and Diptera increased from GI to DR (**Table 3.4**). For both class B and C, percent abundance of Ephemeroptera increased from GI to DR. Furthermore, for class B, the percent abundance of Plecoptera decreased from GI to DR and that of Trichoptera slightly increased. For class C both Trichoptera and TOP percent abundances decreased from GI to DR.

In the Sabor river, both diet diversity and evenness index values increased from GI to DR for classes A and B (**Figure 3.5**) while for class C diversity decreased. In the Baceiro river, from GI to DR, there was a decrease in diet diversity for class A only.

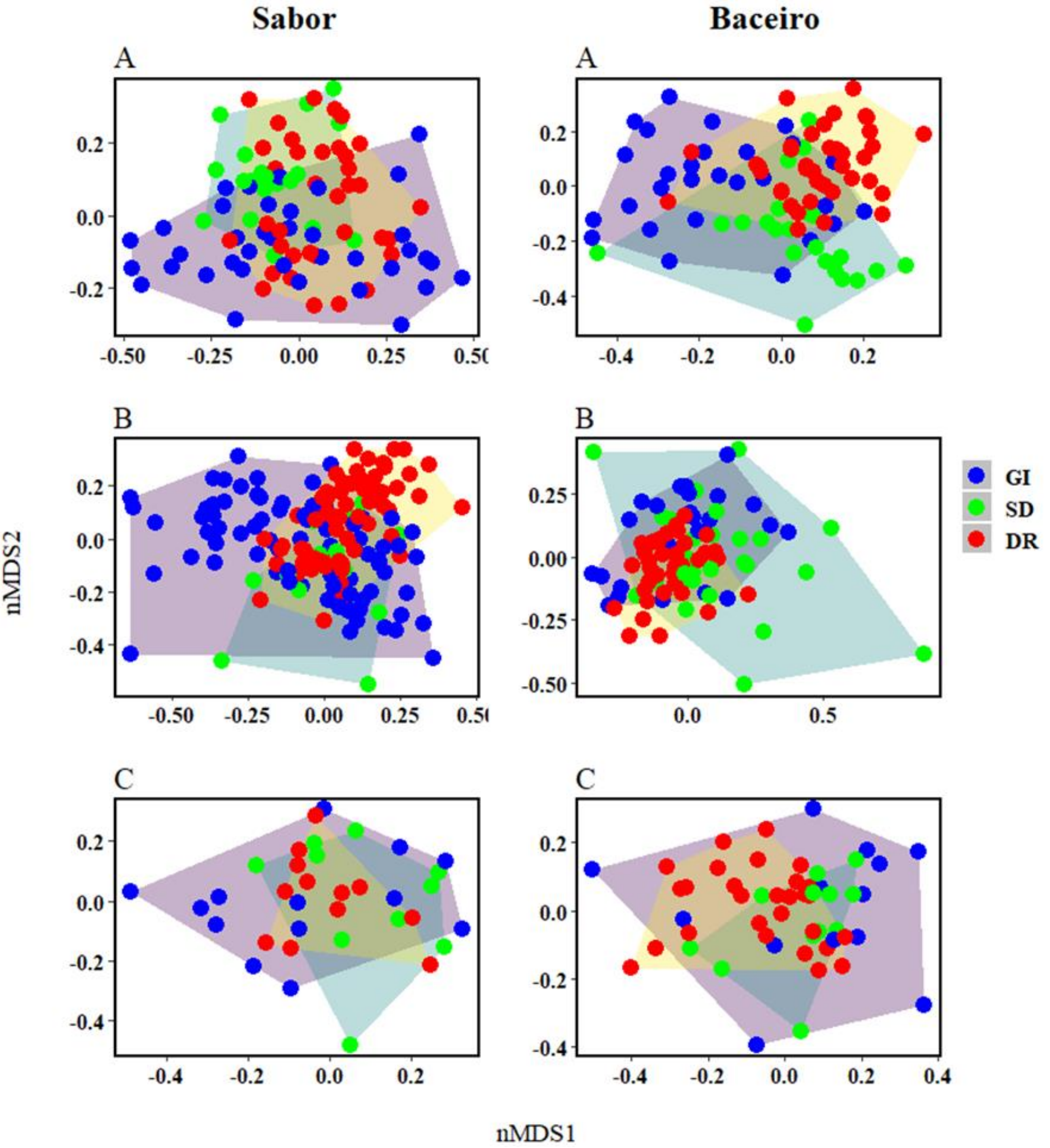

Figure 3.4 nMDS for diet of each size class (A, B and C) of brown trout from the Sabor and Baceiro rivers. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

Table 3.2 Results of ANOSIM and SIMPER analyses showing prey categories with the cumulative contribution (%) to average dissimilarity (AvD) in the diet of each size class (A, B and C) from Sabor river between the 3 defined riparian condition zones: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation. Rank order of contribution is represented in parentheses. Prey codes are as follows: Ephe. – Ephemeroptera; Plec. – Plecoptera; Tric. – Trichoptera; Dipt. – Diptera; TOP – Terrestrial Origin Prey; Other – all other identified prey.

Riparian Condition Zones	AvD	Ephe.	Plec.	Tric.	Dipt.	TOP	Other
Small trout A ≤ 130 mm (R= 0.108; P = 0.0002)							
GI vs. SD (R = 0.094; P = 0.045)	25.6	39.9 (2)		20.3 (1)		57.7 (3)	
GI vs. DR (R = 0.131; P = 0.0002)	**26.1**	**20.9 (1)**		**56.7 (3)**		**40.0 (2)**	
SD vs. DR (R = 0.054; P = 0.109)	22.8			20.9 (1)	37.7 (2)		52.3 (3)
Medium trout B 130 < TL < 200 mm (R= 0.215; P = 0.0002)							
GI vs. SD (R = 0.017; P = 0.376)	26.4	40.0 (2)				21.6 (1)	58.1 (3)
GI vs. DR (R = 0.258; P = 0.0002)	**27.4**	**39.7 (2)**		**21.4 (1)**		**57.3 (3)**	
SD vs. DR (R = 0.201; P = 0.007)	**24.1**			**25.3 (1)**		**45.0 (2)**	**64.6 (3)**
Large trout C ≥ 200 mm (R= 0.077; P = 0.054)							
GI vs. SD (R = 0.106; P = 0.064)	25.8			41.2 (2)		23.8 (1)	56.9 (3)
GI vs. DR (R = 0.039; P = 0.195)	21.7			63.4 (3)		23.7 (1)	44.9 (2)
SD vs. DR (R = 0.110; P = 0.054)	21.3			20.7 (1)		56.3 (3)	38.8 (2)

Table 3.3 Results of ANOSIM and SIMPER analyses showing prey categories with the cumulative contribution (%) to average dissimilarity (AvD) in the diet of each size class (A, B and C) from Baceiro river between the 3 defined riparian condition zones: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation. Rank order of contribution is represented in parentheses. Prey codes are as follows: Ephe. – Ephemeroptera; Plec. – Plecoptera; Tric. – Trichoptera; Dipt. – Diptera; TOP – Terrestrial Origin Prey; Other – all other identified prey.

Riparian Condition Zones	AvD	Ephe.	Plec.	Tric.	Dipt.	TOP	Other
Small trout A ≤ 130 mm (R= 0.307; P = 0.0002)							
GI vs. SD (R = 0.228; P = 0.0002)	**24.7**	**63.3 (3)**	**26.0 (1)**		**48.9 (2)**		
GI vs. DR (R = 0.355; P = 0.0002)	**22.6**		**28.5 (1)**	**49.5 (2)**	**66.6 (3)**		
SD vs. DR (R = 0.293; P = 0.0002)	**21.2**	**61.4 (3)**		**45.6 (2)**	**23.1 (1)**		
Medium trout B 130 < TL < 200 mm (R= 0.184; P = 0.0002)							
GI vs. SD (R = 0.118; P = 0.001)	**29.5**		**19.9 (1)**	**37.3(2)**	**51.4 (3)**		
GI vs. DR (R = 0.264; P = 0.0002)	**25.3**	**55.4 (3)**	**20.5 (1)**	**40.5 (2)**			
SD vs. DR (R = 0.155; P = 0.0006	**26.0**	**46.1 (3)**		**18.2 (1)**	**32.7 (2)**		
Large trout C ≥ 200 mm (R= 0.139; P =0.015)							
GI vs. SD (R = 0.018; P = 0.260)	21.8			49.0 (3)		17.0 (1)	33.7 (2)
GI vs. DR (R = 0.271; P = 0.001)	**23.2**	**19.7 (1)**		**52.6 (3)**		**37.1 (2)**	
SD vs. DR (R = 0.040; P = 0.253)	18.6	20.9 (1)				39.8 (2)	58.3 (3)

Table 3.4 Differences in percent abundance (%) of prey in the diet of brown trout size classes between good integrity sites (GI) and other riparian zones for Sabor (S) and Baceiro (B) river. SD stands for sites with signs of disease. DR stands for sites with dead riparian vegetation. Only the 3 main dissimilarity contributors are represented. Negative differences are highlighted in red and positive in green. Prey codes are as follows: Ephe. – Ephemeroptera; Plec. – Plecoptera; Tric. – Trichoptera; Dipt. – Diptera; TOP – Terrestrial Origin Prey; Other – all other identified prey.

S

Riparian Condition Zones	**Ephe.**	**Tric.**	**TOP**
Small trout A ≤ 130 mm			
GI vs. SD			
GI vs. DR	- 28.2	12.7	- 4.9
Medium trout B 130 < TL < 200 mm			
GI vs. SD			
GI vs. DR	-28.2	27.7	-17.3
Large trout C ≥ 200 mm			
GI vs. SD			
GI vs. DR			

B

Riparian Condition Zones	**Ephe.**	**Plec.**	**Tric.**	**Dipt.**	**TOP**
Small trout A ≤ 130 mm					
GI vs. SD	-24.5	- 20.7		33.9	
GI vs. DR		- 25.3	16.0	6.7	
Medium trout B 130 < TL < 200 mm					
GI vs. SD		-16.2	-14.9	7.8	
GI vs. DR	33.4	-18.6	0.3		
Large trout C ≥ 200 mm					
GI vs. SD					
GI vs. DR	12.6		-2.1		-1.6

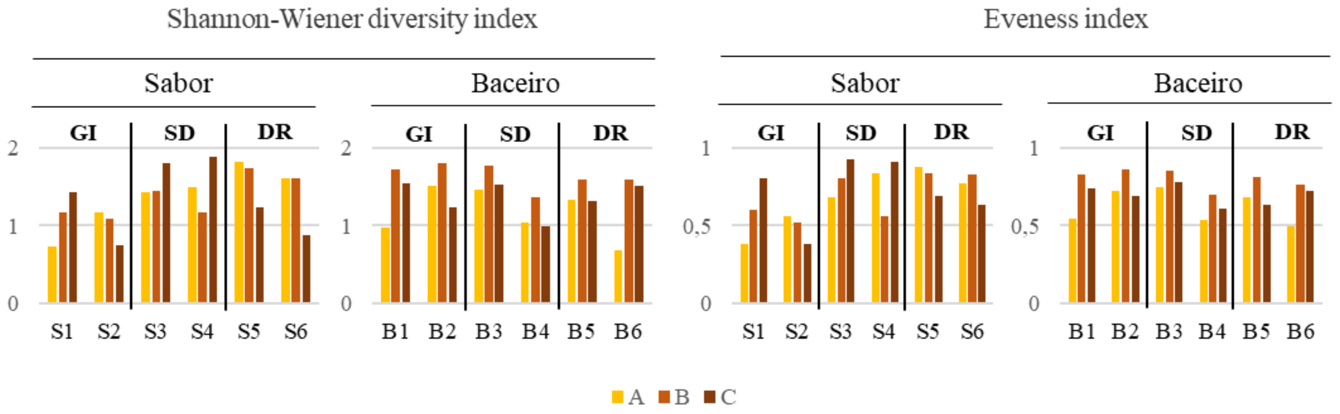

Figure 3.5 Shannon-Wiener diversity and evenness of the diet for each size class (A, B and C) in the Sabor and Baceiro rivers. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

Electivity values for the Sabor and Baceiro rivers are presented in **Table 3.5** and **Table 3.6**, respectively. TOP was positively selected in both rivers in all sites and by all size classes.

In the Sabor river, class A showed, in GI, a negative selectivity towards Trichoptera and positive towards Ephemeroptera. In both SD and DR it showed a positive selectivity towards Trichoptera. Additionally, class A showed a negative selectivity towards Ephemeroptera in DR.

Class B showed, in GI, a positive selectivity towards Ephemeroptera. In both SD and DR it showed a negative selectivity towards Ephemeroptera. Additionally, class B showed a positive selectivity towards Trichoptera in DR.

Class C showed a negative selectivity towards Ephemeroptera in all locations. Furthermore, it showed a positive selectivity towards Trichoptera in DR.

In the Baceiro river, class A showed, in GI, a positive selectivity towards Ephemeroptera and Diptera and negative towards Trichoptera. In SD, it showed a positive selectivity towards Diptera, and negativite towards Ephemeroptera, Plecoptera and Trichoptera. In DR, class A showed a positive selectivity towards Ephemeroptera and Trichoptera, and negative towards Plecoptera.

Class B showed, in GI, a positive selectivity towards trichoptera and negative towards Ephemeroptera and Plecoptera. In SD, it showed a negative selectivity towards Ephemeroptera, Plecoptera and Trichoptera. Additionally, in DR, it showed a positive selectivity towards Trichoptera and negative towards Plecoptera.

Class C showed a negative selectivity towards Ephemeroptera, Plecoptera, Trichoptera and Diptera in both GI and SD. In DR, class C showed negative selectivity towards Plecoptera and Diptera.

Table 3.5 Electivity values for Sabor river for each class and sites. Only the 3 main dissimilarity contributors are represented. Only values above 0.5 and below -0.5 are presented. Positive values (preference) are highlighted in green and negative values (avoidance) in red. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

Riparian Condition Zones	Location	Ephe.	Tric.	TOP
Small trout A ≤ 130 mm				
GI	**S1**	0.92	-0.90	
	S2			1.00
SD	**S3**			
	S4		0.66	0.70
DR	**S5**	-0.62	0.77	0.81
	S6			1.00
Medium trout B 130 < TL < 200 mm				
GI	**S1**	0.85		0.82
	S2			1.00
SD	**S3**			0.97
	S4	-0.83		0.99
DR	**S5**	-0.83	0.79	0.96
	S6	-0.71	0.81	1.00
Large trout C ≥ 200 mm				
GI	**S1**		0.50	0.98
	S2	-0.98	-0.86	1.00
SD	**S3**	-0.56		0.94
	S4	-0.70		0.92
DR	**S5**	-0.93	0.55	0.99
	S6	-1.00		1.00

Table 3.6 Electivity index values for Baceiro river for each class and sites. Only the 3 main dissimilarity contributors are represented. Only values above 0.5 and below -0.5 are presented. Positive values (preference) are highlighted in green and negative values (avoidance) in red. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

Riparian Condition Zones	Location	Ephe.	Plec.	Tric.	Dipt.	TOP
Small trout A ≤ 130 mm						
GI	B1	0.79		-0.62		
	B2				0.52	0.54
SD	B3					-1.00
	B4	-0.62	-0.78	-0.94	0.57	0.79
DR	B5		-0.84	0.73		
	B6	0.81	-1.00			0.63
Medium trout B 130 < TL < 200 mm						
GI	B1		-0.64	0.77		
	B2	-0.93				0.98
SD	B3	-0.57		-0.58		0.91
	B4	-0.64	-0.86	-0.79		0.98
DR	B5		-0.86	0.69		0.93
	B6		-0.71			0.89
Large trout C ≥ 200 mm						
GI	B1		-0.98			0.84
	B2	-1.00		-0.59	-1.00	1.00
SD	B3		-0.86	-0.55		0.97
	B4	-0.87	-1.00	-0.83	-1.00	1.00
DR	B5		-0.95		-0.84	0.99
	B6		-0.84			0.88

3.5.3. *Diet overlap between size classes*

High diet overlaps, i.e. over 60%, were observed between class B and both classes A and C in 5 sites (S1, S2, S3, S5 and S6) in the Sabor river and 4 sites (B2, B4, B5 and B6) in the Baceiro river (**Figure 3.6**). Classes A and C overlapped in diet in S2 and S6 in the Sabor river and in B1 and B6 in the Baceiro river. However, no significant changes in diet overlap were found between riparian condition zones (P > 0.05).

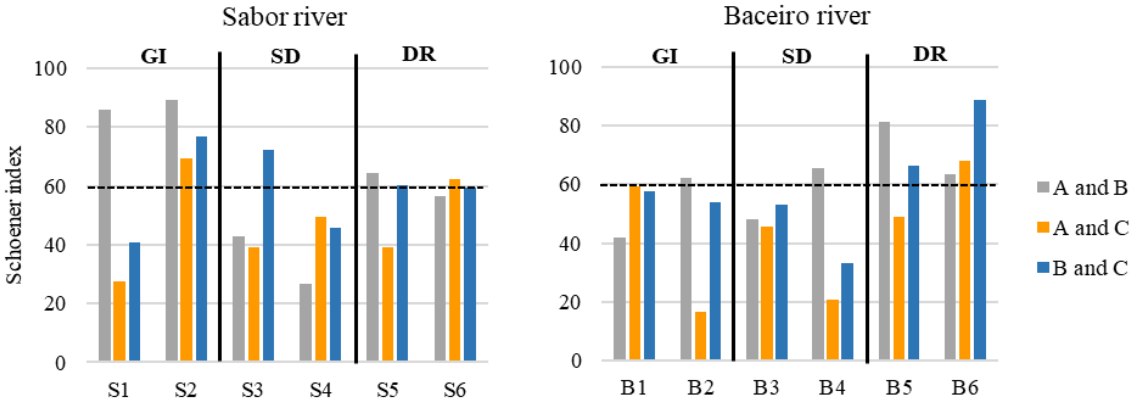

Figure 3.6 Schoener overlap index between each size class (A, B and C) from Sabor and Baceiro rivers. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

3.5.4. Habitat availability and use

A total of 746 brown trout were observed through snorkelling, 419 in the Sabor river and 327 in the Baceiro river. **Figure 3.7** shows the number of brown trout in each size class observed.

Total depth, dominant and subdominant substrate, and cover availability significantly changed between most sites in both rivers (P < 0.01) (Annexes, **Table 6.5**). Nevertheless, some pairs of similar sites (P > 0.05) were detected, namely:

- In the Sabor river: S1 and S2;
- In the Baceiro river: B1 and B2; B3 and B4; B5 and B6.

Furthermore, total depth availability was similar (P > 0.05) between S3 and S4 in the Sabor river and B3, B4, B5 and B6 in the Baceiro river.

In both rivers, lower depths were more available than higher depths. In the Sabor river, depths between 0 and 50 cm were more available in SD than in other sites while depths above 50 cm were less (**Figure 3.8**). In the Baceiro river, GI had little to no availability of depths above 75 cm while in SD and DR these made up to 20% of the overall availability.

No water current made over 65% of total availability in all sites.

In the Sabor river, in GI fine sediment was the most available dominant substrate followed by bedrock. In SD, boulders and big cobbles were the most available substrate while in DR were fine sediment, gravel and small and big cobble. In the Baceiro river fine sediments availability as dominant substrate decreased with alder decay while there was an increase in the availability of higher caliber substrate (i.e. big cobbles, boulders and bedrock).

The most available subdominant substrate were fine sediments, small and big cobbles in both rivers. In the Sabor river, fine sediments were the most available category in GI while in SD and DR was small cobbles. In the Baceiro river, fine sediment availability as subdominant substrate increased with alder decay while small cobbles availability decreased.

In the Sabor river no cover was predominant in GI, boulders in SD and cobbles in DR. In the Baceiro river, cobbles were the most available cover in GI and SD while in DR was boulders. In both rivers, availability of Rus (aquatic roots, undercut banks, submerged logs) decreased.

In terms of dissimilarity between availability and use of total depth, chi-square tests revealed significant results in all riparian zones and for all size classes, with exception of class A in GI in the Sabor river and in DR in the Baceiro river (Annexes, **Table 6.6 and Table 6**.7).

Subdominant substrate use was significantly different from availability in all sites and for all size classes except for class C in GI in the Sabor river and for class B in DR and class C in SD in the Baceiro river. Similar results were found for SS, with the addition of class B in SD and class C in DR in the Baceiro river which also showed no significant difference between use and availability.

Cover use was found to be significantly different from its availability in all sites and for all size classes except for class A in GI in Sabor river.

Microhabitat use significantly changed between most sites for total depth, dominant and subdominant substrate, and cover (P < 0.01) (Annexes, **Table 6.8** to **Table 6**.11).

In both rivers, class A mostly used depths between 0 and 75 cm while class B and C moslty used depths between 25 and 100 cm (**Figure 3.10** and **Figure 3**.11). Furthermore, in the Sabor river, class A used depths between 25 and 50 cm in GI, below 25 cm in SD and between 25 and 50 cm in DR. Class B used depths between 25 and 100 cm in GI, between 50 and 100 cm in SD and between 75 and

100 cm in DR. Class C used depths between 50 and 100 cm in GI, between 50 and 75 cm in SD and between 75 and 100 cm in DR. In the Baceiro river, class A seleted depths between 25 and 75 cm in GI, between 0 and 50 cm in SD and between 25 and 75 cm in DR. Class B used depths between 50 and 100 cm in GI and SD, and above 100 cm in DR. Class C seleted depths between 50 and 75 in GI and SD, and between 75 and 100 cm in DR.

In both rivers, class A used more areas with current, increasing its use with alder decay (Anex, **Figure 6.1**). Furthermore, classes B and C used more areas with no current. In the Sabor river, classes B and C use of areas with no current increased with alder decay while in the Baceiro river the lowest percentage of individuals detected in areas with current was in SD.

In the Sabor river, big cobbles were the dominant substrate mostly used by brown trout of all sizes and in all three riprian zones (Annexes, **Figure 6.2**). In the Baceiro river, class A mainy used fine sediments in GI, fine sediments and big cobbles in SD and small cobbles in DR (Annexes, **Figure 6.3**). Class B used boulders and bedrock in GI and boulders and fine sediment in SD and DR. Class C used boulders in GI, boulders and fine sediment in SD and gravel, fine sediments and bedrock in DR.

In terms of subdominant substrate, in the Sabor river, class A used fine sediments and small and big cobble in GI, fine sediments and small cobble in SD and fine sediment, gravel and small cobble in DR (Annexes, **Figure 6.2**). Class B mainly used fine sediments and small and big cobble in all three riparian zones. Class C used fine sediments in GI, small cobble in SD and fine sediments and small and big cobbles in DR. In the Baceiro river, class A mainly used fine sediments and gravel in GI and DR and fine sediments in SD (Annexes, **Figure 6.3**). Both class B and C used primarly fine sediments in all three riparian zones.

Percentage of brown trout detected in no cover was high in both rivers but decreased from GI to SD and DR (**Figure 3.10** and **Figure 3**.11). In the Sabor river, class A used mainly cobbles and boulders as cover in GI and cobbles in SD and DR. Class B used mainly cobbles in GI, SD and DR, and use of this feature as cover increased with alder decay. Class C mainly used Rus in GI, coobles and Rus in SD and coobles in DR. In the Baceiro river, class A used Rus in GI and SD and cobbles in DR. Overhanging vegetation was also a used cover feature in SD. Class B used Rus in all three riparian zones. Aditionally, many individuals used boulders as cover in GI and SD and boulders and overhanging vegetation in DR. Class C mainly used boulders in GI and SD and Rus in DR.

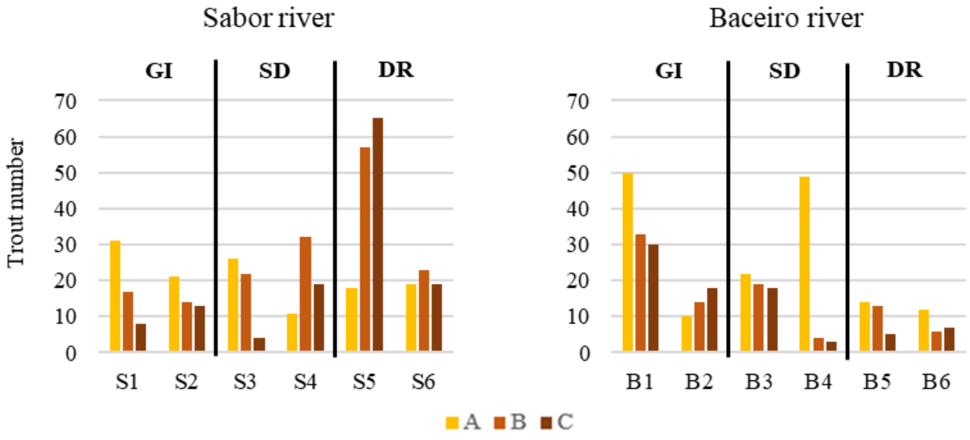

Figure 3.7 Number of brown trout of each size class (A, B and C) from Sabor and Baceiro rivers. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

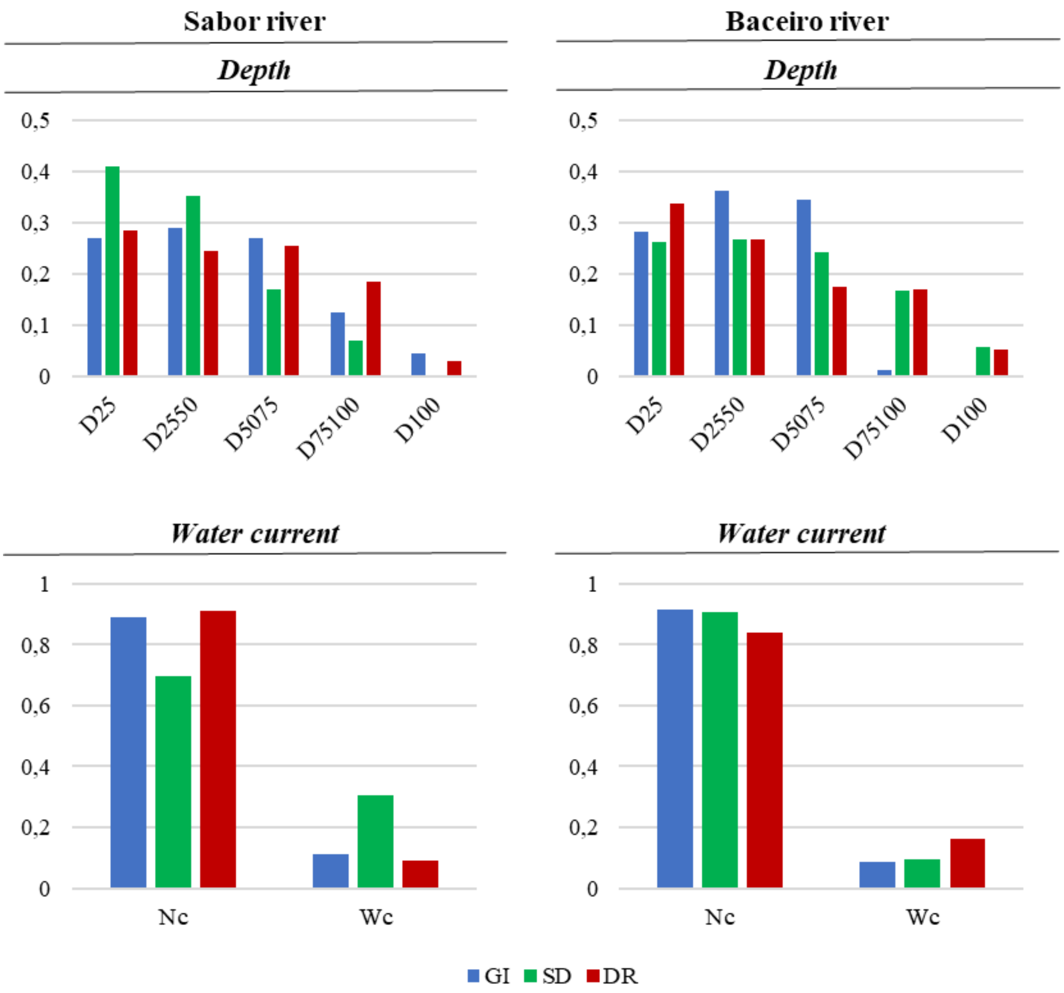

Figure 3.8 Availability of total depth and water current in Sabor (left) and Baceiro river (right). Variable classes are described in Table 3.1. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

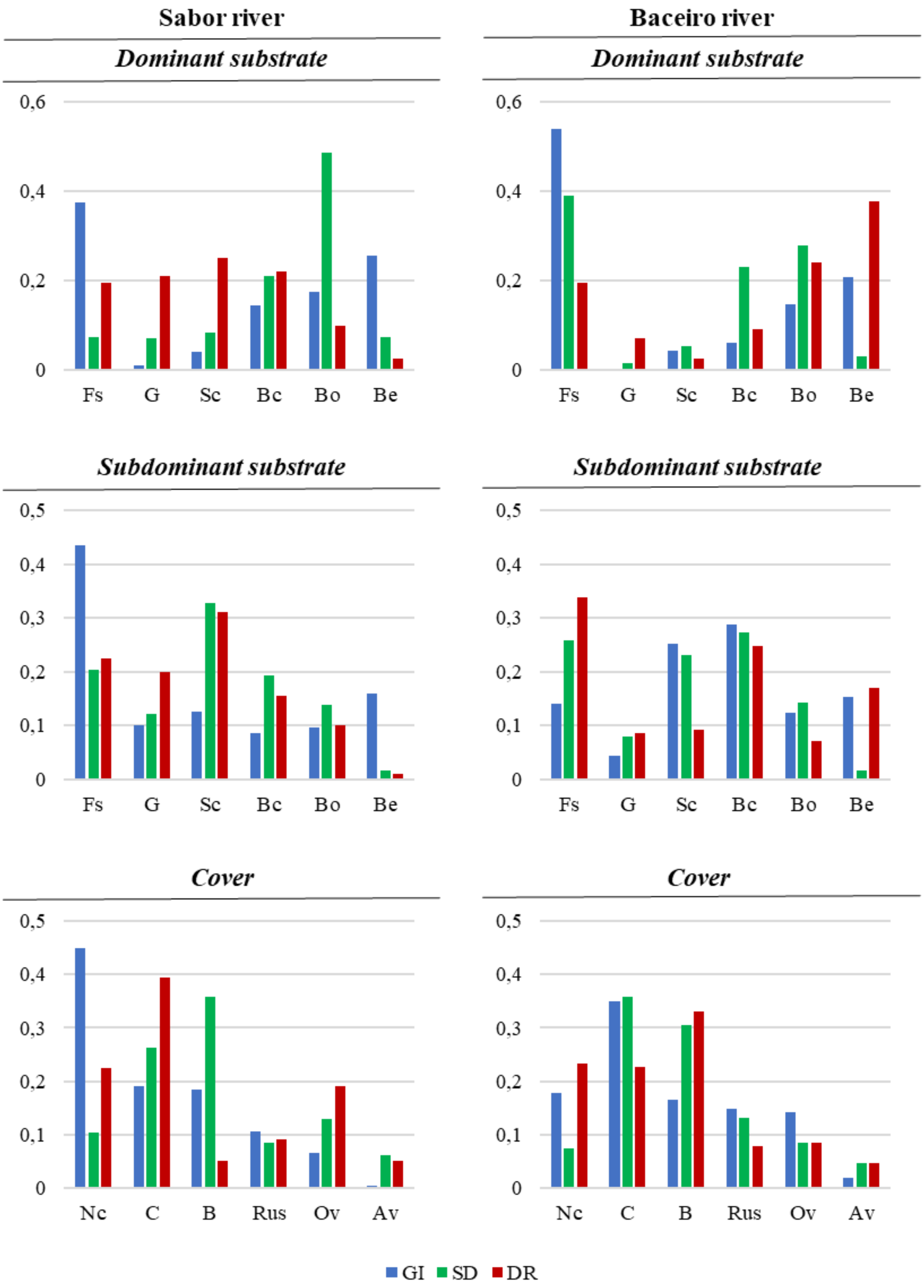

Figure 3.9 Availability of dominant substrate, subdominant substrate, and cover and water current in Sabor (left) and Baceiro river (right). Variable classes are as described in Table 3.1. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

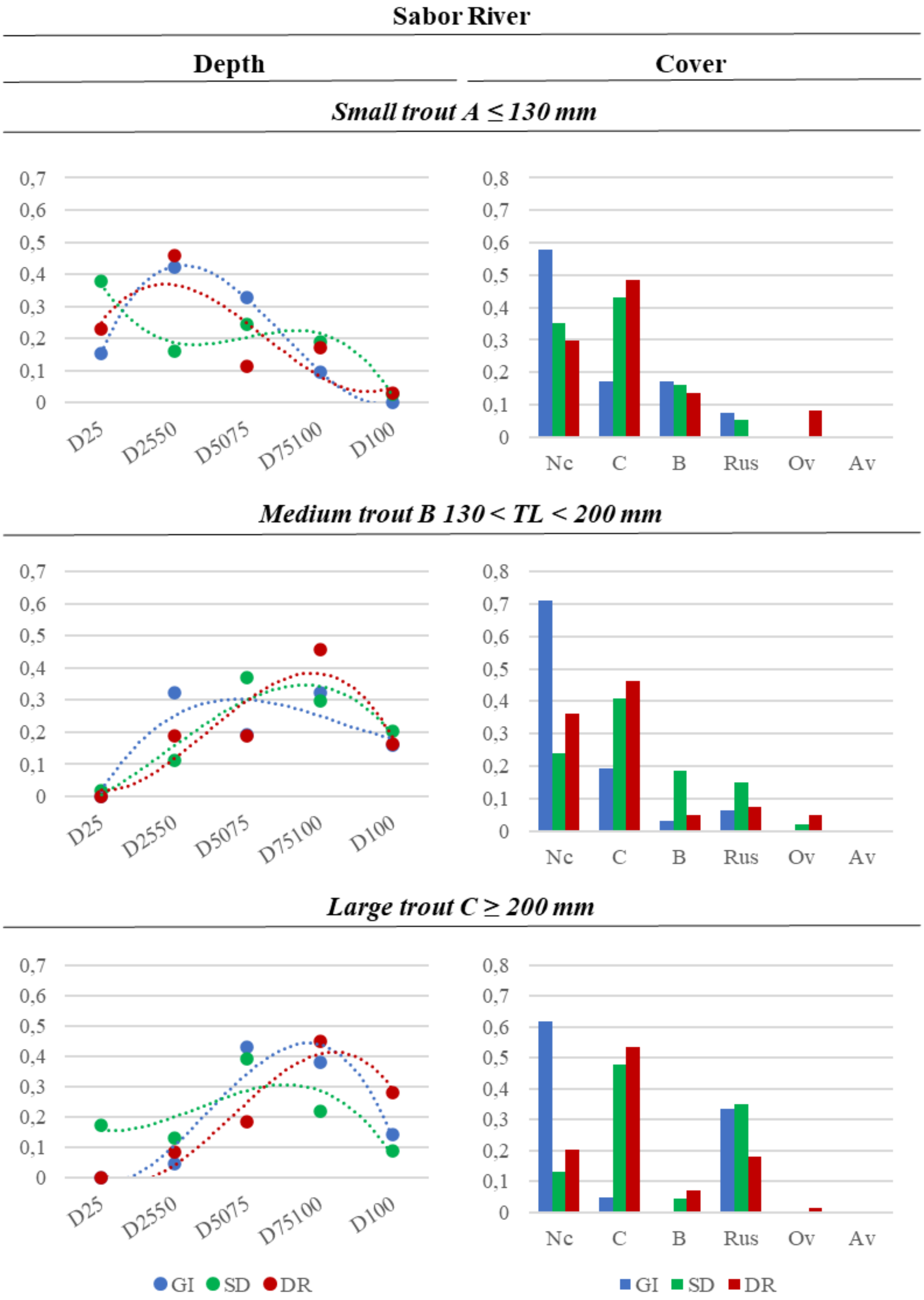

Figure 3.10 Total depth (left) and cover (right) use in Sabor river. Variable classes are described in Table 3.1. A third-degree polynomial regression line was fitted to better represent total depth data variation. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

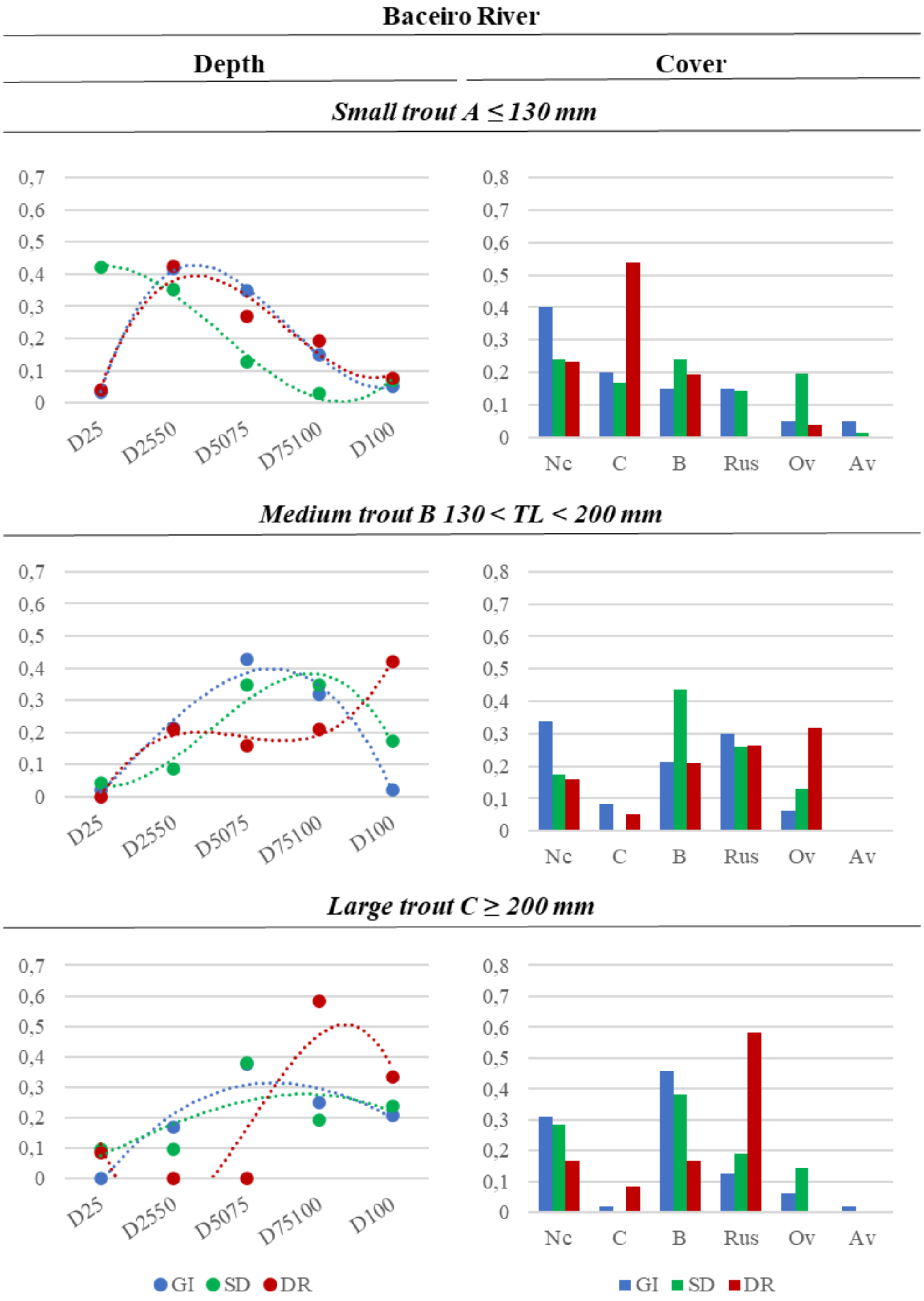

Figure 3.11 Total depth (left) and cover (right) use in Baceiro river. Variable classes are as described in Table 3.1. A third-degree polynomial regression line was fitted to better represent total depth data variation. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

3.6. Discussion

Diet composition of the three size classes of brown trout changed among riparian zones mainly in terrestrial origin prey, and aquatic insects, such as Ephemeroptera, Plecoptera and Trichoptera orders. These changes are likely connected with the direct impact of alder decay on the availability of terrestrial origin prey (Wipfli and Musslewhite, 2004), leading to a decreasing consumption by brown trout and indirectly increasing the consumption of other prey. However, these changes were not reflected in significantly increased overlap between size classes.

Both availability and use of microhabitat varied differently between locations. Microhabitat availability shifts did not show a clear pattern with changes in riparian vegetation condition, possibly being explained by other factors. However, changes in microhabitat use were clear. Specifically, changes in the use of cover were noticeable and possibly reflected indirect effects of changes in the riparian condition.

Although further studies needed to clarify perceived patterns, current results show that alder decay associated with alder disease may have considerable effects on brown trout diet and microhabitat use.

3.6.1. Diet

Brown trout consumed less terrestrial origin prey in sites with dead alder than in other sites, consistent with what was expected. This is apparently inconsistent with the small, changes in macroinvertebrate availability among riparian zones found in chapter 2. However, the method used to sample macroinvertebrates focused on benthic forms, likely leading to underestimation of the availability of drifting and terrestrial origin prey. As such, differences in terrestrial prey availability due to alder decay may have remained largely undetected and should be deserve further research.

In the Sabor river, terrestrial origin prey represented a substantial portion of brown trout diet for all size classes. Decrease in its consumption with increase in alder decay was only detected for small and medium size brown trout. This may be because bigger brown trout monopolize the available terrestrial origin prey, forcing smaller brown trout to explore other resources like Trichoptera and becoming more euryphagous. Indeed, size has been found to be a good predictor of feeding success for brown trout (Harwood et al., 2002).

Changes in terrestrial origin prey consumption in the Baceiro river were not as pronounced as in the Sabor river. Terrestrial origin prey was mainly consumed by larger brown trout, which was the only apparently responsive to alder decay. Furthermore, since Ephemeroptera were locally abundant (chapter 2), its increased consumption by larger brown trout was expected.

A clear difference in diet was observed between size classes, consistent with results from previous studies (Santos, 2014). Small brown trout prey mostly on Ephemeroptera and Diptera while larger brown trout prey mainly on terrestrial origin prey. Medium sized brown trout showed a more even diet when compared to the other classes, showcasing its transitional characteristics (Santos, 2014).

Negative selection of Plecoptera by brown trout may be related to its abundance, size and behavioral habits. The most abundant family of Plecoptera in the study streams was Leuctridae (Annexes, **Table 6.4**). These insects are small shredders that spend a large portion of their time burrowed in the substrate, namely in leaf accumulations (Bouchard, 2004), and may thus not be easily detected by trout, making that its consumption does not keep up with its availability.

Although diet overlap was mainly detected between medium size brown trout and other size classes, and remained constant between riparian zones, the significant changes found in diet are an indicator that alder decay is having an impact on brown trout feeding behavior. The alder tree disease and the observed decrease in the riparian ecotone quality is affecting gradually, from downstream to upstream zones, these low productivity freshwater ecosystems, highly dependent on allochthonous energetic sources, potentially contributing for substantial changes on water quality, hydromorphology and biodiversity which may be increase further. Besides brown trout, several threatened species occurring in the Montesinho National Park, including mammals (e.g., *Lutra lutra, Galemys pyrenaicus*), amphibians (e.g. *Lacerta schreiberi*), and freshwater mussels (*Margaritifera margaritifera*) may be sensitive to changes in ecological integrity of the riparian buffer strips.

3.6.2. Habitat availability and use

Microhabitat availability changed between sites, consistent with the pre-determined riparian zones portraying alder decay. However, in the Sabor and Baceiro rivers microhabitat availability changed differently.

In terms of depth availability, in the Sabor river, there was a marked variability between riparian zones with sites with signs of disease having more availability of lower depths than the remaining. In the Baceiro river, variability between zones was lower and sites with good riparian integrity were found to have a much lower availability of high depths. Due to the loss of bank stability associated with alder root decay (Bjelke et al., 2016), lower depth availability would be expected in areas with decreased riparian condition. Results for the Baceiro river are consistent with that but those for the Sabor river not. Although root decay is one of the first symptoms of infection by the alder disease (Streito, 2003), many decaying alder in the Sabor remained standing possibly maintaining a big portion of radicular system. As such, habitat changes intimately associated with alder roots may be limited or even not yet be in effect.

Available substrate was very diverse, including fine sediments, cobbles, boulders, and bedrock. Variability was high between sites but there were no clear patterns among riparian zones The same was found for cover, with areas with no cover being highly available and cobbles and boulders being the most available cover features.

Microhabitat use by brown trout changed between sites and appeared to be independent of changes in local habitat availability. The observed diversity of available microhabitat likely contributes to this, stressing the importance of analysing other potential factors that may take effect such as food availability and predatorial risk.

Overall, small brown trout mainly used shallow depths (up to 75 cm) while medium and large brown trout made use of deeper areas (above 50 cm). In both rivers, small brown trout used shallower areas in sites with signs of disease than in other sites. Medium, and large size brown trout used deeper areas in sites with signs of disease and with dead alder. Since availability influence was low, the reasons for this pattern of use are unclear. Nevertheless, it might suggest that habitat segregation is higher in sites with signs of disease.

Changes in the use of water current followed patterns described for depth, as presence of current was typically confirmed in shallower areas. Areas with water current were mainly used by smaller brown trout while medium and large brown trout used areas with no current reflecting the differential use of riffles and pools throughout ontogeny described in other studies (Grey, 2001).

In terms of substrate, different sized brown trout used a mixture of fine sediments and cobbles in all sites in the Sabor river, and the same was observed for small brown trout in the Baceiro river. However, in the later river, medium and large brown trout mainly used fine sediments and higher calibre substrate like boulders and bedrock. Nevertheless, changes in substrate use between riparian zones were small. Substrate preferences are generally accentuated during the reproductive season, when brown trout females require gravel to build beds where to lay its eggs (Collares-Pereira et al., 2021). Because this study was conducted before the reproductive season, substrate use was most likely related to cover needs.

Many brown trout of small and medium size were detected without any cover. However, cover use increased with alder decay, with use of cobbles and boulders being higher in sites with dead alder. This increased use of cover features may be related to increased predation of large brown trout on small brown trout in association with changes in terrestrial origin prey and to the increase in consumption of Trichoptera, which are commonly found in such cobbles and boulders (Bouchard, 2004).

Patterns of use of aquatic roots, undercut banks and submerged logs differed between rivers, decreasing with alder decay in the Sabor river and increasing in the Baceiro river. The extent to which these patterns may indeed be related with alder decay is unclear since, as mentioned previously, most of the dead alder remained standing, and should be explored further.

3.6.3. Conclusion and future directions

Despite the alder disease spreads, its consequences on the aquatic communities remain poorly studied. This study sheds a light on the matter focusing on a sensitive species with high ecological and socioeconomic value.

Brown trout *Salmo trutta* depends on a specific set of environmental conditions which alder decay threatens. In the Montesinho Natural Park, current changes on river integrity are still small, but they will likely increase in severity with the spread and development of the disease and its symptoms. Consequently, close monitoring of brown trout populations is required in these areas, and. further studies are needed to help clarify how the impacts of the disease may develop in the future.

In this study, decreased terrestrial origin prey consumption was the major change likely associated with alder decay having indirect consequences on brown trout diet and microhabitat use. Further evaluations of drifting and outsourced macroinvertebrates availability are thus important for a more comprehensive understanding of the alder disease impacts . Additionally, input of other forms of allochthonous matter should simultaneously should also be considered and measured, given the strong connections between detritus and benthic macroinvertebrates like Trichoptera (Murphy and Giller, 2000).

With the advancement of the alder disease, severe consequences on river habitat that were not currently at play will likely occur (see Bjelke et al. 2016). Therefore, continued efforts to quantify microhabitat availability and use should be encouraged to understand as impact progress until the later stages of the alder disease.

3.7. References

Almodóvar, A., Nicola, G.G., Ayllón, D., Elvira, B., 2011. Global warming threatens the persistence of Mediterranean brown trout. Glob. Chang. Biol. 18, 1549–1560. https://doi.org/10.1111/j.1365-2486.2011.02608.x

Amundsen, P., 1996. A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello (1990) method. J. Fish Biol. 48, 607–614. https://doi.org/10.1006/jfbi.1996.0060

Bjelke, U., Boberg, J., Oliva, J., Tattersdill, K., Mckie, B.G., 2016. Dieback of riparian alder caused by the Phytophthora alni complex: Projected consequences for stream ecosystems. Freshw. Biol. 61, 565–579. https://doi.org/10.1111/fwb.12729

Bouchard, R.W.J., 2004. Guide to Aquatic Invertebrates of the Upper Midwest, Water Resources Center. University of Minnesota, St. Paul. https://doi.org/10.1525/9780520320390-009

Bovee, K.D., 1982. A guide to stream habitat analysis using the instream flow incremental methodology. Western Energy and Land Use Team, Office of Biological Services, Fish and Wildlife Service, US Department of the Interior, Washington, DC.

Bovee, K.D., Milhous, R., Turow, J., 1978. Hydraulic simulation in instream flow studies: theory and techniques, 5th ed. Department of the Interior, Fish and Wildlife Service, Office of Biological Services, Western Energy and Land Use Team, Cooperative Instream Flow Service Group, Colorado.

Clarke, K., Gorley, R., 2006. "PRIMER v6: User Manual/Tutorial." PRIMER-E: Plymounth.

Clarke, K.R., Gorley, R.N., 2015. Getting started with PRIMER v7. PRIMER-E. PRIMER-E: Plymounth.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation., 2nd ed. PRIMER-E: Plymounth. Plymouth.

Collares-Pereira, M.J. (coord.), Alves, M.J., Ribeiro, F., Domingos, I., Almeida, P.R., da Costa, P., Gante, H., Filipe, A.F., Aboim, M.A., Rodrigues, P.M., Magalhães, M.F., 2021. Guia dos Peixes de Água Doce e Migradores de Portugal Continental. Edições Afrontamento, Porto.

Creque, S.M., Czesny, S.J., 2012. Diet overlap of non-native alewife with native yellow perch and spottail shiner in nearshore waters of southwestern Lake Michigan, 2000-2007. Ecol. Freshw. Fish 21, 207–221. https://doi.org/10.1111/j.1600-0633.2011.00538.x

De Silva, S., 2012. Aquaculture: A newly emergent food production sector and perspectives of its impacts on biodiversity and conservation. Biodivers. Conserv. 21, 3187–3220. https://doi.org/10.1007/s10531-012-0360-9

Enefalk, Å., Huusko, A., Louhi, P., Bergman, E., 2019. Fine stream wood decreases growth of juvenile brown trout (Salmo trutta L.). Environ. Biol. Fishes 102, 759–770. https://doi.org/10.1007/s10641-019-00869-4

Feio, M.J., Ferreira, V., 2019. Rios de Portugal: comunidades, processos e alterações. Coimbra University Press, Coimbra. https://doi.org/https://doi.org/10.14195/978-989-26-1624-7

Gosselin, M.-P., Maddock, I., Petts, G., 2012. Mesohabitat use by brown trout (Salmo trutta) in a small groundwater-dominated stream. River Res. Appl. 28, 390–401. https://doi.org/10.1002/rra.1464

Grey, J., 2001. Ontogeny and dietary specialization in brown trout (Salmo trutta L.) from Loch Ness, Scotland, examined using stable isotopes of carbon and nitrogen. Ecol. Freshw. Fish 10, 168–176. https://doi.org/10.1034/j.1600-0633.2001.100306.x

Halkhoums, W., 2017. Management of brown trout populations (Salmo trutta L .) in northeastern Portugal (Douro basin): Analysis of habitat use and feeding strategies. Escola Superior Agrária do Instituto Politécnico de Bragança, Bragança.

Harwood, A.J., Armstrong, J.D., Griffiths, S.W., Metcalfe, N.B., 2002. Sympatric association influences within-species dominance relations among juvenile Atlantic salmon and brown trout. Anim. Behav. 64, 85–95. https://doi.org/10.1006/anbe.2002.3039

Hyslop, E.J., 1980. Stomach contents analysis—a review of methods and their application. J. Fish Biol. 17, 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x

INAG I.P., 2008c. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água - Protocolo de amostragem e análise para a fauna piscícola. Ministério do Ambient. do Ordenam. do Territ. e do Desenvolv. Reg. Inst. da Água, I.P.

Jacobs, J., 1974. Quantitative measurement of food selection. Oecologia 14, 413–417. https://doi.org/10.1385/1-59259-055-1:51

Jonsson, B., 1989. Life history and habitat use of Norwegian brown trout (Salmo trutta). Freshw. Biol. 21, 71–86. https://doi.org/10.1111/j.1365-2427.1989.tb01349.x

Larios-López, J.E., Tierno de Figueroa, J.M., Alonso, C., Nebot, B., 2015. Distribution of brown trout (Salmo trutta Linnaeus, 1758) (Teleostei: Salmonidae) in its southwesternmost European limit: Possible causes. Ital. J. Zool. 82, 404–415.

Macedo-Veiga, A., De Sostoa, A., 2011. Observational evidence of the sensitivity of some fish species to environmental stressors in Mediterranean rivers. Ecol. Indic. 11, 311–317. https://doi.org/10.1016/j.ecolind.2010.05.009

Miranda, J.F.V., 2012. A Pesca Lúdica e Desportiva no Nordeste Transmontano (Bacia do Douro , Portugal). Escola Superior Agrária do Instituto Politécnico de Bragança, Bragança.

Mouton, A.M., Alcaraz-Hernández, J.D., De Baets, B., Goethals, P.L.M., Martínez-Capel, F., 2011. Data-driven fuzzy habitat suitability models for brown trout in Spanish Mediterranean rivers. Environ. Model. Softw. 26, 615–622. https://doi.org/10.1016/j.envsoft.2010.12.001

Murphy, J.F., Giller, P.S., 2000. Seasonal dynamics of macroinvertebrate assemblages in the benthos and associated with detritus packs in two low-order streams with different riparian vegetation. Freshw. Biol. 43, 617–631. https://doi.org/10.1046/j.1365-2427.2000.t01-1-00548.x

Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World, 5th ed. John Wiley & Sons, Inc., New Jersey.

Oscoz, J., Leunda, P.M., Campos, F., Escala, M.C., Miranda, R., 2005. Diet of 0+ brown trout (Salmo trutta L., 1758) from the river Erro (Navarra, north of Spain). Limnetica 24, 319–326.

Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13, 131–144. https://doi.org/10.1016/0022-5193(66)90013-0

R Development Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria. http://www.r-project.org

Sánchez-Hernández, J., Cobo, F., 2015. Adaptive flexibility in the feeding behaviour of brown trout: Optimal prey size. Zool. Stud. 54, 1–9. https://doi.org/10.1186/s40555-015-0107-x

Sánchez-Hernández, J., Cobo, F., 2013. Foraging behaviour of brown trout in wild populations: Can population density cause behaviourally-mediated foraging specializations? Anim. Biol. 63, 425–450. https://doi.org/10.1163/15707563-00002423

Sánchez-Hernández, J., Cobo, F., 2012. Summer differences in behavioural feeding habits and use of feeding habitat among brown trout (Pisces) age classes in a temperate area. Ital. J. Zool. 79, 468–478. https://doi.org/10.1080/11250003.2012.670274

Santos, M.H., 2014. Bioecologia e conservação das populações de Salmo trutta (L.) na bacia hidrográfica do rio Tua (NE Portugal). Instituto Politécnico de Bragança, Bragança.

Streito, J.-C., 2003. Phytophthora disease of alder: identification and distribution, in: Gibbs, J., van Dijk, C., Webber, J. (Eds.), Phytophthora Disease of Alder in Europe. Forestry Commission, Edinburgh, pp. 25–38.

Tachet, H., Bournaud, M., Richoux, P., 1981. Introduction à l'étude des macroinvertebrés d'eaux douces. Université Lyon I - Association Française de Limnologie., Lyon.

Tachet, H., Richoux, P., Bournard, M., Usseglio-Polatera, P., 2010. Invertébrés d'eaux douces. Systématique, biologie, écologie. CNRS Éditions, Paris.

Teixeira, A., Cortes, R.M.V., 2007. PIT telemetry as a method to study the habitat requirements of fish populations: Application to native and stocked trout movements. Hydrobiologia 582, 171–185. https://doi.org/10.1007/s10750-006-0551-z

Teixeira, A., Cortes, R.M.V., 2006. Diet of stocked and wild trout, Salmo trutta: Is there competition for resources? Folia Zool. 55, 61–73.

Teixeira, A., Cortes, R.M.V., Oliveira, D., 2006. Habitat use by native and stocked trout (Salmo trutta L.) in two northeast streams, Portugal. BFPP - Bull. Fr. la Pech. la Prot. des Milieux Aquat. 1–18. https://doi.org/10.1051/kmae:2006004

Teixeira, A., Fonseca, T., Oliveira, J., 2013. Melhoria de habitat e repovoamentos Ppiscícolas em rios de aAptidão salmonícola do Nordeste de Portugal. Silva Lusit. 21, 185–200.

Wallace Jr., R.K., 1981. An assessment of diet-overlap indexes. Trans. Am. Fish. Soc. 110, 72–76.

Wipfli, M.S., Musslewhite, J., 2004. Density of red alder (Alnus rubra) in headwaters influences invertebrate and detritus subsidies to downstream fish habitats in Alaska. Hydrobiologia 520, 153–163. https://doi.org/10.1023/B:HYDR.0000027734.95586.24

4. General discussion

The current spread of the alder disease and the consequential decay of alder in the Sabor and Baceiro rivers in the Montesinho Natural Park, resulted in considerable hydromorphological and biological changes. Hydromorphological quality decreased and fish communities changes showed similar patterns to alder decay suggesting the importance of alder in the riparian context and river functioning. Furthermore, although brown trout was the dominant species in both rivers, it showed only fair to good overall condition possibly resulting at least in part from the current environmental context including alder decay. Where alder decay was high, brown trout also decreased the consumption of terrestrial origin insects and used more cover features. Changes in habitat use were not related with microhabitat availability, being likely reflecting predatory risk and prey changes associated with alder decay.

Alder is a key component of the landscape, reaching large sizes and considerable canopies (Houston Durrant et al., 2016), and dominating the riparian galleries of the Montesinho Natural Park (Castro et al., 2010). Alder decline implies significant changes in riparian structure and lower levels of hydromorphological quality of rivers as already observed in this study.

Composition, abundance and integrity of benthic macroinverbrate communities were still little impacted by alder decay in the natural park, probably due to river connectivity and matter exchange from upper stream zones. Although local allochthonous matter inputs may have decreased, healthy upstream areas may provide enough matter input to sustain benthic macroinvertebrates, including shredders, mainly Trichoptera and Plecoptera, in areas more exposed to alder disease. However, this may not be the case of drifting and terrestrial origin prey which were not evaluated here. As a key prey for brown trout and given its strong connection with riparian vegetation, terrestrial origin prey can also be sensitive species of the ecological quality of the ecosystem, encouraging future studies to include evaluations of prey inputs from canopies (see Wipfli and Musslewhite, 2004),

Overall quality of fish communities was high, but brown trout condition ranged between fair and good, with very few individuals in excellent condition. This likely reflects the influence of multiple factors including alder decay. Because populations occurring at the limit of the distribution range, are more susceptible to environmental changes and local extinction (Almodóvar et al., 2011; Collares-Pereira et al., 2021), the lack of individuals in excellent condition should be dully considered in brown trout management, considering the development of sustainable measures, including conservation and exploitation (recreational fisheries) purposes.

Brown trout diet mainly consisted of Ephemeroptera, Diptera and terrestrial origin prey, followed by Trichoptera and Plecoptera. However, alder decay caused significant shifts in brown trout diet with decrease in terrestrial origin prey consumption being the most noteworthy. Likely related to this, there was an increase in the consumption of other prey types including Trichoptera. However, in the future, with the spread of alder disease throughout the river, it is predictable that allochthonous matter and detritus input will decrease, which can impact shredders like Trichoptera, further impacting brown trout diet.

Besides the changes in diet, microhabitat use by brown trout also changed with alder decay. These changes were however unrelated with variations in habitat availability and did not follow the patterns expected with alder decay, being likely influenced by other factors. Alder infected with *Phytophthora* can remain alive for long periods of time (Bjelke et al., 2016), and thus total depth, water current and substrate availability may only be significantly impacted in the latter stages of the disease when root decay is more predominant and bank stability is compromised. Nevertheless, in sites where alder decay was more severe, brown trout used more cover structures, possibly reflecting the increase of predation by large on small brown trout and the increased consumption of Trichoptera which are commonly found in cobbles and boulders (Bouchard, 2004), which were highly used by brown trout as cover.

In conclusion, alder disease is recent in northern Portugal and does not yet imply substantial ecological impacts in river structure and biological communities. However, the decay of alder has already led to some changes in riparian quality and brown trout ecology. With disease advancement, negative consequences may grow and threaten sensitive species and habitats. This study shed a light on how changes that may be expected to occur and points to the importance of understanding ecological relationships between communities and infectious diseases, providing guidelines for future studies.

Because current impacts of the alder decay in the Montesinho National Park were not severe, mitigation measures might still be implemented to control its consequences in this region. Specifically, coppicing or removal of infected trees and replacement by similar species using suitable techniques is highly recommended. Although species like willow and ash trees do cannot fully perform the services provided by alder, they may help maintain riparian gallery functions (Bjelke et al., 2016). Furthermore, identification of resistant alder could help predict areas where the disease will have lower and higher impacts on local communities, allowing for a more effective management and better use of resources. Finally, additional factors known to contribute to increase of disease spread should also be considered. Specifically, establishment of natural flow regime is highly recommended (Rodríguez González et al., 2021), given in regulated river, temporary floods can increase alder vulnerability to the Alder disease. Finally, monitoring of local populations of brown trout is essential, and it will serve as an indicator of the effectiveness of the active management.

5. References

Alexander, H.M., Mauck, K.E., Whitfield, A.E., Garrett, K.A., Malmstrom, C.M., 2014. Plant-virus interactions and the agro-ecological interface. Eur. J. Plant Pathol. 138, 529–547. https://doi.org/10.1007/s10658-013-0317-1

Almodóvar, A., Nicola, G.G., Ayllón, D., Elvira, B., 2011. Global warming threatens the persistence of Mediterranean brown trout. Glob. Chang. Biol. 18, 1549–1560. https://doi.org/10.1111/j.1365-2486.2011.02608.x

Bjelke, U., Boberg, J., Oliva, J., Tattersdill, K., Mckie, B.G., 2016. Dieback of riparian alder caused by the Phytophthora alni complex: Projected consequences for stream ecosystems. Freshw. Biol. 61, 565–579. https://doi.org/10.1111/fwb.12729

Bouchard, R.W.J., 2004. Guide to Aquatic Invertebrates of the Upper Midwest, Water Resources Center. University of Minnesota, St. Paul. https://doi.org/10.1525/9780520320390-009

Brasier, C.M., Rose, J., Gibbs, J.N., 1995. An unusual Phytophthora associated with widespread alder mortality in Britain. Plant Pathol. 44, 999–1007.

Castro, J., De Figueiredo, T., Fonseca, F., Castro, J.P., Nobre, S., Pires, L.C., 2010. Montesinho Natural Park: General description and natural values. Nat. Herit. from East to West Case Stud. from 6 EU Ctries. 119–132. https://doi.org/10.1007/978-3-642-01577-9_15

Cheatham, M.R., Rouse, M.N., Esker, P.D., Ignacio, S., Pradel, W., Raymundo, R., Sparks, A.H., Forbes, G.A., Gordon, T.R., Garrett, K.A., 2009. Beyond yield: Plant disease in the context of ecosystem services. Phytopathology 99, 1228–1236. https://doi.org/10.1094/PHYTO-99-11-1228

Collares-Pereira, M.J. (coord.), Alves, M.J., Ribeiro, F., Domingos, I., Almeida, P.R., da Costa, P., Gante, H., Filipe, A.F., Aboim, M.A., Rodrigues, P.M., Magalhães, M.F., 2021. Guia dos Peixes de Água Doce e Migradores de Portugal Continental. Edições Afrontamento, Porto.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G., Brasier, C.M., 2000. A molecular phylogeny of phytophthora and related oomycetes. Fungal Genet. Biol. 30, 17–32. https://doi.org/10.1006/fgbi.2000.1202

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biol. Rev. Camb. Philos. Soc. 81, 163–182. https://doi.org/10.1017/S1464793105006950

Fry, W.E., Grünwald, N.J., 2010. Introduction to Oomycetes [WWW Document]. Plant Heal. Instr. https://doi.org/10.1094/PHI-I-2010-1207-01 (accessed 14 January 2021)

Gibbs, John, Cech, T., Jung, T., Streito, J.-C., 2003. Field studies on dissemination of the alder Phytophthora and disease development, in: Gibbs, J., van Dijk, C., Webber, J. (Eds.), Phytophthora Disease of Alder in Europe. Forestry Commission, Edinburgh, pp. 55–64.

Gozlan, R.E., Karimov, B.K., Zadereev, E., Kuznetsova, D., Brucet, S., 2019. Status, trends, and future dynamics of freshwater ecosystems in Europe and Central Asia. Inl. Waters 9, 78–94. https://doi.org/10.1080/20442041.2018.1510271

Grêt-Regamey, A., Brunner, S.H., Kienast, F., 2012. Mountain ecosystem services: Who cares? Mt. Res. Dev. 32. https://doi.org/10.1659/MRD-JOURNAL-D-10-00115.S1

Grizzetti, B., Lanzanova, D., Liquete, C., Reynaud, A., Cardoso, A.C., 2016. Assessing water ecosystem services for water resource management. Environ. Sci. Policy 61, 194–203. https://doi.org/10.1016/j.envsci.2016.04.008

Houston Durrant, T., Rigo, Daniele, Caudullo, Giovanni, 2016. Alnus glutinosa in Europe: distribution, habitat, usage and threats, in: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds. . (Eds.), European Atlas of Forest Tree Species. European Publication Office, Luxembourg, pp. 64–65. https://doi.org/10.2788/4251

Jung, T., Downing, M., Blaschke, M., Vernon, T., 2007. Phytophthora root and collar rot of Alders caused by the invasive Phytophthora alni: actual distribution, pathways, and modeled potential distribution in Bavaria. Alien Invasive Species Int. Trade 10, 10–18.

Kanoun-Boulé, M., Vasconcelos, T., Gaspar, J., Vieira, S., Dias-Ferreira, C., Husson, C., 2016. Phytophthora ×alni and Phytophthora lacustris associated with common alder decline in Central Portugal. For. Pathol. 46, 174–176. https://doi.org/10.1111/efp.12273

Lake, P., Palmer, M., Biro, P., Cole, J., Covich, A., Dahm, C., Gibert, J., Goedkoop, W., Martens, K., Verhoeven, J., 2000. Global change and the biodiversity of freshwater ecosystems: impacts on linkages between above-sediment and sediment biota. Bioscience 50, 1099–1107.

Malmqvist, B., Rundle, S., 2002. Threats to the running water ecosystems of the world. Environ. Conserv. 29, 134–153. https://doi.org/10.1017/S0376892902000097

Millenium Ecosystem Assessment, 2005. Ecosystems and human well-being: wetlands and water. World Resources Institute, Washington, DC.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol. Rev. 94, 849–873. https://doi.org/10.1111/brv.12480

Rodríguez González, P.M., Jung, T., Jansson, R., Dufour, S., Cupertino, A., Gomes Marques, I., Vieites Blanco, C., 2021. Riparian Forests - Restoration perspectives under biotic , abiotic and social pressures.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L.R., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science (80-.). 287, 1770–1774. https://doi.org/10.1126/science.287.5459.1770

Wipfli, M.S., Musslewhite, J., 2004. Density of red alder (Alnus rubra) in headwaters influences invertebrate and detritus subsidies to downstream fish habitats in Alaska. Hydrobiologia 520, 153–163. https://doi.org/10.1023/B:HYDR.0000027734.95586.24

6. Annexes

Table 6.1 Absolute and relative (in parenthesis) abundance of each available prey in Sabor river. (1/3)

Prey group	Sites					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Ephemeroptera						
BAETIDAE	38 (0.10)	206 (0.07)	70 (0.08)	42 (0.03)	17 (0.02)	20 (0.08)
CAENIDAE		1 (0.00)				2 (0.00)
EPHEMERELLIDAE	4 (0.01)	86 (0.03)	55 (0.06)	249 (0.22)	28 (0.04)	
HEPTAGENIIDAE		214 (0.08)	89 (0.10)	34 (0.03)	73 (0.12)	5 (0.02)
LEPTOPHLEBIIDAE		144 (0.05)	9 (0.01)	42 (0.03)	150 (0.24)	17 (0.07)
Plecoptera						
LEUCTRIDAE	32 (0.09)	426 (0.16)	273 (0.32)	171 (0.15)	126 (0.20)	76 (0.32)
PERLIDAE	21 (0.06)	137 (0.05)	58 (0.06)	15 (0.01)	6 (0.00)	1 (0.00)
Trichoptera						
BERAEIDAE		5 (0.00)	3 (0.00)		3 (0.00)	
CALAMOCERATIDAE	1 (0.00)	4 (0.00)				
GLOSSOSOMATIDAE			8 (0.00)			
GOERIDAE		3 (0.00)				
HYDROPSYCHIDAE	10 (0.02)	312 (0.11)	42 (0.05)	19 (0.01)	15 (0.02)	
HYDROPTILIDAE		3 (0.00)	3 (0.00)	4 (0.00)	5 (0.00)	
LEPTOCERIDAE		2 (0.00)		70 (0.06)	11 (0.01)	
PHRYGANEIDAE			2 (0.00)			
POLYCENTROPODIDAE		4 (0.00)				9 (0.03)
PSYCHOMYIIDAE						1 (0.00)
SERICOSTOMATIDAE	7 (0.02)	10 (0.00)	1 (0.00)	8 (0.00)	1 (0.00)	

Table 6.1 Absolute and relative (in parenthesis) abundance of each available prey in Sabor river. (2/3)

Prey group			**Sites**			
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Diptera						
ANTHOMYIIDAE				1 (0.00)		
ATHERICIDAE	15 (0.04)	22 (0.00)	15 (0.01)	16 (0.01)	17 (0.02)	3 (0.01)
CERATOPOGONIDAE	1 (0.00)	7 (0.00)				
CHIRONOMIDAE	71 (0.20)	229 (0.08)	25 (0.02)	67 (0.05)	20 (0.03)	37 (0.15)
DIXIDAE		28 (0.01)				
LIMONIIDAE	3 (0.00)	2 (0.00)			9 (0.01)	
SIMULIIDAE	1 (0.00)	48 (0.01)	55 (0.06)	24 (0.02)	10 (0.01)	2 (0.00)
TABANIDAE	2 (0.00)	8 (0.00)		7 (0.00)		1 (0.00)
TIPULIDAE				1 (0.00)		1 (0.00)
Coleoptera						
CURCULIONIDAE		2 (0.00)	1 (0.00)			
DYTISCIDAE	13 (0.03)		1 (0.00)	1 (0.00)		
ELMIDAE	73 (0.20)	520 (0.19)	69 (0.08)	59 (0.05)	45 (0.07)	2 (0.00)
GYRINIDAE	7 (0.02)	37 (0.01)	2 (0.00)	2 (0.00)	8 (0.01)	
HALIPLIDAE						2 (0.00)
HYDRAENIDAE	26 (0.07)	39 (0.01)	7 (0.00)	1 (0.00)		
Odonata						
AESHNIDAE	3 (0.00)	6 (0.00)	8 (0.00)	1 (0.00)	3 (0.00)	
CALOPTERYGIDAE	1 (0.00)	41 (0.01)	6 (0.00)	22 (0.01)	9 (0.01)	
COENAGRIONIDAE						3 (0.01)
CORDULEGASTRIDAE	3 (0.00)	7 (0.00)	1 (0.00)	1 (0.00)		
GOMPHIDAE		3 (0.00)	3 (0.00)	54 (0.04)	23 (0.03)	10 (0.04)

Table 6.1 Absolute and relative (in parenthesis) abundance of each available prey in Sabor river. (3/3)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Heteroptera						
CORIXIDAE				11 (0.00)		
GERRIDAE	2 (0.00)	10 (0.00)	2 (0.00)			3 (0.01)
NAUCORIDAE				1 (0.00)		
NOTONECTIDAE				3 (0.00)		1 (0.00)
Hirudinea						
ERPOBDELLIDAE			1 (0.00)	57 (0.05)	1 (0.00)	5 (0.02)
GLOSSIPHONIIDAE			1 (0.00)	1 (0.00)		
Gastropoda						
ANCYLIDAE		49 (0.01)	3 (0.00)	2 (0.00)	8 (0.01)	4 (0.01)
LYMNAEIDAE		2 (0.00)	3 (0.00)	48 (0.04)	2 (0.00)	
PHYSIDAE				51 (0.04)		4 (0.01)
Bivalvia						
SPHAERIIDAE	3 (0.00)		2 (0.00)	7 (0.00)		1 (0.00)
Crustacea						
ASTACIDAE						3 (0.01)
Turbellaria						
PLANARIIDAE					1 (0.00)	
Pupae						
	6 (0.01)	28 (0.01)	2 (0.00)	7 (0.00)	5 (0.00)	
Terrestrial origin prey						
	3 (0.00)	3 (0.00)	11 (0.01)	9 (0.00)	4 (0.00)	
Anfibian						
	1 (0.00)					
Fish						
		1 (0.00)	1 (0.00)	9 (0.00)	1 (0.00)	
Other						
	2 (0.00)	1 (0.00)	2 (0.00)	13 (0.01)	6 (0.00)	23 (0.09)

Table 6.2 Absolute and relative (in parenthesis) abundance of each available prey in Baceiro river. (1/3)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Ephemeroptera						
BAETIDAE	27 (0.04)	55 (0.02)	161 (0.11)	28 (0.00)	271 (0.15)	9 (0.03)
CAENIDAE						
EPHEMERELLIDAE		42 (0.02)	86 (0.06)	236 (0.06)	146 (0.08)	6 (0.02)
HEPTAGENIIDAE	2 (0.00)	210 (0.11)	155 (0.10)	148 (0.03)	139 (0.08)	6 (0.02)
LEPTOPHLEBIIDAE	90 (0.16)	548 (0.28)	130 (0.09)	399 (0.10)	238 (0.13)	64 (0.23)
Plecoptera						
LEUCTRIDAE	133 (0.24)	268 (0.14)	82 (0.05)	336 (0.08)	282 (0.16)	49 (0.18)
PERLIDAE	15 (0.02)	60 (0.03)	185 (0.13)	200 (0.05)	141 (0.08)	17 (0.06)
Trichoptera						
BERAEIDAE	4 (0.00)	11 (0.00)	14 (0.00)	2 (0.00)	3 (0.00)	
GLOSSOSOMATIDAE		27 (0.01)		1 (0.00)		
GOERIDAE	2 (0.00)	4 (0.00)		10 (0.00)		
HYDROPSYCHIDAE	11 (0.01)	5 (0.00)	31 (0.02)	617 (0.15)	67 (0.03)	2 (0.00)
HYDROPTILIDAE		1 (0.00)	1 (0.00)	2 (0.00)		
LEPTOCERIDAE	11 (0.01)	1 (0.00)	2 (0.00)			2 (0.00)
PHRYGANEIDAE	11 (0.01)		4 (0.00)	1 (0.00)		
POLYCENTROPODIDAE		8 (0.00)	17 (0.01)	6 (0.00)	3 (0.00)	21 (0.07)
RHYACOPHILIDAE				3 (0.00)	3 (0.00)	
SERICOSTOMATIDAE	4 (0.00)	27 (0.01)	13 (0.00)	15 (0.00)	2 (0.00)	
UENOIDAE				3 (0.00)		

Table 6.2 Absolute and relative (in parenthesis) abundance of each available prey in Baceiro river. (2/3)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Diptera						
ATHERICIDAE	2 (0.00)	20 (0.01)	5 (0.00)	8 (0.00)	5 (0.00)	
CERATOPOGONIDAE		28 (0.01)	10 (0.00)	13 (0.00)	7 (0.00)	
CHIRONOMIDAE	86 (0.15)	143 (0.07)	215 (0.15)	1206 (0.31)	230 (0.13)	24 (0.08)
DIXIDAE				2 (0.00)	3 (0.00)	5 (0.01)
DOLICHOPODIDAE			1 (0.00)			
EMPIDIDAE					1 (0.00)	
LIMONIIDAE		14 (0.00)	2 (0.00)	24 (0.00)	19 (0.01)	4 (0.01)
SIMULIIDAE			64 (0.04)	23 (0.00)	4 (0.00)	
TABANIDAE	1 (0.00)	3 (0.00)	4 (0.00)	5 (0.00)	3 (0.00)	
TIPULIDAE				1 (0.00)		
Coleoptera						
DYTISCIDAE	4 (0.00)		3 (0.00)	5 (0.00)		
ELMIDAE	74 (0.13)	262 (0.13)	113 (0.07)	212 (0.05)	84 (0.04)	35 (0.12)
GYRINIDAE	5 (0.00)	26 (0.01)		119 (0.03)	4 (0.00)	
HALIPLIDAE	3 (0.00)			4 (0.00)		
HYDRAENIDAE		21 (0.01)	25 (0.01)	43 (0.01)	8 (0.00)	
HYDROCHIDAE	1 (0.00)					
Odonata						
AESHNIDAE	1 (0.00)		1 (0.00)	2 (0.00)		4 (0.01)
CALOPTERYGIDAE	3 (0.00)	23 (0.01)	31 (0.02)	38 (0.00)	4 (0.00)	3 (0.01)
CORDULEGASTRIDAE	1 (0.00)	2 (0.00)				
GOMPHIDAE			1 (0.00)	4 (0.00)	2 (0.00)	1 (0.00)

Table 6.2 Absolute and relative (in parenthesis) abundance of each available prey in Baceiro river. (3/3)

Prey group	Sites					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Heteroptera						
CORIXIDAE		33 (0.01)				1 (0.00)
GERRIDAE	29 (0.05)		1 (0.00)		2 (0.00)	
HYDROMETRIDAE					1 (0.00)	
NOTONECTIDAE					1 (0.00)	
Hirudinea						
ERPOBDELLIDAE	2 (0.00)	2 (0.00)		10 (0.00)		1 (0.00)
Gastropoda						
ANCYLIDAE		16 (0.00)	7 (0.00)		11 (0.00)	1 (0.00)
Crustacea						
CAMBARIDAE		1 (0.00)	1 (0.00)			1 (0.00)
Turbellaria						
PLANARIIDAE			3 (0.00)	44 (0.01)		
Pupae						
	7 (0.01)	26 (0.01)	31 (0.02)	86 (0.02)	30 (0.01)	11 (0.04)
Terrestrial origin prey						
	15 (0.02)	6 (0.00)	15 (0.01)	6 (0.00)	8 (0.00)	4 (0.01)
Anfibian						
	5 (0.00)					
Other						
	3 (0.00)	11 (0.00)	2 (0.00)	7 (0.00)	2 (0.00)	

Table 6.3 Absolute and relative (in parenthesis) abundance of each consumed prey in Sabor river. (1/4)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Ephemeroptera						
BAETIDAE	7 (0.00)	123 (0.07)	42 (0.16)	13 (0.02)	46 (0.06)	45 (0.09)
CAENIDAE				8 (0.01)		
EPHEMERELLIDAE		5 (0.00)		1 (0.00)	1 (0.00)	
HEPTAGENIIDAE		7 (0.00)	15 (0.06)	2 (0.00)	6 (0.00)	2 (0.00)
LEPTOPHLEBIIDAE	752 (0.62)	93 (0.05)		13 (0.02)	11 (0.01)	1 (0.00)
Plecoptera						
CAPNIIDAE				1 (0.00)		
LEUCTRIDAE	6 (0.00)	9 (0.00)	9 (0.03)	4 (0.00)	40 (0.05)	7 (0.01)
NEMOURIDAE	1 (0.00)	2 (0.00)	1 (0.00)		2 (0.00)	
PERLIDAE					1 (0.00)	
Trichoptera						
BRACHYCENTRIDAE		7 (0.00)	3 (0.01)		15 (0.02)	2 (0.00)
CALAMOCERATIDAE	5 (0.00)		4 (0.01)	10 (0.02)	104 (0.14)	
GLOSSOSOMATIDAE		4 (0.00)		1 (0.00)		1 (0.00)
GOERIDAE		5 (0.00)			5 (0.00)	
HYDROPSYCHIDAE		11 (0.00)	1 (0.00)	1 (0.00)	2 (0.00)	1 (0.00)
HYDROPTILIDAE	1 (0.00)		5 (0.02)	11 (0.02)	14 (0.01)	4 (0.00)
LEPIDOSTOMATIDAE						
LEPTOCERIDAE	6 (0.00)	22 (0.01)	3 (0.01)	9 (0.01)	53 (0.07)	58 (0.11)
LIMNEPHILIDAE	10 (0.00)	4 (0.00)	7 (0.02)	5 (0.01)	6 (0.00)	
PHILOPOTAMIDAE						7 (0.01)

Table 6.3 Absolute and relative (in parenthesis) abundance of each consumed prey in Sabor river. (2/4)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Trichoptera						
POLYCENTROPODIDAE	6 (0.00)	7 (0.00)	2 (0.00)	2 (0.00)	15 (0.02)	1 (0.00)
PSYCHOMYIIDAE			2 (0.00)	8 (0.01)	6 (0.00)	4 (0.00)
RHYACOPHILIDAE	1 (0.00)	1 (0.00)	3 (0.01)	2 (0.00)	1 (0.00)	2 (0.00)
SERICOSTOMATIDAE	16 (0.01)	16 (0.00)	4 (0.01)		12 (0.01)	
UENOIDAE		12 (0.00)	3 (0.01)			
Diptera						
ATHERICIDAE	2 (0.00)	1 (0.00)			4 (0.00)	
BLEPHARICERIDAE		1 (0.00)			1 (0.00)	
CERATOPOGONIDAE		1 (0.00)	1 (0.00)			
CHIRONOMIDAE	222 (0.18)	49 (0.02)	57 (0.22)	18 (0.03)	11 (0.01)	11 (0.02)
CULICIDAE			1 (0.00)	1 (0.00)		
DIXIDAE	1 (0.00)		1 (0.00)		5 (0.00)	4 (0.00)
DOLICHOPODIDAE					1 (0.00)	
EMPIDIDAE	1 (0.00)					
LIMONIIDAE	1 (0.00)					
MUSCIDAE				2 (0.00)		1 (0.00)
PSYCHODIDAE		2 (0.00)				
SIMULIIDAE	1 (0.00)	5 (0.00)	29 (0.11)	13 (0.02)	6 (0.00)	18 (0.03)
TABANIDAE	1 (0.00)	1 (0.00)	1 (0.00)	1 (0.00)	1 (0.00)	
TIPULIDAE		1 (0.00)		1 (0.00)	5 (0.00)	4 (0.00)

Table 6.3 Absolute and relative (in parenthesis) abundance of each consumed prey in Sabor river. (3/4)

Prey group	Sites					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Coleoptera						
CURCULIONIDAE		3 (0.00)				1 (0.00)
DRYOPIDAE		10 (0.00)				
DYTISCIDAE	13 (0.01)		1 (0.00)	3 (0.00)	1 (0.00)	4 (0.00)
ELMIDAE		1 (0.00)	2 (0.00)	1 (0.00)	1 (0.00)	
GYRINIDAE		1 (0.00)	1 (0.00)			
HELOPHORIDAE						1 (0.00)
HYDRAENIDAE		2 (0.00)			1 (0.00)	
HYDROCHIDAE					1 (0.00)	
HYDROPHILIDAE		3 (0.00)			1 (0.00)	2 (0.00)
SCIRTIDAE					1 (0.00)	
Odonata						
AESHNIDAE		3 (0.00)		2 (0.00)	11 (0.01)	4 (0.00)
CALOPTERYGIDAE				4 (0.00)	2 (0.00)	1 (0.00)
COENAGRIONIDAE					1 (0.00)	
GOMPHIDAE		1 (0.00)		2 (0.00)	8 (0.01)	2 (0.00)
PLATYCNEMIDIDAE				1 (0.00)		
Heteroptera						
CORIXIDAE					4 (0.00)	2 (0.00)
GERRIDAE	4 (0.00)	2 (0.00)		5 (0.01)	10 (0.01)	13 (0.02)
Gastropoda						
ANCYLIDAE		21 (0.01)	1 (0.00)	8 (0.01)	82 (0.11)	23 (0.04)
LYMNAEIDAE				7 (0.01)	3 (0.00)	1 (0.00)
PHYSIDAE				27 (0.05)	1 (0.00)	

Table 6.3 Absolute and relative (in parenthesis) abundance of each consumed prey in Sabor river. (4/4)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Crustacea						
ASTACIDAE		1 (0.00)			2 (0.00)	1 (0.00)
CAMBARIDAE					1 (0.00)	
COROPHIIDAE					1 (0.00)	
Annelidae						
ERPOBDELLIDAE				9 (0.01)		1 (0.00)
Hydracarina						
		2 (0.00)	1 (0.00)	1 (0.00)	3 (0.00)	
Megaloptera						
SIALIDAE	6 (0.00)			1 (0.00)	4 (0.00)	
Nemathelmintha						
		3 (0.00)				1 (0.00)
Oligochaeta						
	2 (0.00)			1 (0.00)	1 (0.00)	1 (0.00)
Pupae						
	22 (0.01)	66 (0.03)	15 (0.06)	16 (0.03)	18 (0.02)	28 (0.05)
Terrestrial origin prey						
	119 (0.09)	1235 (0.70)	35 (0.14)	235 (0.51)	191 (0.26)	208 (0.42)
Teleostei						
	1 (0.00)	1 (0.00)		3 (0.00)	7 (0.00)	16 (0.03)
Other						
	2 (0.00)			1 (0.00)		2 (0.00)

Table 6.4 Absolute and relative (in parenthesis) abundance of each consumed prey in Baceiro river. (1/3)

Prey group	**Sites**					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Ephemeroptera						
BAETIDAE	5 (0.01)	44 (0.08)	44 (0.08)	21 (0.03)	161 (0.25)	99 (0.32)
EPHEMERELLIDAE			1 (0.00)			3 (0.00)
EPHEMERIDAE			1 (0.00)	1 (0.00)		
HEPTAGENIIDAE	1 (0.00)	7 (0.01)	7 (0.01)	9 (0.01)	82 (0.12)	40 (0.13)
LEPTOPHLEBIIDAE	195 (0.43)	2 (0.00)	38 (0.07)		8 (0.01)	4 (0.01)
Plecoptera						
LEUCTRIDAE	24 (0.05)	142 (0.27)	55 (0.11)	7 (0.01)	10 (0.01)	6 (0.01)
NEMOURIDAE	1 (0.00)	3 (0.00)			6 (0.00)	4 (0.01)
PERLIDAE		1 (0.00)			2 (0.00)	
PERLODIDAE						2 (0.00)
Trichoptera						
BERAEIDAE		2 (0.00)				
BRACHYCENTRIDAE	1 (0.00)	12 (0.02)	4 (0.00)		2 (0.00)	4 (0.01)
CALAMOCERATIDAE	7 (0.01)					
GLOSSOSOMATIDAE	1 (0.00)	1 (0.00)				
GOERIDAE	1 (0.00)					
HYDROPSYCHIDAE		2 (0.00)	1 (0.00)	6 (0.01)	10 (0.01)	5 (0.01)
HYDROPTILIDAE					12 (0.01)	8 (0.02)
LEPIDOSTOMATIDAE						1 (0.00)
LEPTOCERIDAE	1 (0.00)	2 (0.00)	5 (0.01)	1 (0.00)	5 (0.00)	5 (0.01)
LIMNEPHILIDAE	30 (0.06)	1 (0.00)		3 (0.00)		
POLYCENTROPODIDAE	4 (0.00)	3 (0.00)	1 (0.00)		16 (0.02)	5 (0.01)
PSYCHOMYIIDAE		3 (0.00)	2 (0.00)		64 (0.10)	3 (0.00)
RHYACOPHILIDAE	1 (0.00)				11 (0.01)	3 (0.00)
SERICOSTOMATIDAE	22 (0.04)	7 (0.01)	2 (0.00)			

Table 6.4 Absolute and relative (in parenthesis) abundance of each consumed prey in Baceiro river. (2/3)

Prey group	Sites					
	S1	S2	S3	S4	S5	S6
Diptera						
ATHERICIDAE			2 (0.00)	1 (0.00)		
BLEPHARICERIDAE		1 (0.00)			1 (0.00)	
CERATOPOGONIDAE					1 (0.00)	1 (0.00)
CHIRONOMIDAE	47 (0.10)	91 (0.17)	60 (0.12)	148 (0.26)	76 (0.11)	8 (0.02)
DIXIDAE			3 (0.00)	1 (0.00)		
EMPIDIDAE					1 (0.00)	
MUSCIDAE			1 (0.00)		1 (0.00)	1 (0.00)
PSYCHODIDAE					3 (0.00)	1 (0.00)
SIMULIIDAE		6 (0.01)	35 (0.07)	58 (0.10)	47 (0.07)	8 (0.02)
TABANIDAE	1 (0.00)					
THAUMALEIDAE					2 (0.00)	
TIPULIDAE		1 (0.00)	2 (0.00)			
Coleoptera						
DRYOPIDAE	1 (0.00)	8 (0.01)			1 (0.00)	
DYTISCIDAE	4 (0.00)	1 (0.00)		1 (0.00)		
ELMIDAE					1 (0.00)	
GYRINIDAE		2 (0.00)		2 (0.00)		
HELOPHORIDAE	1 (0.00)					
HYDRAENIDAE					1 (0.00)	
HYDROPHILIDAE						1 (0.00)

Table 6.4 Absolute and relative (in parenthesis) abundance of each consumed prey in Baceiro river. (3/3)

Prey group	**Sites**					
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Odonata						
AESHNIDAE			1 (0.00)		1 (0.00)	
CALOPTERYGIDAE			1 (0.00)			
GOMPHIDAE		1 (0.00)				
LESTIDAE			2 (0.00)			
Heteroptera						
GERRIDAE	1 (0.00)			5 (0.00)	2 (0.00)	
Gastropoda						
ANCYLIDAE	14 (0.03)	7 (0.01)	105 (0.21)		5 (0.00)	3 (0.00)
Bivalvia						
SPHAERIIDAE	6 (0.01)					
Crustacea						
ASTACIDAE					15 (0.02)	18 (0.05)
CAMBARIDAE		5 (0.00)	5 (0.01)		2 (0.00)	
Hydracarina						
		1 (0.00)	2 (0.00)	1 (0.00)		
Isopoda						
ASELLIDAE					1 (0.00)	
Megaloptera						
SIALIDAE	4 (0.00)					
Nemathelmintha						
		4 (0.00)				
Neuroptera						
SISYRIDAE			1 (0.00)	1 (0.00)		
Pupae						
	4 (0.00)	49 (0.09)	25 (0.05)	200 (0.35)	6 (0.00)	7 (0.02)
Terrestrial origin prey						
	76 (0.16)	107 (0.20)	87 (0.17)	90 (0.16)	79 (0.12)	64 (0.21)
Other						
		1 (0.00)				

Table 6.5 Estimates of chi-square goodness-of-fit tests between sites for each microhabiat variable availability (except water current) for Sabor (S) and Baceiro (B) river. DS and SS stand for dominant and subdominant substrate, respectively. Non-significant values are shown as "-".

S	Site	Total depth	DS	SS	Cover	B	Site	Total depth	DS	SS	Cover
	S1 vs. S2	-	-	-	-		**B1 vs. B2**	-	-	-	-
	S1 vs. S3	18.9	64.4	31.6	45		**B1 vs. B3**	68.2	75.9	34.4	27.1
	S1 vs. S4	37.9	172	97.5	117		**B1 vs. B4**	74.7	110	58.2	39.7
	S1 vs. S5	53.9	206	130	145		**B1 vs. B5**	84.5	203	89	62.8
	S1 vs. S6	67.4	366	193	197		**B1 vs. B6**	91.9	249	111	78.3
	S2 vs. S3	16.5	43.5	17.3	29.5		**B2 vs. B3**	37.4	54.9	29.1	19.8
	S2 vs. S4	33.9	131	60.5	96.6		**B2 vs. B4**	40.1	77.5	47.6	26.6
	S2 vs. S5	52.5	172	81.6	121		**B2 vs. B5**	47.5	173	77.7	50.4
	S2 vs. S6	68.9	310	147	167		**B2 vs. B6**	52.6	213	97.4	64.1
	S3 vs. S4	-	46	14.4	27.2		**B3 vs. B4**	-	-	-	-
	S3 vs. S5	47.3	101	27.2	63.6		**B3 vs. B5**	-	136	61.5	32.2
	S3 vs. S6	66.6	213	83.9	107		**B3 vs. B6**	-	167	79.1	44.6
	S4 vs. S5	30.5	47.4	14.2	51.2		**B4 vs. B5**	-	83.5	39.6	22.3
	S4 vs. S6	48	138	74.6	95.8		**B4 vs. B6**	-	100	49.8	28.4
	S5 vs. S6	11.1	31.9	64.9	12.4		**B5 vs. B6**	-	-	-	-

Table 6.6 Estimates of chi-square goodness-of-fit tests between use by each brown trout size class and availability for each microhabitat variable (except water current) and site in Sabor river. DS and SS stand for dominant and subdominant substrate, respectively. Non-significant values are shown as "-". Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

Riparian Condition Zones	Location	Total depth	DS	SS	Cover
Small trout A ≤ 130 mm					
GI	**S1**	-	14.4	24	-
	S2	-	-	15.4	-
SD	**S3**	9.26	-	-	-
	S4	27.5	29.4	17.97	19.5
DR	**S5**	-	31	12.3	-
	S6	7.95	-	-	15.6
Medium trout B 130 < TL < 200 mm					
GI	**S1**	16.2	15	23.9	18.48
	S2	13.6	9.51	20.2	-
SD	**S3**	45.5	11.1	13.6	11.6
	S4	77.7	76.5	29.53	39.5
DR	**S5**	18.4	62	32.7	13.2
	S6	65	18.4	-	11.5
Large trout C ≥ 200 mm					
GI	**S1**	-	-	-	12.36
	S2	15.8	-	-	-
SD	**S3**	29.2	-	-	25.6
	S4	31.4	40.48	25	18.1
DR	**S5**	34.2	61.7	30.5	15.5
	S6	54.3	19.8	-	57.2

Table 6.7 Estimates of chi-square goodness-of-fit tests between use by each brown trout size class and availability for each microhabitat variable (except water current) and site in Baceiro river. DS and SS stand for dominant and subdominant substrate, respectively. Non-significant values are shown as "-". Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

Riparian Condition Zones	Location	Total depth	DS	SS	Cover
Small trout A ≤ 130 mm					
GI	**B1**	33.6	39.6	53.1	18.4
	B2	44.8	37.9	-	-
SD	**B3**	12.8	-	15.6	29.6
	B4	33	21.2	35.3	18.9
DR	**B5**	-	27.8	16.1	-
	B6	-	51.7	35.9	17.1
Medium trout B 130 < TL < 200 mm					
GI	**B1**	56.9	26.3	15.7	19
	B2	34.08	52.7	35.5	12.4
SD	**B3**	18	11.9	-	14.8
	B4	-	-	-	-
DR	**B5**	16.7	-	-	23.4
	B6	32.4	-	-	-
Large trout C ≥ 200 mm					
GI	**B1**	81.4	38.7	35.5	19.3
	B2	55	36	13.7	24.9
SD	**B3**	16.2	-	-	20.2
	B4	-	-	-	-
DR	**B5**	21.3	-	-	-
	B6	28.6	12.2	-	29.2

Table 6.8 Total depth use estimates of chi-square goodness-of-fit tests between sites for each size class in Sabor (S) and Baceiro (B) river. Non-significant values are shown as "-".

S

Site	Class		
	A	B	C
S1 vs. S2	-	9.1	-
S1 vs. S3	10.2	18.6	-
S1 vs. S4	27	-	-
S1 vs. S5	30.1	26.8	36.8
S1 vs. S6	38.9	41.3	43.4
S2 vs. S3	-	11	-
S2 vs. S4	18.2	-	-
S2 vs. S5	21.9	-	31.8
S2 vs. S6	29.7	35.9	38
S3 vs. S4	-	-	-
S3 vs. S5	15.9	-	27
S3 vs. S6	24	28.2	33
S4 vs. S5	10.6	-	23.3
S4 vs. S6	19.4	10.2	29.5
S5 vs. S6	10.1	11.7	-

B

Site	Class		
	A	B	C
B1 vs. B2	14.5	-	-
B1 vs. B3	19.8	-	-
B1 vs. B4	79.4	-	-
B1 vs. B5	90	26.8	29.9
B1 vs. B6	93.5	39.7	35.8
B2 vs. B3	-	-	-
B2 vs. B4	45.5	16.4	-
B2 vs. B5	55.3	23.3	21.9
B2 vs. B6	60.6	32.6	29.4
B3 vs. B4	35.6	-	-
B3 vs. B5	51.8	-	15.9
B3 vs. B6	56.8	22.3	21.8
B4 vs. B5	30	-	-
B4 vs. B6	38.6	-	-
B5 vs. B6	-	-	6.1

Table 6.9 Dominant substrate use estimates of chi-square goodness-of-fit tests between sites for each size class in Sabor (S) and Baceiro (B) river. Non-significant values are shown as "-".

S

Site	Class		
	A	B	C
S1 vs. S2	-	-	-
S1 vs. S3	-	21.5	-
S1 vs. S4	33.6	57.4	24.2
S1 vs. S5	46.5	88.4	45.8
S1 vs. S6	75.6	104	43
S2 vs. S3	-	10.2	-
S2 vs. S4	27	43.9	19.2
S2 vs. S5	38.6	73.8	37
S2 vs. S6	66.2	88.6	100
S3 vs. S4	14.8	28.4	-
S3 vs. S5	23.7	62.3	23.1
S3 vs. S6	44.3	75.5	34.9
S4 vs. S5	-	-	12.6
S4 vs. S6	31.2	43.8	71.9
S5 vs. S6	20	30.2	70.3

B

Site	Class		
	A	B	C
B1 vs. B2	-	-	-
B1 vs. B3	-	-	-
B1 vs. B4	-	-	-
B1 vs. B5	-	26.8	29.9
B1 vs. B6	37.8	39.7	35.8
B2 vs. B3	-	-	-
B2 vs. B4	-	16.4	-
B2 vs. B5	-	23.3	21.9
B2 vs. B6	32.2	32.6	29.4
B3 vs. B4	-	-	-
B3 vs. B5	-	-	15.9
B3 vs. B6	-	22.3	21.8
B4 vs. B5	12.1	-	-
B4 vs. B6	19.8	-	-
B5 vs. B6	-	-	6.1224

Table 6.10 Subdominant substrate use estimates of chi-square goodness-of-fit tests between sites for each size class in Sabor (S) and Baceiro (B) river. Non-significant values are shown as "-".

S

Site	Class		
	A	B	C
S1 vs. S2	-	-	-
S1 vs. S3	-	21.5	-
S1 vs. S4	27.5	57.4	24.2
S1 vs. S5	28.6	88.4	45.8
S1 vs. S6	53.6	104	43
S2 vs. S3	-	10.2	-
S2 vs. S4	24.5	43.9	19.2
S2 vs. S5	25.1	73.8	37
S2 vs. S6	48.5	88.6	100
S3 vs. S4	16.5	28.4	-
S3 vs. S5	18.3	62.3	23.1
S3 vs. S6	36.2	75.5	34.9
S4 vs. S5	7.98	-	12.6
S4 vs. S6	30.9	43.8	71.9
S5 vs. S6	13.4	30.2	70.3

B

Site	Class		
	A	B	C
B1 vs. B2	13.2	-	-
B1 vs. B3	29.2	-	-
B1 vs. B4	45.9	-	-
B1 vs. B5	49.1	26.8	29.9
B1 vs. B6	61.5	39.7	35.8
B2 vs. B3	-	-	-
B2 vs. B4	-	16.4	-
B2 vs. B5	-	23.3	21.9
B2 vs. B6	39.4	32.6	29.4
B3 vs. B4	-	-	-
B3 vs. B5	-	-	15.9
B3 vs. B6	26.1	22.3	21.8
B4 vs. B5	-	-	-
B4 vs. B6	17.2	-	-
B5 vs. B6	-	-	6.1224

Table 6.11 Cover use estimates of chi-square goodness-of-fit tests between sites for each size class in Sabor (S) and Baceiro (B) river. Non-significant values are shown as "-".

S

Site	Class		
	A	B	C
S1 vs. S2	-	-	-
S1 vs. S3	-	21.5	-
S1 vs. S4	22.6	57.4	24.2
S1 vs. S5	30.7	88.4	45.8
S1 vs. S6	35.2	104	43
S2 vs. S3	-	10.2	-
S2 vs. S4	13	43.9	19.2
S2 vs. S5	19.3	73.8	37
S2 vs. S6	22.2	88.6	100
S3 vs. S4	10.1	28.4	-
S3 vs. S5	15.7	62.3	23.1
S3 vs. S6	19.1	75.5	34.9
S4 vs. S5	-	-	12.6
S4 vs. S6	-	43.8	71.9
S5 vs. S6	9.6871	30.2	70.3

B

Site	Class		
	A	B	C
B1 vs. B2	-	-	-
B1 vs. B3	-	-	-
B1 vs. B4	29.3	-	-
B1 vs. B5	38.2	26.8	29.9
B1 vs. B6	58.6	39.7	35.8
B2 vs. B3	-	-	-
B2 vs. B4	-	16.4	-
B2 vs. B5	-	23.3	21.9
B2 vs. B6	43.3	32.6	29.4
B3 vs. B4	12.8	-	-
B3 vs. B5	21.4	-	15.9
B3 vs. B6	39.7	22.3	21.8
B4 vs. B5	-	-	-
B4 vs. B6	16.5	-	-
B5 vs. B6	27.478	-	6.1224

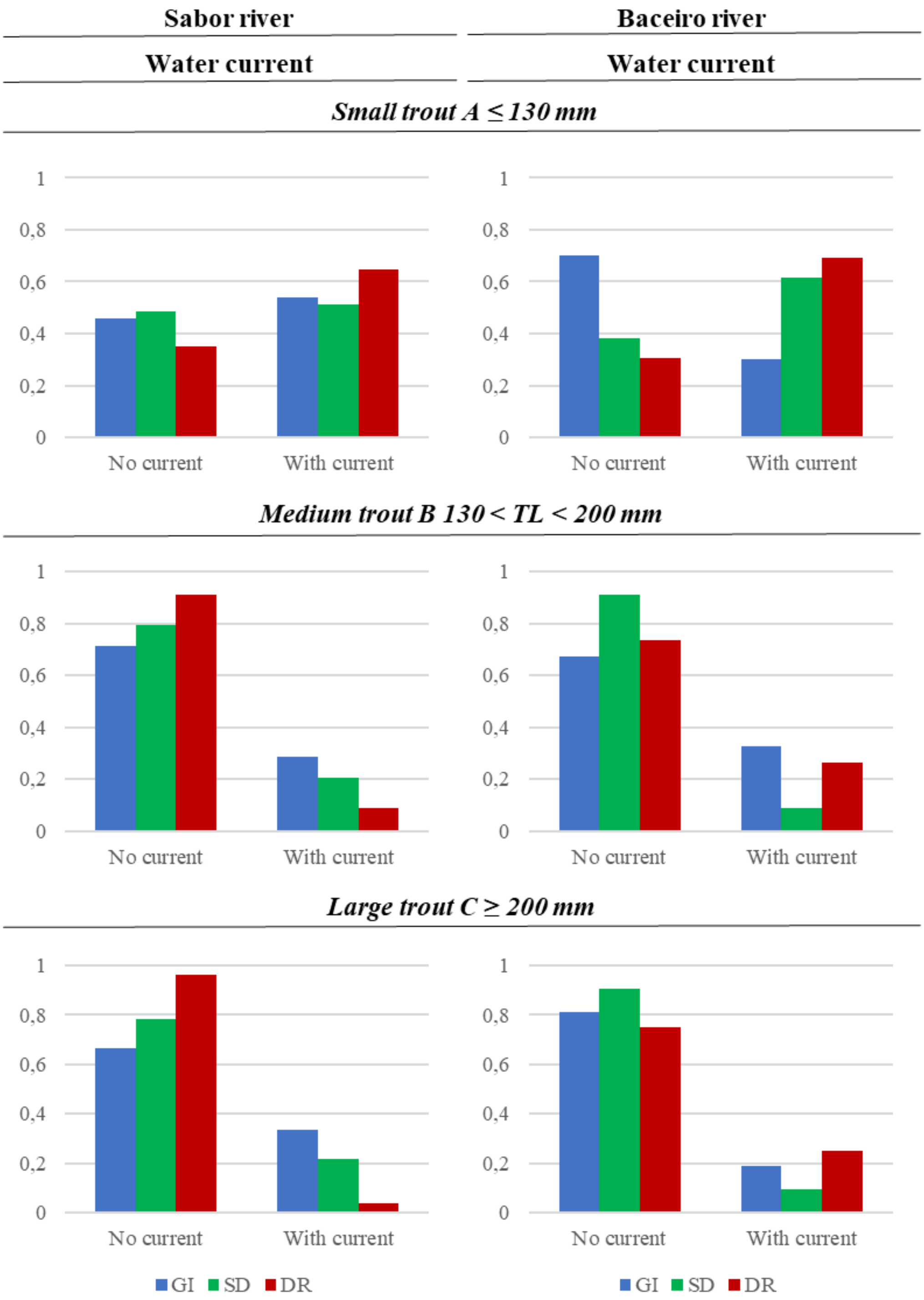

Figure 6.1 Water current use in Sabor (left) and Baceiro river (right). Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

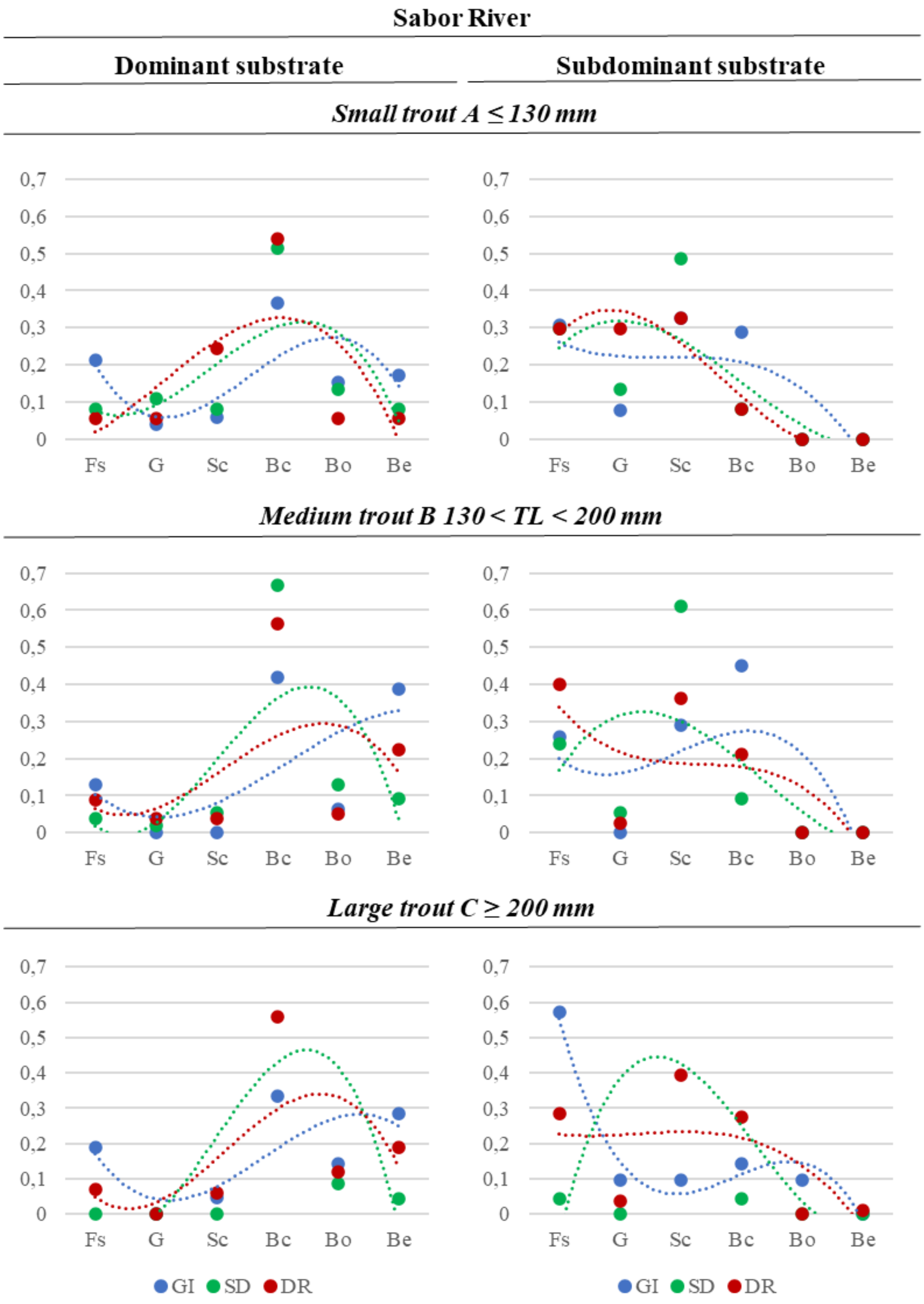

Figure 6.2 Dominant (left) and subdominant substrate (right) use in Sabor river. Variable classes are as described in Table 3.1. A third-degree polynomial regression line was fitted to better represent total depth data variation. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.

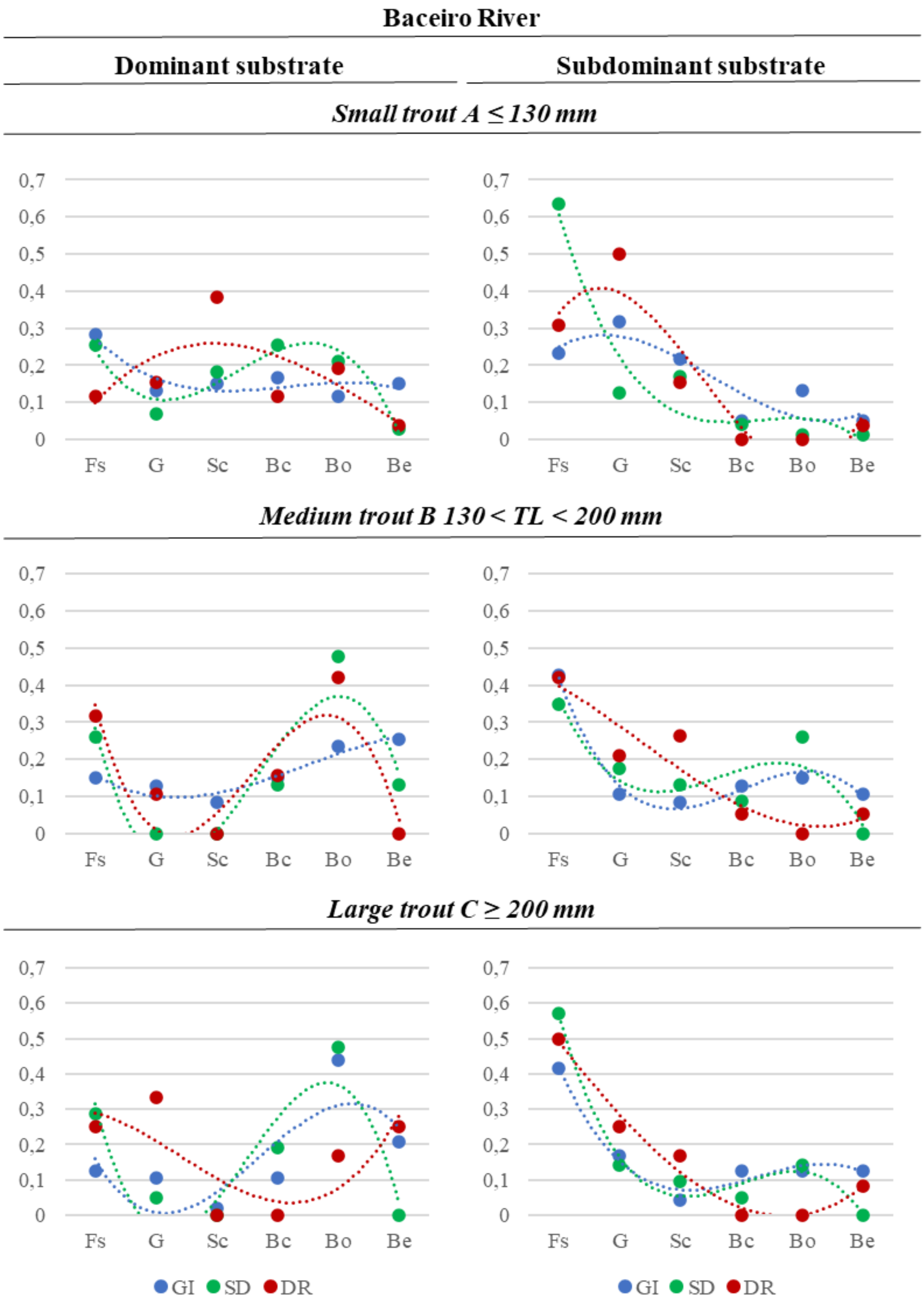

Figure 6.3 Dominant (left) and subdominant substrate (right) use in Baceiro river. Variable classes are as described in Table 3.1. A third-degree polynomial regression line was fitted to better represent total depth data variation. Riparian condition is defined as considering 3 defined riparian conditions: GI – Good integrity; SD – Signs of disease; DR – Dead riparian vegetation.