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Master thesis in Biodiversity, Ecology and Global Changes

Work made under supervision of:
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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Estará o lagostim sinal a afetar as comunidades de peixes em rios pristinos?

Resumo

Os ecossistemas de água doce fornecem inúmeros serviços essenciais para a nossa sociedade. No entanto, estão entre os ecossistemas mais ameaçados pelos seres humanos, incluindo a exploração excessiva de recursos, poluição, perda e fragmentação de habitats, mudanças climáticas e introdução de espécies não nativas. Este estudo teve como objetivo caracterizar as comunidades de peixes nas bacias dos Rios Rabaçal e Tuela (Parque Natural de Montesinho; Nordeste de Portugal), com foco nos possíveis impactos do lagostim sinal *Pacifastacus leniusculus.* Como omnívoros, os lagostins têm a capacidade de modificar significativamente o seu ambiente, o que, por sua vez, pode influenciar a dinâmica e o comportamento de vários grupos de organismos que compartilham o mesmo habitat, incluindo espécies de peixes. Neste estudo, foram amostrados 34 (18 locais invadidos e 16 não invadidos) nas bacias dos Rios Rabaçal e Tuela para avaliar possíveis diferenças na abundância, biomassa, riqueza e diversidade das comunidades de peixes, bem como entender quais os fatores abióticos e bióticos (por exemplo, presença do lagostim sinal) são responsáveis pelas possíveis diferenças. A caracterização da condição fisiológica das espécies amostradas também foi realizada, assim como a caracterização da dieta da truta *Salmo trutta*. Com o presente estudo esperava-se encontrar evidências do impacto negativo da presença do lagostim sinal nas comunidades de peixes. Esse não foi o caso, pois apenas foram encontrados impactos da presença da espécie invasora ao nível da diversidade da comunidade de peixes. Ao nível da espécie observámos impactos negativos na abundância de *Squalius carolitertii* e na condição fisiológica de *Salmo trutta*. Por outro lado, diferenças significativas foram observadas na dieta de *S. trutta* entre locais invadidos e não invadidos. Com base nesses resultados, concluímos que estudos de longo prazo devem ser realizados para avaliar se os impactos causados pela presença da espécie invasora podem mudar, uma vez que a invasão ainda está em fase de expansão, devido à introdução recente do lagostim sinal na área estudada.

Palavras-chave: Áreas protegidas, ecossistemas de água doce, impactos ecológicos, Parque Natural de Montesinho.

Is the invasive signal crayfish affecting the fish communities in pristine rivers?

Abstract

Freshwater ecosystems provide numerous services that are essential to our society. However, they are also among the ecosystems most threatened by humans, including overexploitation, pollution, habitat loss and fragmentation, climate change and introduction of non-native species. This study aims to characterize the fish communities in the Rabaçal and Tuela River basins (Montesinho Natural Park; NE of Portugal), focusing on the possible impacts of the invasive signal crayfish *Pacifastacus leniusculus*. As omnivores, crayfish possess the ability to significantly modify their environment, which in turn can influence the dynamics and behaviors of various groups of organisms sharing the same habitat, including fish species. In this study we sampled 34 (18 invaded sites and 16 non-invaded) in the Rabaçal and Tuela River basins to evaluate possible differences in abundance, biomass, richness and diversity of the fish communities, as well as to understand which abiotic and biotic factors (e.g. presence of the signal crayfish) are responsible for the possible differences. The characterization of the physiological condition of the sampled species was also conducted, as well as the characterization of diet of the brown trout *Salmo trutta.* With this study it would be expected to find evidence of the negative impact of the presence of signal crayfish on fish communities. That was not the case since we only found impacts of the presence of the invasive species on the fish community diversity. At the species level we observed negative impacts in the abundance of *Squalius carolitertii*, and on the physiological condition of *Salmo trutta*. In the other hand significant differences in the diet of the brown trout *S. trutta* were detected between invaded and non-invaded sites. Given these results we concluded that long term studies must be performed to assess if the impacts caused by the presence of the invasive species may change, since the invasion is still progressing, given the recent introduction of the signal crayfish in the studied area.

Keywords: Ecological impacts, freshwater ecosystems, Montesinho Natural Park, protected areas.

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1. Introduction

One of the greatest challenges facing humanity today is the loss of biodiversity (McCauley et al., 2015; Ceballos et al., 2017; Rosenberg et al., 2019; Tickner et al., 2020). With the decrease in the number of species, or reductions in their abundance, ecosystems undeniably become more fragile and less resilient, calling into question their stability and, consequently, affecting the services generated by them (Tilman, 1999; Srivastava & Vellend., 2005; Dirzo et al., 2014). Aquatic ecosystems such as rivers, lakes, ponds, swamps, among others, have played a key role in human societies throughout history (Wantzen et al., 2016). These diverse ecosystems support a wide range of species, including fish, mammals, reptiles, birds, insects, amphibians, invertebrates, fungi, and plants, which collectively provide essential ecosystem services, including provision of water, climate regulation, food production, energy production, tourism, and recreational opportunities that benefit society (Hanna et al., 2018). These ecosystem services, more than having a high economic value, are essential to maintain the quality of life we know today. However, freshwater ecosystems are among the most vulnerable ecosystems on Earth, and despite covering only about 1% of the planet's surface, they harbor approximately 6% of all described species (Dudgeon et al., 2006; Balian et al., 2008; Dudgeon, 2019). Even so, over the past decades, there has been a significant decline in the area covered by these ecosystems, with Europe alone witnessing a 50% decrease between 1970 and 2008 (Costanza et al., 2014; Gozlan et al., 2019). This decline is mainly due to direct habitat loss. However, other anthropogenic actions, such as the construction of hydroelectric power plants that alter the dynamics of rivers and pose a major threat, especially to migratory species, by fragmenting habitats; the introduction of non-native species, which destabilize the balance of ecosystems through predation, competition, and the introduction of parasites and diseases; intensive fishing, which reduces the number of individuals in populations; the excessive use of water for agriculture, which in itself represents a double problem due to the excessive discharge of nutrients, which causes the eutrophication of waters (Dudgeon, 2019). Indirect anthropogenic actions, such as global warming may threaten, for example, species that are sensitive to temperature variations (Dudgeon, 2019).

From the many taxonomic groups that colonize freshwater ecosystems, fish are without a doubt one of the most charismatic and have been being used by man since immemorial times. Fish are a diverse group, with approximately 15.000 described species, even surpassing the marine fauna diversity (Manel et al., 2020). Even so, fish are also affected by the humans actions described above. To get a sense of how these actions affect fish populations worldwide, according to the International Union for Conservation of Nature (IUCN), 52% of endemic species populations are currently facing some form of threat. Within Europe, the Iberian Peninsula stands out as one of the richest in terms of species diversity, with approximately 73% of the fish species described in this area being endemic (Clavero et al., 2004). This high level of endemism can be attributed to several factors, including the presence of numerous hydrographic basins, the influence of the Mediterranean climate, and the geographical isolation that fish communities experienced during the last glaciation (Clavero et al., 2004). However, and despite the high level of endemism, the Iberian Peninsula lacks studies on its ichthyofauna (Alexandre & Almeida., 2010). This lack of information on endemic species may be an obstacle to the implementation of effective conservation plans since basic data on the ecology of species are still lacking.

In freshwater ecosystems, fish play crucial roles in maintaining ecological health and river biodiversity (Closs et al., 2015). Fish serve as vital links in the food chain, preying on smaller organisms while being preyed upon by larger predators. Nonetheless, these organisms are highly susceptible to population declines. In the Iberian Peninsula the decline of endemic fish species is due to a number of factors acting synergistically (Hermoso & Clavero., 2011), which may include the introduction of non-native species. Non-native species are known to impact negatively freshwater ecosystems. Introduced species tend to establish and occupy the new habitats successively, homogenizing the communities and disrupting natural ecosystems processes, contributing to a decrease of the quality of the ecosystem and the services provided by them (Kiruba et al., 2018). For example, the introduction of the invasive water hyacinth *Eichhornia crassipes*, one of the most invasive aquatic plant, is known to cause significant ecological and socio-economic impacts on the ecosystems, altering the water quality, decreasing dissolved oxygen, nitrogen phosphorus and heavy metals, affecting ecological communities, that includes aquatic invertebrates and fishes (Villamagna & Murphy., 2010). A study in Lake Cluster, Nepal, precisely points the impacts that the presence of the water hyacinth has on native fish diversity and abundance (Basaula et al., 2023). Another good example is the introduction of the American beaver *Castor canadensis* in South America. The introduction of this mammal in Tierra del Fuego, an archipelago at the tip of South America, has been causing serious impacts on those ecosystems, through reshaping the habitats due the presence of beaver damns. The beavers damns create lentic habits, that are rare in the region. Their presence changes the hydrology, induces sediment deposition, alters nutrient cycling by increasing the retention and accumulation of nutrients and organic matter, while also destroying riparian trees (Gibson et al., 2014). Even though they cause negative impacts they can also impact positively some organisms. In this case, American beavers are also helping another invasive species to succeed, the brown trout, *Salmo trutta*, by increasing macroinvertebrates abundance helping to increase the growth rate of the trout (Arismendi et al., 2020). In Portuguese freshwater ecosystems, there are also dozens of non-native species. In total there are 84 non-native species that were successfully introduced (Anastácio et al., 2019), and some examples are: Amphibians like the African clawed frog, *X. laevis*; mammals like the American mink, *Mustela vison*; mollusks like the *C. fluminea*; fish like the bleak, *Alburnus alburnus*, the pumpkinseed sunfish, *L. gibbosus*, the Chameleon cichlid, *A. facetus,* the largemouth bass, *Micropterus salmoides,* the common carp, *Cyprinus carpio*, and crustaceans, like the *Procambarus clarkii* and *Pacifastacus leniusculus.*

The signal crayfish *Pacifastacus leniusculus* (Figure 1), a freshwater decapod, that is native to the west coast of North America and was introduced to Portugal from Spain, together with the Louisiana crayfish *Procambarus clarkii*, around 1970. The introduction of this species in Iberia, produced a series of negative impacts on populations of macrophytes, macroinvertebrates, reptiles, benthic fish and amphibian larvae (Vedia & Miranda, 2013). It is known that the presence of the non-native crayfish can alter the aquatic biota directly and indirectly through complex interactions (Strayer, 2010; Jackson et al., 2014). Certain studies also point out that signal crayfish is an effective predator of freshwater mussels (Meira et al., 2019; Sousa et al., 2019) and other macroinvertebrates (Carvalho et al., 2022). In relation to native European crayfish, this invasive species has competitive advantages, in addition to being a vector for the transmission of a parasite (*Aphanomyces astaci* Schikora), responsible for the crayfish plague, of which it is resistant, but is lethal to native crayfish (Dunn et al., 2009). Regarding its impacts on fish fauna, there are studies proving that signal crayfish can outcompete benthic fishes in relation to micro-habitats for hiding, resulting in increased mortality of these fishes (Guan & Wiles., 1997). The effects of their presence tend to be worse when the populations are well established, with the larger individuals having an extensive range of action compared with the smaller ones (Strayer, 2010; Galib et al., 2022). However, the number of studies assessing the possible impacts of signal crayfish on fish communities are rare and we are not aware of a similar study in Portuguese ecosystems.

1.1 Study objectives

Considering the lack of information about the topic, this research focused on characterizing the fish communities in Iberian pristine mountain rivers, considering their ecological role and cultural significance. Additionally, assessed the potential impact of the invasive signal crayfish on these communities. Given the presence of a substantial number of known endemic fish species and the limited information available in the Iberian Peninsula about their basic autecology, this study is highly relevant and timely. The selected sampling sites encompass the Tuela, Rabaçal, Mente, and Baceiro Rivers, all part of the Douro River basin. To assess fish communities, the study examined possible differences in abundance, biomass, richness at the various sampling sites. Additionally, we investigate the biotic and abiotic factors responsible for these differences. The physiological condition of the collected fish species was also characterized. Considering the available information (Galib et al., 2021), we expected to find evidence of the negative impact of the presence of signal crayfish on fish communities. This crayfish species poses a significant threat to freshwater ecosystems due to their aggressive behavior and broad trophic regime (Bernardo et al., 2011). This study seeks to contribute to a better understanding of fish communities in pristine mountain rivers and shed light on the potential impacts of the recent invasion of the signal crayfish on these communities. The results obtained from this study may inform future conservation actions and help protect these delicate ecosystems from further degradation caused by invasive species.

Figure 1 – The non-native signal crayfish *Pacifastacus leniusculus.*

2. Material and methods

2.1 Study area and its biodiversity

This study was conducted in the Montesinho Natural Park and the adjacent downstream areas, located in the northeast region of Portugal. This protected area, created in 1979, is located from 41° 43’47’’ to 41° 59’ 24’’ N and 6° 30’ 53’’ to 7° 12’ 9’’ W and is classified as category V (i.e. a protected landscape covering an entire body of land or ocean with an explicit natural conservation plan, but usually also accommodates a range of for-profit activities) by the IUCN. The landscape of the park is characterized by gentle reliefs with rounded peaks, separated by valleys of winding rivers. There is a predominance of schist, limestone (in plateau areas), and granite in the Montesinho mountain range. The climate is Mediterranean with influence from the Atlantic Ocean, with medium annual temperatures of 12.5°C (Castro et al., 2010). In terms of precipitation the park has an annual average of 1000 – 1600 mm. The Montesinho Natural Park covers a total area of around 75 thousand hectares and was designed to focus specially on the protection of birds, terrestrial vertebrates and plants.

Regarding flora, the autochthonous forest consists of oaks *Quercus pyrenaica*, holm oaks *Quercus rotundifolia* and riparian trees, like alder *Alnus lusitanica*, the ash *Fraxinus sp* and the willow *Salix sp*. In boglands Mat-grass *Nardus stricta* and the crossleaf heath *Erica tetralix* dominate the landscape, having both species high value in terms of conservation. The scrubland is composed of the rockrose *Cistus Ladanifer* or *Genista Tridentata*, while meadows are constituted by the ryegrass *Lolium rigidum* and many other species.

In terms of fauna, the Montesinho Natural Park is home to a diverse range of species. The Iberian wolf *Canis lupus signatus*, the wild boar *Sus scorfa*, the red deer *Cervus elaphus*, the roe deer *Capreolus capreolus*, the wild cat *Felis silvestris*, the water desman *Galemys pyrenaicus*, the otter *Lutra lutra*, the European water vole *Arvicula amphibius*, among many others, are some examples of terrestrial and aquatic mammals found. The park also has a high diversity of birds, with about 125 of the 150 species described known to nidify there. The Golden eagle *Aquila chrysaetos,* the Black stork *Ciconia nigra,* the Hen herrier *Circus cyaneus* or the red-backed shrike *Lanius collurio* are good examples of rare bird species that are stil present in this protected area. Located within this protected area are important aquatic species that hold significant conservation value such as the freshwater pearl mussel *Margaritifera margaritifera* and the Iberian loach *Cobitis* *calderoni*. The Montesinho Natural Park is traversed by multiple rivers and streams, which provide essential habitats for a diverse range of aquatic species. These habitats are home to numerous species of aquatic invertebrates, amphibians, and fish including Brown trout *Salmo trutta* (Figure 2A), the Douro nase *Pseudochondrostoma duriense* (Figure 2B), the Iberian barbel *Luciobarbus bocagei* (Figure 2C), the Iberian chub *Squalius carolitertii* (Figure 2D), the Iberian roach *Squalius alburnoides,* (Figure 2E) and the Northern Iberian spined loach *Cobitis calderoni* (Figure 2F).

Figure 2 – Fish species sampled in the Montesinho Natural Park. The brown trout *Salmo trutta* (A), the Douro nase *Pseudochondrostoma duriense* (B),the Iberian barbel *Luciobarbus bocagei* (C), the Iberian chub *Squalius carolitertii* (D), the Iberian roach *Squalius alburnoides* (E) and the Northern Iberian spined loach *Cobitis calderoni* (F).

2.2 Sampling sites

The sampling of the fish community was done in Montesinho Natural Park. The sampling sites were located in Mente, Rabaçal, Tuela and Baceiro Rivers (Figure 3), with the sampling being done during July 2022. Among these sites, 18 are already invaded by the signal crayfish, while the remaining 16 currently show no evidence of its presence. The sampling sites were defined as: M1-M4 all located in Mente River; R1 - R13 all located In Rabaçal River; T1 - T11 all located in Tuela River and B1 - B6 all located in Baceiro River. The sampled rivers belong to the Douro basin. Those rivers have the particularity of all having very low human pressure (Sousa et al., 2012, 2015, 2018), what makes them perfect for this study since the results obtained will have the least bias possible when comparing the fish communities in invaded and non-invaded sites. As stated above and to assess the impact of signal crayfish on fish communities, we selected 18 sampling points (M1, M2, M3, M4, T1, T2, T3, T4, R6, R7, R8, R9, R10, R11, B1, B2, B3 and B4) where the invasive species was confirmed to be present, and 16 sampling points (T5, T6, T7, T8, T9, T10, T11, R1, R2, R3, R4, R5, R12, R13, B5 and B6) where it is absent (see results section). The sampling was done utilizing electrofishing equipment (see below for further description).

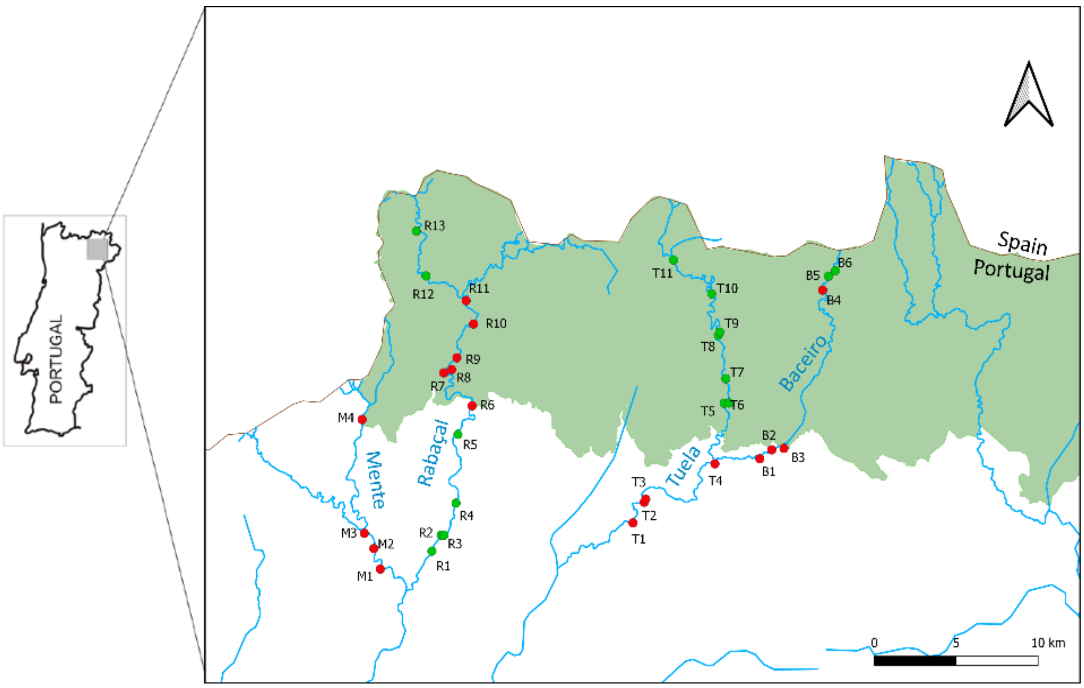


Figure 3 – Map of the study area showing the location of the 34 sampling sites in Mente (M), Rabaçal (R), Tuela (T) and Baceiro (B) Rivers.

2.3 Abiotic Characterization

For the abiotic characterization, water temperature, dissolved oxygen, water conductivity, total dissolved solids, and pH were analyzed *in situ* at all sampling sites in the middle of the river and near the bottom in all sites with a HACH HQ 40d multi-parameter probe (Hach Company, Colorado, USA). These measurements were always performed at the end of the morning.

We used the River Habitat Survey (RHS) (Raven et al., 1998, 2000) for the assessment of hydromorphological quality and the conservation status of aquatic and riparian habitats. This methodology encompasses the combination of two indices: the Habitat Modification Score (HMS) and the Habitat Quality Assessment (HQA). This methodology was selected for the hydromorphological characterization of the studied sites since it was the one chosen by Portuguese authorities and other European countries as one of the most comprehensive approaches for river hydromorphological characterization and is adaptable to the specific conditions of different watercourses. By applying this methodology, it was possible to evaluate the degree of naturalness and/or disturbance existing and obtain the current hydromorphological status of the analyzed sites.

The HMS index measures the extent to which the natural characteristics of the sampled section have been anthropogenically modified through the presence and impact of artificial structures (cross-sectional and longitudinal structures) in river habitats. This includes parameters such as the quantity and size of specific types of cross-sectional structures (dams, crossings, hydraulic passages, groynes, bridges), anthropogenic transformations of bank profiles (reinforcement, channelization, embankments, trampling of banks by livestock, cutting of riparian vegetation), and modifications to the bed (reinforcement, channelization, dredging, artificial bottom materials, among other possibilities). The values obtained for the HMS index do not depend on the characteristics of the river system, allowing for the comparison of results obtained in different types of watercourses. To calculate the HMS, sites are characterized in relation to the presence and extent of these artificial resources (Habitat Modification Score Rules 2003). Greater and more severe modifications result in a higher score. The total accumulated points provide the habitat modification score (HMS).

Table 1 – HMS index and respective sub-indices

HMS Outfall/intakes
HMS artificial berms and raised banks
HMS Bridges
HMS Culverts
HMS Ford
HMS Poached
HMS Reinforced
HMS Resectioned
HMS Weirs/Dams/Sluices
Score HMS
Class HMS

The Habitat Modification Class (HMC) assigns the channel condition of a site to one of five modification classes based on the total score (1 = nearly natural; 5 = severely modified). Table 1 presents the different sub-indices of the HMS, and Table 2 shows the respective scores and classifications.

Table 2 – Categories of artificialization of the riverbed and banks of watercourses and their respective scores on the HMS index according to the Environment Agency (2003).

HMS Score	Categories	Quality classes
0 – 16	Pristine - Seminatural	1
17 – 199	Predominantly unmodified	2
200 – 499	Obviously modified	3
500 – 1399	Significantly modified	4
≥ 1400	Severely modified	5

The HQA index, a measure of the richness, rarity, and diversity of river habitats, is composed of the aggregation of several sub-indices based on the relevance of certain habitat characteristics for biological communities (Table 3). The sub-indices pertain to flow type, substrate, channel characteristics, bank characteristics, marginal vegetation structure, sediment deposition, aquatic vegetation, riparian vegetation, land use, special characteristics, and overall habitat quality. The physical characteristics of an unmodified channel are primarily determined by erosional forces and the erodibility of the bed and bank material. When these two characteristics are combined, they determine the shape of the channel, the frequency, and spatial occurrence of erosion and deposition sites. The frequency of riffles, pools, sand accumulations, and eroded banks is important for determining the type, distribution, and abundance of aquatic biological communities.

Table 3 – HQA Index and respective sub-index

HQA Current type
HQA bed substrate
HQA bed characteristics
HQA shoreline features
HQA structure of riparian vegetation
HQA sediment accumulation in the inner meander zone
HQA bed vegetation
HQA soil use
HQA tree-associated characteristics
HQA special characteristics
HQA score

Through this index, it is possible to obtain an indication of the overall habitat diversity provided by natural features in the channel and the river corridor. Sampling sites are scored based on the presence of channel accumulation features, eroded cliffs, large woody debris, waterfalls, backwater areas, and floodplain wetlands. Additional points reflect the variety of channel substrates, flow types, in-channel vegetation, as well as the distribution of trees along the banks and the extent of land use near the natural river adjacent area. The points are summed to provide the HQA score, and the higher the value, the better the habitat quality, representing more diverse sites. For the hydromorphological assessment based on this index, it is necessary to have a database of reference sites (without disturbance) by river typology (Table 4) that have similar physical characteristics (e.g., gradient, distance from the source, and geology) to the sites being evaluated. In the following table, only the boundary values for Small Northern Rivers (N1≤100) and Medium-Large Northern Rivers (N1>100) typologies are presented since these are the typologies corresponding to the studied sites. In contrast to the HMS (Habitat Modification Score), lower HQA (Habitat Quality Assessment) scores reflect more artificial intervention and modification of the river channel at a given location, affecting the quality of natural habitats. It is worth noting that two ecologically similar river segments can be characterized by different anthropogenic influences (Kiraga, 2020), which do not necessarily lead to a loss in the hydromorphological quality of a river. After determining the HQA and HMS quality indices, the classification of hydromorphological quality elements for the sampling sites will correspond to the more penalizing class of the two. In addition to these two indices, the Riparian Quality Index (RQI) was also calculated using the same software used for the calculation of the HQA and HMS indices (The River Habitat Survey Toolbox). This index, which is more recent than the previous two, represents the complexity, naturalness, and continuity of the riparian zone. This zone is defined as the area that encompasses the bank slope, the top of the bank, and the 5-meter buffer from the top of the bank assessed during the RHS. The RQI comprises 3 sub-indices corresponding to complexity, naturalness, and continuity, which are calculated separately for each bank and then added together to produce the final classification, ranging from 0 to 120 points. The final RQI score is categorized into 5 equal classes representing increasing riparian quality, ranging from "Very Low" quality (1st quintile) to "Very High" quality (last quintile).

Table 4 – Quality boundaries of the HQA index applicable to rivers. Classification according to APA (2021).

National type	HQA limits for the excellent class
N1≤100	>68
N1>100	>60

2.4 Biotic Characterization

The survey aiming to characterize the fish communities was done by electrofishing, performed by three experienced researchers using a portable equipment (Hans Grassl) with a pulsed DC-300-600 V generator. The fisheries were always performed for 20 minutes. The fish were stunned and collected in order to measure their weight and length, identify the species and count how many individuals per species were sampled by site. After each fisheries the fish were released without any harm. For the fish condition, the Fulton's condition factor was used, and it was calculated with the equation $K_c=100*W/L^3$, where W is the total weight of the fish and L Is the total length.

For the characterization of the diet of the brown trout, the stomach content of each individual was obtained by regurgitation, a non-lethal method that consists in using a squeeze bottle, insert it inside the fish mouth and press is stomach area in order to retrieve the content from inside the fish. Later the samples were processed, the individuals were separated and counted using a laboratory magnifying glass. The identification of the invertebrates was done until the family level using the guidebook "Invertébrés d'eau douce" by Henri Tachet (Tachet et al., 2010). The abundance of the signal crayfish was assessed in the same 34 sites surveyed to characterize the fish communities in August 2022. Crayfishes were captured by placing 6 to 8 funnel traps, four-five rectangular (50 × 30 × 20 cm; 0.5 cm mesh) and one-three cylindrical (43 cm diameter; 22 cm height; 1.5 cm mesh), per site for 24 h. Therefore, relative abundance of crayfish per site was expressed as the total number of individuals per catch per unit of effort (ind. CPUE/24 h). The crayfishes collected were also measured from the rostrum tip to telson rear edge and their sex was determined (following Sousa et al., 2013).

2.5 Data analysis

For the abiotic characterization 8 variables were analyzed: Temperature, Dissolved oxygen, Water conductivity, Total dissolved solids (TDS), pH, Altitude, Habitat Modification Score (HMS) and the Habitat Quality Assessment (HQA). The data was fourth routed transformed to perform a Principal Component Analysis (PCA), in order to sort the different sites according to the abiotic variables measured. This analysis was performed on Primer 6 (version 1.0.3, Primer-E Ltd, Plymouth). To analyze the fish communities a non-metric Multi-Dimensional Scaling (nMDS) was performed, using the abundance data previously Square root transformed, to create a matrix of similarity using Bray-Curtis distance. A two-way PERMANOVA test (9999 permutations), was performed to evaluate the influence of the basin (Rabaçal and Tuela) as a fixed factor and the presence of the signal crayfish (Yes and No) as a random factor in the fish communities and in the diet composition of the brown trout, *S. trutta*. If the number of permutations was lower than 150 the Monte Carlo test P-value was considered. In order to appraise the species most contributing for the dissimilarity between basins and between invaded and non-invaded sites a SIMPER analysis was conducted. As well as the analysis just described, the Richness (S), abundance (N), Shannon-Wiener diversity index (H') and the Pielou's evenness (J') were also calculated on Primer 6 using the Diversity option (version 1.0.3, Primer-E Ltd, Plymouth). To test possible differences between abundance, biomass, richness, Shannon-Wiener and Pielou's evenness on Fulton's condition nonparametric Kruskall-Wallis multiple comparison tests were performed, since normality or homogeneity of variance were not met, even using several transformations. To test possible differences in the diet composition of the brown trout, abundance (N), Richness (S), Pielou's evenness (J') and Shannon-Wiener diversity index (H') were analyzed using nonparametric Kruskall-Wallis multiple comparison tests, since normality and homogeneity of variance were not met. When the values of the diversity indices were 0 they were removed from the analysis. These statistical tests were carried out on R studio (Version 2023.06.1-524).

3. Results

3.1 Abiotic characterization

The abiotic characterization of all the sampled sites is available in Table 5. The temperature varied between 16.1°C (B5) and 23 °C (R7/R8); dissolved oxygen between 7.98mg/L (B1) and 9.47 mg/L (T8); water conductivity between 24.5 µS/cm (R11) and 62.4 µS/cm (T7); total dissolved solids between 5 mg/L (B6) and 28.9 mg/L (B1); while pH varied between 6.45 (B4) and 7.1 (R6) and altitude between 385m (M1 and R1) and 843m (B6).

Table 5 – Physico-chemical characterization of all the sampling sites of the Montesinho Natural Park (July 2022).

	M1	**M2**	**M3**	**M4**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
Temperature (ºC)	19.9	19.6	20.8	19.7	21.4	20.6	21.5	21.4	22.4	22.5	23	23
Oxygen (mg/L)	8.21	8.46	7.99	8.17	8.44	8.39	8.28	8.27	8.27	8.24	8.67	8.45
Conductivity (µS/cm)	29.7	30.9	31.6	28.9	28.3	28.1	28.2	28.1	28.1	26.1	26.3	26.3
TDS (mg/L)	16.43	16.28	16.19	15.15	14.18	14.45	14.18	14.41	13.93	12.75	12.67	12.67
pH	7.01	7.07	6.89	6.78	6.78	7.04	6.95	6.53	6.48	7.1	7.08	7.08
Altitude (m)	385	395	398	452	385	395	398	409	459	471	487	490

	R9	**R10**	**R11**	**R12**	**R13**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Temperature (ºC)	22.4	21.1	20.8	21	21.1	22	20.7	20.7	20.2	20.1	19.9
Oxygen (mg/L)	8.5	8.48	8.52	8.55	8.58	8.05	8.06	8.81	8.1	8.06	8.75
Conductivity (µS/cm)	26.5	25	24.5	25.6	25.8	57.7	48.6	48.6	42.6	41.3	39.1
TDS (mg/L)	13.3	12.42	12.3	12.93	12.89	28.8	25	25	21.5	21.5	20.78
pH	6.66	6.5	6.65	6.71	6.61	6.92	6.98	6.98	7.02	7.01	6.93
Altitude (m)	493	520	525	551	587	421	427	430	532	630	634

	T7	**T8**	**T9**	**T10**	**T11**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Temperature (ºC)	19.5	20.3	22.22	21.4	19.2	18.9	18.1	17.2	16.2	16.1	16.3
Oxygen (mg/L)	8.89	9.47	9.33	9.34	9.46	7.98	8.02	8.05	8.12	8.18	8.2
Conductivity (µS/cm)	62.4	36.7	38.9	38	45.7	55.2	51.5	49.6	32.3	31.5	30.5
TDS (mg/L)	7.14	18.7	19.29	19.1	8.75	28.9	27.9	27.3	5.58	5.45	5
pH	6.96	6.79	6.72	6.69	6.79	6.75	6.74	6.83	6.45	6.69	6.6
Altitude (m)	643	655	656	684	750	594	608	612	831	835	843

The PCA analysis is shown in Figure 4. Sites were mainly separated in PC1 by the abiotic factors altitude, HMS index (positive direction) and TDS (negative direction) while in the PC2 TDS, conductivity, altitude (positive direction) and temperature and HQA index (negative direction) were the most important abiotic factors distinguishing sites.

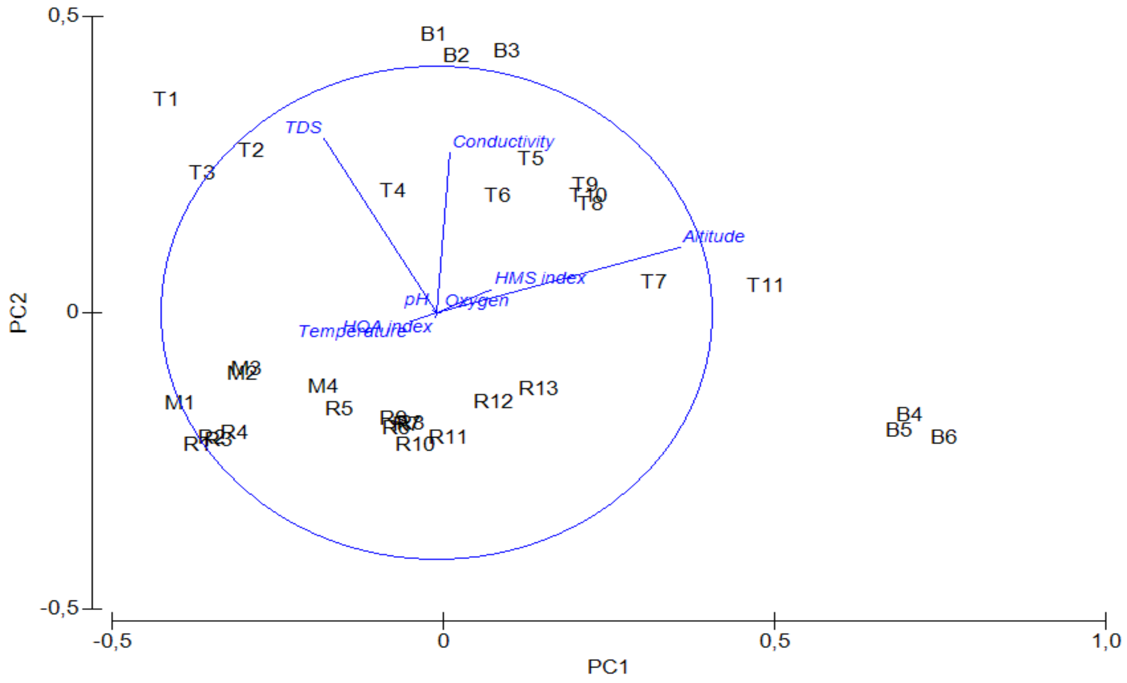


Figure 4 – Principal Components Analysis (PCA) showing the 34 sampling sites disposition based on the abiotic factors measured. PC1 explains 53.4% of all variance and PC2 explains 28.4%.

Based on the surveys conducted at different sampling sites, following the River Habitat Survey (RHS) methodology, the collected data is summarized in Tables 6 to 11, which pertain to the Habitat Quality Assessment (HQA) and sub-indices (Tables 6 and 7), Habitat Modification Score (HMS) (Tables 8 and 9), Hydromorphological quality (HQ) (Table 10) and Riparian Quality Index (RQI) (Table 11). In general terms, in the Rabaçal River basin, HQA scores (Table 6) were quite high. All locations, except Rab 6 (Class 2 - "Good"), achieved the "Excellent" class (95% of locations). On average, the Mente River had higher values (69.5) than the Rabaçal River (67.8), although the highest value was reached at location Rab 2 (76). Regarding the Tuela River basin (Table 7), all locations, without exception, exhibited "Excellent" quality. Overall, although both basins mostly have "Excellent" habitat quality, it was in the Tuela River basin where the highest HQA values were achieved (average of 73.8).

Table 6 – Partial HQA index values and respective sub-indices for the locations distributed throughout the Rabaçal River basin. Classification according to APA criteria (2021).

SIte_reference	HQA Score	HQA Class *	HQA flow type	HQA channel substrate	HQA channel features	HQA bank features	HQA bank vegetation structure	HQA point bars	HQA channel vegetation	HQA land use	HQA trees	HQA special features
M1	68	1	11	5	7	8	12	1	6	4	13	2
M2	71	1	14	8	7	3	12	0	6	5	13	3
M3	70	1	13	7	6	9	12	1	6	4	12	1
M4	69	1	11	6	7	8	12	1	6	4	13	2
Minimum Mente River	**68**	**1**	**11**	**5**	**6**	**3**	**12**	**0**	**6**	**4**	**12**	**1**
Maximum Mente River	**71**	**1**	**14**	**8**	**7**	**9**	**12**	**1**	**6**	**5**	**13**	**3**
Mean	**69.5**		**12.3**	**6.5**	**6.8**	**7.0**	**12.0**	**0.8**	**6.0**	**4.3**	**12.8**	**2.0**
Standard deviation	**1.3**		**1.5**	**1.3**	**0.5**	**2.7**	**0.0**	**0.5**	**0.0**	**0.5**	**0.5**	**0.8**
R1	67	1	12	8	6	6	12	0	6	4	11	2
R2	76	1	14	8	7	5	12	0	6	9	12	3
R3	62	1	11	9	5	3	12	0	6	4	10	2
R4	66	1	13	8	8	4	12	0	6	4	9	2
R5	73	1	13	6	8	5	12	0	6	9	13	1
R6	59	2	14	6	3	3	12	0	6	4	10	1
R7	68	1	13	7	8	6	12	1	6	2	12	2
R8	69	1	11	9	7	6	12	0	6	3	11	4
R9	68	1	13	7	6	4	12	0	6	4	11	5
R10	66	1	12	6	6	5	12	1	6	4	13	2
R11	72	1	13	7	7	3	12	0	6	9	14	1
R12	70	1	12	7	7	4	12	0	6	4	14	4
R13	63	1	12	7	7	4	12	0	6	4	10	1
Minimum Rabaçal River	**59**	**1**	**11**	**6**	**3**	**3**	**12**	**0**	**6**	**2**	**9**	**1**
Maximum Rabaçal River	**76**	**2**	**14**	**9**	**8**	**6**	**12**	**1**	**6**	**9**	**14**	**5**
Mean	**67.6**		**12.5**	**7.3**	**6.5**	**4.5**	**12.0**	**0.2**	**6.0**	**4.9**	**11.5**	**2.3**
Standard deviation	**4.6**		**1.0**	**1.0**	**1.4**	**1.1**	**0.0**	**0.4**	**0.0**	**2.4**	**1.6**	**1.3**
Minimum Rabaçal River basin	**59**	**1**	**11**	**5**	**3**	**3**	**12**	**0**	**6**	**2**	**9**	**1**
Maximum Rabaçal River basin	**76**	**2**	**14**	**9**	**8**	**9**	**12**	**1**	**6**	**9**	**14**	**5**
Mean	**68.1**		**12.5**	**7.1**	**6.6**	**5.1**	**12.0**	**0.3**	**6.0**	**4.8**	**11.8**	**2.2**
Standard deviation	**4.1**		**1.1**	**1.1**	**1.2**	**1.9**	**0.0**	**0.5**	**0.0**	**2.1**	**1.5**	**1.2**

Table 7 – Partial HQA index values and respective sub-indices for the locations distributed throughout the Tuela River basin. Classification according to APA criteria (2021).

SIte_reference	HQA Score	HQA Class *	HQA flow type	HQA channel substrate	HQA channel features	HQA bank features	HQA bank vegetation structure	HQA point bars	HQA channel vegetation	HQA land use	HQA trees	HQA special features
B1	74	1	13	8	7	8	12	0	6	4	12	4
B2	74	1	13	8	8	6	12	1	6	4	12	5
B3	72	1	13	8	9	11	11	1	5	3	12	0
B4	73	1	14	6	7	11	12	2	5	3	12	3
B5	91	1	13	9	9	18	12	1	6	4	17	3
B6	79	1	13	6	7	15	12	0	6	4	12	4
Minimum Baceiro River	**72**	**1**	**13**	**6**	**7**	**6**	**11**	**0**	**5**	**3**	**12**	**0**
Maximum Baceiro River	**91**	**1**	**14**	**9**	**9**	**18**	**12**	**2**	**6**	**4**	**17**	**5**
Mean	**77.2**		**13.2**	**7.5**	**7.8**	**11.5**	**11.8**	**0.8**	**5.7**	**3.7**	**12.8**	**3.2**
Standard deviation	**7.2**		**0.4**	**1.2**	**1.0**	**4.4**	**0.4**	**0.8**	**0.5**	**0.5**	**2.0**	**1.7**
T1	67	1	13	7	6	8	12	1	7	2	10	2
T2	66	1	15	6	4	6	12	0	6	6	9	2
T3	63	1	12	7	5	10	9	1	6	3	10	1
T4	74	1	15	10	7	7	12	1	6	4	10	3
T5	73	1	15	8	8	5	12	0	6	4	10	5
T6	73	1	12	8	7	13	12	1	7	4	10	0
T7	69	1	12	8	4	7	12	0	7	4	13	2
T8	76	1	14	8	8	9	12	0	6	4	12	3
T9	78	1	12	9	8	14	12	1	6	4	12	1
T10	82	1	13	8	6	13	12	0	6	9	13	2
T11	74	1	13	7	8	9	12	0	6	3	12	4
Minimum Tuela River	**63**	**1**	**12**	**6**	**4**	**5**	**9**	**0**	**6**	**2**	**9**	**0**
Maximum Tuela River	**82**	**1**	**15**	**10**	**8**	**14**	**12**	**1**	**7**	**9**	**13**	**5**
Mean	**72.3**		**13.3**	**7.8**	**6.5**	**9.2**	**11.7**	**0.5**	**6.3**	**4.3**	**11.0**	**2.3**
Standard deviation	**5.6**		**1.3**	**1.1**	**1.6**	**3.0**	**0.9**	**0.5**	**0.5**	**1.8**	**1.4**	**1.4**
Minimum Tuela River basin	**63**	**1**	**12**	**6**	**4**	**5**	**9**	**0**	**5**	**2**	**9**	**0**
Maximum Tuela River basin	**91**	**1**	**15**	**10**	**9**	**18**	**12**	**2**	**7**	**9**	**17**	**5**
Mean	**74.0**		**13.2**	**7.7**	**6.9**	**10.0**	**11.8**	**0.6**	**6.1**	**4.1**	**11.6**	**2.6**
Standard deviation	**6.4**		**1.0**	**1.1**	**1.5**	**3.6**	**0.8**	**0.6**	**0.6**	**1.5**	**1.8**	**1.5**

Analyzing both basins together, it is evident that the characteristics that contributed most to these high ratings were associated with flow types (HQA flow Type), bank vegetation structure (HQA bank vegetation Structure), characteristics related to trees (HQA trees), channel substrate type (HQA channel substrate), and features of the channel (HQA channel features) and banks (HQA bank features). Regarding the HMS index, the scores obtained ranged from 0 (Class 1 in both studied basins) to 955 in the Rabaçal River basin (Class 4 - Rab 6) and 1635 in the Baceiro River (Class 5 - Bac 3).

In Rabaçal River basin (Table 8), there is a distribution of sites into 4 quality classes, ranging from "Pristine" (Class 1) to "Significantly modified" (Class 4). However, the "Pristine" and "Predominantly unmodified" classes were achieved in 63.2% of the studied sites. The worst class was only reached at one site (Rab 6, Class 4), mainly due to the presence of an impermeable weir and two highly impactful bridges. The moderate percentage of sites with high HMS values (31.6%) reflects some anthropogenic influence on these riparian habitats, through the construction of structures such as bridges, hydraulic passages, bank reinforcement, and channelization, as well as the presence of weirs/transverse barriers. This situation of habitat alteration due to human intervention slightly worsens in the Tuela River basin (Table 9), where the number of "Obviously modified," "Significantly modified," or "Severely modified" sites represents 38.9% of the total sampled sites.

Table 8 – Partial HMS index values and their respective sub-indices for the locations distributed within the Rabaçal River basin.

Site_reference	HMS Score	HMS Class	HMS Outfall/ Deflector subscore	HMS Berms Embankments subscore	HMS Bridges subscore	HMS Culverts subscore	HMS Fords subscore	HMS Poaching subscore	HMS Reinforced Bank Bed subscore	HMS Resectioned Bank Bed subscore	HMS Weirs dams and sluices subscore
M1	0	1	0	0	0	0	0	0	0	0	0
M2	300	3	0	0	0	0	0	0	180	120	0
M3	430	3	150	0	0	0	200	0	0	80	0
M4	180	2	0	0	0	0	0	0	100	80	0
Minimum River Mente	0	1	0	0	0	0	0	0	0	0	0
Maximum River Mente	430	3	150	0	0	0	200	0	180	120	0
Mean	227.5		37.5	0.0	0.0	0.0	50.0	0.0	70.0	70.0	0.0
Standard deviation	182.8		75.0	0.0	0.0	0.0	100.0	0.0	87.2	50.3	0.0
R1	0	1	0	0	0	0	0	0	0	0	0
R2	0	1	0	0	0	0	0	0	0	0	0
R3	0	1	0	0	0	0	0	0	0	0	0
R4	0	1	0	0	0	0	0	0	0	0	0
R5	80	2	0	0	0	0	0	0	40	40	0
R6	955	4	0	0	500	0	0	0	80	0	375
R7	320	3	25	0	0	0	40	0	0	0	255
R8	330	3	150	0	0	0	0	0	0	0	180
R9	90	2	0	0	0	0	0	0	50	40	0
R10	10	1	0	0	0	0	0	10	0	0	0
R11	35	2	25	0	0	0	0	10	0	0	0
R12	355	3	0	0	0	0	40	10	50	0	255
R13	200	3	0	0	0	0	200	0	0	0	0
Minimum River Rabaçal	0	1	0	0	0	0	0	0	0	0	0
Maximum River Rabaçal	955	4	150	0	500	0	200	10	80	40	375
Mean	182.7		15.4	0.0	38.5	0.0	21.5	2.3	16.9	6.2	81.9
Standard deviation	269.5		41.5	0.0	138.7	0.0	55.7	4.4	27.8	15.0	134.1
Minimum River Rabaçal basin	0	1.0	0	0	0	0	0	0	0	0	0
Maximum River Rabaçal basin	955	4.0	150	0	500	0	200	10	180	120	375
Mean	193.2		20.6	0.0	29.4	0.0	28.2	1.8	29.4	21.2	62.6
Standard deviation	247.2		49.4	0.0	121.3	0.0	66.0	3.9	50.4	37.7	121.5

Table 9 – Partial HMS index values and their respective sub-indices for the locations distributed within the sub-basin of the Tuela River.

SIte_reference	HMS Score	HMS Class	HMS Outfall/ Deflector subscore	HMS Berms Embankments subscore	HMS Bridges subscore	HMS Culverts subscore	HMS Fords subscore	HMS Poaching subscore	HMS Reinforced Bank Bed subscore	HMS Resectioned Bank Bed subscore	HMS Weirs dams and sluices subscore
B1	160	2	0	0	0	0	0	0	80	80	0
B2	100	2	0	0	100	0	0	0	0	0	0
B3	1635	5	200	0	250	0	0	0	490	320	375
B4	435	3	0	0	0	0	0	0	0	0	435
B5	60	2	0	0	0	0	40	20	0	0	0
B6	505	4	0	0	250	0	0	20	80	80	75
Minimum Baceiro River	**60**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Maximum Baceiro River	**1635**	**5**	**200**	**0**	**250**	**0**	**40**	**20**	**490**	**320**	**435**
Mean	**482.5**		**33.3**	**0.0**	**100.0**	**0.0**	**6.7**	**6.7**	**108.3**	**80.0**	**147.5**
Standard deviation	**593.3**		**81.6**	**0.0**	**122.5**	**0.0**	**16.3**	**10.3**	**191.0**	**123.9**	**202.5**
T1	0	1	0	0	0	0	0	0	0	0	0
T2	685	4	0	0	250	0	0	0	100	80	255
T3	0	1	0	0	0	0	0	0	0	0	0
T4	20	2	0	0	0	0	0	0	20	0	0
T5	305	3	0	0	0	0	0	0	50	0	255
T6	0	1	0	0	0	0	0	0	0	0	0
T7	0	1	0	0	0	0	0	0	0	0	0
T8	690	4	0	0	0	0	0	10	40	40	600
T9	560	4	0	0	0	0	0	0	40	40	480
T10	20	2	0	0	0	0	0	20	0	0	20
T11	80	2	0	0	0	0	40	0	0	40	80
Minimum Tuela River	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Maximum Tuela River	**690**	**4**	**0**	**0**	**250**	**0**	**40**	**20**	**100**	**80**	**600**
Mean	**214.5**		**0.0**	**0.0**	**22.7**	**0.0**	**3.6**	**2.7**	**22.7**	**18.2**	**153.6**
Standard deviation	**292.0**		**0.0**	**0.0**	**75.4**	**0.0**	**12.1**	**6.5**	**32.3**	**27.5**	**216.2**
Minimum Tuela River basin	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Maximum Tuela River basin	**1635**	**5**	**200**	**0**	**250**	**0**	**40**	**20**	**490**	**320**	**600**
Mean	**309.1**		**11.8**	**0.0**	**50.0**	**0.0**	**4.7**	**4.1**	**52.9**	**40.0**	**151.5**
Standard deviation	**425.1**		**48.5**	**0.0**	**98.4**	**0.0**	**13.3**	**8.0**	**117.6**	**78.7**	**205.0**

Regarding hydromorphological quality, the Rabaçal River basin stands out for generally having better quality compared to the Tuela River basin, as can be observed in Table 10. The Rabaçal River basin has a higher number of sites with "Excellent" quality (6), whereas the Tuela River basin only reached this quality level in 4 sites. Additionally, it was in the Tuela River basin where one site was classified with the worst quality ("Bad quality") and 4 sites had "Mediocre quality".

Table 10 – Hydromorphological quality for the locations distributed across the Rabaçal and Tuela River basin.

Rabaçal River basin			
SIte_reference	**HQA Class ***	**HMS Class**	**Hydromorphological Quality**
M1	1	1	1
M2	1	3	3
M3	1	3	3
M4	1	2	2
R1	1	1	1
R2	1	1	1
R3	1	1	1
R4	1	1	1
R5	1	2	2
R6	2	4	4
R7	1	3	3
R8	1	3	3
R9	1	2	2
R10	1	1	1
R11	1	2	2
R12	1	3	3
R13	1	3	3

Tuela River basin			
SIte_reference	**HQA Class ***	**HMS Class**	**Hydromorphological Quality**
B1	1	2	2
B2	1	2	2
B3	1	5	5
B4	1	3	3
B5	1	2	2
B6	1	4	4
T1	1	1	1
T2	1	4	4
T3	1	1	1
T4	1	2	2
T5	1	3	3
T6	1	1	1
T7	1	1	1
T8	1	4	4
T9	1	4	4
T10	1	2	2
T11	1	2	2

Regarding the RQI index (Table 11), a similar pattern to the one observed previously emerges, where the Rabaçal River basin exhibits better riparian quality, with the majority of evaluated sites having "Very High" quality (73.7%). In contrast, the Tuela River basin only has 44.4% of sites with "Very High" quality, and one site has "Moderate" quality (Bac 3). It is worth noting that the component that contributed the most to this difference between basins was the complexity related to the structure of vegetation on the bank and the first meter of the top of the bank. The other components (naturalness and continuity), although having similar average values in both studied basins, exhibit higher standard deviations in the Tuela River basin, indicating greater variability in both the naturalness of the banks and the continuity of vegetation structure. It should be highlighted that in the Tuela River basin, riparian vegetation is more affected by the death of alders (particularly due to *Phytophthora lacustris* and *Phytophthora x alni*), leading to more discontinuities in the riparian gallery.

Table 11 – RQI index values and their respective sub-indices for the locations distributed across the Rabaçal and Tuela River basin.

Rabaçal River basin					
SIte_reference	Riparian Quality Index Score	RQI_cat	Complexity SubScore	Naturalness SubScore	Continuity SubScore
M1	110	1	50	40	20
M2	104	1	47	37	20
M3	85	2	34	32	19
M4	102	1	46	36	20
Minimum Mente River	85	1	34	32	19
Maximum Mente River	110	2	50	40	20
Mean	100.25		44.3	36.3	19.8
Standard deviation	10.7		7.0	3.3	0.5
R1	111	1	51	40	20
R2	115	1	55	40	20
R3	110	1	50	40	20
R4	112	1	54	38	20
R5	114	1	56	38	20
R6	93	2	37	38	18
R7	89	2	32	37	20
R8	101	1	41	40	20
R9	111	1	52	39	20
R10	113	1	53	40	20
R11	103	1	44	40	19
R12	96	1	39	37	20
R13	92	2	35	37	20
Minimum Rabaçal River	89	1	32	37	18
Maximum Rabaçal River	115	2	56	40	20
Mean	104.6		46.1	38.8	19.8
Standard deviation	9.4		8.4	1.3	0.6
Minimum Rabaçal River basin	85	1	32	32	18
Maximum Rabaçal River basin	115	2	56	40	20
Mean	103.6		45.6	38.2	19.8
Standard deviation	9.6		7.9	2.1	0.6

Tuela River basin					
SIte_reference	Riparian Quality Index Score	RQI_cat	Complexity SubScore	Naturalness SubScore	Continuity SubScore
B1	104	1	46	40	18
B2	100	1	40	40	20
B3	65	3	30	19	16
B4	95	2	35	40	20
B5	104	1	44	40	20
B6	99	1	41	38	20
Minimum Baceiro River	65	1	30	19	16
Maximum Baceiro River	104	3	46	40	20
Mean	94.5		39.3	36.2	19.0
Standard deviation	14.8		5.9	8.4	1.7
T1	94	2	35	39	20
T2	79	2	29	36	14
T3	84	2	27	39	18
T4	102	1	44	39	19
T5	87	2	30	38	19
T6	99	1	40	40	19
T7	100	1	40	40	20
T8	90	2	34	36	20
T9	87	2	34	33	20
T10	109	1	50	39	20
T11	95	2	37	38	20
Minimum Tuela River	79	1	27	33	14
Maximum Tuela River	109	2	50	40	20
Mean	93.3		36.4	37.9	19.0
Standard deviation	8.9		6.8	2.1	1.8
Minimum Tuela River basin	65	1	27	19	14
Maximum Tuela River basin	109	3	50	40	20
Mean	93.7		37.4	37.3	19.0
Standard deviation	10.9		6.5	5.1	1.7

3.2 Biotic characterization

For this study, a total of 3157 individuals of the invasive species signal crayfish, *Pacifastacus leniusculus*, were collected. They were found at M1, M2, M3 and M4, R6, R7, R8, R9, R10 and R11, T1, T2, T3, T4, B1, B2, B3 and B4. Regarding abundance, the average value at Rabaçal River basin was 16.4 (ind. CPUE), while at Tuela River basin was 28.3 (ind. CPUE) (Figure 5). Significant differences were found between basins ($p<0.005$).

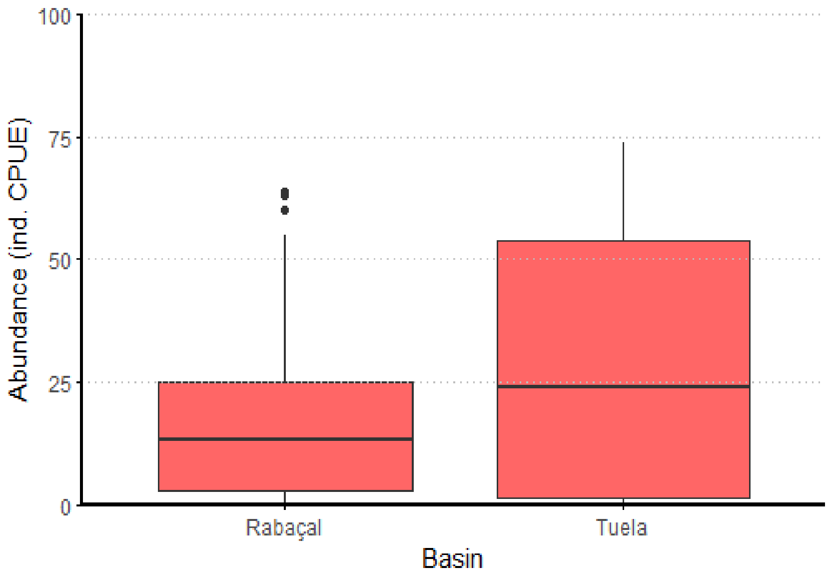


Figure 5 – Abundance (ind. CPUE of the signal crayfish (*Pacifastacus leniusculus*) in Rabaçal and Tuela River basins. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

Regarding the fish community, a total of 2307 fishes belonging to six different species were sampled. The six species were *Salmo trutta*, *Pseudochondrostoma duriense*, *Luciobarbus bocagei*, *Squalius alburnoides*, *Squalius carolitertii* and *Cobitis calderoni*. *S. trutta* was found in all sites expect R1; *P. duriense* was found in all sampling sites from Mente, Rabaçal, Tuela, while on Baceiro was only found at B1, B2 and B3; *L. bocagei* individuals were found in M1, M2 and M3, R1, R2, R3, R4, R5 and R12, T1, T2, T3, and T4; *S. alburnoides* was found in M2, M3 and M4, R1, R2 and R3 and T1 and T3; *S. carolitertii* was present in all sampling sites with the exception of R13, T1, T2, T3 and T4 and B2, B4, B5 and B6; *C. calderoni* was only found on R3. The nMDS of the fish communities is shown in Figure 6.

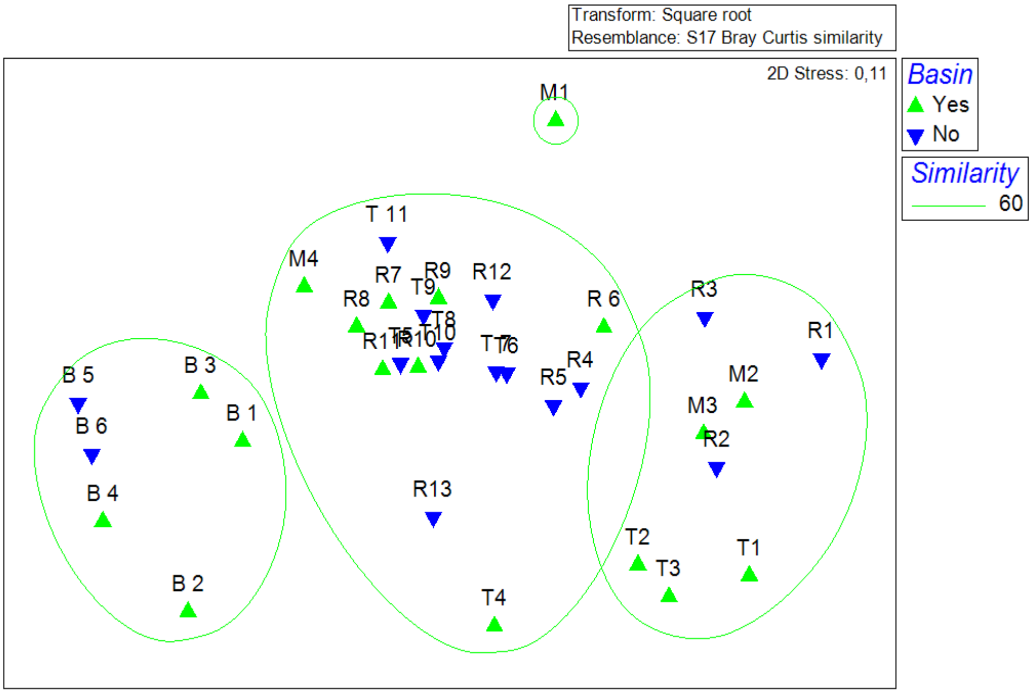


Figure 6 – Non-metric Multi-Dimensional Scaling (nMDS) of the fish communities showing sampling sites and basins.

The Permanova results showed that were no significant differences in the fish communities between basins (Pseudo F=2.13; p=0.10), and between invaded and non-invaded sites (Pseudo F=1.41; p(MC)=0.39). According to the SIMPER analysis the species contributing the most to the dissimilarity between the Rabaçal and Tuela River basins were *Pseudochondrostoma duriense* (15.30%), *Salmo trutta* (10.95%), *Squalius carolitertii* (7.37%), *Squalius alburnoides* (6.71%) and *Luciobarbus bocagei* (5.94%). The species contributing the most to the dissimilarity between invaded and non-invaded sites by the signal crayfish were *Pseudochondrostoma duriense* (34.58%), *Squalius carolitertii* (21.93%) *Salmo trutta* (19.19%), *Squalius alburnoides* (12.54%) and *Luciobarbus bocagei* (10.75%).

Concerning abundance of the fish community (Figure 7A), Rabaçal River basin had an average abundance value of 11.2 (ind. CPUE) at invaded sites and 17.4 (ind. CPUE) at non-invaded sites, while at Tuela River basin the average value was 9.21 (ind. CPUE) at invaded sites and 10.0 (ind. CPUE) at non-invaded sites. The lowest value was found at B2 with 9 (ind. CPUE) individuals sampled, while the highest value was at T3 with 147 (ind. CPUE) individuals sampled. Significant differences in abundance were found between fish species ($p<0.005$) and between basins ($p<0.05$), but not between invaded and non-invaded sites ($p=0.29$). Regarding species richness (Figure 7B), Rabaçal River basin had an average richness value of 3.6 at invaded sites and 4.14 at non-invaded sites, while at Tuela River basin the average value was 2.88 at invaded sites and

2.56 at non-invaded sites. The highest value was at R6 with 6 species and is lowest were at B4, B5 and B6 with only 1 species found. Significant differences between basins were found ($p < 0.005$), but not between invaded and non-invaded sites ($p=0.9$). Regarding Biomass (Figure 7C), Rabaçal River basin had an average biomass value of 206 (g. CPUE) at invaded sites and 343 (g. CPUE) at non-invaded sites, while at Tuela River basin the average value was 212 (g. CPUE) at invaded sites and 223 (g. CPUE) at non-invaded sites. The highest values were at M1, T3 and B1 with 1972.6 (g. CPUE), 1923.3 (g. CPUE) and 1845.7 (g. CPUE) respectively, while the lowest values were at R4, B3 and T1 with 7.48 (g. CPUE), 9.7 (g. CPUE) and 13.62 (g. CPUE) respectively. Significant differences were found between basins ($p<0.05$), but not between invaded and non-invaded sites ($p=0.35$). The Evenness index (Figure 7D), at Rabaçal River basin had an average value of 0.80 at invaded sites and 0.74 at non-invaded sites, while at Tuela River basin the average value was 0.40 at invaded sites and 0.72 at non-invaded sites. The highest values were at T8, T10 and T9 with 0.997, 0.986 and 0.980 respectively, while the lowest were at B4, B5 and B6 with 0. Significant differences were found between invaded and non-invaded sites ($p<0.05$), but not between basins ($p=0.16$). Shannon-Wiener diversity index (Figure 7E), at Rabaçal River basin had an average value of 0.99 both or invaded and non-invaded sites, while at Tuela River basin the average value was 0.45 at invaded sites and 0.80 at non-invaded sites. The highest values were at R1, M3 and R3 with 1.12, 1.14 and 1.43 respectively, while the lowest values were at B4, B5 and B6 with 0. Significant differences were found between basins ($p<0.05$) and between invaded and non-invaded sites ($p<0.05$).

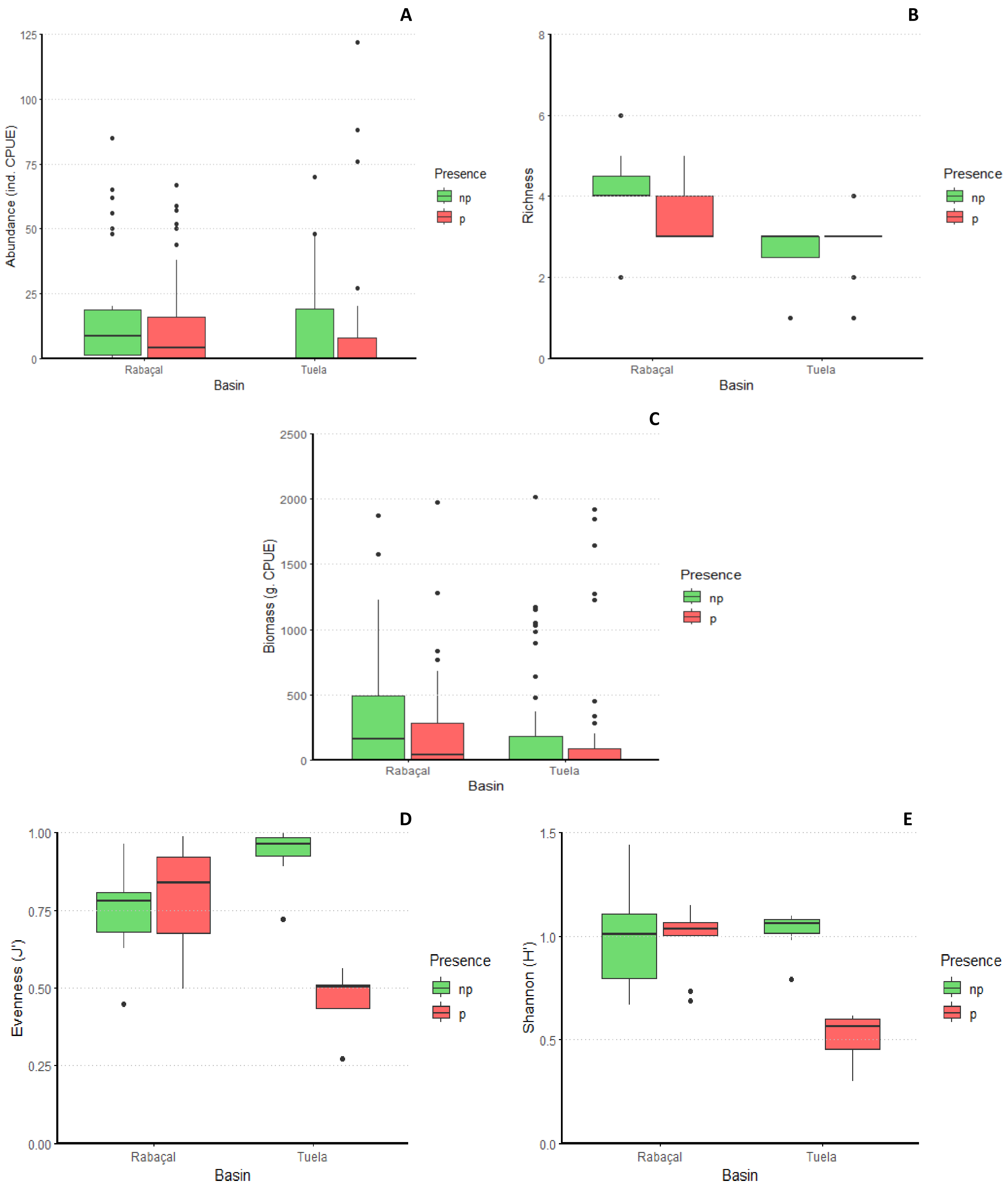
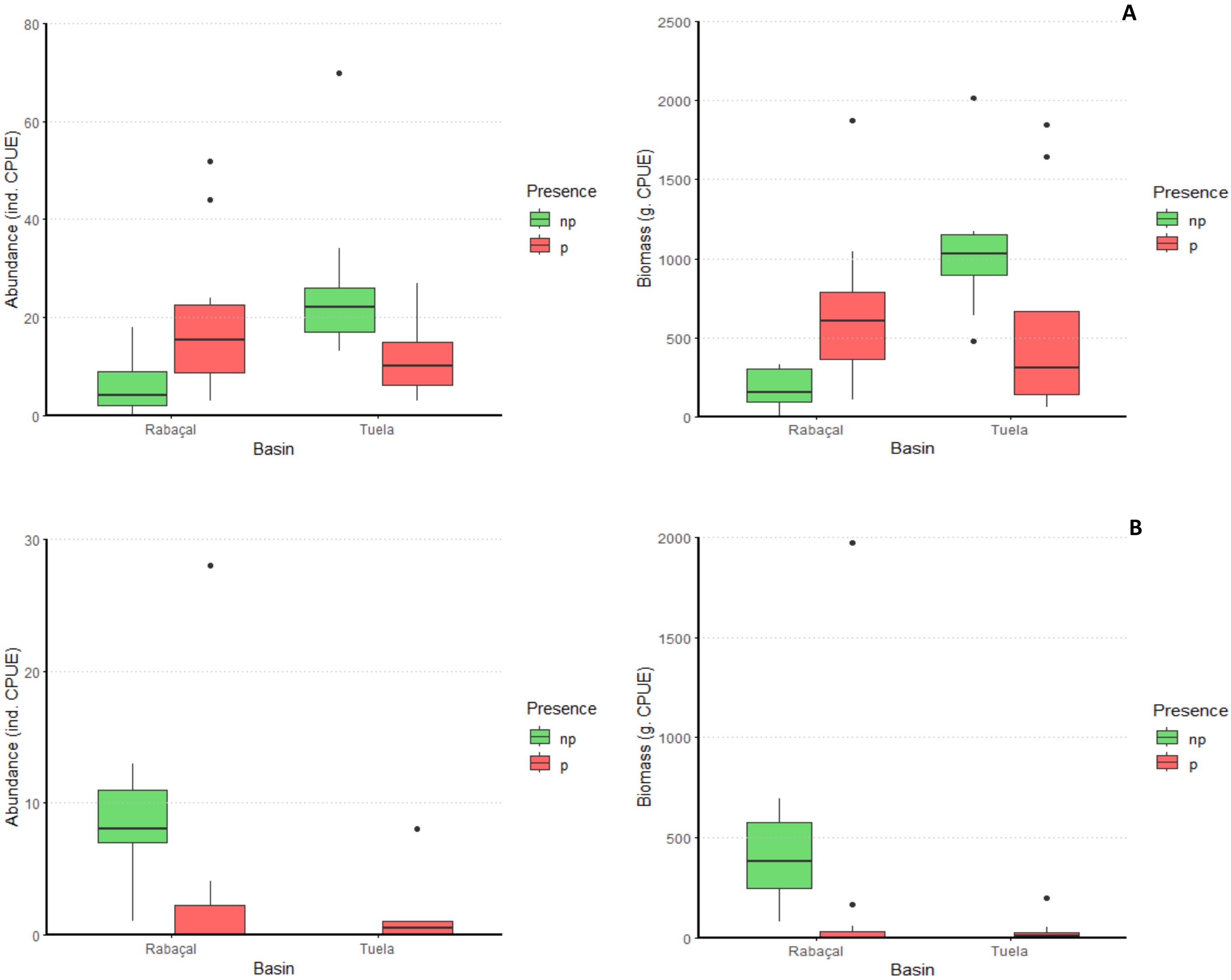


Figure 7– Abundance (A), Richness (B), Biomass (C), Evenness (D) and Shannon index (E) of the fish community on Rabaçal and Tuela River basins. Green indicates no presence of the invasive species while red means presence. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

Analyzing species by species, at Rabaçal River basin, *Salmo trutta* (Figure 8A) had an average abundance value of 18.8 (ind. CPUE) at invaded sites and 6.6 (ind. CPUE) at non-invaded, while at Tuela River basin the average value was 11.4 (ind. CPUE) at invaded sites and 27.0 (ind. CPUE) at non-invaded. No significant differences were found between basins (p=0.1) and between invaded and non-invaded sites (p=0.8). The average biomass, at Rabaçal River basin, was 663 (g. CPUE) at invaded sites and 176 (g. CPUE) at non-invaded, while at Tuela River basin the average was 596 (g. CPUE) at invaded sites and 1045 (g. CPUE) at non-invaded. No significant differences were found between basins (p=0.09) and between invaded and non-invaded sites (p=0.6). *Luciobarbus bocagei* (Figure 8B), at Rabaçal River basin, had an average abundance value of 3.1 (ind. CPUE) at invaded sites and 8.0 (ind. CPUE) at non-invaded, while for Tuela River basin the average value was 1.4 (ind. CPUE) at invaded sites and 0.0 (ind. CPUE) at non-invaded. Significant differences were found between basins (p<0.05), but not between invaded and non-invaded sites (p=0.9). The average biomass, at Rabaçal River basin, was 184.0 (g. CPUE) at invaded sites and 395.0 (g. CPUE) at non-invaded, while for Tuela River basin the average value was 34.7 (g. CPUE) at invaded sites and 0.0 (g. CPUE) at non-invaded. Significant differences were found between basins (p<0.05), but not between invaded and non-invaded sites (p=0.7). *Pseudochondrostoma duriense* (Figure 8C), at Rabaçal River basin, had an average abundance value of 23.3 (ind. CPUE) at invaded sites and 60.8 (ind. CPUE) at non-invaded, while at Tuela River basin the average value was 38.6 (ind. CPUE) at invaded sites and 19.4 (ind. CPUE) at non-invaded. No significant differences were found between basins (p=0.2) and between invaded and non-invaded sites (p=0.4). The average biomass, at Rabaçal River basin, was 344.0 (g. CPUE) at invaded sites and 1142.0 (g. CPUE) at non-invaded sites, while for Tuela River basin the average value was 621.0 (g. CPUE) at invaded sites and 181.0 (g. CPUE) at non-invaded sites. No significant differences were found between basins (p=0.09) and between invaded and non-invaded sites (p=0.7). *Squalius alburnoides* (Figure 8D), at Rabaçal River basin, had an average abundance value of 10.4 (ind. CPUE) at invaded sites and 14.8 (ind. CPUE) at non-invaded sites, while for Tuela River basin the average value was 3.6 (ind. CPUE) at invaded sites and 0.0 (ind. CPUE) at non-invaded sites. No significant differences were found between basins (p=0.1) and between invaded and non-invaded sites (p=0.7). The average biomass, at Rabaçal basin, was 38.0 (g. CPUE) at invaded sites and 124.0 (g. CPUE) at non-invaded, while for Tuela basin the average value was 17.0 (g. CPUE) at invaded sites and 0.0 (g. CPUE) at non-invaded. No significant differences were found between basins (p=0.1) and between invaded and non-invaded sites (p=0.7). *Squalius carolitertii* (Figure

8E), at Rabaçal River basin, had an average abundance value of 11.4 (ind. CPUE) at invaded sites and 13.0 (ind. CPUE) at non-invaded sites, while for Tuela River basin the average value was 0.25 (ind. CPUE) at invaded sites and 13.6 (ind. CPUE) at non-invaded sites. Significant differences were found between invaded and non-invaded sites ($p<0.05$), but not between basins ($p=0.053$). The average biomass, at Rabaçal River basin, was 160.0 (g. CPUE) at invaded sites and 190.0 (g. CPUE) at non-invaded sites, while for Tuela River basin the average value was 3.2 (g. CPUE) at invaded sites and 112.0 (g. CPUE) at non-invaded. Significant differences were found between basins ($p<0.05$), but not between invaded and non-invaded sites ($p=0.09$).



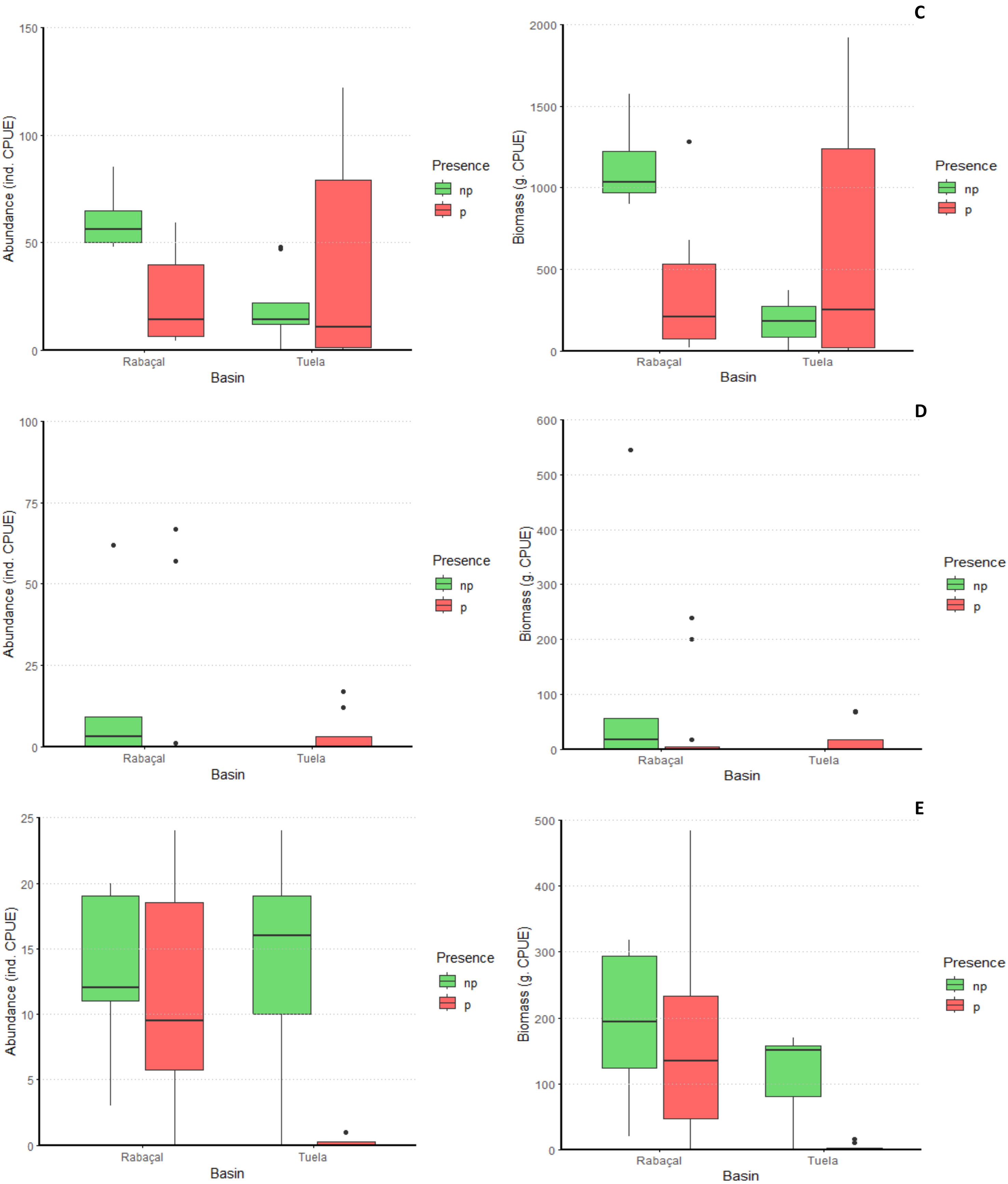


Figure 8 – Abundance and biomass of the *Salmo trutta* (A), *Luciobarbus bocagei* (B) *Pseudochondrostoma duriense* (C), *Squalius alburnoides* (D) and *Squalius carolitertii* (E). Green indicates no presence of the invasive species while red means presence. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

Analyzing the physiological condition through Fulton's condition factor, *Salmo trutta* (Figure 9A) at Rabaçal River basin had an average value of 0.96 at invaded sites and 1.01 at non-invaded sites, while at Tuela River basin the average value was 0.96 at invaded sites and 1.01 at non-invaded sites. Significant differences were observed between invaded and non-invaded sites ($p<0.05$), but not between basins ($p=0.18$). *Luciobarbus bocagei* (Figure 9B) at Rabaçal River basin had an average value of 1.01 at invaded sites and 1.03 at non-invaded sites, while for Tuela River basin the average value was 0.93 at invaded sites, and no specimens were sampled at non-invaded sites. No significant differences were found between basins ($p=0.2$) and between invaded and non-invaded sites ($p=0.3$). *Pseudochondrostoma duriense* (Figure 9C) at Rabaçal River basin had an average value of 0.90 at invaded sites and 0.93 at non-invaded sites, while for Tuela River basin the average values were 1.03 at invaded sites and 0.97 at non-invaded sites. Significant differences were found between basins ($p < 0.005$) and between invaded and non-invaded sites ($p < 0.005$). *Squalius alburnoides* (Figure 9D) at Rabaçal River basin had an average value of 0.87 at invaded sites and 0.88 at non-invaded sites, while for Tuela River basin the average value was 0.94 for the invaded sites, and no specimens were sampled at non-invaded sites. No significant differences were found between basins ($p=0.1$) and between invaded and non-invaded sites ($p=0.97$). *Squalius carolitertii* (Figure 9E) at Rabaçal River basin had an average value of 1.04 at invaded sites and 1.11 at non-invaded sites, while for Tuela River basin, had an average value of 1.36 at invaded sites and 1.1 at non-invaded sites. Significant differences were found between invaded and non-invaded sites ($p < 0.05$), while no difference was found between basins ($p=0.14$).

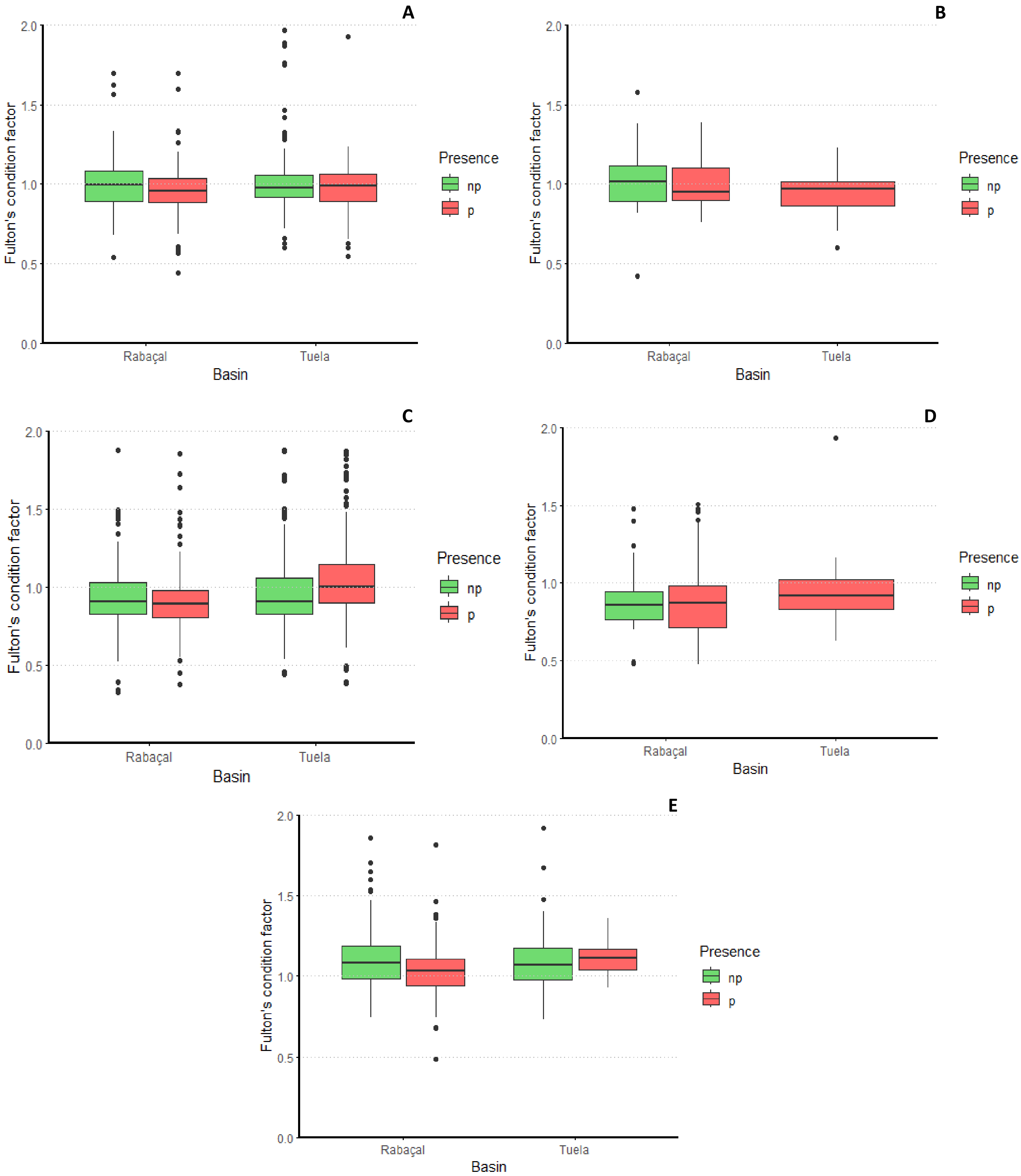


Figure 9 – Fulton's condition factor of *Salmo trutta* (A), *Luciobarbus bocagei* (B), *Pseudochondrostoma duriense* (C), *Squalius alburnoides* (D) and *Squalius carolitertii* (E) on Rabaçal and Tuela River basins. Green indicates no presence of the invasive species while red means presence. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

Analyzing the stomach content of brown trout *S. trutta*, a total of 2206 individuals were sampled and identified (Annex s7), belonging to 47 different *taxa* and 3 different stages of insects. The nMDS of the community sampled is on the figure below (Figure 10).

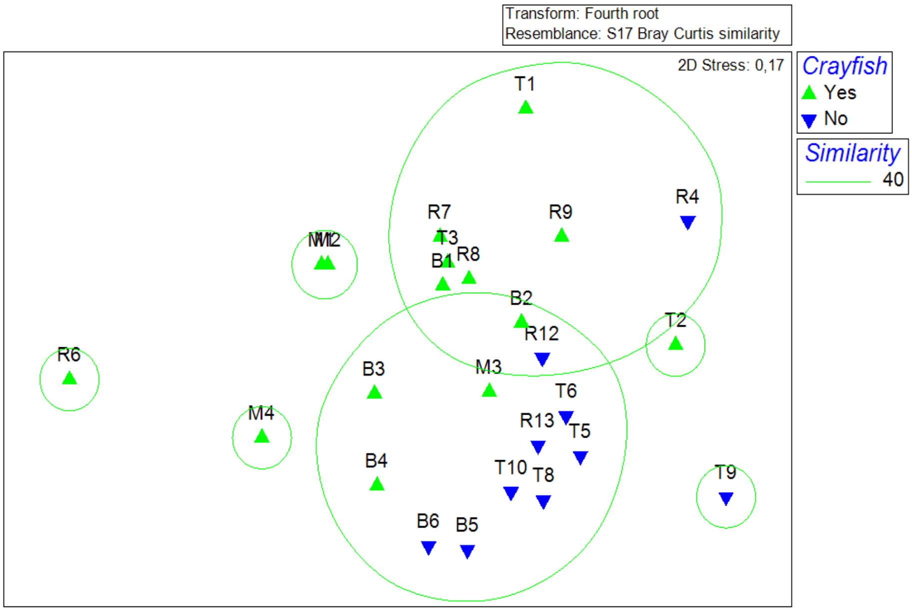


Figure 10 – Non-metric Multi-Dimensional Scaling (nMDS) of the community of the brown trout stomach content showing sampling sites and presence/absence of crayfish.

PERMANOVA results showed no significant differences in prey community between the basins (Pseudo F=1.59; p=0.10) and between the invaded and non-invaded sites (Pseudo F=2.26; p(MC)=0.14). According to the SIMPER analysis the *taxa* contributing the most to the dissimilarity between the Rabaçal and Tuela River basins were Adult insects (35.22%), *Baetidae* (12.94%), *Heptageniidae* (5.37%), *Oligoneuriidae* (5.25%), *Leptoceridae* (4.34%), Pupae (4.12%), *Chironomidae* (3.65%) *Hydropsychidae* (3.05%), *Psychomyiidae* (2.82%), *Simuliidae* (2.53%), *Astacidae* (2.43%). The taxa contributing most to the dissimilarity between invaded and non-invaded sites by the signal crayfish were the *taxa* Adult insects (23.66%), *Baetidae* (12.11%), *Oligoneuriidae* (9.07%), *Leptoceridae* (6.25%), *Heptageniidae* (4.99%), *Chironomidae* (4.76%), Pupae (3.99%), *Simuliidae* (3.23%), *Hydropsychidae* (3.06%), *Leptophlebiidae* (2.98%), *Astacidae* (2.73%), *Psychomyiidae* (2.03%). Concerning abundance (Figure 11A), at Rabaçal River basin, the average value was 12.8 at invaded sites and 9.09 at non-invaded, while at Tuela River basin the average value was 9.24 at invaded sites and 13.1 at non-invaded. No significant differences were found between basins (p=0.06), and between invaded and non-invaded sites (p=0.2). The average richness (Figure11B), at Rabaçal River basin, was 2.71 at invaded sites and 3.55 at non-invaded, while at Tuela River basin, was 2.87 at invaded sites and 5.23 at non-invaded. Significant differences were found between basins ($p<0.005$) and between invaded and non-invaded sites ($p<0.05$). Evenness index (Figure 11C), at Rabaçal River basin had an average value of 0.57 at invaded sites and 0.64 at non-invaded sites, while at Tuela River basin the average value was 0.63 at invaded sites and 0.82 at non-invaded sites. No significant differences were found between basins ($p=0.09$) and between invaded and non-invaded sites ($p=0.08$). Shannon-Wiener diversity index (Figure 11D), at Rabaçal River basin had an average value of 0.66 at invaded sites and 0.89 at non-invaded sites, while at Tuela River basin the average value was 0.73 at invaded sites and 1.32 at non-invaded sites. Significant differences were found between basins ($p<0.05$) and between invaded and non-invaded sites ($p<0.005$).

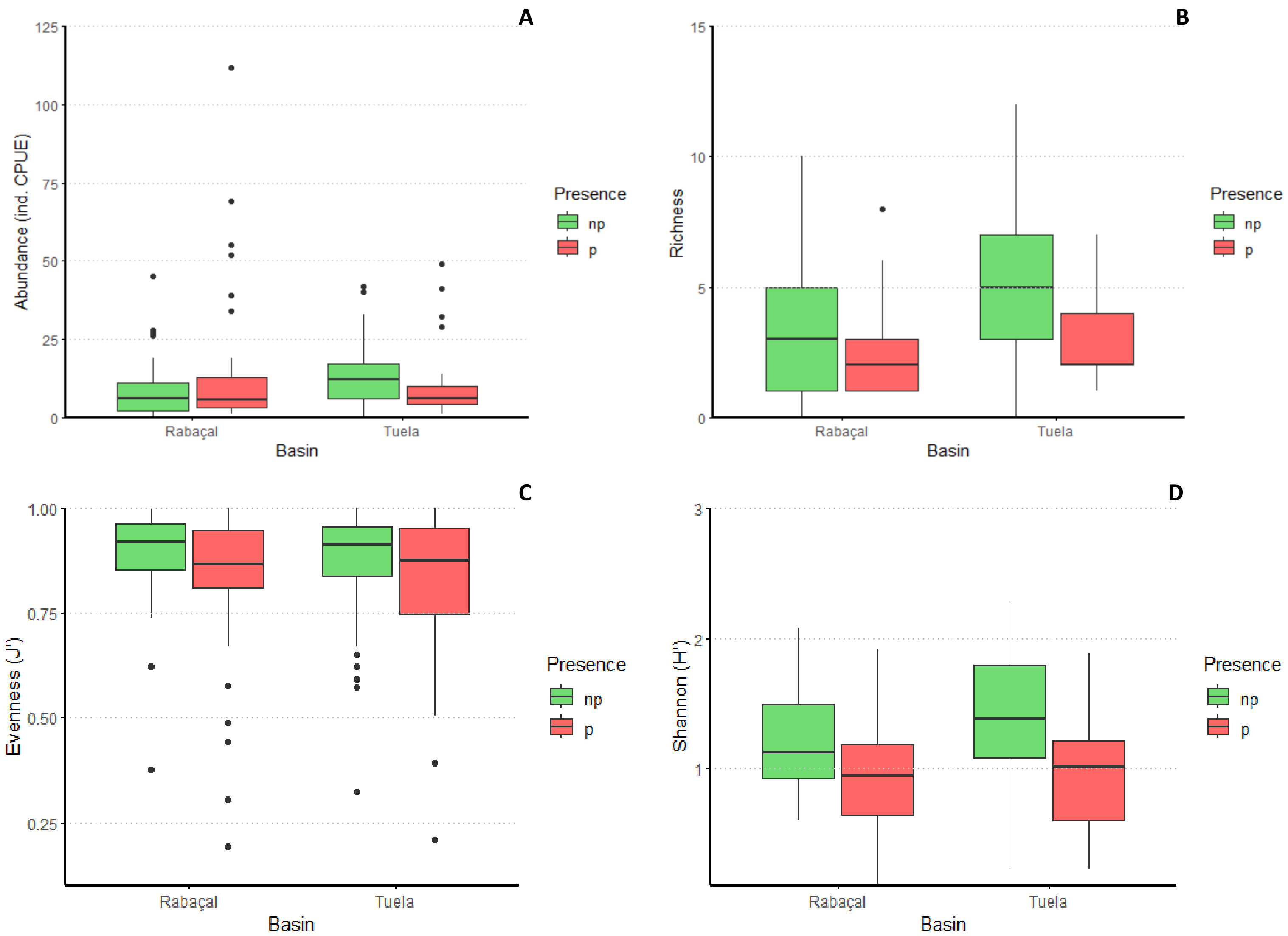


Figure 11– Abundance(A), Richness (B), Evenness index (C) and Shannon index (D) of the community of the brown trout stomach content on Rabaçal and Tuela River basins. Green indicates no presence of the invasive species while red means presence. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

4. Discussion

This study aimed to investigate the ecological effects of the invasive signal crayfish *Pacifastacus leniusculus*, known to produce negative impacts on different organisms (Vedia & Miranda, 2013; Galib et al., 2022; Carvalho et al., 2022), might be having on the fish communities in pristine mountainous rivers of Montesinho Natural Park, Portugal. The lack of studies regarding the ichthyofauna and the invasive signal crayfish in the Iberian Peninsula made it harder to predict the outcomes of this invasion, although studies in other regions point some negative effects on fish populations, especially benthic fish species (Peay et al., 2009; Galib et al., 2021) while others showed no differences (Degerman et al., 2007).

We hypothesized that the presence of the invasive signal crayfish in the Montesinho Natural Park studied rivers would lead to a significant negative impact on abundance, biomass, richness and diversity of fish communities. However, that was not the case, since we only found significant differences in diversity of fish communities present in invaded and non-invaded sites. While we found significant differences in fish communities diversity between invaded and non-invaded sites, there were also significant differences between basins (Rabaçal vs. Tuela). This may indicates that the environmental conditions or the biogeography history of both basins are different enough to distinguish the fish communities in both basins. In fact, the River Habitat Survey performed for this study showed that the Rabaçal River basin has a better overall score than the Tuela River basin and this situation may be responsible for the slightly higher abundance, biomass and species richness retrieved in the Rabaçal River. It should be noted that all the individuals caught in this study belonged to native species, with some of them having high conservation status. This is important to emphasize because the current situation of the fish communities in the Douro River basin is quite precarious since many native fish are disappearing and being displaced by invasive species (Nogueira et al., 2021b). This fact makes the rivers studied and the surrounding areas of great conservation interest for the fish species (and other organisms). Therefore, and despite no negative effects were detected at the community level, the recent introduction of the invasive signal crayfish might pose a serious problem in the future. In fact, and in this study, at the species level we already found differences on the abundance on the species *S. carolitertii* when we compare invaded and non-invaded sites by the signal crayfish. In addition, we also predicted that the presence of the invasive crayfish could impact the physiological condition of the fish, with some studies pointing out a lower condition of native fish species when the invasive species is present

(Light, 2005; Vaeßen & Hollert., 2015). In this study, the species *S. trutta* had a lower physiological condition in invaded sites. Overall, it seems that the invasion of the signal crayfish is already having sub-lethal effects in some fish species as shown by their lower physiological condition, but the impacts were not enough to observe changes at the community level. Therefore, the present study is highly relevant for future comparisons to better understand whether the signal crayfish is affecting the fish communities at different ecological levels (individual, population, species and community) and assess if these impacts will change over the years.

4.1 Abiotic characterization

Rabaçal and Tuela River basins were abiotically similar. Individually, the only sampling sites that stand out, are the ones from Baceiro River, where lower values of water temperature, conductivity and TDS were measured, specially at B4, B5 and B6. Those sites are at higher altitudes compared to the other sampled sites and this situation decreases the water temperature, which will also affect conductivity, since lower water temperature, in natural conditions, is associated to lower values of conductivity. The lower values of conductivity are supported by the lower values of TDS, where the higher number of dissolved ions is an important factor affecting conductivity. All the other sampling sites are differentiated mostly due to altitude, HMS index, TDS, conductivity and water temperature. The sampling sites M1, M2, M3, R1, R2, R3, R4, T1, T2 and T3 are located more downstream, having lower altitudes and wider channels, while R10, R11, R12, R13, T4, T5, T6, T7, T8, T9, T10, T11, B1, B2, B3, B4, B5 and B6 are located more upstream, having higher altitudes and less wider channels. Sites R5, R6, R7, R8 and R9 have intermediate characteristics of altitude and channel width. The Habitat quality index (HQA) on Rabaçal River basin was quite high, with 95% of the sampling sites obtaining "Excellent class", while on Tuela River basin all locations got the "Excellent class". Those scores are attributed to the flow type, bank vegetation structure, characteristics related to trees, channel substrate type, feature of the channel and bank features, that reflects the nearly natural ecological character of the channel habitats and adjacent land use, resulting in good longitudinal and lateral connectivity within the river corridor (Raven et al., 1998, 2002). It is noteworthy that the high scores of each of these sub-indices demonstrate the significant diversity of habitats that the associated characteristics provide to the aquatic and riparian system (Raven et al., 1998, 2002). The Habitat modification score (HMS), regarding both basins, was not so high compared to the HQA, with the "Pristine" and "Predominantly unmodified" classes being achieved in 63.2% of all the sampling sites. For the

Rabaçal River basin, the worst class " Significantly modified" was reached at R6, with the presence of an impermeable weir and two highly impactful bridges clearly contributing to this negative score. While for Tuela River basin, this situation of habitat alteration due to human intervention slightly worsens with the sites B6, T2, T8 and T9 getting the class "Significantly modified" and B3 getting the worst class possible, "Severely modified". This attributed classes reflect anthropogenic influence, through the construction of structures such as bridges, hydraulic passages, bank reinforcement, and channelization, as well as the presence of weirs/transverse barriers (Raven et al., 1998, 2002). Overall, the results from the abiotic characterization supported the claim that the Mente, Rabaçal, Tuela and Baceiro Rivers are in fact worth to be considered almost pristine. In fact, earlier studies, using the same or similar methodologies support the high-quality status of the four studied rivers (Teixeira et al., 2010; Oliveira et al., 2012; Sousa et al., 2015; Nogueira et al., 2021a). Although this high-quality status, recently these basins have been subjected to extreme climatic events such as droughts and heatwaves, that left some parts of the Mente and Baceiro Rivers without water and ended up severely affecting the endangered pearl mussel *Margaritifera margaritifera* (Sousa et al., 2018; Nogueira et al., 2021a). This indicates that, while the results on the River Habitat Survey (RHS) align with the basin's nearly pristine status, all studied regions are still susceptible to extreme climatic events that can have a detrimental impact on the four studied rivers. Overall, the results from the abiotic characterization reflect the almost pristine status of all rivers studied. These results enhance our confidence when assessing the possible effects of the invasive signal crayfish on fish communities, as we recognize that the abiotic conditions and human disturbance are very similar along all the surveyed sites and so possible differences between invaded and non-invaded sites will not be masked by these biases.

4.2 Biotic characterization

Contrary to our expectations, we only observed an impact of the presence of the invasive species signal crayfish on the fish community diversity in the Rabaçal and Tuela River basins. Abundance, biomass and richness did not show any significant difference between invaded and non-invaded sites, with only diversity showing significant differences. These results regarding the fish community are supported by other studies that showed no effects on fish populations (Stenroth & Nystrm., 2003; Degerman et al., 2007). However, other studies found negative effects due to the presence of the signal crayfish (Guan & Wiles., 1997; Peay et al., 2009; Galib et al., 2021), but those are mainly associated with effects on benthic fish. In our studied area most fish are pelagic. When analyzing fish species independently, *S. trutta, L. bocagei*, *P. duriense* and *S. alburnoides* were not affected by the presence of the invasive species signal crayfish, only showing significant differences between basins, while *S. carolitertii* was the only species to exhibit lower abundance with the presence of the invasive species. This result may suggest some interaction between the signal crayfish and *S. carolitertii*, since crayfish species are known to compete with fish for shelter putting them at higher risk of predation (Vaeßen & Hollert, 2015). Therefore, and from all the six fish species, only *S. carolitertii* abundance seems to be affected by the signal crayfish. Anyway, it should be noted that before the introduction, and mainly in the Rabaçal River, *Cobitis calderoni* was much more widespread and abundant (Amílcar Teixeira, personal communication). Being *C. calderoni* a true benthic fish species this recent decline in distribution and abundance occurs at the same time that the invasion of the signal crayfish was progressing in the Rabaçal River. Although our study cannot give a definitive answer about this recent decline in *C. calderoni,* since this species was only found in one sampling site, future studies should address this topic (interaction between *C. calderoni* and *P. leniusculus*) given the high conservation status of this fish species.

At the individual level, and analyzing the physiological condition trough Fulton's condition factor, *S. trutta* exhibited a worst condition when the signal crayfish was present, while *P. duriense* and *S. carolitertii* exhibited better condition in the invaded sites. These results are concordant with (Wood et al., 2017), where these authors assessed the impact of the same invasive species on the growth of the chub (*Squalius cephalus*) and found that the individuals that had the worst growth rate were the younger ones while the older ones seemed to benefit with the presence of the signal crayfish. The same happened in this study, where most of the *S. trutta* individuals that exhibited a lower condition were the younger ones. The possible benefit of the signal crayfish on older fish may make some sense for *S. trutta,* since the older and bigger specimens are known to prey on crayfish (Nyström et al., 2006). The better condition exhibited by *P. duriense* and *S. carolitertii* with the presence of the invasive species might be explained by the location where those individuals were mostly collected, since the invaded sites of the Tuela River basin are located downstream where the rivers are wider, more productive and therefore more suitable for this two species. In addition, these downstream invaded sites can also have a higher concentration of fine particulate organic matter since the signal crayfish is also known to consume leaf litter (Carvalho et al., 2022). In this way both fish species may benefit from this higher availability of food resources. The worse condition of the *S. trutta* individuals might be explained by the fact that crayfish are also known to predate salmonid eggs and alevins (Findlay et al., 2015), while also competing with them for shelter (Griffiths et al., 2004), which exposes them to predation. This decline in the physiological condition of *S. trutta* could also be the result of lesser availability of food, since signal crayfish are known to prey and reduce availability of organisms that are included in fish diets, such as aquatic invertebrates (Crawford et al., 2006; Twardochleb et al., 2013; Ghalib et al., 2021ham), including in the studied rivers (António Nogueira, personal observation). Possibly, this competition for food is higher for the younger individuals that have more difficulty preying than the older ones. The stress induced from competition for shelter and food can also alter the behavior of those fish, while it is also important to not discard the importance of abiotic factors (e.g. physicochemical, physical habitat, water chemistry) that can be changed at a smaller scale by the signal crayfish and this situation somehow affect the fish condition (Nunn et al., 2007). These results concerning the physiological condition of fish must be interpreted with caution since the Fulton's condition factor is affected by the length of the organisms (Blackwell et al., 2000; Froese, 2006). In our defense, we have to say that the fish analyzed between invaded and non-invaded have similar ranges in total length. Anyway, further studies should be done in order to assess these possible differences in the physiological condition of the fish considering their age.

Regarding the analysis of the brown trout diet, the community retrieved from their stomach content showed significant differences in richness and Shannon index between basins and invaded and non-invaded sites. These results make sense because the macroinvertebrate communities analyzed at the same time also showed differences between basins and between invaded and non-invaded sites (António Nogueira, personal observation). Therefore, and since the brown trout mainly consumed invertebrates (Montori et al., 2006; Rocaspana et al., 2016; Piria et al., 2022) it is expected that their diet reflects what is available in the habitat and also the brown trout diet responds to these changes between basins and invaded and non-invaded sites. Of the 2206 individuals sampled from the trout stomach 37 were small signal crayfish, what supports the fact that the invasive species is also preyed by brown trout *S. trutta* (Englund, 1999; Nyström et al., 2006). The groups that were the most consumed were adult insects (700), *Baetidae* (353), *Leptoceridae* (137), *Oligoneuriidae* (132) and *Helptageniidae* (125). Since brown trout is known to be a visual predator, this results are in accordance with other studies done in the Iberian Peninsula (Sánchez et al., 2011; Morante et al., 2012).

The overall results seem to indicate that although we do not detect many significant impacts of the signal crayfish at the community level some sub-lethal effects at the individual level (i.e. physiological condition) were observed. This may be problematic given the recent introduction of the signal crayfish in the studied area (around 10 years since the first individuals were detected in 2013). On the other hand, the low level of disturbance in these rivers, coupled with the abundance of prey, may be contributing to the low impact of this invasive species on the fish populations so far. However, this situation may worsen in the future and so the monitoring of the signal crayfish populations and their possible impacts in the fish (and other organisms) communities should be pursued.

5. Conclusion and future directions

Overall, the analysis of the biotic characterization demonstrated that the presence of the invasive signal crayfish has not yet affected severely the fish communities, with results showing only impacts on diversity. Most of the impacts of their presence were observed at the species level, particularly on *Squalius carolitertii* abundance and the physiological condition of some species (*Salmo trutta*, *Pseudochondrostoma duriense* and *Squalius carolitertii*).

In the future, long term studies must be performed to assess if the impacts caused by the presence of the invasive species may change. To better understand the impacts being caused by the signal crayfish, not only on fish but on other taxonomical groups, periodical surveys and manipulative experiments should be performed. For instance, it will be important analyze the impact of the invasive signal crayfish on the native mammal populations, also present in the Montesinho Natural Park, namely otter (*Lutra lutra*), Pyrenean desman (*Galemys pyrenaicus*) and even in the establishment and spread of other invasive species, such as American mink (*Neovison vison*). In fact it is known that the crayfish, when present, is one of the most consumed preys by otter and American mink (Gonçalves, 2012). This will allow management actions to be taken (e.g. define sites where control measures are more necessary given the higher impacts of the signal crayfish). Studies assessing the diet of the brown trout and the signal crayfish would also be very informative. In particular, studies addressing possible temporal changes since the results reported in this study just addressed the summer situation. Studies at the community level must continue to be performed because, despite the results obtained in this study showing only sub-lethal effects, it is possible that the effects will escalate and produce serious ecological impacts in the ecosystems in the coming years, as shown in other studies (Ghalib et al., 2022). Studies that take on consideration that the invasion is still progressing should assess if the locations where the crayfish is present for longer are actually producing more negative impacts compared with the locations where the crayfish recently established. Finally, in this study we choose to utilize the Fulton's condition factor, despite having some disadvantages, because it is a method that could give us a glimpse of what is happening with the ecophysiology of the fish at the individual level without having to sacrifice them given their conservations status. However, future studies should use other methodologies (biomarkers, transcriptomics, among others) to address this topic in a more accurate way.

A lot of work remains to be done in order to fully understand where and how to control this invasive species and mitigate their ecological effects, but the data collected in this study contributed to increase the knowledge on a topic not well studied and never addressed in Portuguese ecosystems. In fact, the results reported here, concerning a large spatial area and four different rivers with very low human disturbance, can be used as a reference where future studies can compared the progression of the studied impacts. More important than producing more studies about this topic is to use the already available information to design measures to protect fish and other organisms in this protected area and take actions that prevent the further spread and newer introductions of this or other invasive species.

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7. Annex

Table S1 - Contribution of the abiotic variables to each of the two PCA axis.

Variable	PC1	PC2
Temperature	-0.099	-0.037
Oxygen	0.018	0.001
Conductivity	0.048	0.649
TDS	-0.409	0.705
pH	-0.015	0.009
Altitude	0.883	0.266
HQA index	-0.004	-0.021
HMS index	0.201	0.095

Table S2 - Abundance of fish in Mente, Rabaçal, Tuela and Baceiro Rivers.

Species	M1	M2	M3	M4	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13
Salmo trutta fario	5	5	12	44	0	2	4	18	9	3	17	16	10	15	22	52	24
Pseudochondrostoma duriense	4	44	50	5	56	65	48	85	50	59	7	5	12	16	12	28	38
Luciobarbus bocagei	28	3	4	0	11	7	13	8	1	0	0	0	0	0	0	2	0
Squalius alburnoides	0	67	57	1	62	3	9	0	0	0	0	0	0	0	0	0	0
Squalius carolitertii	8	6	3	5	11	3	19	20	12	24	21	13	20	11	8	18	0
Cobitis calderoni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Species	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	B1	B2	B3	B4	B5	B6
Salmo trutta fario	3	7	12	4	20	17	26	17	13	22	70	15	8	27	15	34	24
Pseudochondrostoma duriense	88	76	122	19	14	48	47	20	12	22	12	2	1	1	0	0	0
Luciobarbus bocagei	1	8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Squalius alburnoides	17	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Squalius carolitertii	0	0	0	0	10	19	24	19	19	15	16	1	0	1	0	0	0
Cobitis calderoni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S3 - Results of the analysis of deviance of GLM models, ANOVAs and Kruskal-Wallis tests between the predictor variables (Presence of crayfish and River basin) with the response variables Abundance (N), Richness (S), Pielou's evenness (J') and Shannon-Wiener (H') diversity for the fish community. The asterisk and bold indicates significant values ($p<0.05$).

Fish Community	**Crayfish**	**Basin**
Abundance (N)	X^2=0.95115 p=0.3294	X^2=0.5.2393 **p<0.05***
Richness (S)	X^2=0.00311 p=0.955	X^2=8.8173 **p<0.005** ***
Evenness Index (J')	X^2=5.3004 **p<0.05***	X^2=0.35452 p=0.5516
Shannon index (H')	X^2=4.2605 **p<0.05***	X^2=4.2605 **p<0.05***
Biomass	X^2=0.84461 p=0.3581	X^2=6.1532 **P<0.05***

Table S4 - Results of the analysis of deviance of GLM models, ANOVAs and Kruskal-Wallis tests between the predictor variables (Presence of crayfish and River basin) with the response variables Abundance (N) and biomass of the fish species analyzed individually. The asterisk and bold indicates significant values ($p<0.05$).

Specie by specie	Crayfish	Basin
***S. Trutta* abundance (N)**	t=-0.228 p=0.821	t=1.376 p=0.178
***S. Trutta* biomass**	F=0.258 p=0.615	F=2.931 p=0.0966
***L. bocagei* abundance (N)**	X^2=0.0064 p=0.9361	X^2=4.4502 **p<0.05***
***L. bocagei* biomass**	X^2=0.0784 p=0.0779	X^2=4.7796 **p<0.05***
***P. duriense* abundance (N)**	t=-0.728 p=0.472	t=-1.18 p=0.247
***P. duriense* biomass**	X^2=0.1225 p=0.7263	X^2=2.7923 p=0.0947
***S. alburnoides* abundance (N)**	X^2=0.0935 p=0.7597	X^2=2.4796 p=0.1153
***S. alburnoides* biomass**	X^2=0.0669 p=0.7958	X^2=2.4796 p=0.1153
***S. carolitertii* abundance (N)**	X^2=4.0628 **p<0.05***	X^2=3.7318 p=0.053
***S. carolitertii* biomass**	X^2=2.7554 p=0.0963	X^2=7.4472 **p<0.005****

Table S5 - Results of the analysis of deviance of Kruskal-Wallis tests between the predictor variables (Presence of crayfish and River basin) with the response variable Fulton's condition factor of the fish species analyzed. The asterisk and bold indicates significant values (p<0.05).

Fulton condition factor	Crayfish	Basin
S. Trutta	X^2=6.7083 **p<0.05***	X^2=1.754 p=0.1854
L. bocagei	X^2=1.0559 p=0.3042	X^2=1.576 p=0.2093
P. duriense	X^2=8.9474 **p<0.005****	X^2=37.92 **p<0.0005*****
S. alburnoides	X^2=0.0008 p<0.9769	X^2=2.6626 p=0.1027
S. carolitertii	X^2=7.2226 **p<0.05***	X^2=2.1494 p<0.1426

Table S6 - Results of the analysis of deviance of GLM models and Kruskal-Wallis tests between the predictor variables (Presence of crayfish and River basin) with the response variable Abundance (N), Richness (S), Pielou's evenness (J') and Shannon-Wiener (H') diversity for the trout stomach content community The asterisk and bold indicates significant values ($p<0.05$).

Stomach content community	**Crayfish**	**Basin**
Abundance (N)	t=18.292 p=0.201	t=15.16 p=0.0603
Richness (S)	X^2=4.2956 **p<0.05***	X^2=10.936 **p<0.0005*****
Evenness index (J')	X^2=*2.9176 p=0.087*	X^2=0.0019 p=0.965
Shannon index (H')	X^2=27.482 ***p<0.0005*****	X^2=*5.2496* **p<0.05***

Table S7 - Abundance of the *taxa* collected from the stomach content of brown trout *S. trutta* at the Mente, Rabaçal, Tuela and Baceiro sampling sites.

	M1	M1	M1	M1	M1	M2	M2	M2	M3	M3	M3	M3	M3	M3	M3	M3	M3	M3	M3	M3	M4	M4	M4	M4	M4	M4	M4	M4	M4
Taxa	t1m1	t2m1	t3m1	t4m1	t5m1	t1m2	t2m2	t3m2	t1m3	t2m3	t3m3	t4m3	t5m3	t6m3	t7m3	t8m3	t9m3	t10m3	t11m3	t12m3	t1m4	t2m4	t3m4	t4m4	t5m4	t6m4	t7m4	t8m4	t21m4
AESHNIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANCYLIDAE	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APHELOCHEIRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	1	0	0	0	0
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	0	0	0	0	0	0	0	0	0	7	5	0	0	15	0	3	8	8	2	1	0	0	0	0	0	0	0	0	0
BLEPHARICERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7	1	0	1	1	0	0
CHIRONOMIDAE	0	0	0	0	0	0	0	0	0	2	0	0	0	3	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0
CULICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GERRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	0	0	1	0	1	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE	0	0	0	0	0	0	0	0	0	0	1	0	0	5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
LEPTOCERIDAE	0	0	1	0	0	0	1	0	0	2	1	0	5	16	6	0	0	0	0	0	0	0	0	0	0	0	0	2	0
LEPTOPHLEBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMNEPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMNAEIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	0	0	0	0	0	0	2	0	0	5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POLYCENTROPODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PSYCHOMYIIDAE	0	0	0	0	0	0	0	0	0	11	1	0	1	9	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0
RHYACOPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PUPAE	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4	0	0
ADULT INSECTS	0	0	3	3	3	0	3	2	3	0	4	110	32	0	6	0	0	0	1	0	1	52	26	33	6	9	3	3	0
TERRESTRIAL LARVAE	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0
TELEOSTEI	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R12	R4	R4	R4	R4	R4	R4	R4	R6	R7	R7	R7
Taxa	t4r12	t1r12	t7r12	t21r12	t13r12	t15r12	t6r12	t12r12	t16r12	t18r12	t8r12	t3r12	t5r12	t2r12	t19r12	t17r12	t21r12	t9r12	t11r4	t5r4	t6r4	t2r4	t8r4	t10r4	t9r4	t1r6	t2r6	t1r7	t2r7
AESHNIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANCYLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APHELOCHEIRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	0	3	0	2	2	0	0	1	7	0	1	2	2	1	5	0	1	1	0	5	1	1	2	24	1	0	0	0	0
BLEPHARICERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHIRONOMIDAE	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0
CULICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GERRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	0	0	0	0	0	0
LEPTOCERIDAE	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEPTOPHLEBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
LIMNEPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMNAEIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POLYCENTROPODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PSYCHOMYIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	0
RHYACOPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0
TABANIDAE	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PUPAE	0	0	2	2	0	1	2	0	0	0	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
ADULT INSECTS	1	2	3	5	0	0	1	0	0	0	0	0	5	1	0	0	0	2	0	0	0	0	0	0	0	69	2	0	8
TERRESTRIAL LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TELEOSTEI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxa	R7	R7	R7	R7	R7	R8	R9	R7	R7	R8	R8	R8	R8	R8	R8	R8	R8	R8	R8	R8	R9	R9	R9	R9	R9	R13	R13	R13	R13
	t3r7	t4r7	t5r7	t6r7	t7r7	t8r8	t9r9	t10r7	t11r7	t1r8	t2r8	t3r8	t4r8	t6r8	t7r8	t8r8	t9r8	t10r8	t12r8	t15r8	t1r9	t2r9	t3r9	t4r9	t5r9	t1r13	t2r13	t3r13	t4r13
AESHNIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANCYLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
APHELOCHEIRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	0	0	11	3	6	0	3	3	1	0	5	0	1	2	2	1	0	3	7	8	7	0	0	4	0	2	2	2	0
BLEPHARICERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHIRONOMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
CULICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
GERRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
GOERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	0	1	4	4	8	0	1	0	0	0	1	0	1	1	0	1	0	1	4	3	1	1	0	1	3	2	1	4
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	6
HYDROPTILIDAE	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEPTOCERIDAE	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	3	0	0	6
LEPTOPHLEBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMNEPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMNAEIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	6	22
PERLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	1	0	0	3
POLYCENTROPODIDAE	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PSYCHOMYIIDAE	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	3	0	1	0	0	3	0	0	0	0	0
RHYACOPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	1	0	0	0	0	0	0
TABANIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PUPAE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ADULT INSECTS	1	0	0	0	0	0	0	0	0	0	0	4	0	0	2	2	0	0	0	0	0	1	0	0	0	3	0	3	0
TERRESTRIAL LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TELEOSTEI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	R13	R13	R13	R13	T1	T1	T1	T1	T2	T2	T2	T2	T3	T3	T3	T3	T3	T3	T5	T5	T5	T5	T5	T5	T5	T5	T6	T6	T6
Taxa	t5r13	t6r13	t7r13	t8r13	t1t1	t2t1	t3t1	t5t1	t1t2	t3t2	t4t2	t5t2	t1t3	t2t3	t3t3	t6t3	t7t3	t8t3	t1t5	t2t5	t3t5	t4t5	t5t5	t6t5	t7t5	t8t5	t1t6	t2t6	t3t6
AESHNIDAE	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0
ANCYLIDAE	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	0
APHELOCHEIRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1	0	0	0	1
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	0	0	2	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	0	6	6	0	0	4	2	0	3	0	0	3	0	0	0	6	0	6	0	5	0	10	3	18	3	8	0	0	2
BLEPHARICERIDAE	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	0	2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHIRONOMIDAE	0	0	1	1	0	0	0	0	0	2	0	3	0	0	0	0	0	0	0	1	2	4	3	5	0	5	0	0	0
CULICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0
GERRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	0	0	3	0	0	0	0	1	2	1	2	0	2	0	1	1	0	0	0	0	1	2	0	0	1	5	0	0
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROBIIDAE	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	2	2	4	2	1	1	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	1
HYDROPTILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEPTOCERIDAE	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	7	0	1	0	0	3	6	0
LEPTOPHLEBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMNEPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	5	0	3	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
LYMNAEIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	5	3	15	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0
PERLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	0
PERLODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2	1	0	1
POLYCENTROPODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
PSYCHOMYIIDAE	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	2	0	2	0	0	1	1	0	0	0
RHYACOPHILIDAE	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	1	0	0	0
TABANIDAE	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PUPAE	0	0	0	3	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0	0	5	0	0	10	5	0	2	0	2
ADULT INSECTS	2	0	0	2	0	0	0	0	0	0	0	0	1	2	10	0	0	0	0	1	3	0	0	4	2	0	2	0	0
TERRESTRIAL LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TELEOSTEI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	T6	T6	T6	T6	T6	T8	T8	T8	T8	T8	T8	T8	T8	T9	T9	T9	T10	T10	T10	T10	T10	T10	T10	T10	T10	T10	T10	B1	B1
Taxa	t4t6	t5t6	t6t6	t8t6	t9t6	t1t8	t2t8	t5t8	t6t8	t7t8	t8t8	t9t8	t10t8	t1t9	t2t9	t3t9	t1t10	t2t10	t3t10	t4t10	t5t10	t6t10	t7t10	t8t10	t9t10	t10t10	t12t10	t2b1	t3b1
AESHNIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
ANCYLIDAE	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APHELOCHEIRIDAE	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	2	0	2	12	0	0	0	0	0	2	0	0	0	5	0	2	0	3	0	0	0	2	3	1	0	0	2	1	0
BLEPHARICERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHIRONOMIDAE	1	0	0	4	0	0	0	0	0	0	0	0	2	6	0	0	0	0	0	1	0	0	1	0	0	0	2	0	0
CULICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0	0	0	2	0	1	0	2	0	0	0	0	1	0	0
GERRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	5	2	3	0	0	0	0	0	7	3	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	3
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2	0	0
HYDROBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	1	0	0	0	0	0
HYDROPTILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
LEPTOCERIDAE	0	4	4	7	0	0	0	0	10	8	0	0	0	0	0	0	0	0	0	0	1	3	6	1	2	0	2	0	0
LEPTOPHLEBIIDAE	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMNEPHILIDAE	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMNAEIDAE	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	4	1	2	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	3	0	1	0	0	2	0	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	0	2	0	3	0	0	4	1	1	1	0	0	0	3	4	1	0	4	0	18	9	0	1	3	0	0	1	0	0
PERLIDAE	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLODIDAE	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	0	0	0	0	0	5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POLYCENTROPODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PSYCHOMYIIDAE	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RHYACOPHILIDAE	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIIDAE	0	0	0	3	0	0	8	8	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PUPAE	0	0	0	2	0	0	0	3	0	0	0	0	8	5	0	0	2	0	0	0	0	0	0	2	2	1	0	0	0
ADULT INSECTS	0	0	0	2	0	0	2	2	0	2	1	0	2	0	0	0	8	3	0	0	3	0	3	0	0	5	0	2	1
TERRESTRIAL LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
TELEOSTEI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxa	B1 t4b1	B1 t5b1	B1 t7b1	B1 t9b1	B1 t10b1	B1 t11b1	B2 t1b2	B2 t2b2	B2 t3b2	B2 t4b2	B2 t5b2	B3 t1b3	B3 t2b3	B3 t3b3	B3 t4b3	B3 t5b3	B3 t6b3	B3 t7b3	B3 t8b3	B3 t9b3	B3 t10b3	B3 t11b3	B4 t1b4	B4 t2b4
AESHNIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANCYLIDAE	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APHELOCHEIRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	2	0	2	0	0	1	1	2	0	1	0	3	3	1	1	1	1	0	0	0	0	0
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	0	0	0	0	0	7	22	1	0	2	0	0	0	0	0	0	8	0	0	0	0	0	0	0
BLEPHARICERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHIRONOMIDAE	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
CULICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GERRIDAE	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	0	0	0	0	1	7	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEPTOCERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
LEPTOPHLEBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMNEPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMNAEIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLIDAE	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POLYCENTROPODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
PSYCHOMYIIDAE	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RHYACOPHILIDAE	0	0	0	0	0	0	0	0	2	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SIMULIIDAE	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PUPAE	0	0	0	2	2	0	0	0	5	3	0	0	0	2	0	0	0	0	0	2	0	1	1	0
ADULT INSECTS	2	2	3	6	6	0	1	0	18	2	3	0	2	0	2	5	0	39	12	1	7	4	3	49
TERRESTRIAL LARVAE	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
TELEOSTEI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxa	B4 t3b4	B4 t4b4	B4 t5b4	B4 t6b4	B4 t7b4	B5 t1b5	B5 t2b5	B5 t3b5	B5 t5b5	B5 t6b5	B5 t7b5	B5 t8b5	B5 t9b5	B5 t10b5	B5 t11b5	B5 t12b5	B5 t13b5	B5 t14b5	B6 t1b6	B6 t3b6	B6 t4b6	B6 t5b6	B6 t6b6	B6 t7b6
AESHNIDAE	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANCYLIDAE	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	6	0	1	0	0	0
APHELOCHEIRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASELLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ASTACIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
ATHERICIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE	0	0	0	2	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	2	0	0	0	1
BLEPHARICERIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACHYCENTRIDAE	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CAENIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERATOPOGONIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
CHIRONOMIDAE	0	0	0	4	0	0	2	0	2	6	1	1	0	0	3	1	0	0	0	2	2	0	1	2
CULICIDAE	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
DRYOPIDAE	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
ELMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
EPHEMERELLIDAE	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
GERRIDAE	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
GLOSSOSOMATIDAE	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
GOERIDAE	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GOMPHIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
GYRINIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEPTAGENIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDRACARINA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
HYDROBIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPSYCHIDAE	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LEPTOCERIDAE	0	0	3	1	0	0	0	0	0	3	0	0	0	0	1	0	1	0	2	0	1	0	0	0
LEPTOPHLEBIIDAE	0	0	0	0	0	0	3	0	0	0	0	16	0	0	0	1	1	0	1	3	2	2	0	2
LEUCTRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
LIMNEPHILIDAE	0	0	0	0	0	0	2	2	0	0	0	0	0	1	0	0	2	2	0	0	0	0	0	0
LIMONIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LYMNAEIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMATHELMINTHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NEMOURIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
OLIGONEURIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PERLODIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHILOPOTAMIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POLYCENTROPODIDAE	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1	0	0
PSYCHOMYIIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RHYACOPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0
SERICOSTOMATIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIIDAE	0	0	0	0	0	0	0	0	0	1	0	0	0	0	7	0	0	0	0	0	0	0	0	1
TABANIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UENOIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
PUPAE	0	2	5	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0
ADULT INSECTS	3	0	2	1	5	7	0	0	2	1	0	0	2	3	1	0	10	0	5	1	2	0	2	3
TERRESTRIAL LARVAE	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	1	0	0
TELEOSTEI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0