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Management of Brown trout populations (*Salmo trutta* L.) in northeastern Portugal (Douro basin): Analysis of habitat use and feeding strategies

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GENERAL ABSTRACT

During the spring/summer of 2017, the ecological integrity of salmonid headwater streams and salmonid/cyprinid Transition Rivers of the Upper Tua basin was evaluated. Abiotic parameters (water quality and aquatic and riparian habitats) and biotic (macroinvertebrate community) parameters were evaluated in 14 selected sampling sites, distributed across six different rivers and streams of Upper Tua. Sampling of the fish and invertebrate communities was done according to the protocol defined by the Portuguese Environment Agency. In relation to the target species of the study, the Brown trout (Salmo trutta L.) were determined: 1) Population parameters 2) Ecological guilds 3) F-IBIP index and 4) Use of resources, namely the strategy developed for food and habitat.

The obtained results showed that, in general, aquatic ecosystems have a good ecological integrity, based on the physical and chemical quality of the water (*e.g.*, and low temperature T <20ºC, conductivity EC_{25} <80 µS.cm^{-1} and content of dissolved salts TDS< 100 mg.L^{-1}, high oxygenation (D.O.> 8 mg.L^{-1}) hydromorphological conditions (*e.g.* pronounced riffle/pool sequence, diversity of microhabitats and well structured riparian galleries) and biological (*e.g.* macroinvertebrate communities dominated by stenobiont insects, mostly belonging to Ephemeroptera, Plecoptera, Trichoptera and Diptera orders) . All metrics (*e.g.* Shannon-Wienner's H'Diversity, taxonomic richness) and biotic indexes (e.g. IBMWP, IPTIN) corroborated the good ecological quality found. However, some signs of anthropogenic influence were detected, especially in small hydroelectric dams (eg T4, Rio Tuela, below the Hydroelectric PowerPlants), with a slight decrease in the quality of habitats and the density and structure of fish stocks, in particular Brown trout.

The fish fauna of the sites sampled in the Upper Tua basin is composed of native species, considering populations of: 1) Brown trout (*Salmo trutta*), dominant or exclusive populations present in the small sampled streams; 2) Cyprinids: Northern chub (*Squalius carolitertii*), Northern straight-mouth nase (*Pseudochondrostoma duriense*), common barbel (*Luciobarbus bocagei*) and Calandino roach (*Squalius alburnoides*) and (3) Cobitids: in the case of Northern Iberian Spiny Loach (*Cobitis calderoni*). The biotic integrity based on the F-IBIP showed that all the sampled sites had a GOOD or EXCELLENT classification. Regarding to the *Salmo trutta* populations, significant differences were detected in the physical condition of the fishes, taking into account the different watercourses. In fact, in the Mente river, the Brown trout indivuiduals have a low physical condition, when compared to the other rivers. Differences were also observed in the diet of the trout in the sampling sites, both intra and interpopulational, although the feeding strategies presented by this species, similarly to other studies, is typically microcarnivore with an opportunitic behavior. The obtained results for habitat use allowed to confirm the social hierarchy of these animals, since the best refuges (boulders, undercut banks) are colonized by adult trout, while juveniles were found in microhabitats of lower quality in riffle zones, and with lower degree cover.

For the correct management of Brown trout populations in the Upper Tua, it is necessary the preservation of the actual ecological integrity status, in particular the priority habitats that should benefit of specific fishery management measures. Finally, it should be stressed that the management of any target species can only be successful if it is integrated into an ecosystem-wide strategy involving all groups of potential users and, in particular, find the right solutions to the sustinable combination of the exploitation and conservation of resources.

Key-words: bioecology, conservation, ecological integrity, river, trout

RESUMO GERAL

Durante a primavera/verão de 2017 foi avaliada a integridade ecológica de rios aptidão salmonícola (i.e. dominados por populações de truta) e mista (i.e. as populações de truta coabitam com ciprinídeos endémicos) da bacia hidrográfica do Alto Tua. Foram avaliados parâmetros abióticos (qualidade da água e dos habitats aquáticos e ribeirinhos) e bióticos (comunidade de macroinvertebrados) em 14 locais de amostragem selecionados e distribuídos por seis diferentes rios e ribeiras do Alto Tua. A amostragem das comunidades de peixes e invertebrados foi feita de acordo com o protocolo definido pela Agência Portuguesa do Ambiente. No que respeita à espécie alvo do estudo, a truta-de-rio (*Salmo trutta* L.) foram determinados: 1) Parâmetros populacionais 2) Guildas ecológicas 3) Índice F-IBIP e 4) Uso de recursos, nomeadamente a estratégia desenvolvida na alimentação e habitat.

Os resultados obtidos permitiram verificar que, globalmente, os ecossistemas aquáticos possuem uma boa integridade ecológica, baseada na qualidade físico-química da água (e.g. valores baixos de temperatura, T< 20ºC, condutividade, EC_{25}< 80 µS.cm^{-1} e teor de sais dissolvidos TDS< 100 mg.L^{-1}, elevada oxigenação O.D.> 8 mg.L^{-1}) hidromorfológica (e.g. elevada sequência *riffle/pool*, diversidade de microhabitats e galerias ripícolas bem estruturadas) e biológica (e.g. comunidades de macroinvertebrados dominadas por organismos estenobiontes, maioritariamente pertencentes aos Ephemeroptera, Plecoptera, Trichoptera e Diptera). Todas as métricas (e.g. Diversidade H' de Shannon-Wienner, riqueza taxonómica) e índices bióticos (e.g. IBMWP, IPtIN) calculados corroboram na boa qualidade ecológica encontrada. Foram, contudo, detetados alguns sinais de influência antropogénica, nomeadamente junto de pequenas mini-hídricas (e.g. T4, Rio Tuela, abaixo do Aproveitamento Hidroelétrico das Trutas), registando-se uma ligeira diminuição na qualidade de habitats e a densidade e estrutura das populações de peixes, nomeadamente de truta-de-rio.

A fauna piscícola dos locais amostrados na bacia do Alto Tua está composta por espécies autóctones, caso de populações de 1) Truta-de-rio, dominante ou exclusivamente presentes nas ribeiras amostradas; 2) ciprinídeos, como o escalo-do-Norte (*Squalius carolitertii*), a boga-do-Douro (*Pseudochondrostoma duriense*), o barbocomum (*Luciobarbus bocagei*) e o bordalo (*Squalius alburnoides*) e ainda 3) cobitídeos, caso do verdemã-do-norte (*Cobitis calderoni*). A integridade biótica baseada no F-IBIP mostrou que todos os locais amostrados possuem uma BOA ou EXCELENTE classificação. No que respeita às populações de *Salmo trutta* detetaram-se diferenças significativas na condição física dos peixes dos diferentes cursos de água, i.e. no rio Mente as trutas possuem uma menor condição física, quando comparada com os restantes rios. Foram também observadas diferenças na dieta das trutas nos rios amostrados, quer em termos intra como interpopulacionais, embora a alimentação da espécie , à semelhança doutros estudos, tenha apresentado um comportamento trófico tipicamente generalista e microcarnívoro. Os resultados obtidos relativamente ao uso do habitat permitiram confirmar a hierarquia social patente nestes animais, dada a colonização, dos melhores refúgios (blocos, margens escavadas) pelas trutas adultas, enquanto os alevins e juvenis foram encontrados nos microhabitats de menor qualidade, em zonas de *riffle* e com menor grau cobertura.

Para a correta gestão das populações de truta-de-rio do Alto Tua é necessária a preservação do estado de integridade ecológica, nomeadamente os habitats prioritários que deverão beneficiar, à luz dos conhecimentos existentes de medidas específicas de ordenamento piscícola. Finalmente deve salientar-se que a gestão duma qualquer espécie-alvo só pode ter sucesso caso esteja integrada numa estratégia à escala ecossistémica, que envolva todos os grupos de potenciais utilizadores e muito em particular sejam encontradas soluções para a harmoniosa conjugação da exploração e conservação de recursos.

Palavras-chave: *bioecologia, conservação, integridade ecológica, rios, truta*

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SYMBOLS

ANOSIM - Analysis of Similarities

EC_{25} - Electric Conductivity

EPT- Ephemeroptera, Plecoptera, Trichoptera

GQC- Channel Quality Index

IBMWP - Iberian Biological Monitoring Working Party

INAG - National Institute of Water

$IPtI_N$ - Portuguese Index of North Invertebrates

IUCN - International Union for Conservation of Nature

NMDS - Non-metric Multi Dimensional Scaling Ordination

QBR - Quality Index of Riparian Gallery

RHS - River Habitat Survey

RQE- Ecological quality index

TDS- Total Dissolved Solids

WFD – Water Framework Directive in Portugal

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Chapter 1

GENERAL INTRODUCTION

Freshwater ecosystems have suffered a long history of anthropogenic disturbances that are responsible for the decline of fish populations (Dudgeon et al., 2006). A special focus is put on Brown trout, as it has an outstanding socio-economic importance, both in commercial and sport fisheries, and it is frequently used as tourist attraction (Aas et al., 2000; Butler et al., 2009), being the most important and popular recreational fishery all across Europe (Almodóvar & Nicola, 1998). Owing to stream habitat degradation caused by water regulation, pollution, riparian coverage removal and the introduction of exotic species (Elvira, 1995; Smith & Darwall, 2005; Clavero et al., 2010; Moyle, Katz & Quinones, 2011) local Brown trout stocks are not always sufficient to support the increasing demand of sport fishery. Mortality produced by excessive angling can reduce the spawning stock density to such a low level that natural recruitment becomes insufficient to maintain the fishery (Avery & Hunt, 1981). Studies on the response of fish species to natural and anthropogenic pressures increase our understanding of the ecology of the species involved and are also relevant to international legislation such as the Water Framework Directive in Europe (EU Commission, 2000).

Brown trout (*Salmo trutta* L.) belongs to the family of Salmonidae. Its morphology, population structure and genetics can be highly variable from one location to another. In general, Brown trout has a fusiform body, little and pointed head, large mouth extending mostly after the eye and has well developed, thick and rounded caudal peduncle, little scales, grey-blue body colored with numerous spots, also below the lateral line, blackish colored on upper part of body, usually orange on sides, surrounded by pale halos, adipose fin with red margin (Rochard & Elie, 1994).

Brown trout populations can be found in different aquatic ecosystems, such as streams, ponds, rivers and lakes (Scott & Scott, 1988). There are resident populations that spend all life cycle in headwater streams and anadromous populations. In this case, the individuals spend 1 to 5 years in freshwater ecosystems and 6 months to 5 years in salt water (Rochard & Elie, 1994). Juveniles mature in 3-4 years (Hart, 1973). Lacustrine populations undertake migration to tributaries and lake outlets to spawn, rarely spawning on stone, wave-washed lake shores. Spawning takes place normally more than one time (Rochard & Elie, 1994). Life history and spawning behavior is similar to the salmon, *Salmo salar* (Rochard & Elie, 1994). Each female produces about 10 000 eggs (Muus & Nielsen, 1999; Rochard & Elie, 1994). Female covers the eggs by restirring the sand and fine gravel (Vostradovsky, 1973). After hatching at 12 mm, larval Brown trout remain in the gravel for 2-3 weeks until they are about 25 mm long, when they emerge to begin feeding in the water column. Brown trout are territorial and begin establishing territories as juveniles. Juvenile trout from lake populations move from their natal inlets to lakes during the first two years of life (Kailola et al., 1993).

Brown trout is an indigenous species from Europe, North Africa and Western Asia (Elliott, 1994). It occurs in the rivers of North and Central Portugal. Its distribution is limited to the south by the upper section of the river Zêzere. The anadromous migratory form occurs only in the watersheds of the rivers Âncora, Lima and Minho (Almaça, 1996). The stream elevation, depth and slope are main factors that help us to understand the bioecology of this fish species. In fact, according to the Water Framework Directive, rivers were classified and their typology distinguished between three types of rivers that our study area concerns: North streams with small dimension (N1≤100 km^2), North streams with median and big dimension (N1> 100km^2) and Alto Douro rivers (N2).

The Brown trout species presents a territorial behavior, occurring in exclusively fish populations in the upper sections of the rivers (headwater zone) in Portugal, with steep gradient, fast flowing water and cool temperature. The fast flow rate causes turbulence, which keeps the water well oxygenated, essential for reproduction phase but also for other life cycle phases, since Brown trout has very exigent bioecology requirements. In the transition zone between headwater and depositional zones where the temperature is slightly higher and the slope is relatively lower, Brown trout cohabitates with endemic cyprinids such as: *Pseudochondrostoma duriense*, *Squalius alburnoides*, *Squalius carolitertii* and sometimes with *Luciobarbus bocagei.*

Studies of the feeding ecology of *Salmo trutta* are frequent across the world (e.g., Jensen et al., 2004; Fochetti et al., 2008; Sánchez-Hernández et al., 2011). They report for Brown trout, taking into account ontogenetic dietary variations, a diet based on aquatic and terrestrial insects (Cadwallader & Backhouse, 1983; Rochard & Elie, 1994) but also other preys, while cyprinids comprise a wide variety of specialists and generalists feeding behaviors (Lammens & Hoogenboezem, 1991). Most individuals feed on the secondary producers: zooplankton, macrocrustaceans, larvae, pupae and adults of insects, oligochaetes, bryozoans, snails, and mussels. In both cases of Brown trout or cyprinids, the diet must be related to the size of the fish and to availability (density, size distribution, visibility etc.) of food.

The management of *Salmo trutta* requires an integral analysis of population's structure and their interactions with their environment, comprising its habitat, feeding, ecological behavior, interactions with other species and human impact. Furthermore, Brown trout species can be the unique host fish for threatened mussel populations, like freshwater pearl mussels (*Margaritifera margaritifera*) present in Natural Park of Montesinho. Therefore, the involving of human dimension is a key component in the management of fish populations. Thus, the management of Brown trout is considered as a compromising between the conservation of local populations and their exploitation by anglers using methods such as stocking of domestic Brown trout populations and fishing regulations in order to control angler impacts on fish populations and maintain numbers and sizes of fish in a lake or stream.

OBJECTIVES AND ORGANIZATION OF THE DISSERTATION

To achieve the main objective of the dissertation, which is the conservation and management of Brown trout, in Upper Tua, several specific objectives can be defined in order:

- To characterize the ecological status of salmonid streams of NE Portugal , based on abiotic (water quality, channel and riparian habitat) and biotic (invertebrate and fish) characteristics;
- To estimate fish population parameters (abundance, biomass, growth, condition factor) of fish stocks;
- To evaluate the ichthyological (F-IBIP) index;
- To determine the resources used by trout populations: habitat and feeding strategies and

- To identify disturbed areas and propose measures for better management and planning of sport fishing of salmonid streams.

The dissertation is organized in four chapters. The first (chapter 1) corresponds to the introduction and definition of objectives. The next two chapters (Chapters 2 and 3) are presented with a structure similar to that of scientific articles. In the last one (Chapter 4), general discussion and main conclusions will be done:

- **Chapter 1: General Introduction. Objectives and organization of the dissertation**
- **Chapter 2: Ecological status of salmonid streams in Northeastern Portugal (Douro Basin)**
- **Chapter 3: Management of Brown Trout Populations in Montesinho Natural Park (Tua Basin, Northeastern Portugal)**
- **Chapter 4: General Conclusions and final considerations**

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Chapter 2

ECOLOGICAL STATUS OF SALMONID STREAMS IN NORTHEASTERN PORTUGAL

ABSTRACT

During the spring of 2017 the ecological integrity of salmonid streams of the Upper Tua (River Douro basin, NE Portugal) was evaluated. Abiotic (e.g. water quality and aquatic and riparian habitats) and biotic (e.g. macroinvertebrate community) variables were measured in 14 sampling sites, selected along the different tributaries of low order (e.g. Baceiro, Tuela, S. Cibrão, Mente, Assureira and Rabaçal). It was followed the methodology defined by Water Framework Directive in Europe, and adapted to Portugal by Portuguese Environmental Agency (APA). The sampling sites showed, in general, good or excellent water quality, confirmed by the low values of water temperature (T <15°C), dissolved salts (EC25 <80 µS/cm) and high levels of dissolved oxygen (OD> 8 mg/L). Some heterogeneity of aquatic and riparian microhabitats (RHS and GQC indexes majority rating of Good or Excellent condition) were detected, and allowed the presence of a high diversity of macroinvertebrates and particularly threatened species (e.g. *Margaritifera margaritifera*, *Macromiia splendens*). The proportion of sensible insect taxa, such as Ephemeroptera, Plecoptera and Trichoptera confirmed the good ecological status of the rivers in the Natural Park of Montesinho (PNM). Lower values were detected near a small hydroetectric powerplant (A.H. das Trutas), where substantial but localized shifts in aquatic and riparian habitats contributed to the diminishing of the ecological integrity. The conservation and management of salmonid streams in PNM, considering not only *Salmo trutta* populations but also a threatened cobitid, *Cobitis calderoni* and several endemic cyprinids, like *Pseudochondrostoma duriense*, *Squalius alburnoides*, *Squalius carolitertii* and *Luciobarbus bocagei* must be safeguard in order to maintain the good ecological status and prevent the environmental degradation and the dispersion to upstream of exotic species, some with high invasive potential, such as the detected signal crayfish (*Pacifastacus leniusculus*).

Key-words: *ecological integrity, water quality, habitats, invertebrates*

2.1. INTRODUCTION

Considering geomorphological and hydrological traits, two types of freshwater ecosystems are distinguished *i.e.* lotic ecosystems with running water (e.g. rivers, creeks, streams) and lentic ecosystems with still water (e.g. ponds, reservoirs, lakes, marshes). Although slight differences in temperature can exist between the surface and bottom waters of deep lotic systems, the greatest changes take place as water moves downstream. Therefore, changes in the hydrological and hydraulic conditions of lotic ecosystems can severely affect aquatic communities, whose structure and functioning will have to adapt accordingly (Dolédec et al., 2007; Milner et al., 2001). Increasing anthropogenic influence on lotic environments, as a result of human disturbances, has captured public interest because of the consequent problems associated with deterioration of water quality (Bere and Tundisi, 2010). Especially that activities or disturbances at one location affect processes and organisms downstream, this fundamental feature characterizing lotic environments complicates the management of these systems (Tundisi and Matsumura, 2008).

The implementation of Water Framework Directive requires tools for measuring and monitoring the ecological status of aquatic ecosystems, namely physical (channel and riparian habitat assessment), chemical (water quality) and biological methods (Lobo et al., 2004). In the WFD, ecological status is defined as ‘‘an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters’’ and for biological quality elements is mainly measured as changes in the composition and abundance of different taxonomic groups (Benejam et al., 2008)

Largely diverse, occupying a wide range of habitats, macroinvertebrates are an excellent indicator of the quality of habitats where they occur, hence their increasing use in different types of biotic indices (Tachet et al. 2010). Macroinvertebrates include a variety of organisms which are all critical in maintaining the functional integrity of lotic ecosystems due to the key roles they play (Wallace & Webster, 1996; Kagalou et al., 2006). The presence of certain macroinvertebrates over others can suggest varying water qualities and can assist researchers in proposing solutions. For example, shredder species are not tolerant of high nitrogen areas; chronimids are tolerant of pollution and toxins; and Ephemeroptera, Plecoptera, and Trichoptera (EPT) are intolerant of toxins (Heino, 2000; Lenat, 1988; Kagalou et al., 2006; Deacon & Lavoie, 2010).

This chapter aims to contribute to the knowledge of the integrity of salmonid streams of Tua Basin, located in northeastern Portugal. In detail, the specific objectives are:

1) **Assess the water quality**, based on several physical and chemical parameters;
2) **Determine the hydromorphological quality**, based on aquatic and riparian habitats;
3) **Evaluate the biota quality**, in particular related with the macroinvertebrates communities.

2.2. METHODOLOGY

2.2.1. Study Area

The study area is located in northeastern Portugal (Figure 2.1). One of the main tributaries is River Tua. The headstreams, Tuela, Rabaçal and Mente rivers, are located in the Natural Park of Montesinho, near the border with Spain.

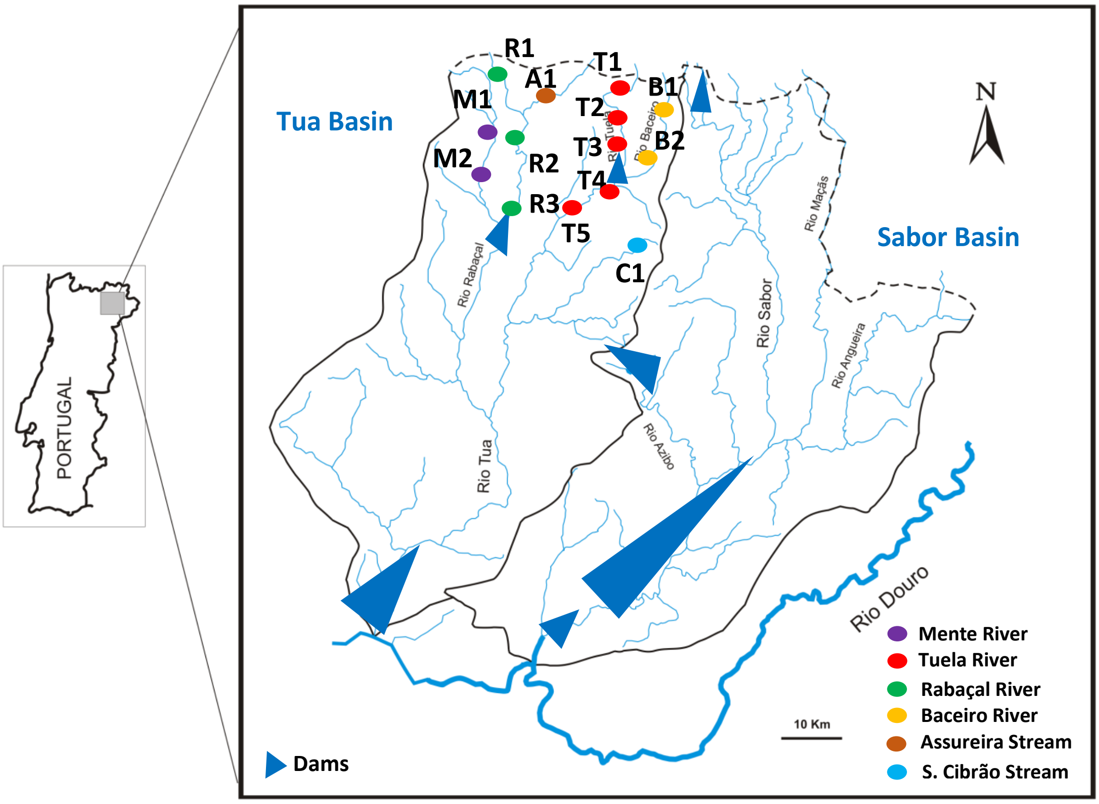

Figure 2. 1. Map of the watercourses in Tua and Sabor Basins and location of sampling sites

A total of fourteen sampling sites were selected and distributed along four rivers: Tuela (T1, T2, T3, T4 and T5), Baceiro (B1, B2), Rabaçal (R1, R2 and R3), and Mente (M1, M2) and two streams: S. Cibrão (C1) and Assureira (A1) (Table 2.1, Figure 2.1).

Sampling sites were selected in salmonid streams, taking into consideration the presence of exclusively Brown trout (*Salmo trutta*) populations, located in small headwater streams and the cohabitation in downstream zones with endemic cyprinids, like Iberian northern chub (*Squalius caroliterii*), Douro nase (*Pseudochondrostoma duriense*), calandino roach (*Squalius alburnoides*) and Iberian northern Barbel (*Luciobarbus bocagei*). All these rivers benefit of a good ecological status and threatened autochthounous mussel (e.g. *Margaritifera margaritifera*) and dragonflies (e.g. *Macromiia splendens*) populations can be indentified in several river streches.

Table 2. 1. Location (geographical coordinates) of sampling stations in Tua Basin

Basin	River	Location (village)	Symbol	Latitude	Longitude	Altitude
Tua	Baceiro	Cheira	B1	41°55'47.86"N	6°51'18.73"W	836
Tua	Baceiro	Ornal	B2	41°53'20.72"N	6°51'14.43"W	744
Tua	Tuela	Pontões Dine	T1	41°55'30.06"N	6°56'30.17"W	684
Tua	Tuela	Soeira	T2	41°50'52.90"N	6°55'59.35"W	601
Tua	Tuela	Moinho Melro	T3	41°50'26.76"N	6°56'20.48"W	579
Tua	Tuela	Downstream Dam	T4	41°50'3.88"N	6°56'36.41"W	564
Tua	Tuela	Vinhais	T5	41°48'36.53"N	6°59'48.01"W	441
Tua	Rabaçal	Pinheiro Novo	R1	41°57'28.57"N	7° 9'26.79"W	592
Tua	Rabaçal	Gestosa	R2	41°53'4.39"N	7° 8'13.94"W	487
Tua	Rabaçal	Palas	R3	41°47'43.03"N	7° 8'40.92"W	404
Tua	Mente	Segirei	M1	41°51'56.59"N	7°11'40.29"W	451
Tua	Mente	S. Jomil	M2	41°47'33.94"N	7°11'36.65"W	396
Tua	Assureira	Contim	A1	41°56'14.56"N	7° 5'47.19"W	599
Tua	S. Cibrão	S. Cibrão	C1	41°43'24.46"N	6°54'53.55"W	750

Figures 2.2 to 2.15 showed images of the selected sampling sites.

Figure 2. 2. Location of the sampling station B1- River Baceiro (near the border-Spain)

Figure 2. 3. Location of the sampling station B2- River Baceiro (near Ornal)

Figure 2. 4. Location of the sampling station T1- River Tuela (near Pontões de Dine)

Figure 2. 5. Location of the sampling station T2- River Tuela (near Soeira)

Figure 2. 6. Location of the sampling station T3- River Tuela (upstream of AH Trutas)

Figure 2. 7. Location of the sampling station T4- River Tuela (downstream of AH Trutas)

Figure 2. 8. Location of the sampling station T5- River Tuela (near Vinhais)

Figure 2. 9. Location of the sampling station R1- River Rabaçal (near Pinheiro Novo)

Figure 2. 10. Location of the sampling station R2- River Rabaçal (near Gestosa)

Figure 2. 11. Location of the sampling station R3- River Rabaçal (near Palas)

Figure 2. 12. Location of the sampling station M1- River Mente (near Segirei)

Figure 2. 13. Location of the sampling station M2- River Mente (near S. Jomil)

Figure 2. 14. Location of the sampling station A1- Assureira stream (near Contim)

Figure 2. 15. Location of the sampling station C1- S. Cibrão stream (near S. Cibrão)

The water quality was assessed by measuring some physical and chemical parameters. These measurements were done ***in situ***, using portable probes (Figure 2.16) namely for: 1) Dissolved Oxygen (mg O_2/L); 2) Temperature (°C); 3) Total Dissolved Solids (TDS mg/L), 4) Electrical Conductivity EC25 (µS/cm), and 5) pH. All procedures were performed according to the APHA (2005) procedures. The interpretation of the results was based on Portuguese legislation (Decreto-Lei 236/98 of 1 August). The parameters were measured in the spring/summer seasons of 2017.

Figure 2. 16. In situ measurement of conductivity in River Baceiro (B2) (spring 2017)

The habitat evaluation was made in spring season (May 2017), through the calculation of two indexes, adapted to the rivers of the Iberian Peninsula that allowed to classify the hydromorphology and riparian quality of all the sampling sites. The following indexes were used:

1) Index of Riparian Quality- QBR (MUNNÉ *et al.* 2003)

The QBR index, developed for the use of environmental managers and planners at national and regional levels, is an important tool to characterize the riparian condition of streams. This index is based on four components of riparian habitat: total riparian vegetation cover, cover structure, cover quality and channel alterations. The differences are measured in a simple and quantitative way and the final index score varies between 0 and 100 points (see Appendix I, for details), considering five quality classes for riparian habitats (Table 2.2).

Table 2. 2. Quality classes according to the QBR index (MUNNÉ et al. 2003)

QBR range	Colour/Class	Riparian habitat quality class
≥ 95	I	Riparian habitat in natural condition
75 – 90	II	Some disturbance, good quality
55 – 70	III	Important disturbance, fair quality
30 – 50	IV	Strong alteration, poor quality
0 – 25	V	Extreme degradation, bad quality

2) Index of Channel Quality- GQC (CORTES et al. 1999)

The Channel Quality, GQC index, is a measure of the physical structure, taking into consideration not only the channel conditions but also some river corridor features. This index is based on eight components: Presence of artificial obstacles (e.g. weirs, dams), in-channel structure, sediments and stability of the channel, bank structure, and artificial alterations of the banks, channel heterogeneity, river bottom structure and embedness. The final index score varies between 8 and ≥ 31 points (see Appendix II, for details) and there are five quality classes to characterize the channel habitats (Table 2.3).

Table 2. 3. Quality classes according to the GQC index (CORTES et al. 1999)

GQC range	Colour/Class	Channel habitat quality class
≥ 31	I	Channel habitat in natural conditions, excellent quality
26 – 30	II	Some disturbance, good quality
20 – 25	III	Initial of important alteration of the channel, fair quality
14 – 19	IV	Strong alteration, poor quality
8 – 13	V	Extreme degradation (channelization, regulation) bad quality

2.2.2. Biota: Benthic Macroinvertebrate Communities

2.2.2.1. Sampling Procedures

The sampling of benthic macroinvertebrate communities was based on the protocol established by the Environment Portuguese Agency (APA), according to the implementation of the Water Framework Directive (WFD) in Portugal (INAG 2008). It was done as recommend by the protocol during spring season. In each sampling site, a section of 50 m was selected taking into consideration the representativeness of different habitats (riffle, pool and run) and microhabitats (e.g. fine and coarse materials, leaves, aquatic plants) identified in erosion units (turbulent flow) and adjacent sedimentation units (laminar flow). Six subsamples were collected in each sampling site, using a kick handnet (25*25 cm dimensions and with 500 µm of mesh size), removing the substrata with the foot (1 meter extension) displacing the invertebrates from the river bottom to the net (Figure 2.17).

Figure 2. 17. Sampling procedures of benthic invertebrates collection (spring 2017)

Attached invertebrates were also collected using appropriate brushes. Invertebrates were immediately preserved adding alcohol (70%). In the laboratory, invertebrates were sorted and subsequently identified and counted, using a stereomicroscope SZX10 with 10-132*x* zoom magnification. Appropriate dichotomous keys were used (e.g. TACHET et al. 1981, 2010) (Figure 2.18) and the invertebrates identified until the taxonomic level of Family level, with the exception of the Oligochaeta and Acari Subclasses.

Figure 2. 18. Laboratorial procedures: macroinvertebrate sorting and identification

2.2.2.2. Metrics used to environmental quality evaluation

The environmental quality based on macroinvertebrate communities was evaluated considering several uni and multimetric variables, some of them calculated using the Software AMIIB@ (***http://dqa.inag.pt/implementacao_invertebrados_AMIIB.html***). Among the most important metrics can be cited the following ones:

1) Number of individuals (N) and number of *taxa* (S);

2) Diversity (*e.g.* H' index of Shannon-Wienner);

3) Evenness (*e.g.* J' index of Pielou);

4) Relative abundance of Ephemeroptera, Plecoptera and Trichoptera (% EPT);

5) Biotic Index IBMWP (ALBA-TERCEDOR 1996).

The IBMWP index is a simple method to assess the biological quality of freshwater ecosystems. The identification of organisms was only until family taxonomic level. To each family is given a score ranging between 10 and 1, according to a gradient of pollution tolerance (Appendix III). The calculation of IBMWP final score is done by the sum of all the scores of the families present in each sample, classifying the biological quality of water on 5 defined classes (Table 2.4).

Table 2. 4. Quality classes according to the IBMWP index

IBMWP range	Colour/Class	Biological quality class
> 100	I	Clean water, Excellent quality
61 – 100	II	Light polluted, Good quality
36 – 60	III	Moderately Polluted, Fair Quality
16 – 35	IV	Strongly polluted, Poor quality
<15	V	Extremely polluted, Bad quality

6) Portuguese Northern Invertebrate Index- $IPtI_N$ (INAG 2009).

The **multimetric Index $IPtI_N$**, developed and applied according to WFD, integrates different metrics like nº of *taxa*, EPT, evenness of Pielou J', diversity of Shannon-Wienner H', IASPT and Sel. ETD, combined as presented in the following formula:

$IPtI_N$ = Nº *taxa* x 0.25 + EPT x 0.15 + *Evenness* x 0.1 + (IASPT – 2) x 0.3 + Log (Sel. ETD+1) x 0.2

where:

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

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

H' - diversity of Shannon-Wienner

S - number of present *taxa*

Ln - natural or neper logarithm

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

$$H' = -\sum p_i\ Ln\ p_i$$ where:

$p_i = n_i/N$

n_i- nº of individuals of each *taxon i*

N- total nº of individuals present in sample

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

The final score of **$IPtI_N$** depends on the sum of the weighted metrics. Two steps of normalization are performed and the index expressed in terms of Ecological Quality Ratio (RQE). For the normalization process is needed to determine the ratio between the observed value and the reference value of each river type (median reference sites) (INAG 2009). Table 2.5 displays the reference values and boundary values between the quality classes in RQE, according to the type of each sampling site selected in this study, taking into account the boundaries of the adjustment relating to classification criteria, revised in the Management Plan of River Basin in 2016 / 2021 (APA 2015).

Table 2. 5. Median reference values and boundaries for river types of the study (APA 2015)

River Tipology	Reference Values	Excellent	Good	Fair	Poor	Bad
Northern Rivers Small Dimension (N1 < 100 km^2)	1.02	≥0.87	[0.68-0.87[	[0.44-0.68[	[0.22-0.44[	[0-0.22[
Northern Rivers Medium-Large Dimension (N1 > 100 km^2)	1.00	≥0.88	[0.68-0.88[	[0.44-0.68[	[0.22-0.44[	[0-0.22[

2.2.3. Data treatment

Data treatment was made using the software PRIMER 6 & PERMANOVA + (CLARKE & GORLEY 2006) to perform the *non-metric multi dimensional scaling* (NMDS) for the analysis of macroinvertebrate communities. For this analysis the abundance data were previously transformed [Log (x + 1)] and applied the Bray-Curtis similarity coefficient. Analyses of similarity were also done through the calculation of ANOSIM tests.

2.3. RESULTS

The results of abiotic and biotic characterization in the different watercourses of River Tua (Douro Basin, Northeastern Portugal) are presented below.

2.3.1. Physical and chemical quality of water

The analysis of physical and chemical parameters, namely temperature, dissolved oxygen, electric conductivity, pH, total dissolved solids, measured in spring 2017 for the 14 sampling sites, showed a good water quality. In fact, the results suggested that anthropic activities promote very few changes in the physico-chemical parameters. The interpretation of temperature variation (Figure 2.19) among sampling sites can highlighted the following aspects:

- Low temperature of water, varying from extreme values of 9 to 13 °C;
- Increasing tendencies towards downstream zones
- Typical temperature of salmonid streams of NE Portugal

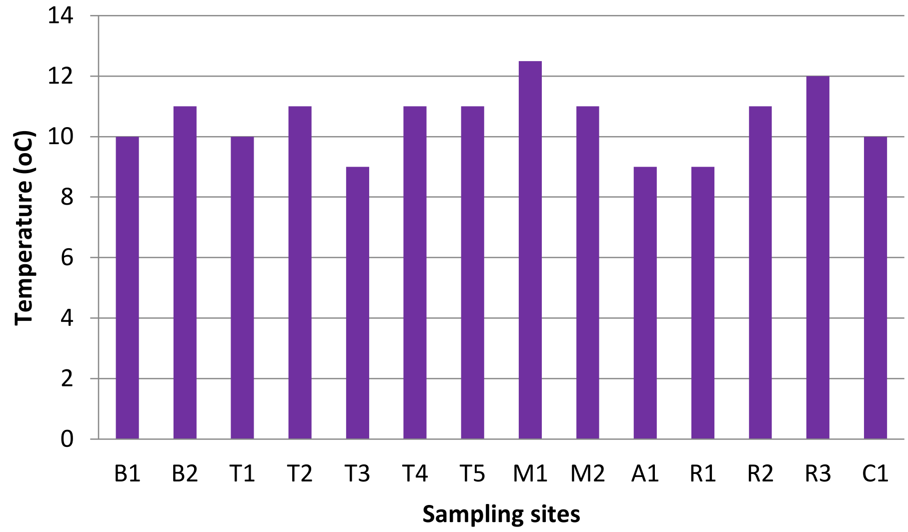

Figure 2. 19. Variation of temperature (°C) among sampling sites (spring 2017)

Relatively to the Dissolved Oxigen (DO), it was observed a good oxygenation rate (> 8.0 mg O_2/L). The content of dissolved oxygen in the upperzones of Tua River are not a problem to the invertebrate and fish communities, inclusively during summer season, where temperature and DO maintain high levels (Figure 2.20).

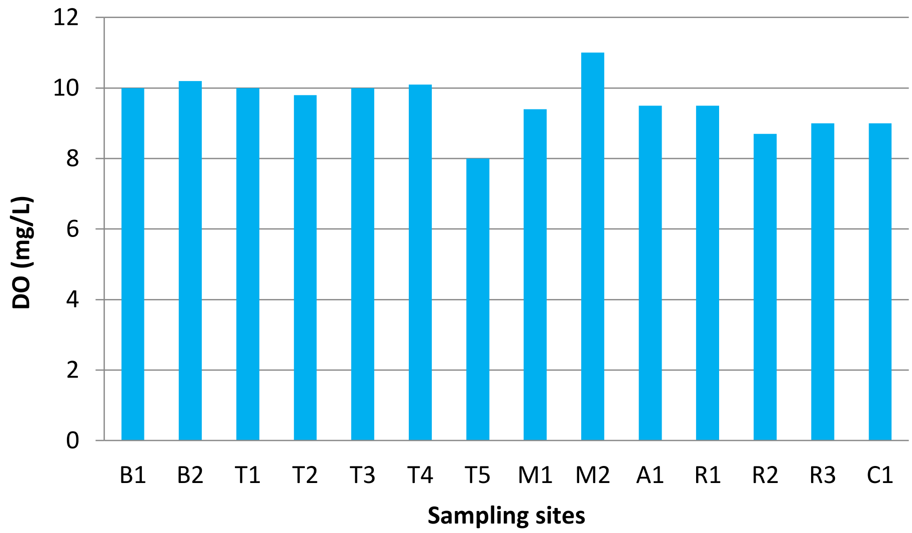

Figure 2. 20. Variation of dissolved oxygen (mg O_2/L) among sampling sites (Spring 2017)

It was detected low values for the electrical conductivity (EC25< 80 µS/cm) in the different sampling sites. In fact, the geology present, mainly schists and granites but also small spots of ultramafic rocks contributed with a very low content of salts for the aquatic ecosystems (Figure 2.21).

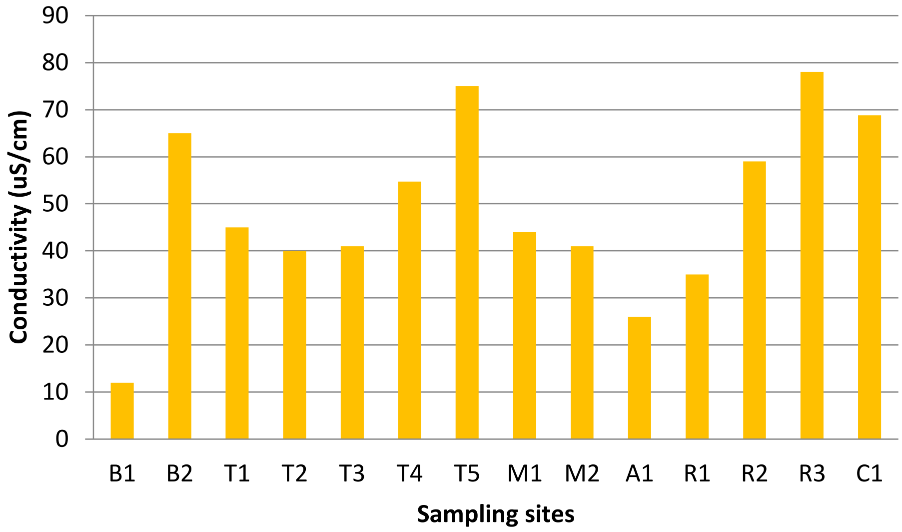

Figure 2. 21. Variation of Electric Conductivity (µS/cm) among sampling sites (Spring 2017)

The pH parameter varied between 6.6 and 7.2, depending, in part, of the dominant rocks present. These values, ranging from subacid to subalcaline, are excellent for aquatic faunal communities (Figure 2.22).

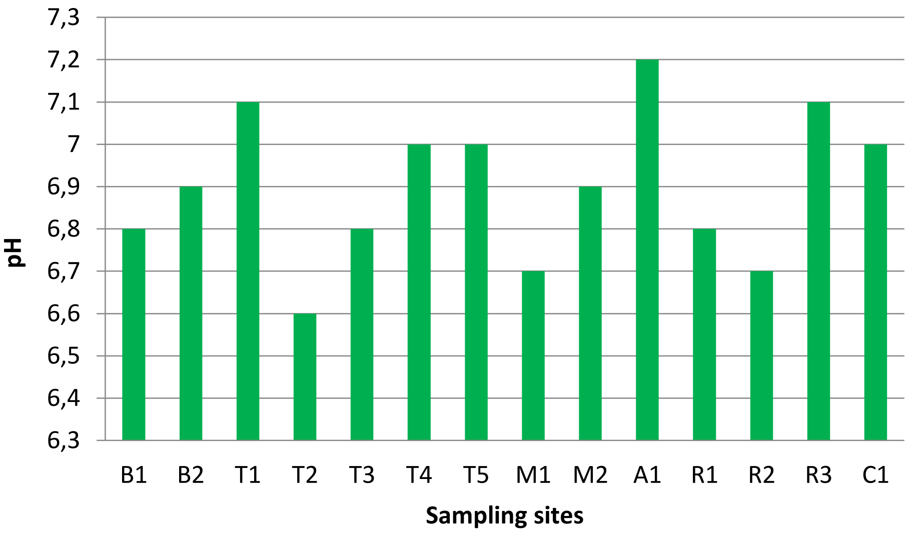

Figure 2. 22. Variation of pH among sampling sites (Spring 2017)

The Total Dissolved Solids (TDS), revealed a similar tendency to EC25, Electric conductivity. The lowest value was detected in the small headwater streams (Assureira). An increasing tendency of TDS levels towards down parts (Figure 2.23).

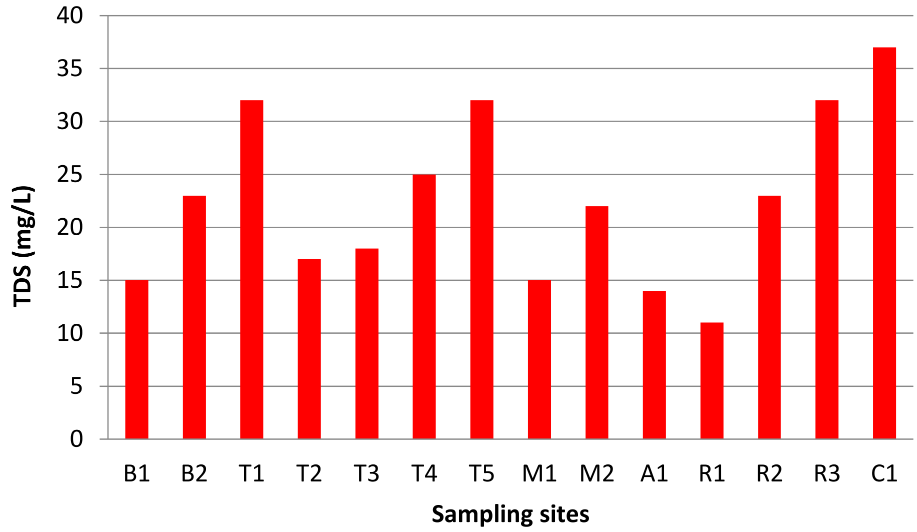

Figure 2. 23. Total dissolved solids (TDS) (mg/L) among sampling sites (Spring 2017).

2.3.2. Quality of aquatic and riparian habitats

The indexes QBR and GQC showed a good hydromorphological quality of the rivers and also the marginal ecosystems- riparian buffer strips. In Natural Park of Montesinho the main impacts are related with small hydroeletrical powerplants, changing substantially the aquatic and riparian habitats (Figures 2.24 and 2.25, Table 2.6).

Figure 2. 24. Good hydromorphological and riparian conditions: R2- R. Rabaçal (Spring 2017)

Comparatively, differences can be detected between non-impacted and impacted sites. However, the impacts are localized and the good condition rapidly recovered.

Figure 2. 25. Bad hydromorphological and riparian conditions: T4 – R. Tuela (Spring 2017).

Table 2. 6. Values of QBR and GQC indexes of Tua Basin (Spring 2017)

River	Sampling site	Final Score		Habitat's index	
		QBR	GQC	QBR	GQC
Baceiro	B1	100	35	I	I
Baceiro	B2	100	35	I	I
Tuela	T1	100	30	I	II
Tuela	T2	90	34	II	I
Tuela	T3	75	25	II	III
Tuela	T4	35	25	IV	III
Tuela	T5	90	31	II	I
Mente	M1	50	29	IV	II
Mente	M2	100	35	I	I
Assureira	A1	100	36	I	I
Rabaçal	R1	75	36	II	I
Rabaçal	R2	90	28	II	II
Rabaçal	R3	95	35	I	I
S. Cibrão	C1	85	29	II	II

2.3.3. Macroinvertebrate Communities

The variation in the total number of individuals captured in the 14 sampling sites can be visualized in Figure 2.26. A total of 6911 individuals of invertebrates were identified, belonging to 67 faunistic groups, mainly families (Figure 2.27).

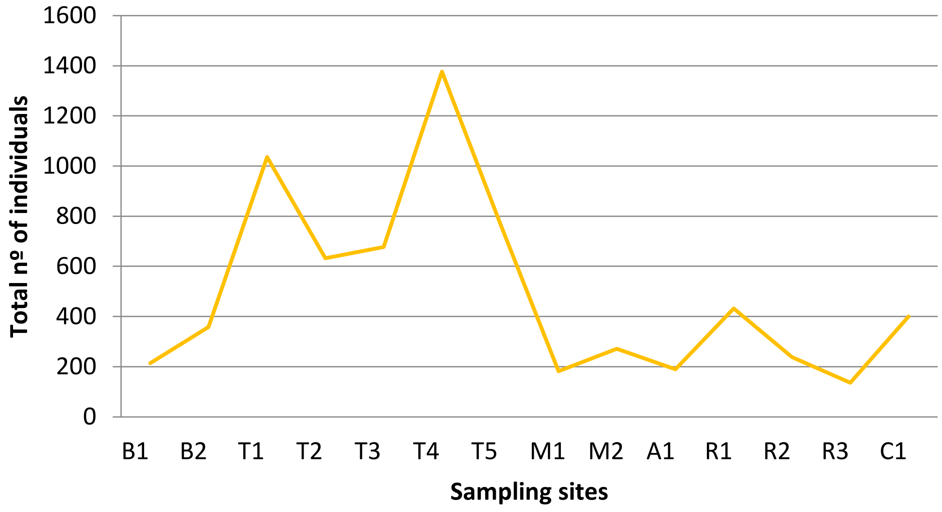

Figure 2. 26. Number of individuals present in each sampling site (Spring 2017)

Most of the samples contained between 200 and 400 individuals, with a maximum of individuals collected in T4 (1376 individuals, most of them belonging to Simuliidae, Diptera) and a minimum in Rab3 (136 individuals, mainly Leuctridae, Plecoptera).

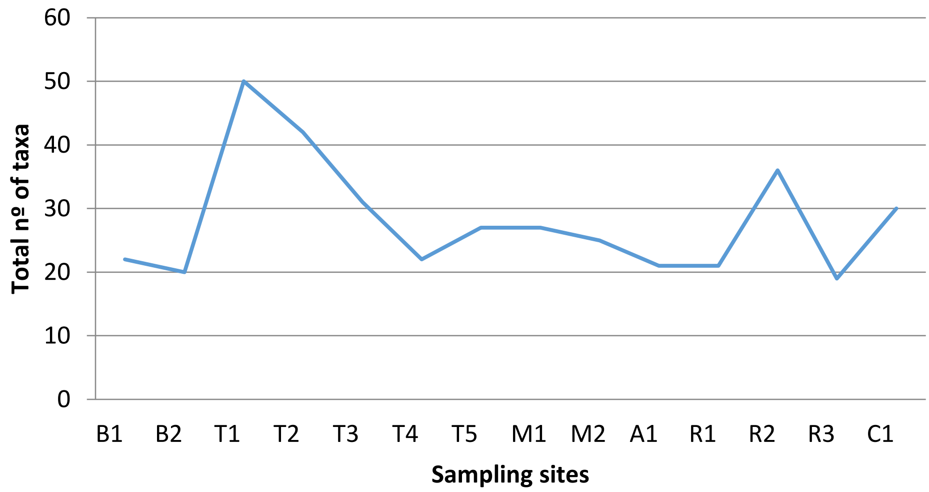

Figure 2. 27. Total number of taxa present in the sampling sites (Spring 2017)

The total number of taxa (e.g. families, with the exception of Oligocaeta and Acari) ranged between 20 and 35. Most sampling sites of Tuela and Rabaçal rivers have the highest taxa richness (Figure 2.27).

2.3.3.1. Diversity (H') and Evenness (J') indexes

The following figures (Figures 2.28 and 2.29) showed the variation of the H' Shannon-Wienner diversity and J'Pielou Evenness indexes.

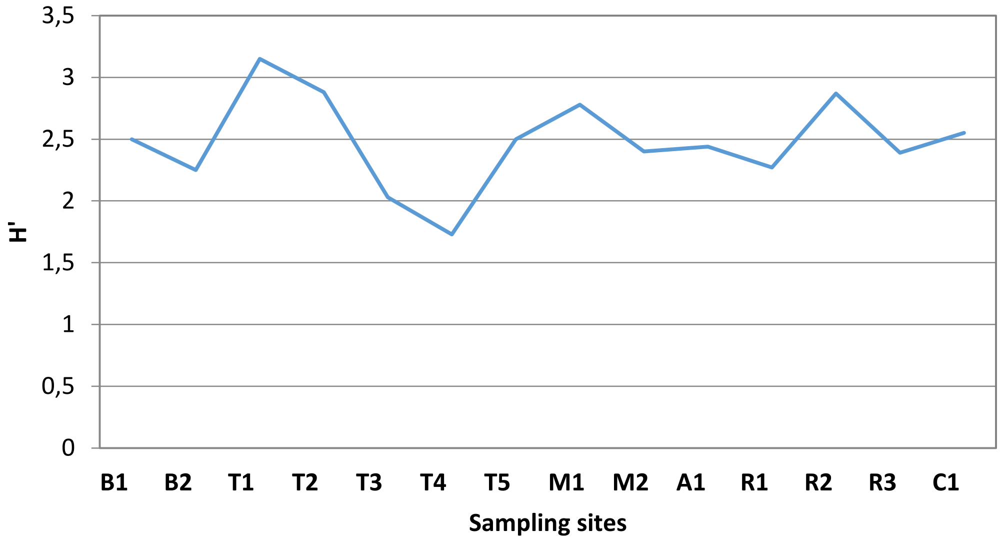

Figure 2. 28. Variation of Shannon-Wienner diversity index (H') (Spring 2017)

The upperzone of Tua River display a high diversity of macroinvertebrate communities. It was not observed substantial differences in terms of evenness (J') among sampling sites, with exception of T4 where the evident dominance of Simuliidae (adapted to that kind of habitats).

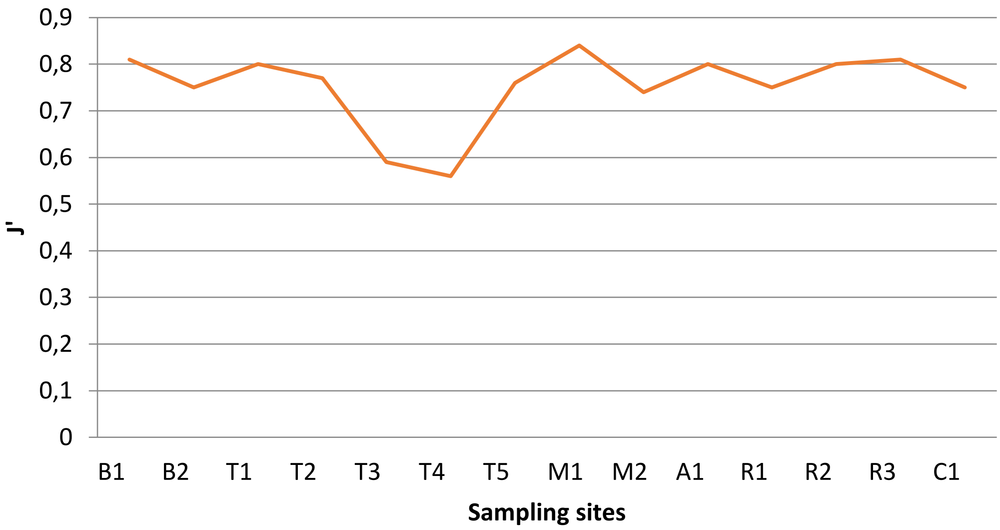

Figure 2. 29. Variation of Pielou evenness index (J') (Spring 2017)

2.3.3.2. Faunal composition

A general image of the global proportion of each faunistic group, considering all sampling sites, can be observed in the Figure 2.30.

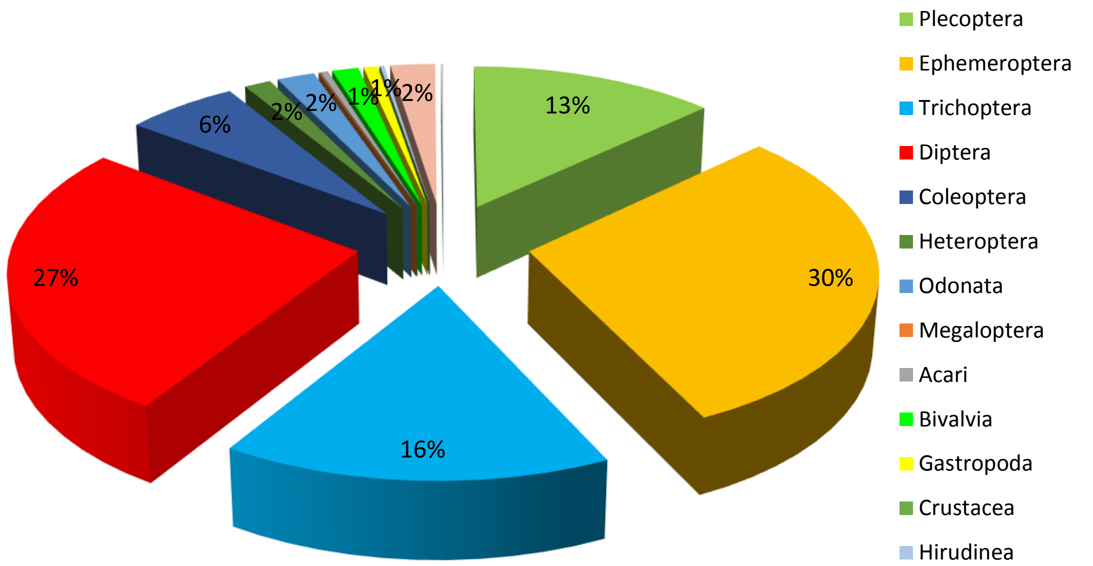

Figure 2. 30. Faunal composition of invertebrates present in all sampling sites (Spring 2017)

The main aspects that must be highlighted are:

- The dominance EPT (Ephemeroptera, Plecoptera, Trichoptera), representing 59% of total abundance;
- The significant presence of Odonata (2%) and Bivalvia (1,5%) groups, taking into considerantion the threatened species (e.g. *Margaritifera margaritifera*, *Macromiia splendens*) listed by IUCN (2017);
- The recent detection of an exotic and invasive species, signal crayfish (*Pacifastacus leniusculus*);

However, in this global analysis is not possible distinguish the intra and intervariation of taxa richness among sampling sites of different rivers. In the following Figures 2.31 and 2.32, the faunal composition variation can be observed and compared amosng sampling sites.

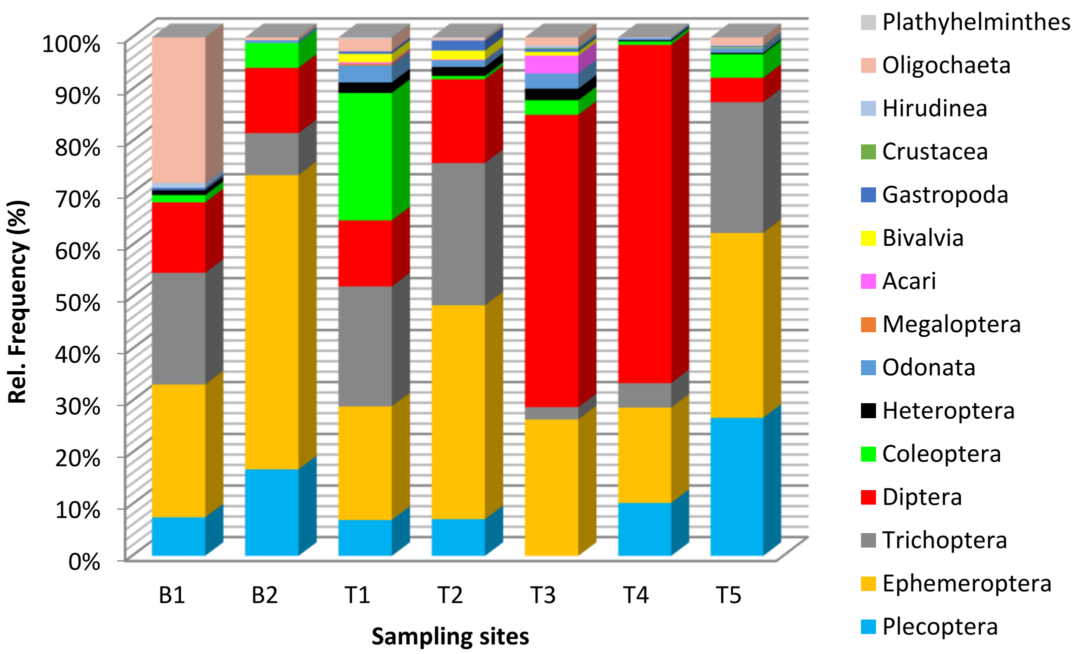

Figure 2. 31. Faunal composition of invertebrates in Baceiro and Tuela rivers (Spring 2017)

In Baceiro and Tuela rivers, the proportion of EPT families must be highlighted, namely in the upper part (B1, B2, T1 and T2). Near the small dam (AH Trutas), in particular in the downstream zone the dominance of Simuliidae (Diptera) contributed to the shift observed in the faunal composition.

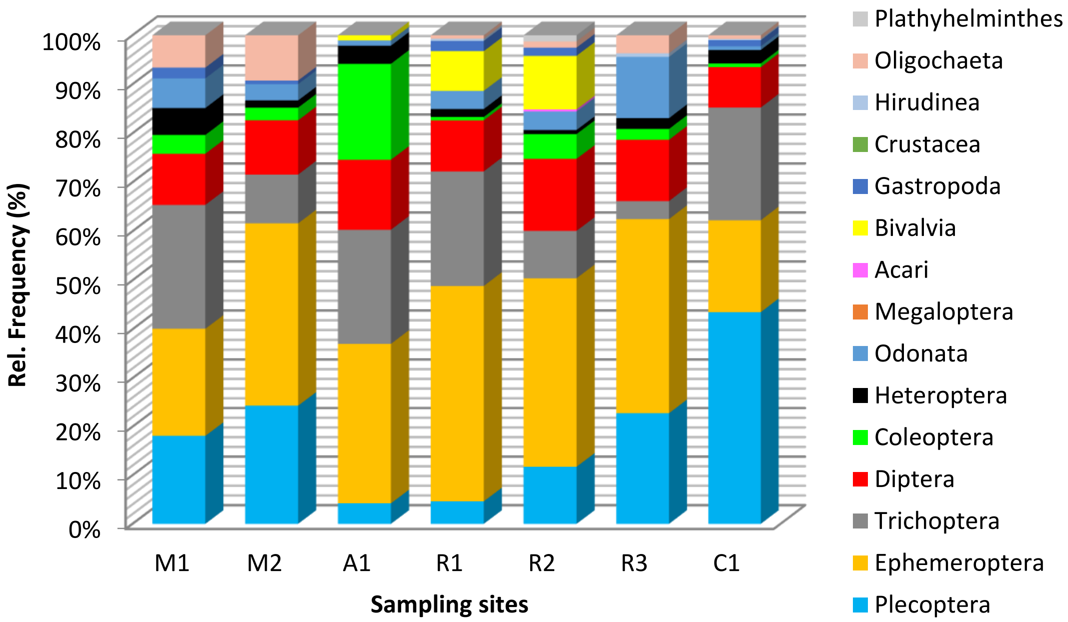

Figure 2. 32. Faunal composition of invertebrates communities in Rabaçal, Mente, Assureira and S. Cibrão rivers (Spring 2017).

In the remaining rivers, i.e. Rabaçal, Ment, Assureira and S. Cibrão the proportion of EPT families represented more than 60% for all sampling sites. Globally, it was detected a notable presence of stenobiont species in all sampling sites.

2.3.3.3. IBMWP and $IPtI_N$ indexes

The results obtained for IBMWP and $IPtI_N$ showed the excellent and good biotic condition for all headwater streams (Table 2.7). Both indexes are important tools in the assessment of the biological quality in the rivers. No significant differences were found between the classification of unimetric (IBMWP) and multimetric ($IPtI_N$) indexes.

Table 2. 7. Values and classification of IBMWP and IPtIN for each sampling site (Spring 2017)

Sampling site	IBMWP		$IPtI_N$	
	Value	Classification	Value	Classification
B1	126	Excellent	0.804	Good
B2	126	Excellent	0.769	Good
T1	318	Excellent	1.466	Excellent
T2	273	Excellent	1.32	Excellent
T3	158	Excellent	0.931	Excellent
T4	130	Excellent	0.95	Excellent
T5	173	Excellent	1.095	Excellent
M1	184	Excellent	1.075	Excellent
M2	164	Excellent	1.004	Excellent
A1	136	Excellent	0.799	Good
R1	130	Excellent	0.956	Excellent
R2	223	Excellent	1.129	Excellent
R3	120	Excellent	0.865	Good
C1	201	Excellent	1.009	Excellent

Other metric calculated by AMIIB software corroborated with the tendency observed (see Annexes IV.1, IV.2 and IV.3).

2.3.3.4. Biotypology of macroinvertebrate communities

The NMDS analysis, based on the abundance of macroinvertebrate communities, considering all sampling sites, showed a good two-dimensional representation (2D stress value of 0.17). All sampling sites are very close since they share a big number of taxa. However, a slight separation can be identified between sampling sites located at the upper and lower parts of the same river, and inclusively among rivers (more visible for Tuela River) (Figure 2.33). The ANOSIM similarity (one-way) tests showed no significant differences (P > 0.05) among rivers, disturbed (e.g. T3, T4) vs. non-disturbed sites (T1, T2), and exclusively trout rivers vs trout and other fish species.

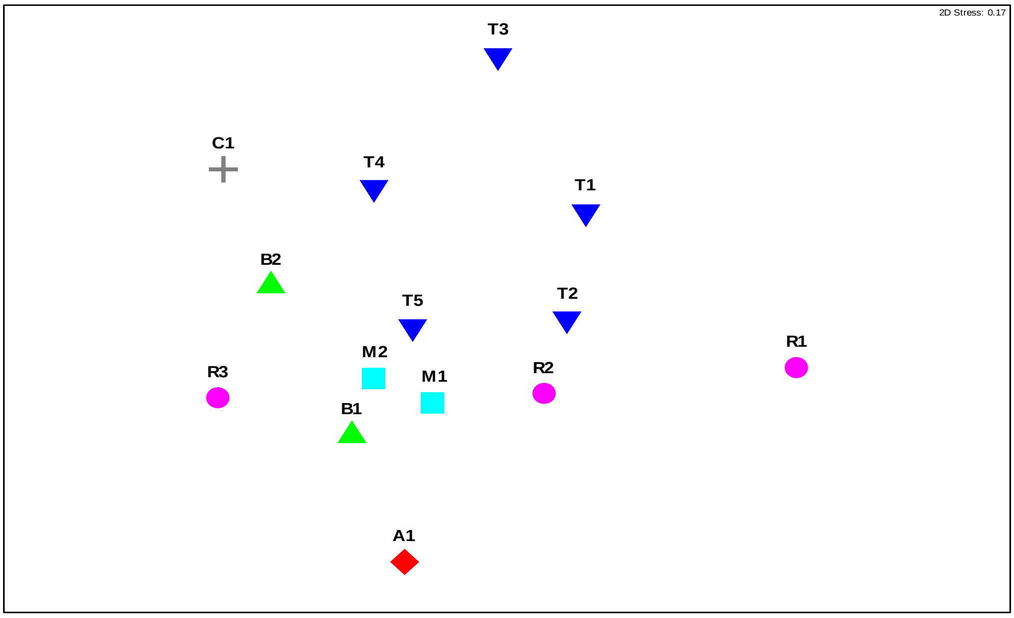

Figure 2. 33. NMDS ordination of sampling sites, based on invertebrate communities (Green Triangles: river Baceiro ; Blue Triangles : river Tuela ; Pink circles: River Rabaçal, Blue squares: river Mente; Plus symbol : River S. Cibrão)

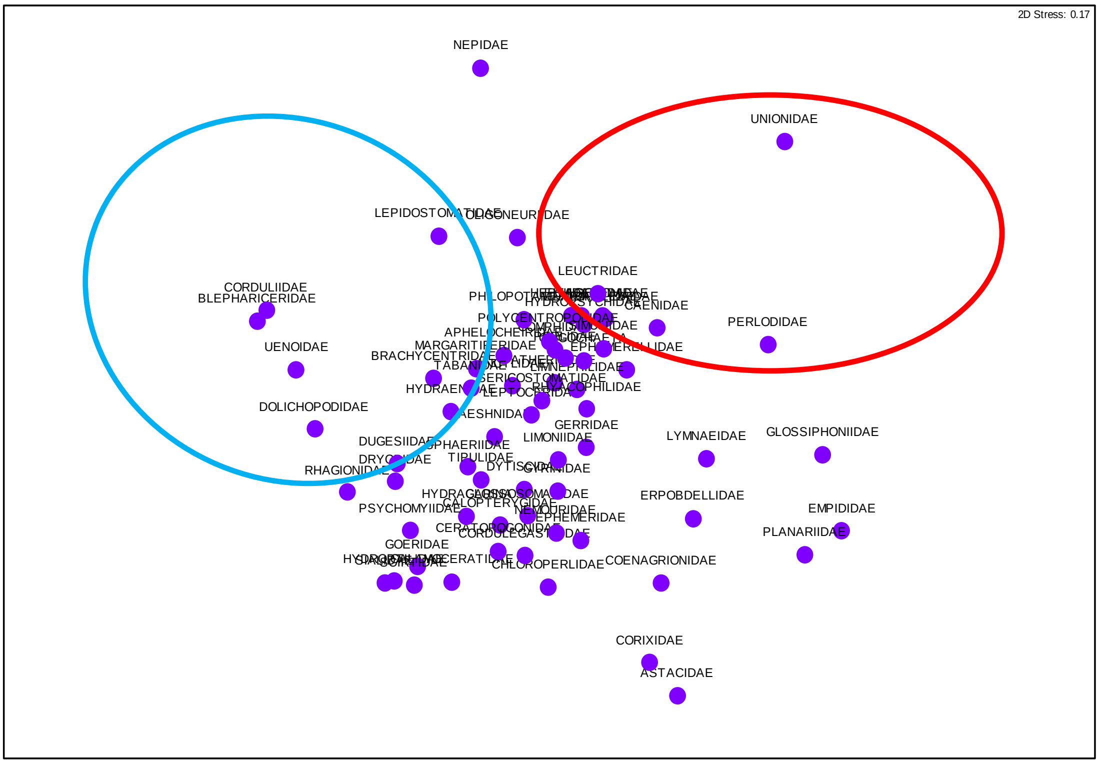

Figure 2. 34. NMDS Ordination of invertebrate communities of Tua Basin (Spring 2017)

The NMDS for invertebrate communities (Figure 2.34) showed a good representation of the different taxa. Some separation between families can be linked to the distribution stenobiont taxa (e.g. Plecoptera: Perlodidae, Leuctridae) which colonizes the High Tua.

2.4. DISCUSSION

The present work allowed the characterization of six watercourses of Tua basin located in Northeastern Portugal (Baceiro, Rabaçal, Tuela, Mente, S. Cibrão and Assureira) in terms of water quality and aquatic and riparian habitats quality basing on physico-chemical parameters and macroinvertabrates communities. This study contributed to update previous information related with the abiotic and biotic quality of these rivers.

In a general perspective, it was detected in the different sampling sites good to excellent quality of water: Low temperature (from 9 to 13 °C), good oxygenation rate (> 8.0 mg O_2/L), low values for the electrical conductivity (EC25< 80 µS/cm), values of pH near to neutral (between 6.6 and 7.2), and low contents of dissolved solids (between 11 to 37 mg/L). Regarding to the quality of aquatic and riparian habitat, the indexes QBR and GQC showed in general a good hydromorphological quality of the rivers, the differences between rivers in terms of quality of habitat are related especially to their exposition to human disturbances that are more detected in downstream zones. Using the classification of unimetric (IBMWP) and multimetric ($IPtI_N$) indexes, no significant differences were found between the indexes, both indicated good to excellent biological quality for all sampling sites. These patterns of good ecological integrity was found by other authors in the same (Claro 2010, Patricio 2013) or contiguous (e.g. Tâmega, Sabor) watershed (Nogueira 2011, Ramos 2011), in previous years.

The typology of sampling sites explains the hydromorphological and biological similarities and differences between sites: In upstream zones, the temperature is cooler, the water is well oxygenated and the electric conductivity and dissolved solids proportion are lower relatively to median and downstream zones, these conditions are typical for North-eastern Portugal and constitute special characteristics for salmonid streams where Brown trout is exclusively found. Rabaçal 1 and Tuela 5 can be considered as the worst sites in terms of quality of aquatic and riparian habitat, the degradation of these aquatic ecosystems is related especially to human disturbances such as construction of dams and pollution. However, the upperzones of Tuela river are examples of excellent quality of habitat. Considering biotic analysis, the upperzone of Tua River display a high diversity of macroinvertebrate communities described by both indexes: Shannon' Diversity (H') and evenness (j'). It was detected a notable presence of stenobiont species (Ephemeroptera, Plecoptera and Trichoptera) especially in upperzones of Tuela and Rabaçal rivers and a decrease of this propotion towards downstream parts favorising the increase of other orders more tolerant to environmental changes such as Diptera. The IBMWP and $IPtI_N$ indexes confirmed this tendency.

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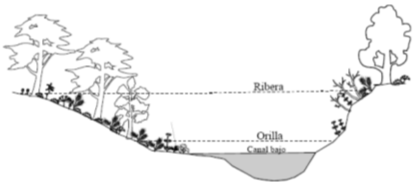
ANNEXES

ANNEXE I : QBR: Riparian Quality Index

QBR INDEX
Riparian habitat quality



The score of each of the four blocks can not be negative or exceed 25 points

Station	
Classification	

1 - Total riparian cover - Score between 0 and 25

Score		
25	>80% of riparian cover (excluding annual plants)	
10	50–80% of riparian cover	
5	10–50% of riparian cover	
0	<10% of riparian cover	
+10 +5	If connectivity between the riparian forest and the woodland is total If the connectivity is higher than 50%	
-5 -10	Connectivity between 25% and 50% Connectivity lower than 25%	

2 - Cover structure -Score between 0 and 25

Score		
25	>75% of tree cover	
10	50–75% of tree cover or 25–50% tree cover but 25% covered by shrubs	
5	Tree cover lower than 50% but shrub cover at least between 10% and 25%	
0	<10% of either tree or shrub cover	
+10 +5 +5	At least 50% of the channel has helophytes or shrubs If 25–50% of the channel has helophytes or shrubs If trees and shrubs are in the same patches	
-5 -5 -10	If trees are regularly distributed and shrubland is >50% If trees and shrubs are distributed in separate patches, without continuity Trees distributed regularly, and shrubland <50%	

3 - Cover quality - Score between 0 and 25 *(the geomorphological type should be first determined[a])*

Score		Type 1	Type 2	Type 3	
25	Number of native tree species	> 1	> 2	> 3	
10	Number of native tree species	1	2	3	
5	Number of native tree species	-	1	1 - 2	
0	Absence of native trees				
+10 +5 +5 +5	If the tree community is continuous along the river and covers at least 75% of the edge riparian area The tree community is nearly continuous and covers at least 50% of the riparian area When the number of shrub species is	> 2	> 3	> 4	
-5 -5 -10 -10	If there are some man-made buildings in the riparian area If there are some isolated species of non-native[b] trees Presence of communities of non-native[b] trees Presence of garbage				

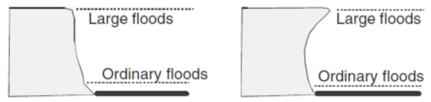
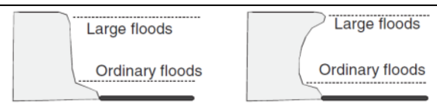
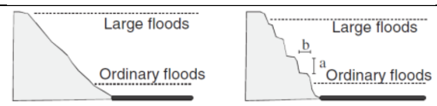
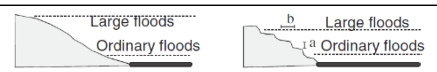
4 - Channel alteration - Score between 0 and 25

Pontuação		
25	Unmodified river channel	
10	Fluvial terraces modified and constraining the river channel	
5	Channel modified by rigid structures along the margins	
0	Channelized river	
-10 -10	River bed with rigid structures (e.g., wells) Transverse structures into the channel (e.g., weirs)	

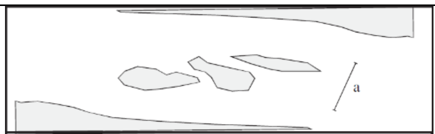
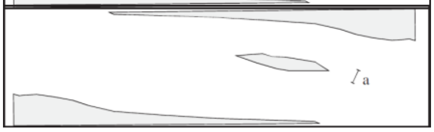
Final score (sum of four section scores)	

[a] **Type of the riparian habitat (to be applied at level 3, cover quality)**

The score is obtained by addition of the scores assigned to left and right river margins according to their slope. This value can be modified if islands or hard substrata are present.

Slope and form of the riparian zone		Score Left	Score Right
Very steep, vertical or even concave (slope >75°), very high, margins are not expected to be exceeded by floods. *Slope is the angle subtended by the line between the top of the riparian area and the edge of the ordinary flooding of the river.*		6	6
Similar to previous category but with a bankfull which differentiates the ordinary flooding zone from the main channel.		5	5
Slope of the margins between 45° and 75°, with or without steps. (*a*>*b*)		3	3
Slope between 20° and 45°, with or , with or without steps. (a < b)		2	2
Slope <20°, large riparian zone.		1	1

Presence of one or several islands in the river

Width of all the islands "a" > 5 m.		-2	
Width of all islands 'a' < 5 m.		-1	

Percentage of hard substrata that can make impossible the presence of plants with roots

> 80%	Not applicable
60 – 80%	+6
30 – 60%	+4
20 – 30%	+2

Total score	

Geomorphological type following the score

> 8	**Type 1**	Closed riparian habitats. Riparian trees, if present, reduced to a small strip. Headwaters.
5 – 8	**Type 2**	Headwaters or midland riparian habitats. Forest may be large and originally in gallery.
< 5	**Type 3**	Large riparian habitats, and potentially extensive forests. Lower courses.

[b] **Non-native tree species in the study area**

(This should be listed for each study area)

e. g. in the studied area of Catalonia the following species are considered non-native: *Populus deltoides, Populus* x *canadensis, Populus nigra* ssp. *italica, Salix babilonica, Ailanthus altissima, Celtis australis, Robinia pseudo-acacia,Platanus* x *hispanica.*

ANNEXE II: GQC INDEX - CLASSIFICATION OF CHANNEL QUALITY

Index of channel quality	Code:
(Conducted in at least three transects distance apart of 20 meters)	

1. Presence of retaining structures

Absence of structures	4
Semi-disaggregated rustic weir	3
Well established rustic weir	2
Dam or concrete dam	1

2. Channel Structure

W/D<7, It does not occur flood of the banks	4
W/D = 8-15, rare flooding of banks	3
W/D = 15-25, frequent flooding of banks	2
W/D> 25, very frequent flooding of banks	1

W - Average width of the wet bed obtained in transects

D – Average of maximum depth obtained in transects.

3. Sediments and stability of the channel

Absence of enlargement channel or accumulations of transported materials; single channel;	4
Some accumulation of transported material; single channel;	3
Lignes of gravel, sand and silt; the bed has full independent channels;	2
Channel divided into multiple lignes of sand and silt (or channelized river).	1

4. Structure of banks

Stable Banks with continuous and structurally complex riparian vegetation (trees and shrubs); without signs of erosion;	4
Stable Banks but with fragmented riparian vegetation; some eroded zones without vegetation;	3
Consolidated little Banks maintained by a sparse vegetation of grasses and shrubs;	2
Banks with very little and uniform vegetation, lowered by erosion along the stretch.	1

5. Artificial alteration of the banks

Almost complete absence of artificial change of banks;	4
One of the banks present moderate changes (e.g. rip-rap> 30% of the length section);	3
Both banks present moderate changes (e.g. rip-rap > 30%), or one of them is significantly altered (e.g. bank linearization)	2
As in the previous case, but the edge of the structure is of reinforced concrete or cyclopic.	1

6. Channel heterogeneity

Sinuosity of the channel and very marked lotic / lentic sequence;	4
Rectilinear channel with reduced lotic / lentic sequence;	3
Substantially constant velocity over the whole section;	2
Artificial lentic zone or channelized river	1

7. Bottom structure

Type 1	Headwater streams, low capability of supporting an extensive riverine forest;
Type 2	Middle zones of rivers, intermediate potential to support a riverine forest;
Type 3	Downstream zones with high potential to support a riverine forest;

Type 1 (Section on which predominates erosion)

> 50% of material comprises a particle size> 25 cm (boulders);	8
> 50% of material comprises particle sizes> 6.5 cm (pebble);	6
> 50% of material comprises particle sizes> 2.0 cm (gravel);	3
Predominates sand and silt (> 50%).	1

Type 2 (section in which predominantes transport)

> 50% of the material comprises boulders and pebbles (> 6.5 cm);	8
50% of material comprises pebble or higher (> 6.5 cm);	6
<25% of the material is larger than gravel (> 1.5 cm);	3
The bed is exclusively silt and sand (> 1.5 cm) is less than 10%.	1

Type 3 (Section on which dominates sedimentation)

> 50% of the material consists of larger than coarse sand (0.5 cm);	8
30-50% of the material consists of larger than coarse sand (0.5 cm) and the rest is formed by silt and sand;	6
<30% of the material consists of larger than coarse sand (0.5 cm) and the rest is formed by silt and sand;	3
The bed is only of silt and fine sand (<0.125 cm).	1

8. Deposition of fine interstitial sediments

% fines and <5%;	4
% fines is 5-25%;	3
% fines is 25-50%;	2
% fines is> 50%.	1

• For Type 1 rivers, fines are considered <0.5 cm

• For Type 2 and 3 rivers, fines are considered <0.125 cm.

ANNEXE III: Scores assigned to different families of aquatic macroinvertebrates to calculate the IBMWP (adapted from ALBA-TERCEDOR 2000).

Families	Score
E: Siphlonuridae, Heptageniidae, Leptophlebiidae, Potamanthidae, Ephemeridae P: Taeniopterygidae,Leuctridae, Capniidae, Perlodidae, Perlidae,Chloroperlidae T: Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae D: Athericidae, Blephariceridae H: Aphelocheiridae	10
T: Psychomyiidae, Philopotamidae, Glossosomatidae O: Lestidae, Calopterygidae, Gomphidae, Cordulegasteridae, Aeschnidae, Corduliidae, Libellulidae C: Astacidae	8
E: Ephemerellidae, Prosopistomatidae P: Nemouridae T: Rhyacophilidae, Polycentropodidae, Limnephilidae, Ecnomidae	7
M: Neritidae, Viviparidae, Ancylidae, Thiaridae, Unionidae T: Hydroptilidae C: Gammaridae, Atyidae, Corophiidae O: Platycnemididae, Coenagrionidae	6
E: Oligoneuriidae, Polymitarcidae C: Dryopidae, Elmidae, Helophoridae, Hydrochidae, Hydraenidae, Clambidae T: Hydropsychidae, Helicopsychidae D: Tipulidae, Simuliidae Pl: Planariidae, Dendrocoelidae, Dugesiidae	5
E: Baetidae, Caenidae C: Haliplidae, Curculionidae, Chrysomelidae D: Tabanidae, Stratiomyidae, Empididae, Dolichopodidae, Dixidae, Sciomyzidae Ceratopogonidae, Anthomyidae, Limoniidae, Psychodidae, Rhagionidae Mg: Sialidae Pl: Piscicolidae A: Hidracarina	4
H: Mesovellidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Pleidae, Veliidae, Notonectidae, Corixidae C: Helodidae, Hydrophilidae, Higrobiidae, Dytiscidae, Gyrinidae M: Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae, Bithyniidae, Bythinellidae, Sphaeriidae Hr: Glossiphoniidae, Hirudidae, Erpobdellidae C: Asellidae, Ostracoda	3
D: Chironomidae, Culicidae, Muscidae, Thaumaleidae, Ephydridae	2
O: Oligochaeta (All Families) D: Syrphidae	1

ANNEXE IV.1: Metrics and indexes determinated with the *amiib software* (INAG) for each samping site in Tuela River (spring 2017).

Metrics	Sampling sites (river Tuela)				
	T1	T2	T3	T4	T5
BMWP	267	218	152	118	162
Nº Taxa BMWP	38	31	26	18	24
ASPT	7.03	7.03	5.85	6.56	6.75
IASPT	6.49	6.66	5.27	5.91	6.41
EPT Taxa	22	19	8	11	15
Number of individuals - EPT	539	479	195	460	674
% of individuals – EPT	52.03	75.79	28.8	33.43	87.53
N Fam. Hirudinea	0	0	1	1	1
% Ind. Hirudinea	0	0	0.44	0.29	0.13
N Fam. Gastropoda	1	1	2	1	1
% Ind. Gastropoda	0.48	1.9	0.59	0.29	0.26
N Fam. Bivalvia	2	2	2	0	1
% Ind. Bivalvia	1.64	1.74	0.74	0	0.13
N Fam. Crustacea	0	0	1	0	0
% Ind. Crustacea	0	0	0.15	0	0
N Fam. Coleoptera	6	3	3	2	1
% Ind. Coleoptera	24.52	0.63	2.81	0.65	4.55
N Fam. Ephemeroptera	5	4	5	5	6
% Ind. Ephemeroptera	22.01	41.3	26.44	18.46	35.58
N Fam. Diptera	9	7	5	6	4
% Ind. Diptera	12.74	16.14	56.28	65.12	4.68
N Fam. Heteroptera	2	2	2	1	1
% Ind. Heteroptera	2.03	1.74	2.22	0.22	0.26
N Fam. Odonata	4	5	5	0	2
% Ind. Odonata	3.28	1.27	2.95	0	0.91
N Fam. Plecoptera	4	3	0	3	2
% Ind. Plecoptera	6.95	7.12	0	10.25	26.75
N Fam. Trichoptera	13	12	3	3	7
% Ind. Trichoptera	23.07	27.37	2.36	4.72	25.19

ANNEXE IV.2: Metrics and indexes determinated with the *amiib software* (INAG) for each samping site in Mente and Rabaçal rivers (Spring 2017).

Metrics	Sampling sites (Mente and Rabaçal rivers)				
	M1	M2	R1	R2	R3
BMWP	176	146	127	177	108
Nº Taxa BMWP	25	21	18	26	16
ASPT	7.04	6.95	7.06	6.81	6.75
IASPT	6.81	6.56	6.5	6.37	6.32
EPT Taxa	14	14	11	14	9
Number of individuals - EPT	119	194	312	143	90
% of individuals - EPT	65.38	71.59	72.22	60.08	66.18
N Fam. Hirudinea	0	0	1	0	1
% Ind. Hirudinea	0	0	0.46	0	0.74
N Fam. Gastropoda	2	2	1	1	0
% Ind. Gastropoda	2.2	0.74	2.08	1.68	0
N Fam. Bivalvia	0	0	1	2	0
% Ind. Bivalvia	0	0	8.1	10.92	0
N Fam. Crustacea	0	0	0	0	0
% Ind. Crustacea	0	0	0	0	0
N Fam. Coleoptera	3	2	1	4	1
% Ind. Coleoptera	3.85	2.58	0.69	5.04	2.21
N Fam. Ephemeroptera	4	6	3	5	5
% Ind. Ephemeroptera	21.98	37.27	44.21	38.66	39.71
N Fam. Diptera	3	4	2	7	3
% Ind. Diptera	10.44	11.07	10.42	14.71	12.5
N Fam. Heteroptera	2	1	1	1	1
% Ind. Heteroptera	5.49	1.48	1.62	0.84	2.21
N Fam. Odonata	2	1	2	4	3
% Ind. Odonata	6.04	3.32	3.7	3.78	12.5
N Fam. Plecoptera	2	2	2	1	1
% Ind. Plecoptera	18.13	24.35	4.63	11.76	22.79
N Fam. Trichoptera	8	6	6	8	3
% Ind. Trichoptera	25.27	9.96	23.38	9.66	3.68

ANNEXE IV.3: Metrics and indexes determinated with the *amiib software* (INAG) for each samping site in Baceiro, Assureira and S. Cibrão rivers (Spring 2017).

Metrics	Sampling sites (Baceiro, Assureira and S. Cibrão rivers)			
	B1	B2	A1	C1
BMWP	120	116	120	182
Nº Taxa BMWP	19	17	17	26
ASPT	6.32	6.82	7.06	7
IASPT	5.73	6.3	6.48	6.7
EPT Taxa	11	11	9	17
Number of individuals - EPT	117	292	114	341
% of individuals - EPT	54.67	81.56	60.32	85.25
N Fam. Hirudinea	1	0	0	1
% Ind. Hirudinea	0.93	0	0	0.25
N Fam. Gastropoda	1	0	0	1
% Ind. Gastropoda	0.47	0	0	0.25
N Fam. Bivalvia	0	0	1	0
% Ind. Bivalvia	0	0	1.06	0
N Fam. Crustacea	0	0	0	0
% Ind. Crustacea	0	0	0	0
N Fam. Coleoptera	3	2	3	1
% Ind. Coleoptera	1.4	4.75	19.58	0.75
N Fam. Ephemeroptera	3	5	5	5
% Ind. Ephemeroptera	25.7	56.7	32.8	18.75
N Fam. Diptera	4	5	5	5
% Ind. Diptera	13.55	12.57	14.29	8.25
N Fam. Heteroptera	1	0	2	1
% Ind. Heteroptera	0.93	0	3.7	2.75
N Fam. Odonata	0	1	1	2
% Ind. Odonata	0	0.56	1.06	0.75
N Fam. Plecoptera	2	4	1	5
% Ind. Plecoptera	7.48	16.76	4.23	43.5
N Fam. Trichoptera	6	2	3	7
% Ind. Trichoptera	21.5	8.1	23.28	23

Chapter 3

MANAGEMENT OF BROWN TROUT POPULATIONS IN MONTESINHO NATURAL PARK (TUA BASIN, NORTHEASTERN PORTUGAL)

ABSTRACT

Brown trout (*Salmo trutta*) is an emblematic fish species of Northeastern Portugal and in particular in Montesinho Natural Park, due to their interest for conservation and exploitation purposes. In fact, there is a big demand of Brown trout for fishing activity (*i.e.* leisure and/or sport fishing) in well preserved mountainous rivers that, nowadays, still maintain several endangered vertebrate (*e.g. Galemys pyrenaicus*, *Lutra lutra*) and invertebrate (*e.g. Margaritifera margaritifera*, *Macromia splendens*) species.

During spring/summer season of 2017 in 14 sampling sites distributed by 6 different watercourses of Upper Tua, it were sampled the fish communities, using the protocol defined by the Portuguese Environment Agency. It was found a low density of native fish populations, varying the composition from exclusively Brown trout populations, in salmonid streams, until salmonid/cyprinid transition zones, where target species can cohabit with endemic cyprinids (*Pseudochondrostoma duriense*, *Squalius carolitertii*, *Squalius alburnoides* and *Luciobarbus bocagei*) and cobitids (*Cobitis calderoni*). The absence of exotic species contributed to the good/excellent classification of all sampling sites using the fish integrity F-IBIP index. The ecological guilds confirmed the good integrity of these rivers, showing typical functional relationships among fish species present in these undisturbed ecosystems. It was observe an isometric coefficient (b=3) and a good physical condition factor (K> 1.0) for the majority of sampled Brown trout populations, except for Mente River, where significant differences were found (K< 0.8). The riffle/pool sequence, the diverse mosaic of aquatic microhabitats and the riparian buffer strips are contributing to the maintenance of good conditions food and habitat for the conservation of Brown trout populations. The results suggested that the ecological integrity of Upper Tua must be maintained, conditioning the fishing activity as the main management measure to preserve not only the wild Brown trout populations but also other communities.

Key-words: *Brown trout, bioecology, conservation, management*

3.1. INTRODUCTION

Brown trout (*Salmo trutta* L.) is classified as a species of least concern by the IUCN (2017). This species are not specifically listed for protection by EU Directives, although they are widely managed throughout the world because of their socioeconomic value and their ecological importance (Sanchez-Hernandez & Cobo, 2012).

Knowledge of feeding habits is essential to understand the ecological role and the productive capacity of fish populations, and the understanding of these mechanisms is critical to the development of conservation and management plans (Teixeira & Cortes, 2006). Furthermore, the knowledge of habitat use and territorial behavior is another consideration in management of trout populations. Studies of the ontogenetic shifts about feeding behavior (e.g. Steingrimsson & Gislason, 2002; Montori et al., 2006; Fochetti et al., 2008) and habitat preference (e.g. Haury et al., 1991; Ayllón et al., 2010; Parra et al., 2011) in Brown trout populations are carried out in order to clarify the mechanisms involved during the ontogeny, since ontogenetic shifts may reduce competition facilitating the partitioning of resources (e.g. Elliott, 1967; Hyndes et al., 1997; Amundsen et al., 2003; Oscoz et al., 2006).

3.1.1. Bioecological requirements of Brown trout

Good water quality will always be a requirement for oxygenation of intra-gravel life stages as well as for general ecology of the free-swimming stages of Brown trout. Gross pollution will lead to high mortalities (King et al., 2011).

3.1.1.1. Life-cycle of Brown trout

Brown trout is a highly successful, polytypic species exhibiting a range of quantitatively complex life cycles. These can be simplified into four typical strategies. In the first and simplest, the trout remain in their natal stream for life, growing slowly and achieving only small size. The second involves migration of juveniles and subadults from the natal stream to the parent river, and the mature adults do not return until they are ready to spawn. The third fourth life cycles are exhibited by trout that migrate as smolts to a lake, or to the estuary or sea. The first year of life is a period of rapid growth for the fish. Consequent associated changes in diet due to an increase in gap size and the ability to handle larger prey, and improved locomotory skills increasing potential for migration, account for considerable ontogenetic change early on in the trout life cycle (Grey 2001).

3.1.1.2. Feeding behavior of Brown trout

Salmonids are top-consumers in freshwater habitats and play an important role as a carrier of energy from lower to higher trophic levels (e.g. Karlsson & Byström, 2005). Dominant fish will feed in the optimal place for feeding (preferences are, normally, in dusk and dawn periods) and may specialize eating in slow-moving riffles near the surface or fast-moving water near the middle of the water column. Smaller and younger trout feed on the bottom of the river, or maybe at the surface or shoreline while larger trout will move about the river (Klemetsen et al., 2003).

3.1.1.3. Habitat use of Brown trout

The preferred habitat for Brown trout is headstreams with moderate water currents, although they can be found in lakes. The depth at which they swim is dependent upon the size and stage of life. Juvenile Brown trout feeding away from the nest, are normally found in the shallow waters less than 30 cm, while adults are deeper in the stream. Larger adult Brown trout occupy the slow moving water closer to the center of waterways, away from the banks. Brown trout also make use of eddies, slow moving pools of water, in the river. The preferred substrate is rocky bottoms that provide cover, but also gravel sand, silt, and mud. Depending on accessibility, some Brown trout are anadromous. Newly hatched Brown trout alevins and fry will experience a gradual habitat shift from their gravel redd to the more open riverbed to feed. Some Brown trout may not migrate and choose to stay within their natal stream throughout their life (Cunjak & Power, 1986; Heggenes, 1996; Klemetsen et al., 2003; Shirvell & Dungey, 1983; Stauffer Jr. et al., 1995). The decision to migrate seems to be a plastic response, influenced by growth opportunities (Olsson et al., 2006).

The objectives of this chapter are organized as following:

- To estimate trout population parameters (abundance, growth, condition factor);
- To evaluate the ichthyological (F-IBIP) index;
- To determine the resources used by trout populations: habitat and feeding strategies.

3.2. METHODOLOGY

3.2.1. Sampling of Fish Fauna

The sampling of fish fauna was made following the Manual for the biological evaluation of water quality in river systems according to the Water Framework Directive - Sampling and analysis protocol for fish fauna (INAG, 2008). According to the procedures expressed there, the samples were captured by electrofishing. The fish species were captured during spring season of 2017 using a portable electrofishing device, with direct (DC) current output (Hans Grassl ELT; 300-600V) (Figure 3.1). Fish were identified according to the book of the AQUARIPORT Project (Oliveira et al., 2007) and considering a later amendment - as outlined in Handbook of European Freshwater Fishes in order to include the genus Luciobarbus. It should be noted that the nomenclature used is from the IUCN (International Union for Conservation of Nature) list, more specifically from the Red List of Threatened Species (available at http://www.iucnredlist.org/).

Figure 3. 1. Field sampling of fish species (Tua basin) (Spring of 2017)

The sampling sites were chosen considering all representative habitats and in particular the presence of a sequence pool/riffle and the diversity of microhabitats. All fish captured were measured using a fish ruler (0.1 cm of precision) and weighed with a digital balance (0.1 g of precision) and then realeased into the river (Figure 3.2).

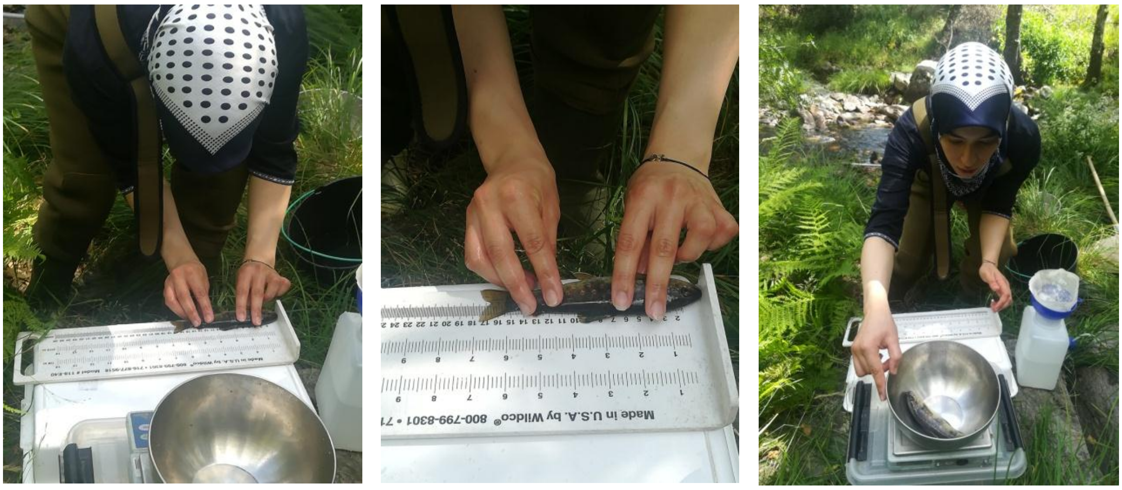


Figure 3. 2. Biometric data of fish species (Tua basin) (spring 2017)

3.2.2. Ecological Guilds

Ecological guilds are used in metrics that reveal the functional composition of communities. An ecological/functional guild can be defined when a group of fish species exploit the same type of environmental resources.The fish species of Tua river basin were classified into ecological groups (or guilds) according to the AQUARIPORT Project (OLIVEIRA et al., 2007). It should be noted that the classification of taxa in different trophic, reproductive and habitat use groups, among others, is the keystone of the biotic indexes for assessing the ecological status of aquatic ecosystems based on fish communities. In this perspective, the guild concept comprises numerous and complex species interactions (OLIVEIRA et al., 2007). In Table 3.1 the ecological guilds are presented for the determination of responses of fish communities.

Table 3. 1. Ecological guilds*, according to the FAME project proposal.

ESPÉCIES	G_HAB_{rp}	G_HAB_{fb}	G_MIG	G_REP	G_TRO	G_TOL
L. bocagei	LIMN	BENT	POTA	LITH	OMNI	TOLE
P. duriense	REOP	BENT	POTA	LITH	HERB	INTO
S. alburnoides	EURI	PELA	RESI	PHYT	INVE	INTO
S. carolitertii	EURI	PELA	RESI	LITH	INVE	INTO
C. calderoni	REOP	BENT	NA	LITH	INVE	INTO
S. trutta	REOP	PELA	PM	LITH	INVE	INTO

*Habitat Guild G_HAB_{rp} (reophilic degree): EURI (Eurytopic), LIMN (Limnophilic), REOP (Reophilic); Habitat Guild G_HAB_{fb} (feeding behavior): BENT (benthic), PELA (pelagic); Migration Guild (G_MIG): GMA (large anadromous), GMC (large catadromous), PM (small migratory), POTA (potamodromous), RESI (Resident); Reproductive guild (G_REP): PHYT (phytolithophilic), GENE (generalist), LITH (lithophilic); Trophic guild (G_TRO): HERB (herbivorous), INVE (invertivorous) OMNI (omnivorous); Tolerance Guild (G_TOL): TOLE (tolerant), INTO (intolerant); NA - not applicable.

3.2.3. Trout population parameters

The relationship between length and biomass was made recurring to the following equation:

$$W = aL^b$$ where:

W - Individual weight in grams (g)

L - Total length in centimeters (cm)

a , b - coefficients of the equation.

The coefficient **b** can be of isometry (b = 3) or allometry (b ≠ 3). According to Cortes & Ferreira (1993), the interpretation of the meaning of the relationship between the parameters of the biomass and the length is clarified in the evaluation of the physical condition of the fish.

For the determination of the K- condition factor of the fish sampled, the following formula was used:

$$K = (100 \times W) / L^3$$ where:

K - Condition factor or coefficient of physical condition

W - Individual weight in grams (g)

L - Total length of the individual in centimeters (cm)

The Condition Factor (K) allows the comparion of physical condition of the fish, and can be used as a productivity index. In the case of salmonids, K typically assumes values between 0.8 and 2, based on the comparison of the K values with the general appearance and fat reserves the following evaluations are given below in Table 3.2 (Barnham & Baxter, 1998).

Table 3. 2. Condition Factor K for salmonid fish (Barnham & Baxter, 1998).

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

3.2.4. F-IBIP Fish-based Index of Biotic Integrity

For the fish fauna, the data analysis was carried out through a classical approach at community level, evaluating its specific composition (with the support of ecological indexes of dominance, uniformity, richness and diversity) and numerical (in particular with the analysis of abundance, density, Capture per Unit of Effort (CPUE) and rarity of species). The CPUE calculation allows a standardization of abundance index between each sampling site for each species and dimensional class. The evaluation of ecological quality of the fish fauna element was carried out by the calculation of F-IBIP Fish-based Index of Biotic Integrity for Portuguese Wadeable Streams (AFN, 2012; APA, 2014) using the WEB application: **https://www.isa.ulisboa.pt/proj/fibip/index.php.**

The F-IBIP is the official index for Portugal for quality evaluation based on the fish community. The F-IBIP is based on the principle that the structures of biological communities respond in a predictable and quantifiable way to human changes in aquatic systems. The alteration of the fish community resulting from disturbances of human origin is measured by metrics that represent components of the community structure. The value of the metrics obtained at a given sampling site is compared to the expected value in a river of the same type under reference conditions, *i.e.* in sites where there is no disturbance or minimal disturbance. The metrics used in the calculation of F-IBIP reflect the wealth and composition (*e.g.* number of native species, percentage of exotic individuals) or functional characteristics (*e.g.* food guild, reproductive guild) of the community. The final value of the index is obtained by the sum of the metrics that compose it, reflecting the global deviation of the sampling location relative to the reference situations.

The application of the F-IBIP takes into consideration the sampling sites belonging to fish clusters, for which a particular community structure is expected, due to the abiotic characteristics that tend to be more similar within each cluster. According to the F-IBIP methodology, a sampling site is characterized using the following abiotic characteristics: drainage basin size, altitude, average air temperature in July, annual mean precipitation and latitude. The F-IBIP considers 6 types of fish groups.

Table 3. 3. Caracterization of used metrics in the calculation of F-IBIP for each fish group

Group	Metric	Type of Metric	Response to pressure
Salmonid- Northern Region	% of intolerant individuals	Tolerance	↓
	% of exotic individuals	Composition	↑
	% of omnivorous individuals	Trophic	↓
Salmonid/Cyprinid Transition- Northern Region	% of exotic individuals	Composition	↑
	% of intolerant + intermediate species	Tolerance	↓
	% of invertivorous (excluding tolerant species)	Trophic	↓
	% of potamodromous individuals	Migratory	↓

The F-IBIP calculation is performed using different sets of metrics for each cluster, considering the ones that best translate the disturbance response to that cluster. Table 3.3 shows the metrics used in the Salmonid- Northern Region and Salmonid-Cyprinid Transition Region groups. The arithmetic mean of the metrics considered in each cluster is used to obtain the final value of the F-IBIP. This is expressed in the form of ecological quality ratio (RQE) ranging from 0, which corresponds to poor quality, and 1, which corresponds to excellent quality. Table 3.4 shows the range of values for each of the quality classes.

Table 3. 4. Values of the Ecological Quality Ratios applied in the F-IBIP index.

Range	Quality
[0.850 – 1.000]	**EXCELLENT**
[0.675 – 0.850[	**GOOD**
[0.450 – 0.675[	**FAIR**
[0.225 – 0.450[	**POOR**
[0.000 – 0.225[	**BAD**

3.2.5. Trout resource use: Food and Habitat

3.2.5.1. Food preferences based on diet studies

The food preferences of Brown trout were made through the technique of regurgitation - Stomach Flushing Technique (KAMLER & POPE, 2001). In fact, for salmonid species, this technique can be used to obtain stomach contents and do not require the death of the fish (Figure 3.3).

Figure 3. 3. Regurgitation of a Brown trout to obtain stomach content (Spring 2017)

In the laboratory, all food items were identified and counted using a stereoscopic microscope, Olympus SZX10, with a 10-132x magnification. The identification was done through dichotomous keys (TACHET et al., 1981, 2010). The aquatic invertebrates were identified until family taxonomic level, except for Oligochaeta and Acari (suborder) and allochthonous preys (terrestrial insects), pupae and imagos, zooplankton and fish and amphibia animals.

The method used for identified prey items in the different stomach contents was based on HYSLOP (1980). For each sample the data were treated in absolute (N), numerical percentage (% N) and frequency of occurrence (% FO). It were considered four size classes (Table 3.5), roughly related with different age classes defined for Brown trout in Northestern rivers of Portugal (Teixeira & Cortes, 2006).

Table 3. 5 .Relationship among size/age classes for Brown trout in NE rivers of Portugal

Class	Total Lenght (cm)	Age	Classification
A	< 10.0	0+	Alevins
B	10.1-15.0	1+	Juveniles
C	15.1-20.0	2+	Subadults
D	≥ 20.0	≥ 3+	Adults

3.2.5.2. Ivlev's Electivity Index (D)

The **Ivlev Electivity Index (D)** modified by Jacobs (1974) analyzes the selectivity of the diet of the species and measures the degree to which a fish selects a particular category of resources relative to the theoretical range of available resources. It is determined by the formula:

$$D = (ri-pi)/(ri+pi-2ripi) \quad \text{where:}$$

ri- relative proportion of a given category (food item) in the stomach contents.

pi- relative proportion of a given category (item) in the available aquatic environment.

The index ranges from a minimum of -1 (the item is completely avoided by the fish) and a maximum of +1 (the item is preferred by the fish). To evaluate the available food, a semi-quantitative sampling of the macroinvertebrate community was carried out in accordance with the protocol established in Portugal by the INAG*b* (2008).

3.2.5.3. Schoener Index (S)

The SCHOENER index (1970) is given by the following formula:

$$S = 100\,(1 - 0.5\, \Sigma\, \text{I}\; p_x,i - p_y,i\; \text{I}) \quad \text{where:}$$

px,i - frequency of item i for size class x

py,i - frequency of item i for size class

According to WALLACE (1981), it is considered that there is overlap of diets between trout size classes when S assumes values higher than 60%.

3.2.6. Habitat Use

3.2.6.1. Available microhabitat

The evaluation of the available microhabitat, several transects, proportional to the representativeness of each identified habitat unit, were made at randomly selected sites. In each transect, with intervals of 50 cm, the following variables were measured:

A) Total depth (cm) - measurement made with a graduated rod;

B) Water column velocity (m/s) – water current Valeport® model;

C) Dominant substrate - codes presented in Table 3.6 and;

D) Cover - defined codes defined presented in Table 3.7.

Table 3. 6. Substrate codes (adapted from BOVEE, 1982)

Code	Description of substrate classes
1	Plant Detritus
2	Fines (sand, silt, clay) < 2.0 mm
3	Gravels (0.2 – 14.9 cm)
4	Pebbles (15.0 – 60.0 cm)
5	Boulders (> 60.0 cm)
6	Bedrock

Table 3. 7. Cover codes (adapted from BOVEE, 1982)

Code	Description of cover classes
1	No cover
2	Gravel
3	Pebbles
4	Boulders
5	Overhanging vegetation (riparian tree)
6	Aquatic roots and undercut banks
7	Aquatic vegetation
8	Turbulent surface

3.2.6.2. Used microhabitat

The microhabitat used by Brown trout was evaluated through the technique of underwater observation (snorkeling). In this technique, fish are observed by snorkelers (Figure 3.4) who move in zig-zag, preferably in upstream direction.

Figure 3. 4. Evaluation of used microhabitat of Brown trout throught snorkeling (Spring 2017)

Whenever a fish considered undisturbed (i.e. when the fish maintains the activity detected) was located, the snorkeler communicated the values assumed by the variables listed below, which are recorded by an operator at the river bank:

A) **Total depth** of the water column (cm);
B) **Water column velocity** (m/s);
C) **Dominant substrate** (i.e. area of 0.2 x 0.2 m under the fish), using the substrate code (see Table 3.6);
D) **Cover**, considering the objects that can promote shelter at least 50% of fish's body, according to the code defined in Table 3.7 and;
E) **Size (cm) of observed fish**. Measurement was done recurring to a graduated rod, making the comparison with objects of the river bottom (e.g. pebble).

3.2.7. Data treatments

Multivariate analyses were used through the software PRIMER 6 & PERMANOVA + (CLARKE & GORLEY, 2006). A non-metric Multi Dimensional Scaling (NMDS) was performed for the analysis of fish communities in the different sampled sites. Abundance data were transformed [Log (x + 1)] and the Bray-Curtis similarity coefficient applied. A multivariate analysis of similarities was carried out, using one-way ANOSIM non-parametric tests, to investigate the similarity between the salmonid and salmonid/cyprinid river streches of the Tua basin. The underwater observations allowed to analyze the behavioral differences among size classes of Brown trout related to the microhabitat used. The preference of a species can be transformed in terms of "preference curves" that relate the values of a variable to a "preference index", which ranges from 0 (minimum preference) to 1 (most preferred values). Their calculation is based on the relationship between the fish habitat use (obtained through underwater observation) and the available habitat data (recurring to transects). The microhabitat variables were measured considering the four size classes defined previously (see section 3.2.6): 1) cover, 2) dominant substrate, 3) total depth, 4) water current. The dependent variable represents the relative probability of use and was standardized on a scale of 0 to 1. Kruskal-Wallis H tests were also applied using the software STATISTICA 7 (STATSOFT, 2004). Non-parametric statistical tests were performed to test independent samples: more than two independent samples (Kruskal-Wallis H test). H tests correspond to an ANOVA, since it is used to test the null hypothesis when the assumptions of ANOVA are not verified (data normality and homogeneity).

3.3. RESULTS

The results showed the composition and abundance of fish species present in the different selected sampling sites of High Tua.

3.3.1. Composition and Abundance of Fish Communities

Six (6) fish native fish species of Portuguese rivers were found in this study, belonging to 3 different families: 1) Salmonidae: Brown Trout (*Salmo trutta*); 2) Cyprinidae: Calandino Roach (*Squalius alburnoides*), Northern Iberian Chub (*Squalius carolitertii*), Douro Nase (*Pseudochondrostoma duriense*), Common Iberian barbel (*Luciobarbus bocagei*), and 3) Cobitidae: Spined Loach of Northern Portugal (*Cobitis calderoni*) (Figure 3.5).

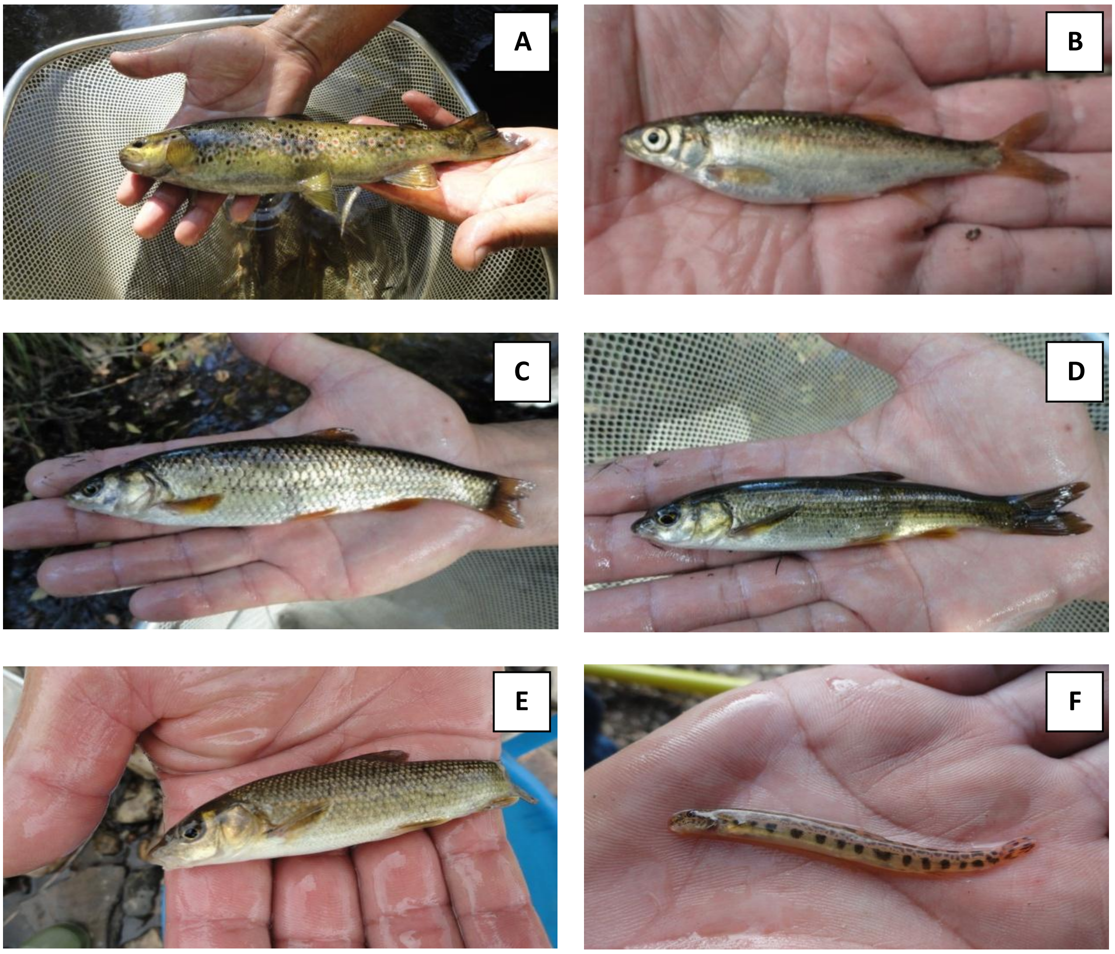


Figure 3. 5. Fish species captured in the High Tua Basin (**A-** ***Salmo trutta***; **B-** ***Squalius alburnoides***; **C-** ***Squalius carolitertii***; **D-** ***Pseudochondrostoma duriense;*** **E-** ***Luciobarbus bocagei;*** **F-** ***Cobitis calderoni***) **(Spring 2017)**

Sampling sites located in the headwater streams (eg B1, B2, T1, M1 and M2), the dominant species is Brown trout, even representing the total catch in sections B1 and M1. The abundance of fish per sampling site can be seen in Figure 3.6.

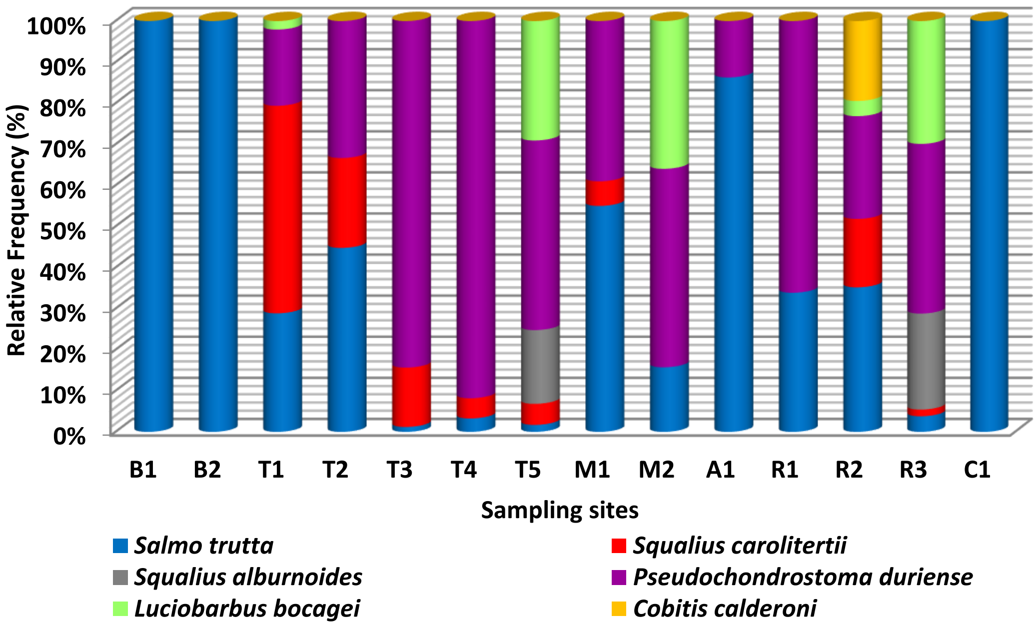


Figure 3. 6. Composition and relative abundance of fish communities (Spring 2017).

3.3.2. Biotipology of Fish Communities

The NMDS analysis based on fish abundances can be observed in Figure 3.7.

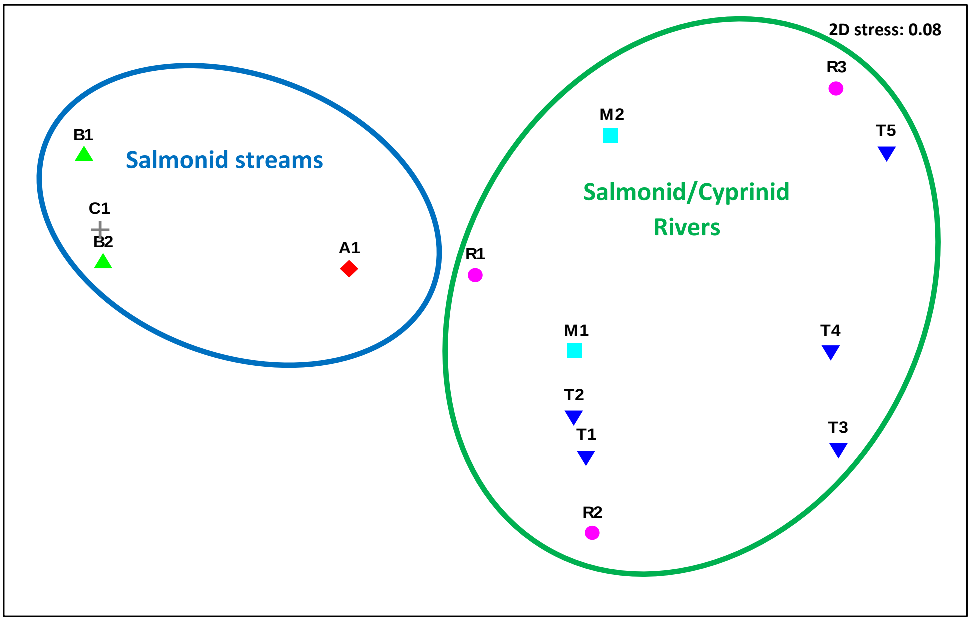


Figure 3. 7. NMDS ordination of sampling sites, based on the abundance of fish communities and considering 6 rivers and 14 samping sites: 1) Green triangles: Baceiro; 2) Blue triangles: Tuela; 3) Pink circles: Rabaçal; 4) Blue squares: Mente; Red square: Assureira; Cross symbol: S. Cibrão

The 2D stress value of 0.08 is indicative of a good two-dimensional representation of the ordination. In this way, there are an effective separation between the sampling sites of Salmonid Group (B1, B2, A1, C1) and the Salmonid/Cyprinid Group (eg M1, M2, R1 to R3 and T1 to T5). Salmonid Group Region is represented by headwater streams, with oligotrophic character, cold and oxygenated waters. Salmonid/Cyprinid Group is represented by rivers with median dimension where Brown trut cohabit with endemic cyprinids and cobitids. Figure 3.8 shows the NMDS ordination of the fish species present in High Tua Basin.

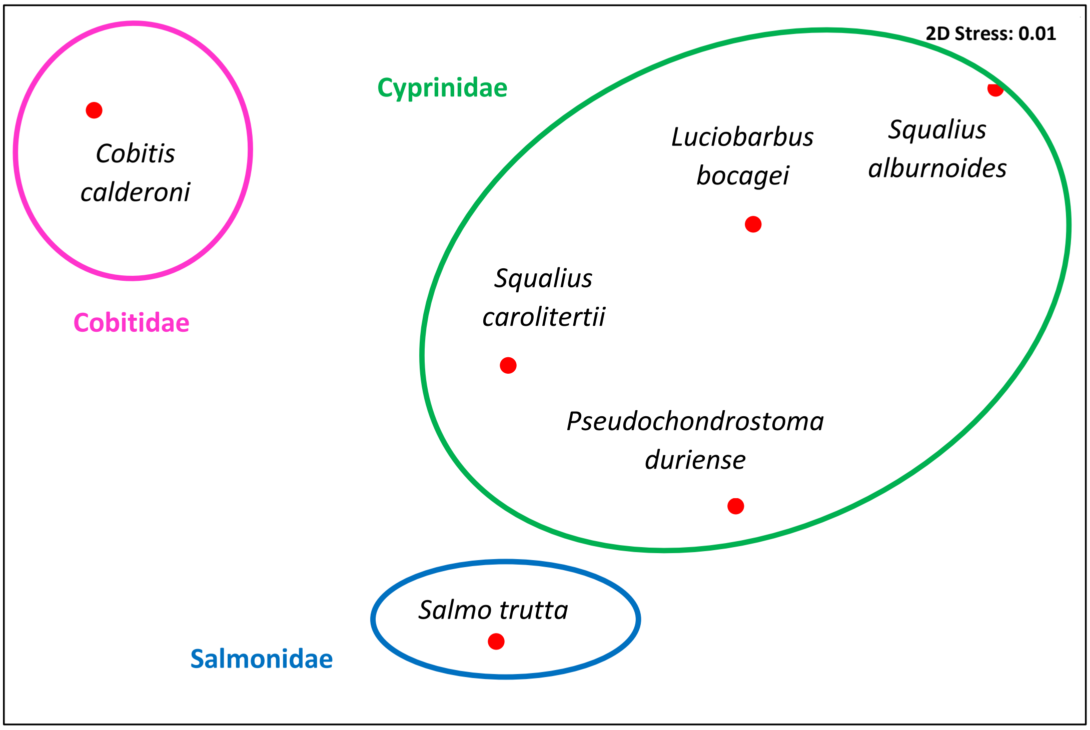


Figure 3. 8. NMDS ordination of fish species in the Tua river basin.

ANOSIM One-Way Tests were performed between the groups considered detected significant differences ($P < 0.05$) between Salmonid *vs.* Salmonid/Cyprinid groups.

3.3.3. Ecological Guilds

In Figure 3.9 the classification for the 15 sampling sites of the Ecological Guilds related to: 1) Habitat- Reophilic Degree; 2) Habitat- Food Zone; 3) Migratory; 4) Reproductive; 5) Trophic and 6) Tolerance level to degradation of the aquatic environment. It can be observed that in the majority of the sampled rivers the percentage of reophilic species is high. In these sections, the fish fauna consists mainly of specimens of the species *Salmo trutta* and *Pseudochondrostoma duriense*, species that have, essentially, reophilic habits. The composition of the fish fauna in the middle sections is composed of a greater percentage of species of euritopics habits.

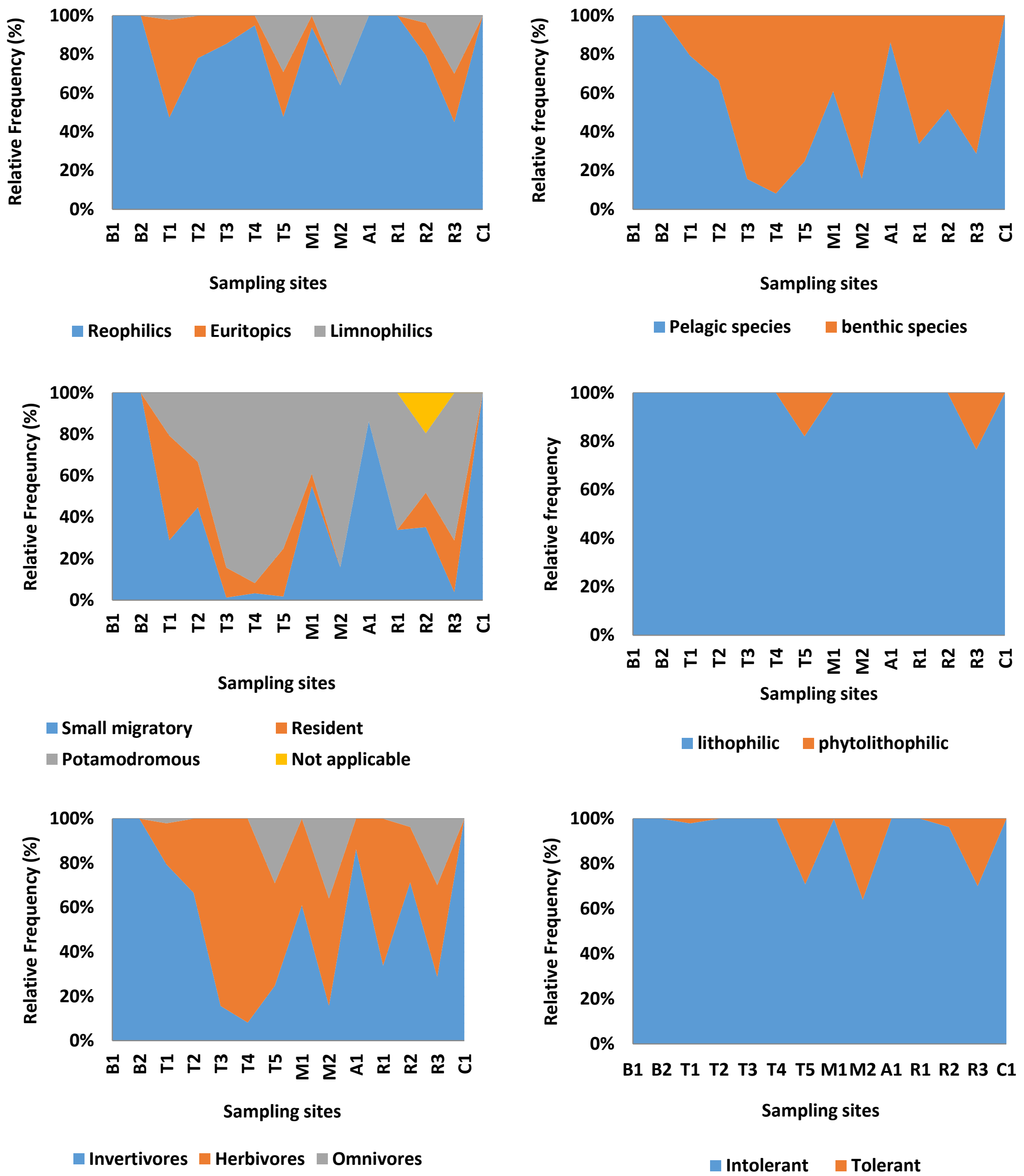


Figure 3. 9. Classification of Ecological Guilds: 1) Habitat- Reophilic Degree; 2) Habitat- Food Zone; 3) Migratory; 4) Reproductive; 5) Trophic and 6) Tolerance level to degradation of the aquatic environment, in the 15 sampling sites, based on the species abundance captured in the Alto Tua basin (Spring 2017).

Most of the fish populations in these river sections were intolerant, mainly invertivores, with small migration (Brown trout) or potamodromous (cyprinids) reproduction behavior in litophilic areas and a more pelagic swimming behaviour in upper parts and more benthic behaviour in lower parts of the sampled rivers.

3.3.4. F-IBIP Index

The calculation of F-IBIP Fish-based Index of Biotic Integrity for Portuguese Wadeable Streams showed an excellent/good status for all sampling sites (Tables 3.8 and 3.9).

Table 3. 8. Classification of sampling sites of Group 2 (Salmonid/Cyprinid Transition-Northern Region of Portugal), based on the fish composition and abundance (Spring 2017)

Sampling site	Group	Exotic Individuals	Intolerant+ intermediate Individuals (%)	Invertivore Individuals (excluding tolerant species) (%)	Potamodromous Individuals (native species) (%)	F-IBIP	Quality Class
T1	2	0	97.9	79.4	49.5	0.845	Good
T2	2	0	100.0	66.7	78.1	0.908	Excellent
T3	2	0	100.0	15.7	85.5	0.758	Good
T4	2	0	100.0	8.2	95.1	0.750	Good
T5	2	0	70.9	24.8	76.9	0.723	Good
M1	2	0	100.0	61.0	94.0	0.908	Excellent
M2	2	0	64.0	15.7	100.0	0.684	Good
R1	2	0	100.0	33.9	100.8	0.811	Good
R2	2	0	96.3	71.0	64.5	0.885	Excellent
R3	2	0	70.1	28.8	75.0	0.735	Good

The sampling sites located in salmonid (Group 1) and salmonid/cyprinid transition (Group 2) zones of Natural Park of Montesinho displayed an excellent condition for the preservation not only of autochthnous fish but also for other organisms, considering invertebrates, reptiles, amphibians and riparian birds. In fact, the environmental conditions and the low impact of human activities contributed, all over the time, to the maintenance of the excellent stat of health of these aquatic and riparian ecosystems.

Table 3. 9. Classification of sampling sites of Group 1 (Salmonid - Northern Region of Portugal), based on the fish composition and abundance (Spring 2017)

Sampli ng site	Group	% Exotic Individuals	% Intolerant Individuals	% Omnivorous Individuals	F-IBIP	Quality Class
B1	1	0	100	0	1	Excellent
B2	1	0	100	0	1	Excellent
A1	1	0	86.3	13.7	0.891	Excellent
C1	1	0	100	0	1	Excellent

3.3.5. Population Parameters of Brown Trout Populations

3.3.5.1. Relationship between Length and Biomass

Based on the 209 individuals of Brown trout, captured in the 6 distinct watercourses of High Tua basin (n_A= 44; n_{R1}= 21; n_{M1}= 54; n_{T1}= 41 n_{B1}= 32; n_{C1}=17), is it possible present (Figure 3.10) the relationship between Total Length and Biomass in each sampling site.

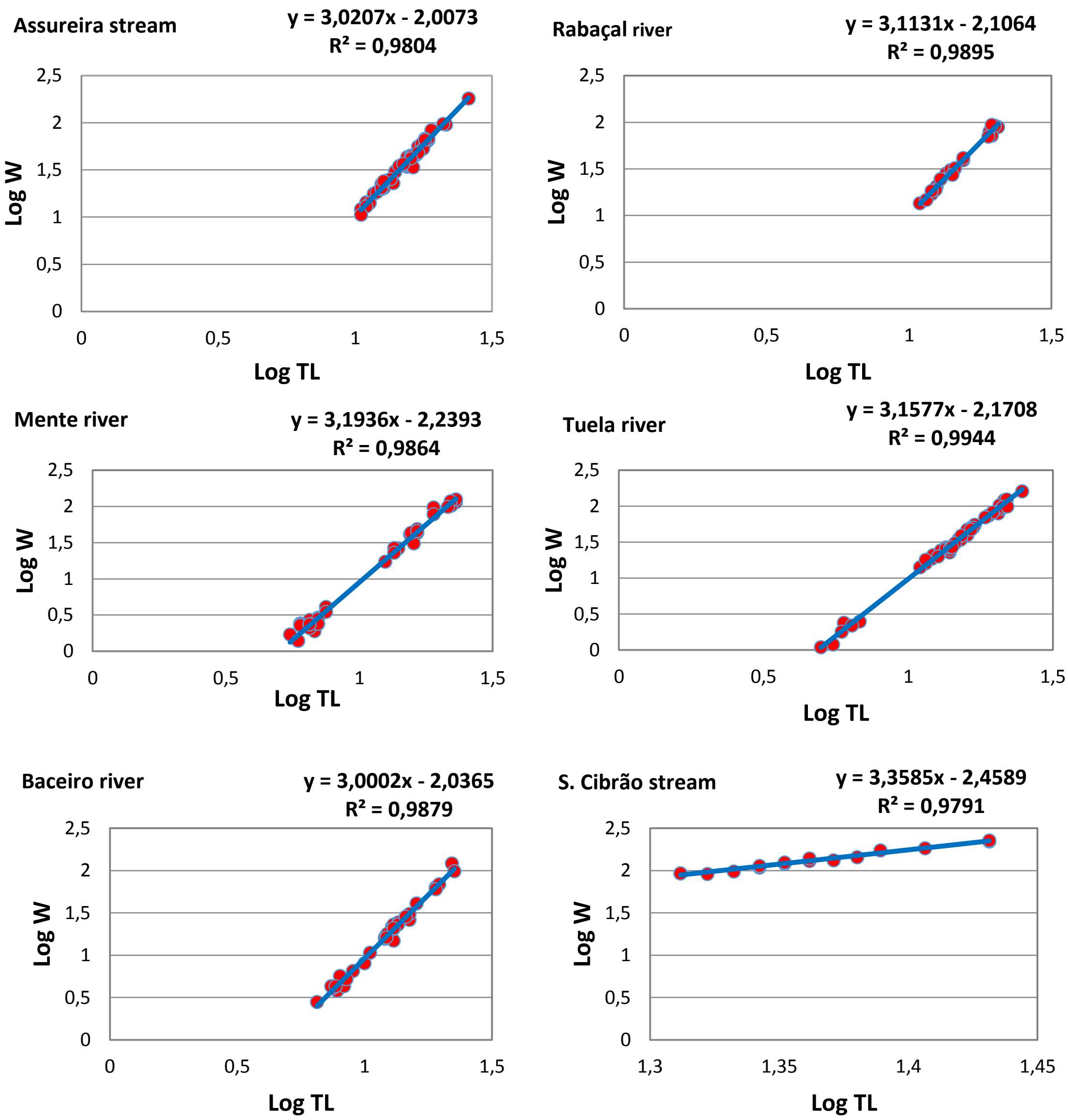


Figure 3. 10. Relationship between Total Length (TL) and Biomass (W) for the individuals of Brown trout captured in Assureira, Rabaçal, Mente, Baceiro, Tuela, and S. Cibrão watercourses of High Tua basin (Spring 2017).

Fish growth in most of the sampling sites (Baceiro, Tuela and Mente rivers) is almost isometric (b = 3), or positive allometric (b > 3) that means that fish are growing, more or less, at same rate in terms of size and biomass. However, in S. Cibrão stream, it was detected a higher coefficient of allometry (b = 3.36). This small population of Brown trout is composed by domestic animals introduced in the previous year in the stream. In this case, stocked Brown trout showed an unusual adaptation and grow up ability in wild conditions. However, no annual recruitment was detected in the stream and the high angling pressure will, apparently, exctint the population in a near future.

3.3.5.2. Physical Condition Factor (K)

Regarding to the Condition Factor K, significant differences were observed between the trout populations captured in the 6 sampling sites of different watercourses in Tua River basin (H (5, n= 208) = 48.342; p <0,05). Analyzing the Figure 3.11, it is verified that the River Rabaçal and S. Cibrão stream were the sampling sites with higher mean Condition Factor (K > 1.0), corresponding to individuals with excellent condition. The lowest value was determined in the Mente river, where the mean Condition Factor (K = 0.9). These results suggested that in the Mente River the biogenic capacity of the aquatic ecosystem can limit the presence of *Salmo trutta*.

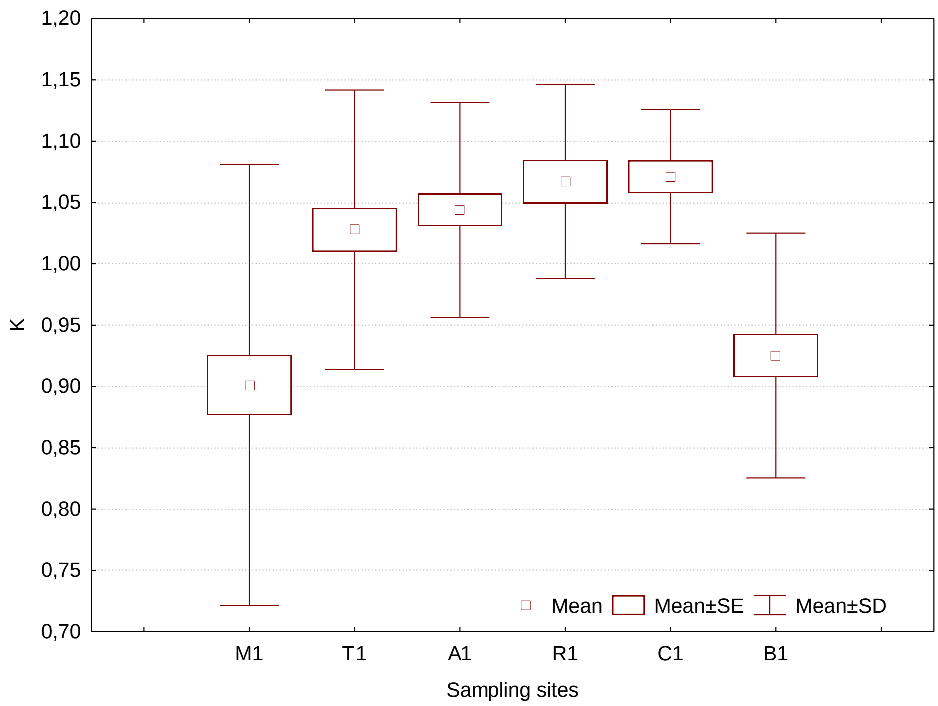


Figure 3. 11. Physical condition Factor K calculated for 6 watercourses of the Tua Basin.

3.3.6. Use of Resources: Feeding Strategies

The diet of Brown trout is composed manly of invertebrates, namely aquatic and terrestrial arthropods, and also by mollusks, crustaceans, annelids and even fish and sporadically other vertebrates (amphibia) as it can be observed for each sampled river of High Tua (Figure 3.12)

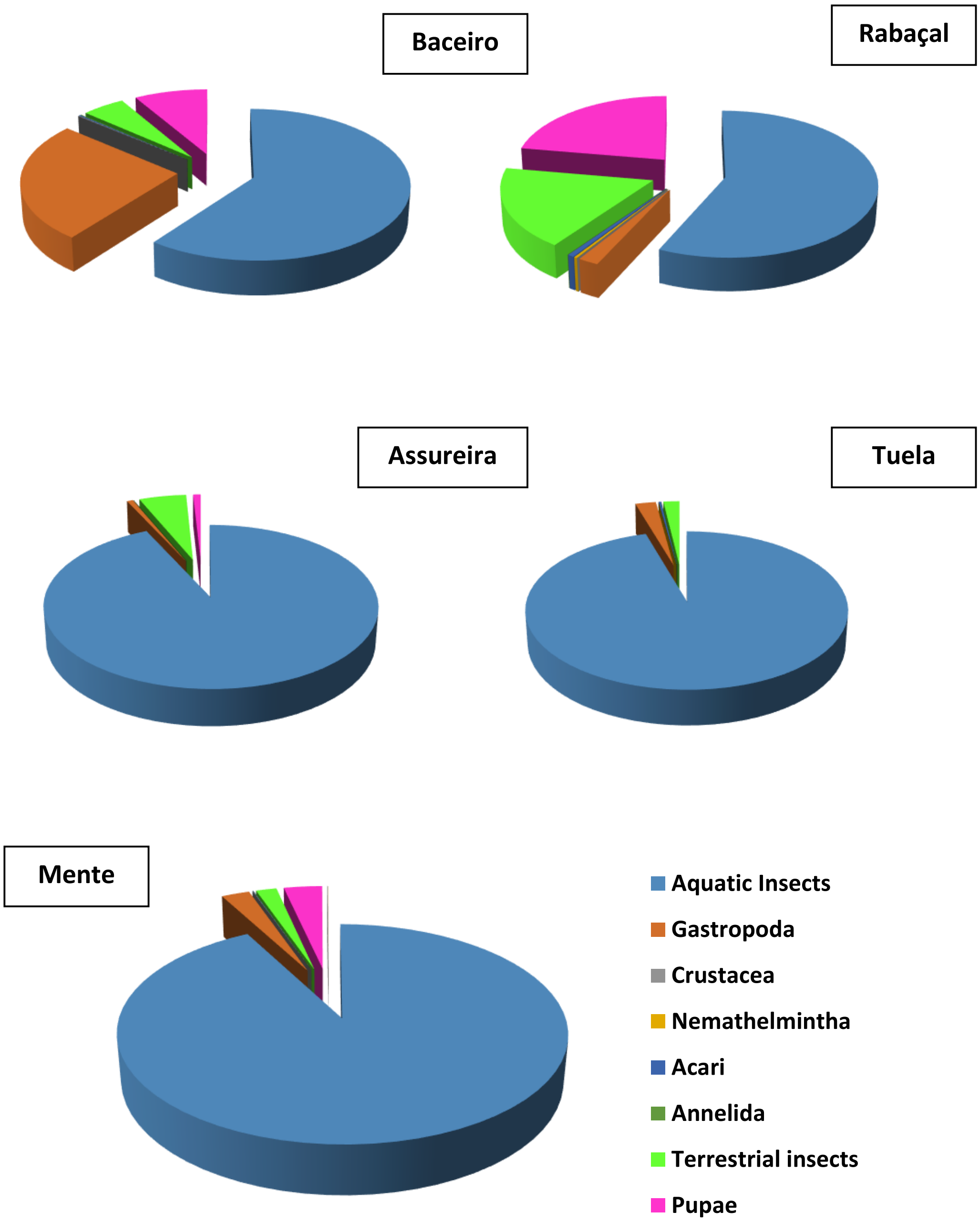


Figure 3. 12. Relative abundance (%) of prey items of Brown trout for 5 watercourses of High Tua (Spring 2017)

3.3.6.1. Ontogenetic variation

The ontogenetic variation is other approach to verify if there are differences in the feeding among size classes/age within the same trout population (Figure 3.13 to 3.17).

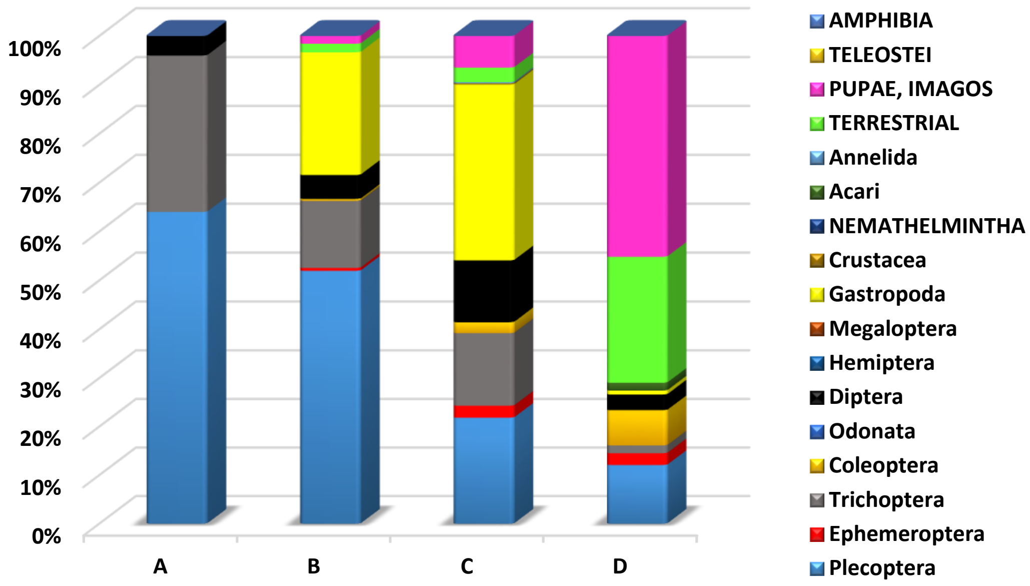


Figure 3. 13. Relative abundance (%) of the prey items of Brown trout for sampling site B2, Baceiro River, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Baceiro stream, the larger the trout size the higher the consumption of terrestrial insects and pupae and the lower the consumption of Plecoptera and Trichoptera taxa.

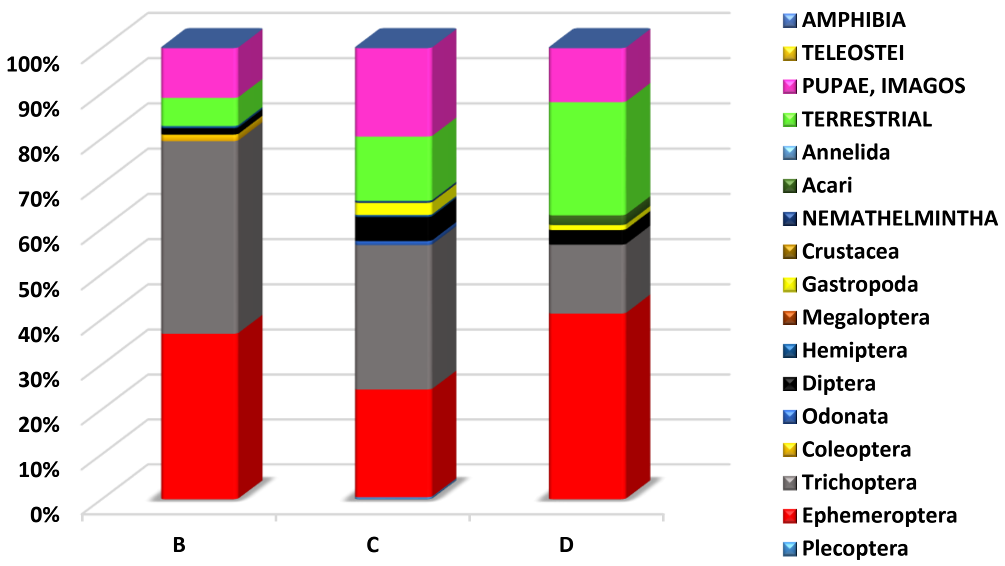


Figure 3. 14. Relative abundance (%) of the prey items of Brown trout for sampling site R1, Rabaçal River, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Rabaçal river, the larger the size the higher the consumption of terrestrial insects and the lower the consumption of Trichoptera. Plecoptera taxa is absent in the diet.

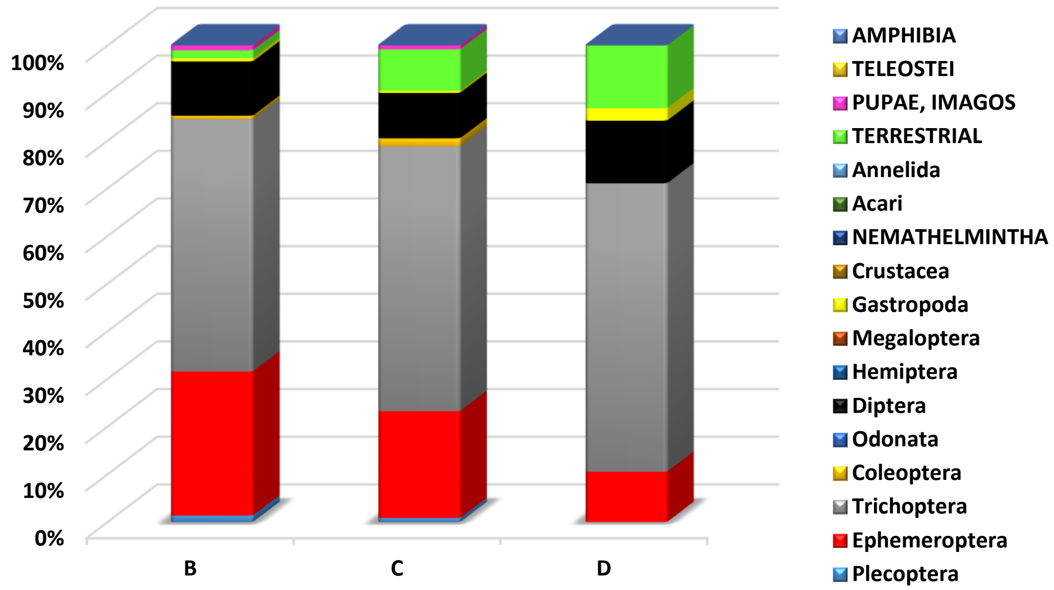


Figure 3. 15. Relative abundance (%) of the prey items of Brown trout for sampling site A1, Assureira stream, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Assureira stream, the larger the size the higher the consumption of terrestrial insects and the lower the consumption of Ephemeroptera preys.

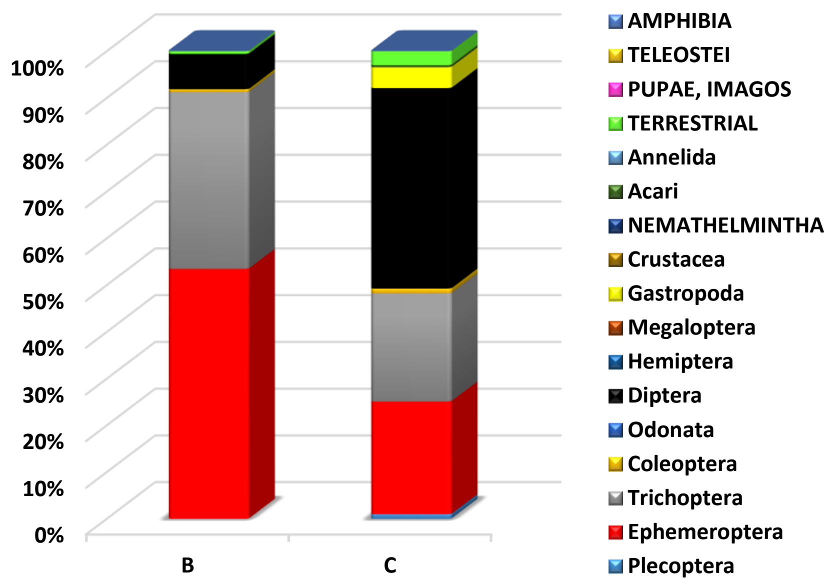


Figure 3. 16. Relative abundance (%) of the prey items of Brown trout for sampling site T1, Tuela River, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Tuela river, EPT are taxa used as main food item by trout, subadults feed also on terrestrial insects.

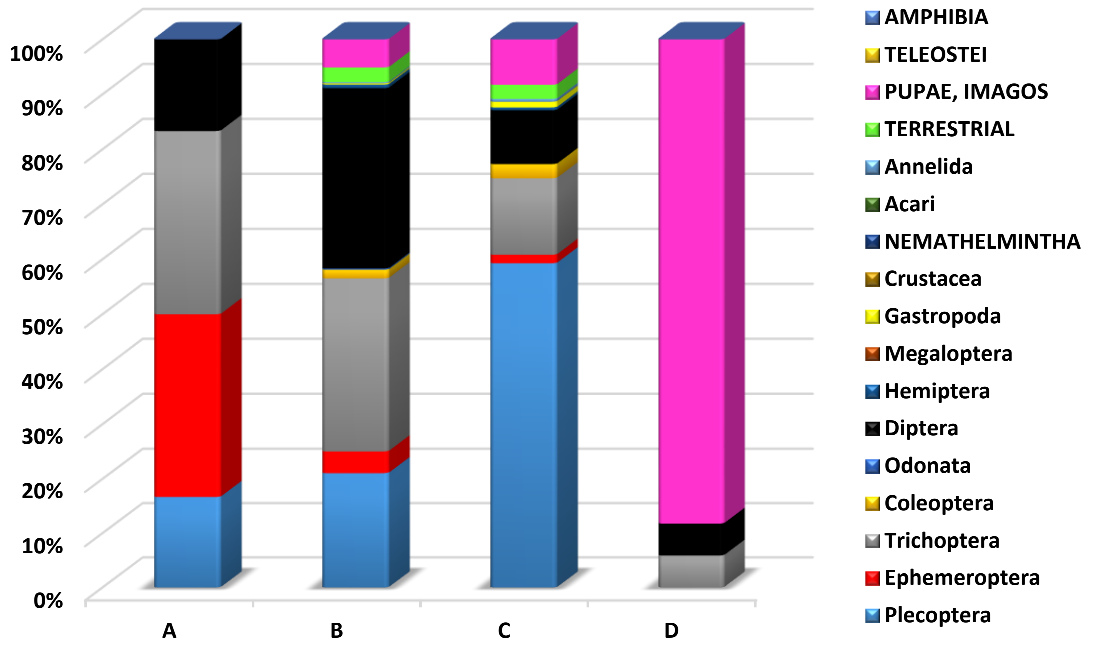


Figure 3. 17. Relative abundance (%) of the prey items of Brown trout for sampling site M1, Mente River, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Mente river, adults tend to feed mainly on pupae, while smaller trout prefer EPT taxa.

3.3.6.2. Ivlev's Electivity Index

The Ivlev's Electivity Index was employed in order to study prey selection which is an important part of fish feeding ecology. (Figure 3.18 to 3.23). The possible values of this index range from –1 to +1, with negative values indicating rejection or inaccessibility of the prey, zero indicating random feeding, and positive values indicating active selection (Sánchez-Hernández et al., 2011).

Ivlev's index shows a preference for Plecoptera taxon in YOY, juveniles and subadults decreasing with age (or size).Terristrial insects are captured by adults and subadults but also by juveniles that succed to reach the surface of the water. Pupae is captured in the column of the river by adults and subadults and sometimes by juveniles.

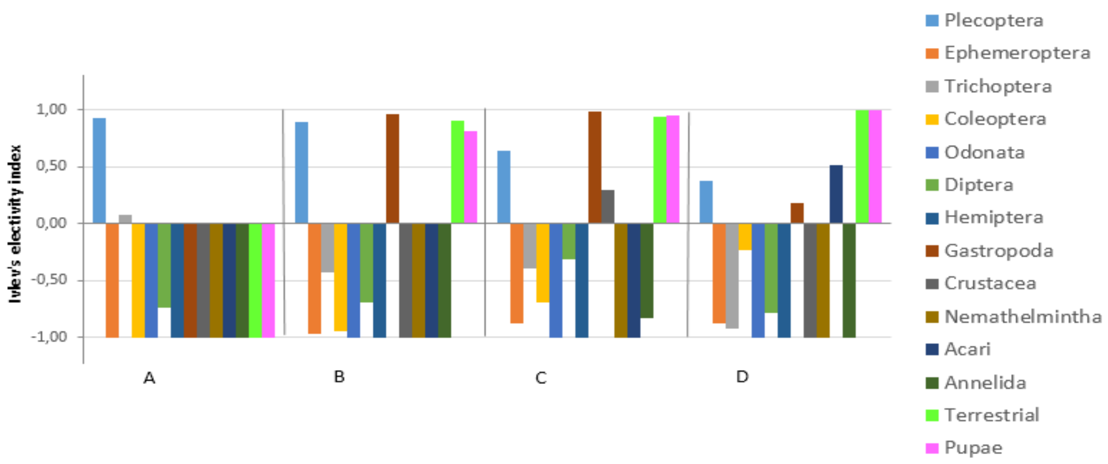


Figure 3. 18. Ivlev's electivity index for Brown trout in Baceiro river- B2, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

A relevant preference for Gastropoda is shown in juveniles and subadults. The other taxa are avoided by population trout. The selectivity of food items is dependent on available ressources in site B2, Baceiro river.

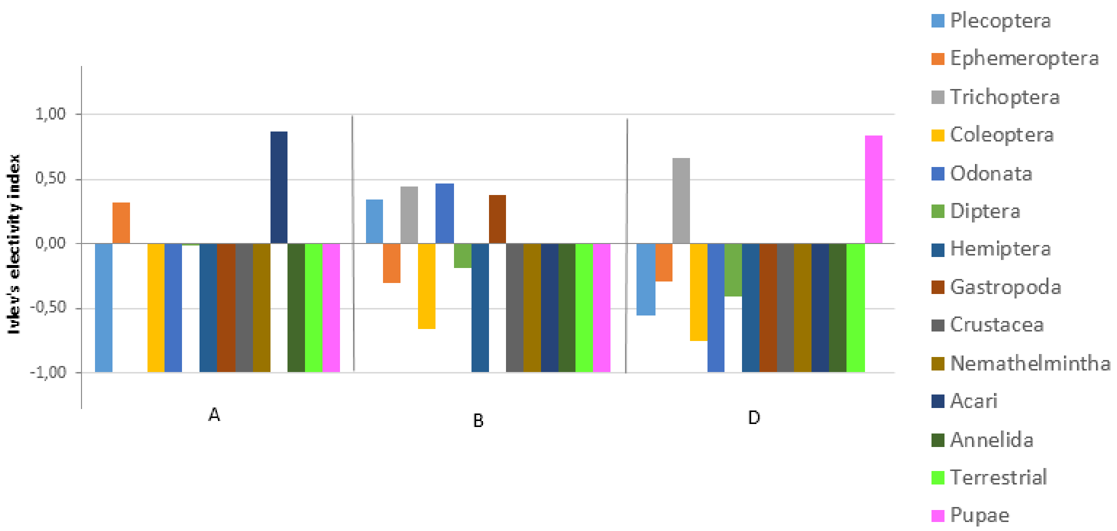


Figure 3. 19. Ivlev's electivity index for Brown trout in Baceiro river- B1, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In B1, Baceiro river, different results are obtained: YOY tend to prefer Hemiptera taxon. Juveniles show more or less an indiference (Ivlev's index between -0.5 and 0.5) for some taxa: EPT, Coleoptera, Odonata, Gastropoda and Diptera and an avoidance of the remaining taxa. Adults prefer pupae and then Trichoptera.

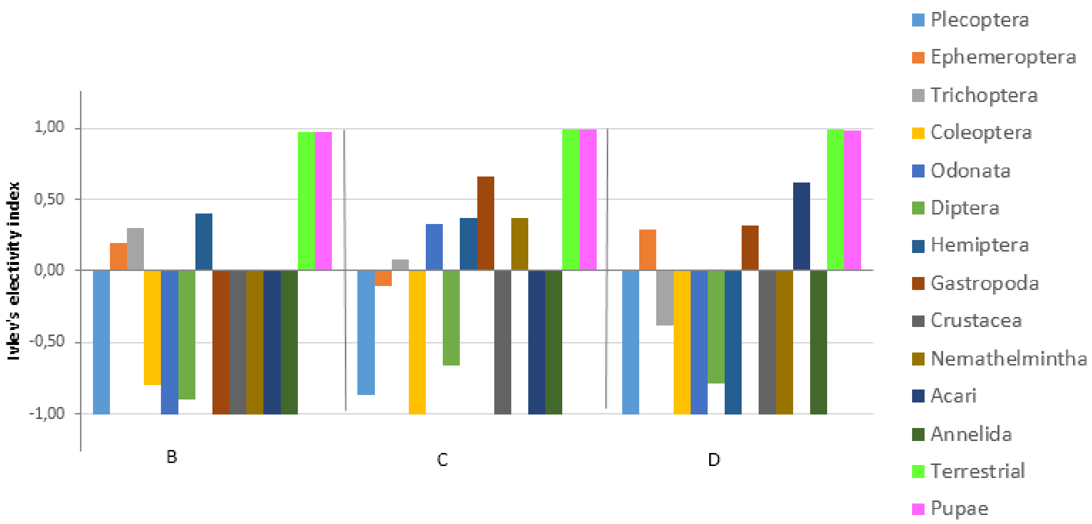


Figure 3. 20. Ivlev's electivity index for Brown trout in Rabaçal river- R1, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In R1, Rabaçal river, each of juveniles, subadults and adults show preference for pupae and terrestrial adults.

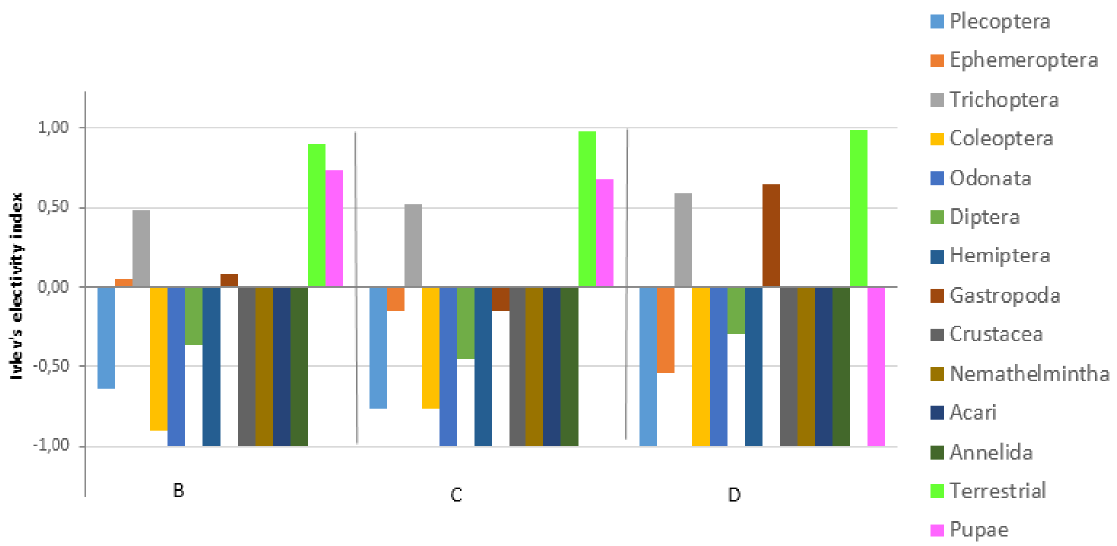


Figure 3. 21. Ivlev's electivity index of Brown trout for sampling site Ass1, Assureira stream, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Tuela headwater river, adults prefer terrestrial insects then Gastropoda taxon, while juveniles show a small preference for Ephemeroptera and bigger preference for terrestrial insects.

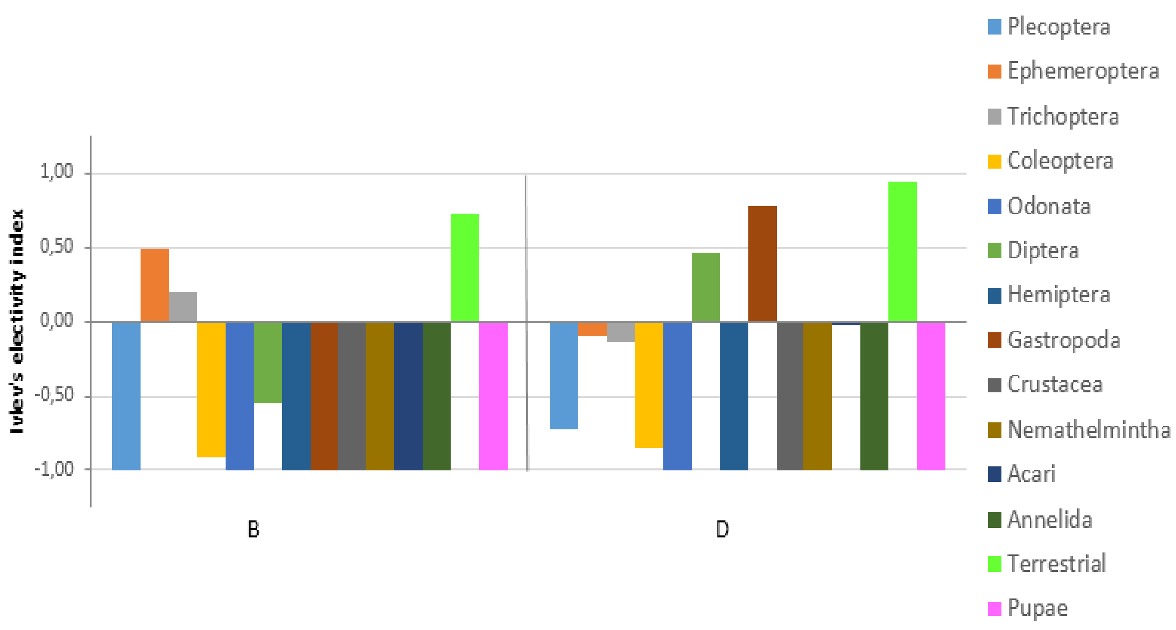


Figure 3. 22. Ivlev's electivity index of Brown trout for sampling site Tue1, Tuela river, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

In Men1, Mente River, pupae is preferred by adults, subadults and juveniles. Terrestrial insects are preferred by juveniles and subadults. The preference of Plecoptera increases with age (with size) until adult stage where plecoptera is avoided.

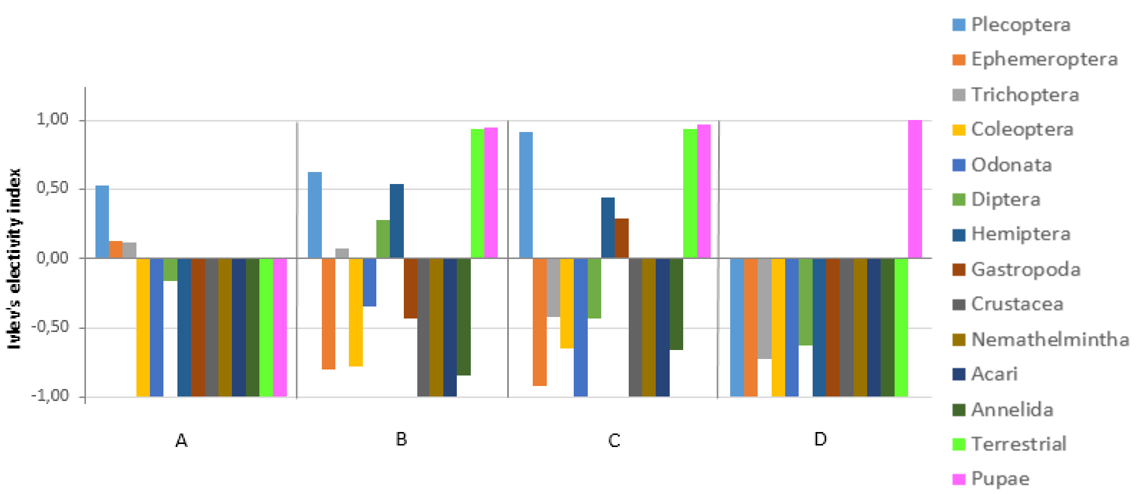


Figure 3. 23. Ivlev's electivity index of Brown trout for sampling site Men1, Mente river, considering 4 size classes (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) (Spring 2017)

3.3.6.3. Overlap Schoener Index

The diet overlap between the different size classes of Brown trout was determined by the Shoener percent overlap index (S) (Table 3.10). Diet overlap between two size classes is highlithed when the value of the index is over 60%.

Table 3. 10. Diet overlap (Schoener index) between Brown trout size class (A< 10.0 cm; B- 10.0-14.9 cm; C- 15.0-20 cm and D> 20.0 cm) for sampling sites: (B1) Baceiro river, (T1) Tuela river, (M1) Mente river, (A1) Assureira stream and (R1) Rabaçal river (Spring 2017). Significant values (S>60%) identified with green color.

River	Baceiro river	Tuela river	Mente river	Assureira stream	Rabaçal river
Site	**B2**	**T1**	**M1**	**A1**	**R1**
A *vs* B	69,7	-	68,8	-	-
A *vs* C	40,7	-	41,9	-	-
A *vs* D	16,9	-	11,8	-	-
B *vs* C	69,9	56,0	56,5	89,4	75,1
B *vs* D	22,1	-	16,9	77,3	70,7
C *vs* D	31,8	-	20,0	84,8	69,7

- Schoener's index showed a tendency to a diet overlap (S> 60%) between successive size classes for Baceiro (B2), Rabaçal (R1) and Assureira (A1) sampling sites.
- In Mente (M1) site, the diet overlap is more relevant between YOY and juveniles.
- In Assureira (A1) and Rabaçal (R1) sites, the diet overlap is highlited mutually between juveniles, subadults and adults.

3.3.7. Use of Resources: Habitat

Habitat used by Brown trout in River Baceiro, during Spring 2017, is presented and discussed for the following variables:

a) Cover

The relative frequency for cover variable and preference curves developed for Brown trout, can be observed in Figures 3.24 and 3.25.

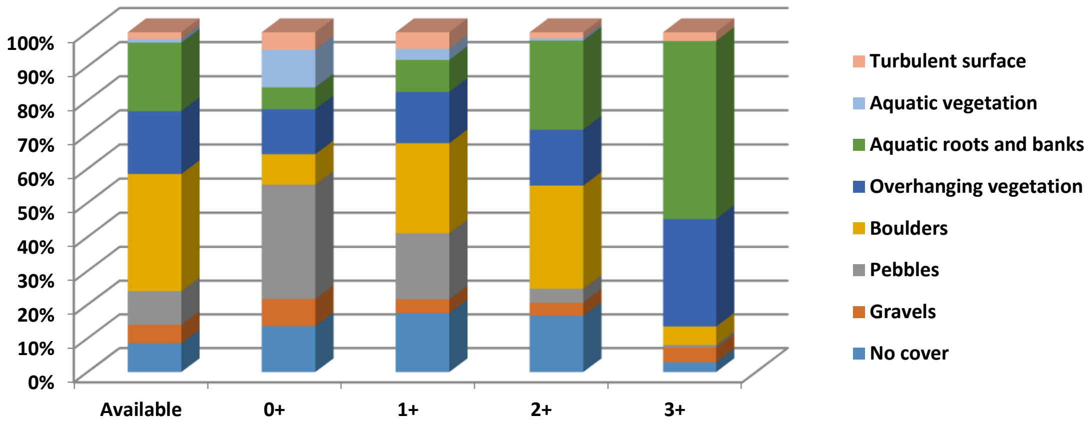


Figure 3. 24. Cover: available and used microhabitat for Brown Trout (Spring 2017)

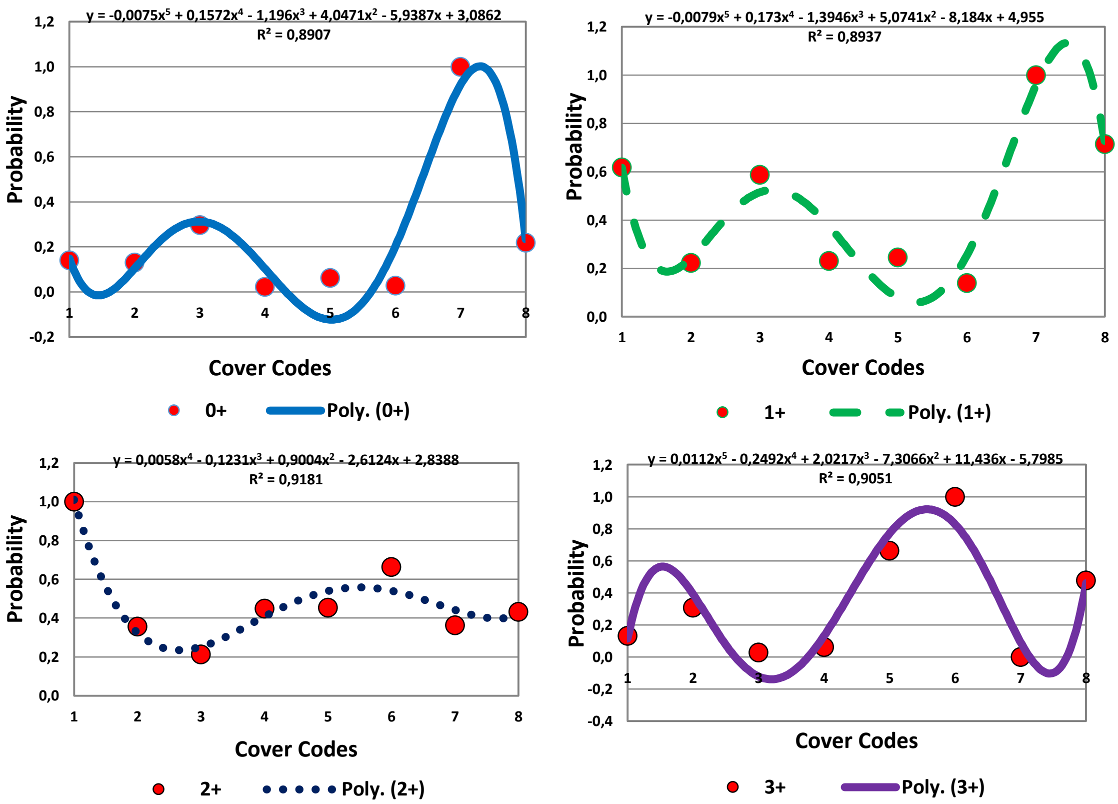


Figure 3. 25. Cover: Preference curves for Brown trout (0+, 1+, 2+, ≥3+) (Spring 2017)

It was detected notable differences in trout behaviour, namely between alevins and juveniles and adults. In fact adult trout were detected in pool zones near undercut bank and boulders, while juveniles and alevins were found, mainly, in riffle zones.

b) Dominant Substrate

Relatively to the dominant substrate variable the available and used microhabitat and preference curves are showed in Figures 3.26 and 3.27.

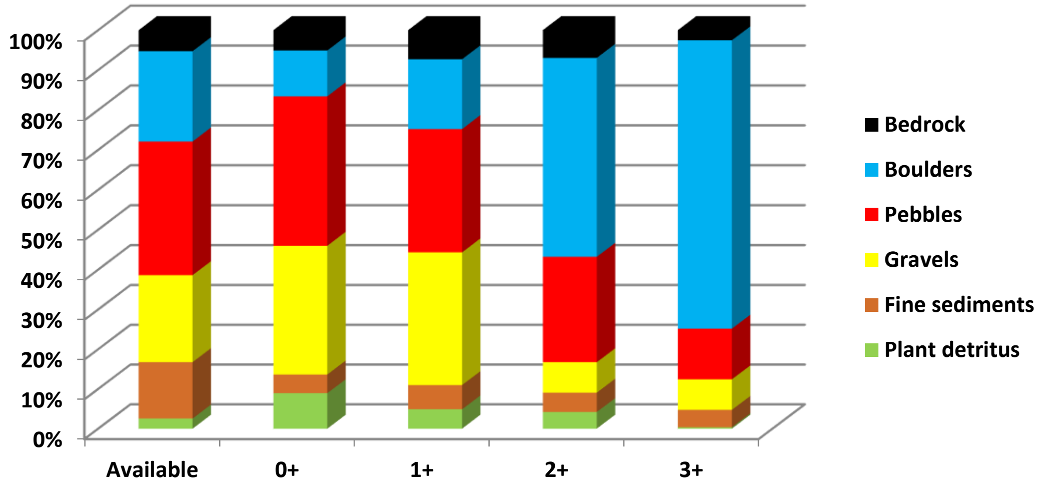


Figure 3. 26. Dominant substrate: Available and used habitat for Brown Trout (Spring 2017).

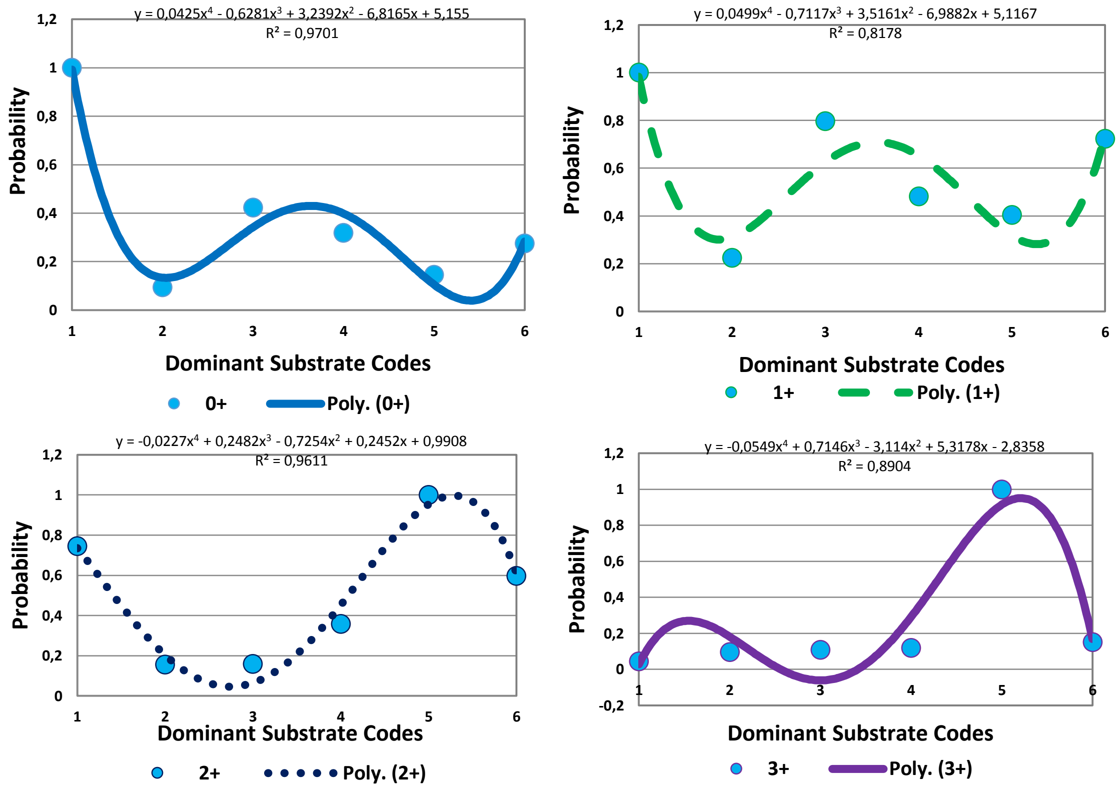


Figure 3. 27. Dominant Substrate: Preference curves for trout (0+, 1+, 2+, ≥3+) (Spring 2017).

The dominant substrate used by Brown trout showed marked differences, again, between YOY/juveniles and adults. Adult trout were mainly present near boulders, and YOY/juveniles were found in gravels and pebbles.

c) Total Depth

The total depth variable, considering the available and used microhabitat and preference curves are showed in Figures 3.28 and 3.29.

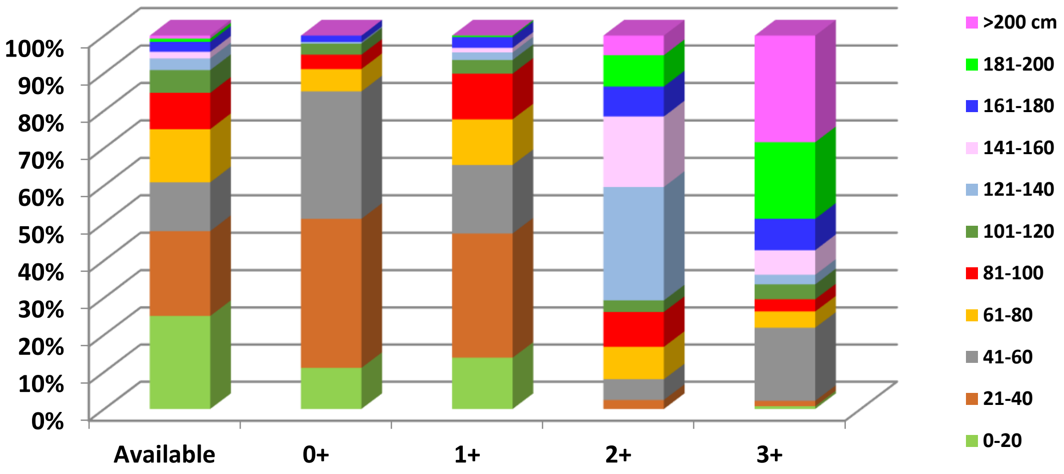


Figure 3. 28. Total Depth: Available and used habitat for Brown Trout (Spring 2017)

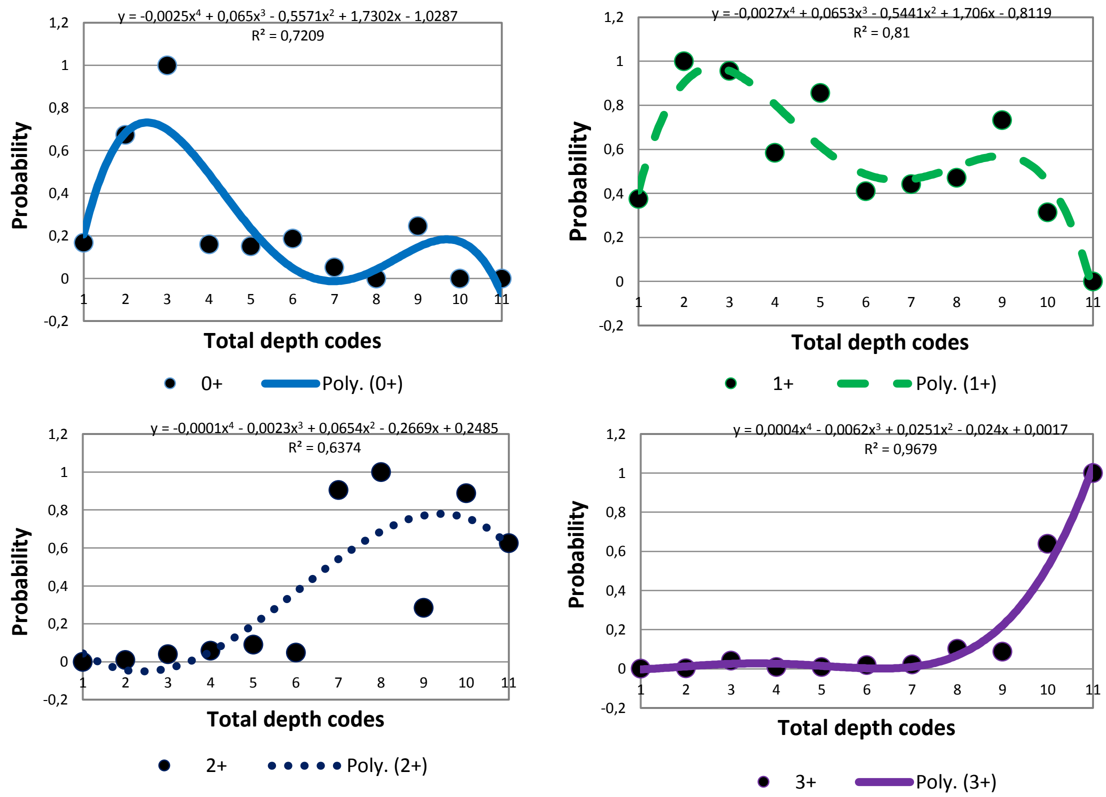


Figure 3. 29. Total Depth: Preference curves for trout (0+, 1+, 2+, ≥3+) (Spring 2017).

Adult Brown trout preferred pool zones where higher depth can be found. YOY and juveniles trout tend to be in riffle zones where the total depth is very low.

c) Water current

For the water current variable, the available and used microhabitat and preference curves are displayed in Figures 3.30 and 3.31.

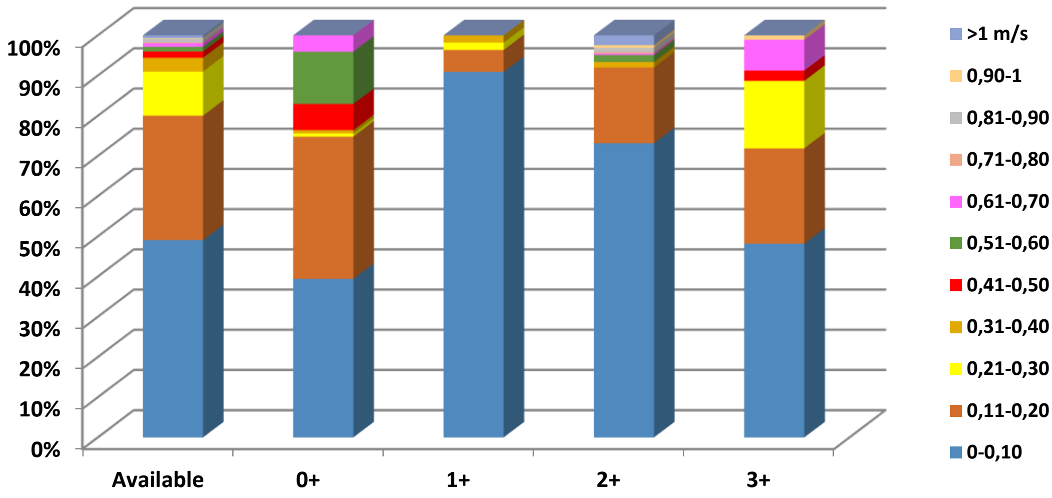


Figure 3. 30. Water current: Available and used habitat for Brown Trout (Spring 2017)

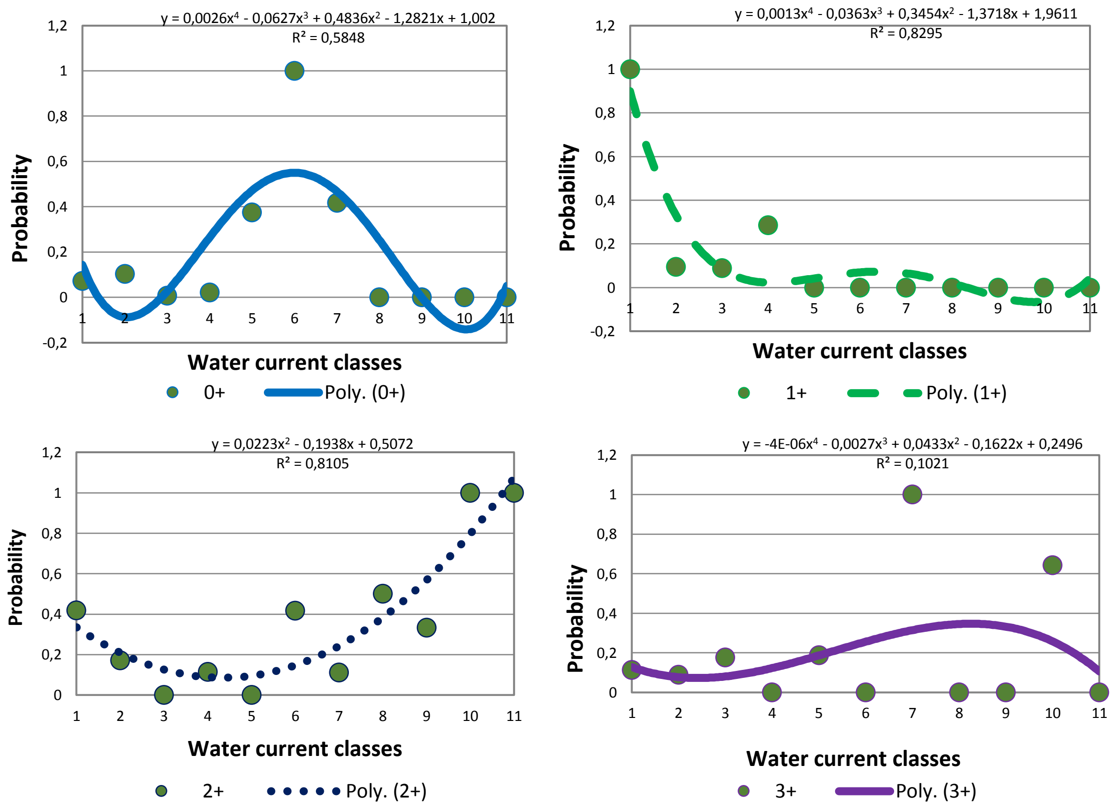


Figure 3. 31. Water current: Preference curves for trout (0+, 1+, 2+, ≥3+) (Spring 2017)

A distinct pattern was found for water current, take into consideration the ontogenic variation. Higher water currents seemed to be preferred by adult trout, probably as a result of their feed strategy, since most of them find their preys near riffle zones. However YOY and Juvenile trout showed differences, based on the obtained results.

3.4. DISCUSSION

The population parameters determined for Brown trout in this study, during 2017, in Northeastern rivers of Portugal are similar to other values found in Iberian salmonid rivers (Martinho, 2008; Sanchez-Hernandez et al., 2012; Ribeiro, 2014; Santos, 2014). In fact, the growth (i.e isometric coefficient, b=3, for most sampling sites), and condition factor (i.e. $0.8 < K < 1.1$) found revealed environmental conditions for the presence of well structured Brown trout populations. The recent F-IBIP index confirmed a good ecological integrity, based on the fish communities. This tendency is also corroborated by other metrics (see chapter 2) highlighting the potential of this headwater streams not only for conservation but also for exploitation purposes. Furthermore the ecological guilds showed the dominance of an intolerant and reophilic fish community to environmental disturbance, where Brown trout, tipically invertivore and litophilic behaviour, can dominate in the upper zones. However, native trout populations can develop different feeding strategies, in order to adapt to available biotic (e.g. fish density, amount of food) and abiotic (e.g. channel and riparian habitats typologies) characteristics. The scarcity of available resources at certain critical periods (e.g. first months after emergence of alevins) may have adverse effects (i.e. bottlenecks) in a given fish population, especially when the carrying capacity of the aquatic system is reached (Armstrong et al., 2003). So, the correct management of Brown trout populations can be done if the bottlenecks are known. For these reasons the knowledge of the available resources used by Brown trout and, in particular, of their feeding strategies and the main habitat use for different life cycle phases is vital information for the managers**.** The diet composition of Brown trout in this study showed a wide range os invertebrate consumption in the 6 watercourses (Assureira, Cibrão, Mente, Rabaçal, Tuela and Baceiro) and is according with the results of other studies previously carried out (García de Jalón & Barceló, 1987; Kara & Alp, 2005). Relatively to habitat use, the preference curves showed different paterns for the microhabitat variables considered (cover, dominant substrate, water current and total depth) more pronounced between alevins and juvenile trouts and subadult and aduls individuals. These patterns were also observed in the same rivers of the region by other authors (Teixeira & Cortes, 2006).

3.5. FINAL CONSIDERATIONS

Management of Brown trout

This study aimed to contribute to the knowledge of the bioecology of Brown trout in rivers and streams of the Northeastern Portugal. In this sense, taking into account the different studies carried out in the past (*e.g.* Teixeira, 2006, Teixeira & Cortes, 2006, 2007, Claro, 2010, Miranda, 2012, Patrício, 2013, Santos, 2014), it is possible to define a set of considerations and future strategic guidelines for the adequate management of Brown trout in salmonid and salmonid/cyprinid transition zones in the Upper Tua basin. Thus, considering that:

- The Northern rivers of Portugal have, naturally, a low fish productivity, especially headwater streams, located in schist/granite watersheds (Teixeira, 2006);
- Trout populations in Southern Europe have high genetic diversity, justifying conservation units that may include the sub-basin level (Antunes et al., 1999);
- The biotic integrity status in lotic ecosystems of this region is high, especially inside the Montesinho Natural Park (Claro, 2010; Patrício, 2013, Santos, 2014);
- The main negative impacts identified resulted from the presence of small hydroelectric powerplants, where it was identified a local decrease in biological quality and a low efficiency of constructed fish passages (Santo, 2005);
- Some recent disturbing signs of degradation of the riparian gallery are occurring, namely resulting from the death of alder trees (*Alnus glutinosa*) that may influence the refuge and thermal regime of the waters, protection against the erosion and functioning of the lotic ecosystems;
- Previous management actions, mostly focused on restocking of Brown trout with domestic animals, have resulted in low success with a limited temporal and spatial effects (Teixeira, 2006; Teixeira & Cortes, 2006, 2007);
- Actual fishery regulations in sudy area are not adjusted to the existing knowledge of the bioecology of the species and the functioning of aquatic ecosystems;
- Training, information and surveillance are not, at present, sufficient for the correct preservation of the natural values of the NE rivers of Portugal.

Strategic lines for the management of trout populations

Based on the present study and taking into account the previous works, we are of the opinion that the main strategic guidelines for the management of trout populations in the Upper Tua basin should include:

- An integrated and global vision, differentiated by each watercourse in terms of fishery management. Free-fishing and fishing limitations should receive scientific and technical support in order to promote the correct exploitation of resources avoiding the distubance of the natural sustainability and biogenic capacity of each ecosystem.The new legislative framework which will regulate the inland fisheries and the development of Management Plans, especially in the Montesinho Natural Park area are essential in a near future to define the best options;
- A specific conservation strategy must be implemented in rivers where coexist sympatric populations of both species, *Salmo trutta* and the freshwater pearl mussel *Margaritifera margaritifera*, a species listed as CR "critically endandgered" for Portugal. In fact, since Brown trout is identified as the only host fish species of the *M. margaritifera* in the PNM, and knowing that the populations of this bivalve have the highest density and viability in the Rabaçal and Tuela rivers, it is fundamental to guarantee their preservation and to act proactively eliminating possible threats;
- Appropriate management tools of long-term application. In fact, stocking was the most commonly used tool, until nowadays, in the management of Brown trout populations. The studies carried out, including in the region, advice the use of stocked trout, only surgically performed and always after rigorously evaluation of the potential ecological and socioeconomic impacts resulting from these actions;
- Training and technical-scientific support that allows a sustainable and efficient management by managers, with an annual redefinition of the stock of trout with dimensions available for catch through recreational and / or sport fishing. At the same time, measures such as improvement of fish habitat should increasingly be implemented whenever bottlenecks are identified in the structure of Brown trout populations. The strengthening of the riparian gallery, the implementation of good agricultural and forestry practices, the efficiency of the mini-ETARs disseminated

by the rural areas seem to be adequate measures for the preservation of the species and the ecosystems;

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Chapter 4

CONCLUSIONS AND GENERAL CONSIDERATIONS

The present study allowed the assessment of salmonid streams in Tua river basin, NE Portugal, by analyzing the hydromorphological (channel and riparian habitats) and water quality of freshwater ecosystems during the spring/summer of 2017. Furthermore, it was studied the bioecology of *Salmo trutta L.* and other fish communities (*e.g.* Cyprinids and Cobitids). The sampling sites were selected along the different tributaries of low order of rivers and streams (Baceiro, Rabaçal, Tuela, Mente, Assureira and S. Cibrão) belonging to Tua river basin. It should be pointed out that most of these sampling sites are located inside a protected area *i.e.* Natural Park of Montesinho, hence the preservation of these natural conditions is required. The assessment of ecological status of these freshwater ecosystems was obtained using abiotic and biotic tools following the methodology required by the Water Framework Directive. The management of the target species, Brown trout, requires a deep knowledge not only of population parameters, habitat use and feeding strategies but also at ecosystem level in order to define the best management and legislation plans.

The main conclusions obtained from this work can be resumed as following:

- **Good to excellent water quality:** based on few parameters, it was observed low temperatures (from 9 to 13 °C), good oxygenation rate (> 8.0 mg O_2/L), low values for the electrical conductivity (EC25< 80 µS/cm), values of pH near to neutral (between 6.6 and 7.2), and low contents of dissolved solids (between 11 to 37 mg/L).
- **Good hydromorphological quality of the rivers/streams**: Through the hydromorphological assessment (QBR and GQC indices) it was observed that most of sampling sites showed good quality in terms of channel and riparian habitat. Different results in terms of habitat quality were detected in downstream zones which are related especially to human disturbances.
- **Good biological quality, based on macroinvertebrate communities:** Using the classification of unimetric (IBMWP) and multimetric ($IPtI_N$) indexes, both indicated good to excellent biological quality for most of sampling sites. Other

metrics obtained by Amiib @ software (e.g. % EPT, number of taxa, Shannon diversity and Eveness) confirm this results.

- **Fish fauna composed mostly by native fish species:** belonging to 3 different families: 1) Salmonidae: Brown Trout (*Salmo trutta*); 2) Cyprinidae: Calandino Roach (*Squalius alburnoides*), Northern Iberian Chub (*Squalius carolitertii*), Douro Nase (*Pseudochondrostoma duriense*), Common Iberian barbel (*Luciobarbus bocagei*), and 3) Cobitidae: Spined Loach of Northern Portugal (*Cobitis calderoni*). Using multivariate analyses was found an effective separation between the sampling sites of Salmonid Group and Salmonid/Cyprinid Group (NMDS ordination). Salmonid Group Region is represented by headwater streams, with oligotrophic character, cold and very oxygenated waters. Salmonid/Cyprinid Group is represented by rivers with median dimension where Brown trut cohabit with endemic cyprinids and cobitids. The calculation of F-IBIP Fish-based Index of Biotic Integrity for Portuguese Wadeable Streams showed an excellent/good status for all sampling sites displaying an excellent condition for the preservation not only of autochthnous fish but also for other organisms, considering invertebrates, reptiles, amphibians and riparian birds.
- **Good growth and condition factor of Brown trout population:** Analysing trout population parameters, it is showed in general a good growth (isometric coefficient b=3) and a good physical condition factor (K> 1.0), with the exception of two cases:
    - In S. Cibrão stream, recently stocked trout showed a low adaptation to wild conditions.
    - In Mente river, ecological conditions are less suitable for Brown trout.
- **The importance of aquatic insects in the diet of Brown trout:** It was observed that Brown trout feed mainly on aquatic insects (over 60%), but also on other macroinvertabrates (mollusks, crustaceans, annelids and terrestrial arthropods) and even small fish. Significant differences were detected in trout diet among the sampling sites, these differences are related to the nature of sampling site (hydromorphological status and available ressources).

- **Ontogenetic diet variations in Brown trout population:** In general, the diet of YOY and juveniles is limited by their small size and their conditional accessibility to resources in order to avoid predation. Therefore their diet is basically composed by aquatic rheophilic taxa (e.g. Plecoptera) captured near the bottom of the river, whereas, adults tend to prefer preys captured in the column of the river (e.g. Pupae and nymphs) or in the surface (terrestrial insects). In addition to the avoidance of predation, this feeding strategy optimizes the use of resources which decreases the competition (e.g. Elliott, 1967; Hyndes et al., 1997; Amundsen et al., 2003; Oscoz et al., 2006).
- **Ontogenitic variation in the use of the microhabitat of Brown trout:** Habitat use allowed to confirm the social hierarchy of these animals, since the best refuges (boulders, undercut banks) are colonized by adult trout, while juveniles were found in microhabitats of lower quality in riffle zones, and with lower degree cover.

Salmo trutta is an emblematic fish species of Tua river basin, located in Natural Park of Montesinho, being an interesting population fish due to its socio-economic value (e.g. sport fishing) and ecological importance (e.g. Unique host fish of the endangered invertebrate species, *Margaritifera margaritifera).*

Basing on good results in terms of aquatic ecosystems' quality, it is pointed out that the ecological integrity of Upper Tua must be maintained. The maintenance of these conditions requires the implementation of regulations conditioning the fishing activity as sustained management compromising between exploitation (i.e. recreational and sport fishing) and conservation of biological resources (i.e. preservation of native species). In fact, several stockings of domestic strains of Brown trout were implemented in these northern rivers with low success and no contribution to the sustainability of local native populations (Teixeira, 2006). For these reasons the effort must be focused on the propmotion of better fish habitat conditions, when disturbance can be detected. However this tools must be applied in particular zones in order to improve, for instances the connectivity between up and downstream zones. Is the case of several small hydoreletric powerplants where fish passages are not in good conditions.

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