

Conservation and management of lotic ecosystems in Northeastern Portugal: the influence of abiotic and biotic factors

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ABSTRACT

Freshwater ecosystems are threatened by several pressures worldwide. Water pollution and eutrophication, the degradation and fragmentation of aquatic and riparian environments, overexploitation of resources, the dispersion of non-native species and more recently climate change have contributed to the loss of biodiversity and ecological integrity of rivers. For this reason, river conservation and management are essential to guarantee all associated ecosystem goods and services, this taking into account the abiotic and biotic factors. Which leads to the objectives of the present study which were: 1) to evaluate the impact of abiotic factors on the biological quality of a watercourse, the river Fervença belonging to the drainage basin of River Sabor (NE of Portugal), subject to anthropic pressures, and 2) to determine the influence of biotic factors, namely the complex relationship between fish and bivalves, in the ecological integrity of lotic systems. In the spring of 2020, abiotic (i.e. water quality and habitats) and biotic (i.e. macroinvertebrates and fish) characterization of 7 sampling sites were made, using the Water Framework Directive protocols. The results showed a marked decrease in the quality of water, habitats and biota in sampling sites located downstream of the city of Bragança, as a result of the input of contaminants from the urban environment, despite the presence of a wastewater treatment plant (WWTP). The degradation of abiotic conditions has led to the disappearance of species sensitive to pollution and to the loss of biodiversity, biotic relationships and ecological functions in the aquatic system. In this context, the relationship between a bivalve, the unionoid *Anodonta anatina*, and the fish fauna present in the river Sabor was studied. Four native Iberian species (*Luciobarbus bocagei*, *Squalius alburnoides*, *Squalius carolitertii* and *Pseudochondrostoma duriense*) and one non-native species (*Alburnus alburnus*) were tested in the laboratory. All fish species functioned as viable hosts for *Anodonta anatina* due to the wide distribution of this bivalve, although the higher rates of metamorphosis were found for a native species, *Squalius carolitertii*. For the conservation and management of NE Portugal's aquatic systems, measures for the rehabilitation of disturbed ecosystems and the conservation of priority habitats are essential, namely for threatened native species.

Keywords: rivers, ecological quality, aquatic fauna, biotic relationships

RESUMO

Os ecossistemas de água doce estão ameaçados por várias pressões à escala mundial. A poluição e eutroficação da água, a degradação e fragmentação de ambientes aquáticos e ribeirinhos, a sobreexploração de recursos, a dispersão de espécies não nativas e mais recentemente as alterações climáticas têm contribuído a perda de biodiversidade e integridade ecológica de rios. Por tal motivo, a conservação e gestão de rios é fundamental para garantir todos os bens e serviços ecossistémicos associados.

O presente estudo teve como objetivos: 1) avaliar o impacto de fatores abióticos na qualidade biológica de um curso de água, o rio Fervença (NE de Portugal), sujeito a pressões antrópicas, e 2) determinar a influência de fatores bióticos, nomeadamente da relação complexa entre peixes e bivalves, na integridade ecológica dos sistemas lóticos. Na primavera de 2020 foi feita a caracterização abiótica (i.e. qualidade da água e habitats) e biótica (i.e. macroinvertebrados e peixes) de 7 locais de amostragem, com recurso aos protocolos da Diretiva Quadro da Água. Os resultados evidenciaram uma diminuição acentuada da qualidade da água, dos habitats e do biota nos locais situados a jusante da cidade de Bragança, como resultado do input de contaminantes do meio urbano, apesar da presença duma estação de tratamento de águas residuais (ETAR). A degradação das condições abióticas levou ao desaparecimento de espécies sensíveis à poluição e à perda de biodiversidade, relações bióticas e funções ecológicas no sistema aquático. Neste âmbito, foi estudada a relação entre um bivalve, o unionídeo *Anodonta anatina*, e a fauna piscícola presente no rio Sabor. Foram testadas, em laboratório, quatro espécies nativas da Ibéria (*Luciobarbus bocagei*, *Squalius alburnoides*, *Squalius carolitertii* e *Pseudochondrostoma duriense*) e uma espécie não nativa (*Alburnus alburnus*). Todas as espécies piscícolas funcionaram como hospedeiros viáveis de *Anodonta anatina* em virtude da distribuição alargada deste bivalve, embora as taxas superiores de metamorfose tivessem sido encontradas para uma espécie nativa, o *Squalius carolitertii*. Para a conservação e gestão dos sistemas aquáticos do NE Portugal são essenciais medidas de reabilitação de ecossistemas perturbados e a conservação de habitats prioritários, nomeadamente para as espécies nativas fortemente ameaçadas.

Palavras-chave: rios, qualidade ecológica, fauna aquática, relações bióticas

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SYMBOLS

ANOSIM - Analysis of Similarities

EC - Electric Conductivity

EPT- Ephemeroptera, Plecoptera, Trichoptera

F-IBIP – Fish biotic integrity index

FFG - Functional Feeding Groups

GQC- Channel Quality Index

ASPT - Average Score by Taxon

IASPT - Iberian Average Score by Taxon

IBMWP - Iberian Biological Monitoring Working Party

INAG - National Institute of Water

IPT_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

TDS- Total Dissolved Solids

WFD – Water Framework Directive in Portugal

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Chapitre 1

GENERAL INTRODUCTION

Every living thing on Earth needs water to survive. Many species including our own need a particular type of water which represent less than 3% of our planet water: Freshwater. At these days, the world's demand for freshwater is high, though there is a limited supply. Additionally, freshwater ecosystems contain an exceptional biodiversity and provide fundamental services but are highly threatened by human activities (VÖRÖSMARTY et al., 2010; SABO et al., 2010). Freshwater environments are among the most threatened ecosystems worldwide (DARWALL et al., 2011) and species extinction rates far exceed their marine or terrestrial counterparts (ABELL et al., 2017). Surface waters in a river basin are usually submitted to pressures and changes such as water pollution and eutrophication, habitat degradation and fragmentation, overfishing and introduction of invasive alien species and more recently by climate change (RICCIARDI & RASMUSSEN, 1999; ALLAN et al., 2005; POFF et al., 2007; WOODWARD et al., 2010; OLDEN et al. 2010; CARPENTER et al., 2011). The degradation of aquatic ecosystems can become dangerous for public health and for aquatic life. For these reasons, there is a need to establish methodologies for systematic data monitoring, for the characterization of surface water quality, hydromorphological and biota elements. In Europe, the protection of water resources and aquatic ecosystems lead to the creation of the Water Framework Directive (WFD), with the objective of reduce pressures and achieve the good ecological status in all countries. Plants, fishes and invertebrates are part of the biological elements that, together with the hydromorphological and physicochemical elements play a relevant role to the assessment of ecological status (DIRECTIVE 2000/60/EC).

Among freshwater fauna, the invertebrates constitute the majority of abundant species in these aquatic ecosystems and are responsible for important ecosystem functions and services. For example, freshwater mussels (Bivalvia: Unionoida), are organisms that have critical trophic and non-trophic functional roles in aquatic environment (STRAYER et al., 1994; VAUGHN et al., 2004; ALLEN & VAUGHN, 2011), such as cycling of nutrients, matter and energy transfer from the water column to the bottom

(that may have strong effects on primary and secondary production, biogeochemical cycles, sedimentation rates, and water clarity (STRAYER et al., 1999)), water purification, bioturbation, among others (VAUGHN & HAKENKAMP, 2001; SPOONER et al., 2013; LOPES-LIMA et al., 2017; VAUGHN, 2018). Furthermore, these bivalves are considered as ecosystem engineers (VAUGHN et al., 2008) contributing for the creation of habitat for other species (HAAG & WILLIAMS, 2014), the removal of large volumes of seston (suspended matter; VANDEN BYLLAARDT & ACKERMAN, 2014), and the modification of bottom habitats, and the enrichment of sediments (HOWARD & CUFFEY, 2006). These are simple functions developed by a particular faunistic group that highlight the role of freshwater mussels in the delivery of ecosystem services (VAUGHN, 2017). However, this faunal group is also among the world's most imperiled taxa (HAAG & WILLIAMS, 2014; LOPES-LIMA et al., 2014), and the most critically threatened groups of animals world-wide (LYDEARD et al., 2004; STRAYER et al., 2004) from a conservation point of view. Like most others in freshwater ecosystems, they have been declining dramatically over the last decades. These declines are mainly caused by habitat loss and fragmentation (e.g. by dams and other type of physical structures), pollution and deterioration of water quality (e.g. from increased fine sediment deposition, excessive nutrient input, heavy metal accumulation, herbicides and fungicides used in agriculture), overexploitation (including exploitation of fish hosts), introduction of invasive alien species (IAS), and climate change (more details available in the review of LOPES-LIMA, SOUSA, GEIST et al., 2017).

With a complex life cycle, freshwater mussels have a short-term larval stage (glochidium) that is obligatory parasitic on the gills or fins of fishes (KAT, 1984). The glochidia of most species can develop successfully into juvenile mussels only on a limited number of host fish species, which influence the reproductive success and dispersal abilities of the mussels (JANSEN, BAUER & ZAHNERMEIKE, 2001; BARNHART, HAAG & ROSTON, 2008; STRAYER, 2008). This trait makes them particularly threatened by invasive alien species (DOUDA et al., 2013) since changes on the host fish population may lead to the decline or co-extinction of the dependent species (MOIR et al., 2010). Several studies documented possible host limitations of specialized freshwater mussels following changes in fish host community composition (STRAYER, 2008). Given that native mussels seem to depend on specific, and usually native fish host species to

complete their life cycle, changes in the fish fauna can also have deep implications on the mussel populations (DOUDA et al., 2013; MODESTO et al., 2018).

Numerous studies have documented the direct negative effects of invading species on local biota via predation, competition or parasitism (BYERS, 2000; KATS & FERRER, 2003; TARASCHEWSKI, 2006; WARD & RICCIARDI, 2007; SOUSA et al., 2011). Indeed, bivalve invasions in freshwater are becoming increasingly common. And several freshwater bivalve species are some of the world's most problematic biological invaders (HIGGINS & VANDER ZANDEN, 2010) and have considerably altered ecosystems worldwide (DOUDA et al., 2012). Recent invasions of *Dreissena polymorpha*, *Corbicula fluminea* and *Limnoperna fortunei* (**Figure 1.1**) have been particularly problematic. These species have altered entire aquatic environments by their filtration and burrowing activities (HIGGINS & VANDER ZANDEN 2010; KARATAYEV et al. 2007; SOUSA et al., 2009; STRAYER, 2009), and they have severely affected native communities by altering habitats and competing for resources (KARATAYEV et al., 1997; SOUSA et al. 2008b; WARD & RICCIARDI, 2007). However, it is difficult to differentiate between the real impact of invasive species from other factors such as habitat degradation and fragmentation (REYJOL et al. 2007, HERMOSO et al., 2011; IUCN, 2015).



Dreissena polymorpha (source: Wikimedia)



Limnoperna fortunei (source: Alchetron)



Corbicula fluminea (source: Alchetron)

Figure 1.1. Bivalvia: Invasive alien species, *Dreissena polymorpha*, *Corbicula fluminea* and *Limnoperna fortunei*

In Portugal especially in the Sabor, Tua and Tâmega rivers (Douro basin, Northern Iberia), can be observed the presence of several native species with high value in terms of conservation. In this aquatic ecosystems, naiad populations can be detected, with great reproductive success and are distributed into two families (TEIXEIRA et al., 2013) (Figure 1.2): 1) *Margaritiferidae*, which includes only the species *Margaritifera margaritifera* (LINNAEUS 1785), a “critically endangered” species at national and european level, being included in Annexes II and V of the Habitats Directive, in Annex II of the Berne Convention and 2) *Unionidae*, composed of the species *Potomida littoralis* (CUVIER 1798), *Anodonta anatina* (LINNAEUS 1785) and *Unio delphinus* (SPENGLER 1793). *Potomida littoralis* is nowadays classified as an “endangered” species by the IUCN Red Book (IUCN, 2018).



Margaritifera margaritifera (Source: miljolare.no)



Potomida littoralis



Anodonta anatina



Unio delphinus

Figure 1.2. Freshwater mussels of the Sabor, Tua and Tâmega rivers: *Margaritifera margaritifera*, *Potomida littoralis*, *Anodonta anatina* and *Unio delphinus* (source: Biodiversidadvirtual.org)

Given their important ecological role, but also high sensitivity to habitat, water, and sediment quality, some freshwater mussel species simultaneously fulfill criteria for indicator, flagship, and umbrella species ((e.g. *Margaritifera margaritifera*), making

them important targets for environmental monitoring and conservation (GEIST, 2010; LOPES-LIMA et al., 2017). Feeding primarily through water filtration (DOUDA et al., 2012), freshwater mussels are very sensitive to human activities, but other intrinsic features increase the probability of extirpation or extinction (LOPES-LIMA et al., 2020).

Unionid bivalves exhibit a varying degree of host specificity (BARNHART et al., 2008). This is the case for *Anodonta anatina* (Linnaeus 1758), listed as threatened and protected in Germany (Van Damme, 2011) with whom have been recorded several populations declines around Europe. Generally considered to have a wide host fish spectrum (BAUER, 2001), there is a risk that *A. anatina* and other species considered to be host generalists may be unable to exploit the majority of individuals in novel host communities that are the result of human-mediated biotic homogenization. In this case, biotic homogenization would have manifold implications for the persistence and conservation of these affiliate species. Nevertheless, the effect of potential host limitation on the conservation status of endangered unionid bivalves remains poorly documented, and this lack of information causes uncertainty in the prioritization of conservation actions (SCHWALB et al., 2011). Other unionid species, *Unio delphinus*, has suffered a 30% range decline over the last decades, mainly due to habitat degradation, including pollution and changes in the hydrologic regime due to the presence of dams or other infrastructures, poor river management and water shortage (ARAUJO, 2011). The Iberian Peninsula, as most of the regions within the Mediterranean biodiversity hotspot (MYERS et al., 2000), is suffering from water scarcity exacerbated by climate change and associated instability (ROBSON et al., 2013; CID et al., 2017). And similar negative impacts were observed on the Iberian populations of other freshwater mussel species (SOUSA et al., 2012, 2018).

However, the National Program for Dams with High Potential Hydroelectric Plant (PNBEPH) which includes three large hydroelectric plants in the construction (Baixo Sabor- AHBS, Foz Tua-AHFT and Cascadas do Tâmega), as well as multiple mini-hydroelectric plants distributed across different hydrographic basins, seriously threaten the survival of many of these populations of naiads (TEIXEIRA et al., 2013). Degradation of water courses and loss of diversity underpin the creation of Water Framework

Directive (WFD) (2000/60/CE) whose objectives include, among others, the preservation and recovery of aquatic ecosystems.

1. OBJECTIVES OF THE DISSERTATION

The main objectives of this dissertation are:

1) **Evaluate, based on the Water Framework Directive, the ecological integrity, of a lotic ecosystem in Northern Portugal**, the River Fervença, located near Bragança city, namely:

- **Assess the water quality**, based on different physical and chemical parameters;
- **Determine the hydromorphological quality**, i.e. aquatic and riparian habitats;
- **Evaluate the biota quality**, in particular macroinvertebrates and fish communities.

2) **Determine, based on laboratory tests, the host-fish relationships between *Anodonta anatina* and fish communities** present in the Sabor River, such as:

- Examine the **ability of the duck mussel species to exploit its host community**;
- Test the **ability between native fish and non-native fish species**;
- **Develop laboratorial procedures** to obtain detailed information about the process.

To achieve these objectives, the organization of the dissertation is presented in four chapters:

- **Chapter 1** - a general introduction is done about main pressures and effects on water quality, hydromorphology and biota of freshwater ecosystems and particularly in a faunistic group, the freshwater mussels.
- **Chapter 2** - is presented like a scientific article, entitled: "Evaluation of ecological status of River Fervença and the human influence on abiotic characteristics and invertebrate and fish communities".
- **Chapter 3** - is also developed like a scientific article, entitled: "Can native and non-native species be host fishes for duck mussel (*Anodonta anatina* L., 1758) in Northern Rivers of Portugal (Douro basin)?"

- **Chapter 4** - present a general conclusion, based on previous chapters and some measures are presented in order to promote the preservation of the ecosystem integrity and the conservation of threatened native populations.

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Chapitre 2

EVALUATION OF ECOLOGICAL STATUS OF RIVER FERVENÇA AND THE HUMAN INFLUENCE ON ABIOTIC CHARACTERISTICS AND INVERTEBRATE AND FISH COMMUNITIES

ABSTRACT

The ecological integrity of River Fervença (River Douro basin, NE Portugal) was evaluated in the spring of 2020. Abiotic (e.g. water quality and aquatic and riparian habitats) and biotic (e.g. macroinvertebrate and fish communities) variables were measured in 7 sampling sites, selected along the watercourse, according with the methodology defined by the Water Framework Directive in Europe. The results showed, in general, good water quality in upstream zones, in contrast with reasonable or poor condition in downstream zones, namely after the urban water cycle of Bragança. In fact, it was observed an increase of dissolved salts ($EC_{25} > 200 \mu S.cm^{-1}$), nutrients ($N_{Total} > 2.0 mg.L^{-1}$ and $P_{Total} > 0.5 mg.L^{-1}$) and lower levels of dissolved oxygen ($DO < 7 mg.L^{-1}$) in downstream sites. In addition, RHS and GQC indexes identified some disturbance in aquatic and particularly in riparian habitats. A higher biodiversity of macroinvertebrates, composed by sensible organisms to pollution (e.g. Ephemeroptera, Plecoptera and Trichoptera) were present in headstream zone. In the downstream section of River Fervença, a dominance of resistant organisms (e.g. Diptera, Oligochaeta, Hirudinea) reflected the lower biological quality. Fish communities are not present in upstream zones, while in lower zones native species coabit with exotic species. The water pollution and eutrophication, the sucession of small weirs (re)constructed during POLIS program, the fragmentation of habitats, the introduction of exotic species, and the recent mortality detected in alder trees are the main factors contributing to the decrease of the ecological integrity of the River Fervença. The ecological integrity of River Fervença must be enhanced and highlighted, considering the ecosystem goods and services provided by these zones to urban tissues. For this reason, is urgent to take measures in order to rehabilitate the most disturbed zones and prevent environmental degradation and the dispersion to upstream of exotic species, some of them with high invasive potential.

Keywords: *ecological integrity, water quality, habitats, invertebrates, fish*

2.1. INTRODUCTION

One of the major problems that humanity is facing is related to water quantity and quality (UNESCO, 2009). In fact, human activities have led to the deterioration of rivers, lakes, groundwaters and coastal waters. Multiple negative pressures such as water pollution and eutrophication, regulation and river channelization, alterations of river flow and habitat fragmentation, and most recently the introduction of invasive alien species are responsible for the decreasing of freshwater biodiversity (GRIZZETTI et al., 2017). These problems will be more severe in the future due to the climate change, and it is expected higher water temperatures, melting of glaciers, and an intensification of the water cycle (HUNTINGTON, 2006), reflected by the occurrence of more floods and droughts (OKI & KANAE, 2006).

The human health is highly related to safe drinking water. Threats can include, for example, exposure to pathogens or to chemical toxicants through the food chain (e.g., bioaccumulation and biomagnification of toxic chemicals by aquatic organisms) or during recreation (e.g., swimming in polluted surface water). Water pollution has become a real problem nowadays since it is estimated that approximately 14000 people worldwide die every day due to water pollution (LECHINGER, 2000; PINK, 2006; LARRY, 2006). Both developed as well as developing countries are facing water pollution problems and global water resource policy is needed (EPA, 2009).

Renewable freshwater is a finite resource that has many uses and conflicts. Actually, aquatic systems can provide different uses including recreation, transportation, hydroelectric power and domestic, industrial and commercial uses (KUMAR, 2007) and can support all forms of life affecting our health, lifestyle, and inclusively social and economic well-being (IGWE et al., 2017). Water quality is influenced by natural factors like 1) precipitation, 2) climate, 3) soil type, 4) vegetation, 5) geology, 6) flow conditions, 7) ground water and antropic factors derived from the human activities, such as 1) Point source pollution of industries and municipalities, mining, urban development and agriculture diminish the water quality, 2) Non-point source pollution also includes nutrients, sediments and toxic contaminants (FLORESCU et al., 2010).

The assessment of water quality in rivers can be made using not only the abiotic factors (physical and chemical variables) but also the established links between

pollutants and biota. It is common the use of aquatic bioindicators that are organisms accumulating toxic substances or responding to environmental stress, such as pollution, nutrient enrichment, habitat loss or overexploitation (ADAMS, 2002). The concept of bioindicator requires standardized procedures including field sampling, sample processing and identification of collected organisms in order to summarise the obtained information in biological metrics (KARR & CHU, 1999). Responses to human-induced disturbances in rivers have frequently been analysed separately for macroinvertebrates (ARCHAIMBAULT et al., 2010), diatoms (BESSE-LOTOTSKAYA et al., 2011), macrophytes (LACOUL & FREEDMAN, 2006) and fish (YATES & BAILEY, 2010). More recently, it has been used the combinations of functional traits (ecological and biological), selected by habitat conditions through the survival ability of individual organisms relative to others (i.e. their fitness). Such integrative approaches were based on the functional structures of fish (Index of Biotic Integrity, IBI: KARR, 1981) and macroinvertebrate assemblages (USSEGLIO-POLATERA et al., 2000).

The protection and enhancement of water resources and aquatic ecosystems was adopted by the European Union, since the year 2000, through the Water Framework Directive (WFD), with the objective of reducing pressures and achieving good ecological status for all European water bodies. The knowledge about the ecological integrity of rivers must consider the evaluation of: 1) chemical and physical elements (e.g. water quality variables); 2) hydromorphological (e.g. riparian and channel habitat characteristics), and 3) biological (e.g. primary producers, benthic macroinvertebrates and fish) elements, according to the requirements defined by the WFD (DIRECTIVE 2000/60/EC).

The objectives of the present study were to evaluate the integrity of lotic ecosystems of River Fervença, located near Bragança city, in northeastern Portugal. In detail, the specific objectives were:

- 1) **Assess the water quality**, based on different physical and chemical parameters;
- 2) **Determine the hydromorphological quality**, i.e. aquatic and riparian habitats;
- 3) **Evaluate the biota quality**, in particular macroinvertebrates and fish communities.

2.2. METHODOLOGY

2.2.1. Study Area

River Ferveça belongs to the drainage basin of River Sabor, one of the main tributaries of Douro River basin. Is located in northeastern Portugal, crossing the main city of the region - Bragança (**Figure 2.1**).



Figure 2.1. River Ferveça near the city of Bragança, northeastern Portugal.

Seven sampling sites were selected and distributed along the longitudinal axis of River Ferveça (F1 to F7) (**Figure 2.2**, **Table 2.1**).

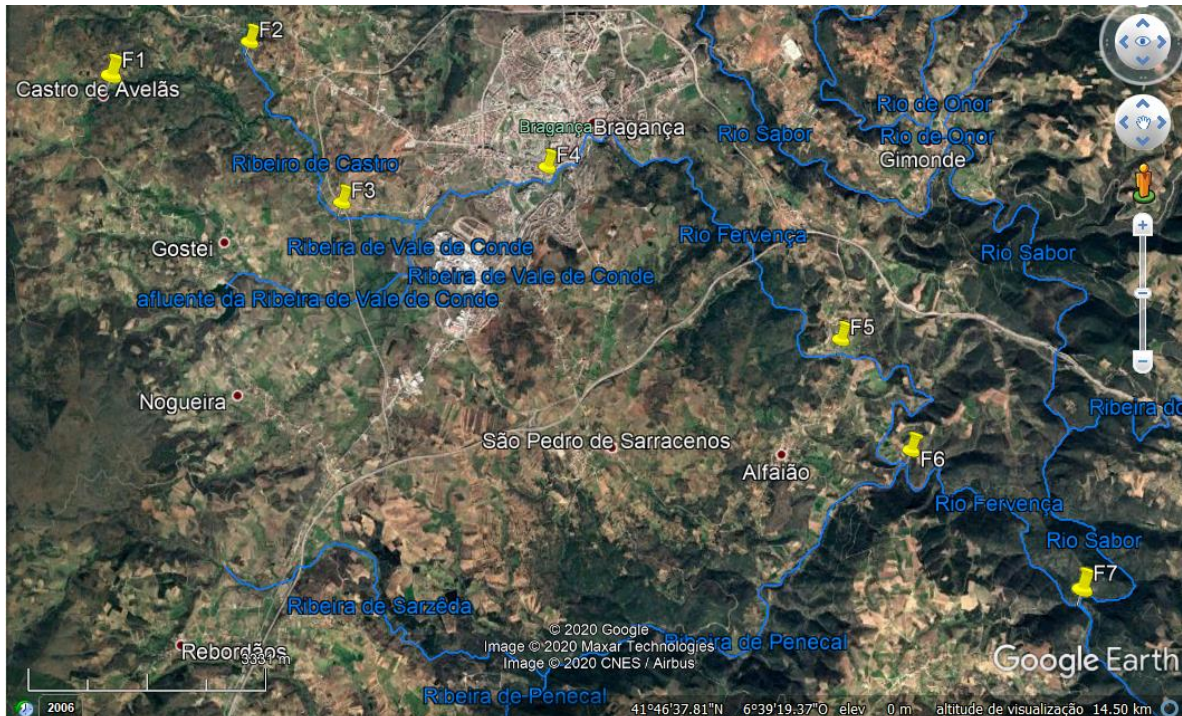


Figure 2.2. Map of the watercourses in Ferveça River and location of sampling sites.

Table 2.1. Location (geographical coordinates) of sampling sites in River Fervença.

Basin	River	Location (village)	Symbol	Latitude	Longitude	Altitude
Sabor	Fervença	Fontes Barrosas	F1	41°48'38.86"N	6°50'24.12"W	860 m
Sabor	Fervença	Grandais	F2	41°48'54.76"N	6°48'58.13"W	738 m
Sabor	Fervença	Castro de Avelãs	F3	41°47'40.07"N	6°48'2.01"W	694 m
Sabor	Fervença	Bragança	F4	41°47'55.16"N	6°45'55.59"W	675 m
Sabor	Fervença	Qta Figueiredos	F5	41°46'34.19"N	6°42'57.06"W	508 m
Sabor	Fervença	Confluence R. Penacal	F6	41°45'43.19"N	6°42'15.43"W	486 m
Sabor	Fervença	Confluence R. Sabor	F7	41°44'37.85"N	6°40'33.37"W	482 m

The sampling sites were selected along the longitudinal gradient of each river taking into consideration reference sites (under mountain conditions, having undergone no disturbance), moderate and highly modified sites affected by several human impacts, such as the construction of a succession of small weirs, pollution and eutrophication of the water and clearcutting of riparian vegetation (**Figure 2.3 to Figure 2.9**).



Figure 2.3. Sampling site F1 in River Fervença (near Fontes Barrosas).



Figure 2.4. Sampling site F2 in River Fervença (near Grandais).



Figure 2.5. Sampling site F3 in River Fervença (near Castro de Avelãs).

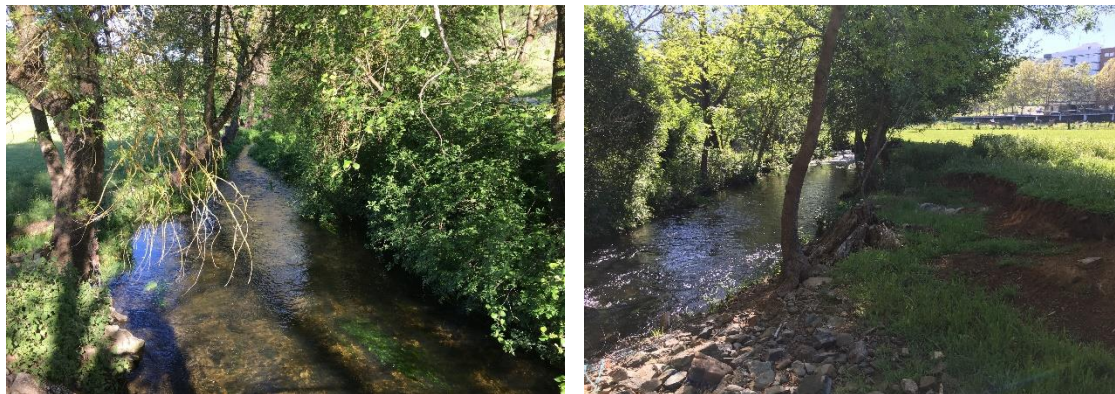


Figure 2.6. Sampling site F4 in River Fervença (Bragança-IPB).



Figure 2.7. Sampling site F5 in River Fervença (near Quinta dos Figueiredos).



Figure 2.8. Sampling site F6 in River Fervença (Confluence with R. Penacal).



Figure 2.9. Sampling site F7 in River Fervença (confluence with R. Sabor).

2.2.2. Water quality: Physical and chemical parameters

The water quality evaluation was made determining several physical and chemical parameters. Some variables were measured *in situ*, using portable probes (Figure 2.10) including: 1) Dissolved Oxygen ($\text{mg O}_2/\text{L}$); 2) Temperature ($^{\circ}\text{C}$); 3) Total Dissolved Solids (TDS mg/L), 4) Electrical Conductivity EC25 ($\mu\text{S}/\text{cm}$), and 5) pH. Other variables were determined in the laboratory, after the collection of water samples (1.5 L) and the transportation in coolers (to maintain the temperature of 4°C). It was only considered the main nutrients, particularly: 1) Total Nitrogen ($\text{mg N}_{\text{total}}/\text{L}$) and 2) Total Phosphorus ($\text{mg P}_{\text{total}}/\text{L}$) contents. All procedures were performed and determined according to the APHA (2005) procedures. The physical and chemical parameters were measured in the spring season (May 2020) and the interpretation of the results was based on Portuguese legislation (Decreto-Lei 236/98 of 1 August).



Figure 2.10. In situ evaluations of water quality in River Fervença, Bragança (Spring 2020).

2.2.3. Habitat evaluation: Channel and Riparian Quality Indexes

The habitat evaluation in this study was made in spring season (May 2020), using two indexes adapted to the Iberian Peninsula Rivers. Both indexes allow the classification of the hydromorphology and riparian quality of the selected sampling sites in River Fervença. The following indexes were used:

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

The QBR index was developed to evaluate the riparian condition of lotic ecosystems. This index is based on the following four components: 1) total riparian vegetation cover; 2) cover structure; 3) cover quality and 4) channel alterations. QBR is sensible to the differences in the geomorphology of the river from upstream to downstream reaches. The final index score varies between 0 and 100 points (see Annex I, for details). There are five quality classes of 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 habitat assessment considering the GQC Channel Quality index, is a broad measurement of the physical structure, considering mainly the channel conditions but also the river corridor characteristics. Eight components must be analyzed: 1) Presence of artificial obstacles (e.g. weirs, dams); 2) In-channel structures; 3) sediments and stability of the channel, 4) bank structure; 5) artificial alterations of the banks; 6) channel heterogeneity; 7) river bottom structure and 8) embedness. The final index score varies between 8 and ≥ 31 points (see Annex II, for details). There are five quality classes of 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

2.2.4. Biota: Benthic Macroinvertebrate Communities

2.2.4.1. Sampling Procedures

The sampling procedures used to capture benthic macroinvertebrate communities were 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). This protocol recommends the spring season as the best period to sample benthic macroinvertebrate communities. The protocol established in each sampling site considered a section of 50 m, and the representativeness of different habitats (riffle, pool and run) and microhabitats (e.g. fine and coarse materials, leaves, aquatic plants) presence of erosion units (turbulent flow) and adjacent sedimentation units (laminar flow) were previously determined. Six subsamples were collected in the sampling selected river zone, using a handnet (25*25 cm dimensions and with 500 µm of mesh size) removing the substrata with the foot (1-meter extension) (**Figure 2.11**).



Figure 2.11. Sampling procedures of benthic invertebrates collection (spring 2020).

Attached invertebrates to stable substrata were also collected using appropriate brushes. Invertebrates were captured and kept alive (in polyethylene bottles with river water coolers transported at low temperature) in order to facilitate the processing of the samples. In the laboratory, invertebrates were sorted and subsequently preserved in 70% alcohol.



Figure 2.12. Sampling procedures of benthic invertebrates collection (spring 2020).

All invertebrates were sorted, counted, and identified using a stereomicroscope SMZ10 with 10-132x zoom magnification, and recurring to appropriate dichotomous keys (e.g. TACHET et al., 1981, 2010) (**Figure 2.13**). The taxonomic level of identification was Family level with the exception of Subclasses Oligochaeta and Acari.

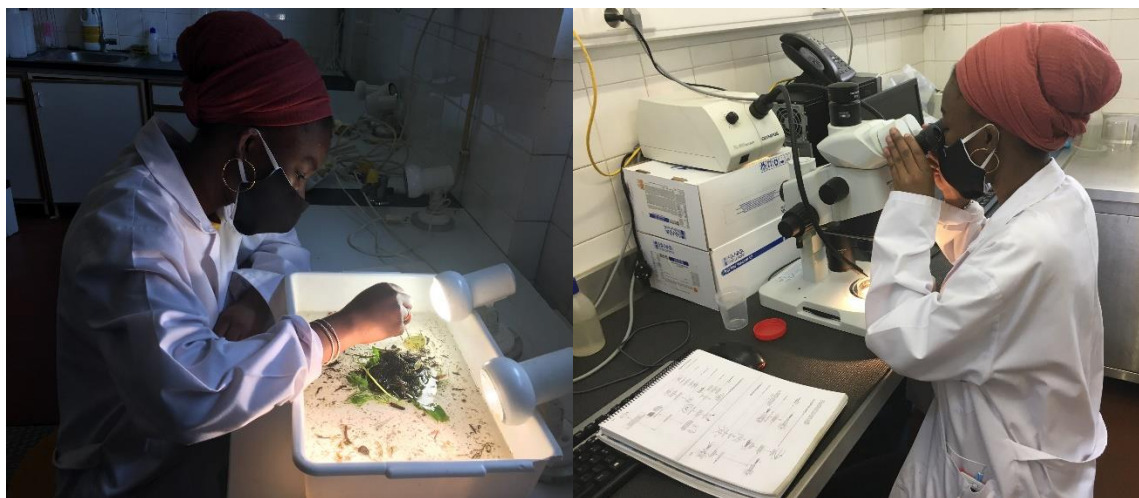


Figure 2.13. Laboratorial procedures: macroinvertebrate sorting and identification.

2.2.4.2. Metrics determined for invertebrate fauna

The evaluation of biological quality based on macroinvertebrate communities was done through a set of uni and multimetric variables, some of them calculated using the Software AMIIB@ (http://dqa.inag.pt/implementacao_invertebrados_AMIIB.html). Several metrics were calculated, highlighting the following ones:

- 1) Number of individuals (N) and number of *taxa* (S);
- 2) Diversity (*e.g.* H' Shannon-Wiener index);
- 3) Evenness (*e.g.* Pielou J' index);
- 4) Relative abundance of Ephemeroptera, Plecoptera and Trichoptera (% EPT);
- 5) Biotic Index IBMWP (ALBA-TERCEDOR, 1996).

The IBMWP index is a simple method for assessing the biological quality of lotic ecosystems. The identification of organisms must be done until the taxonomic level of family. The score ranges between 10 and 1 point, according to a gradient of pollution tolerance, corresponding 10 to the families more sensible to pollution and 1 to the families more resistant to disturbance (see Annex III). The final score of IBMWP is determined by the sum of all the scores of the families present in each sample, and the biological quality classified in 5 defined classes (**Table 2.4**).

Table 2.4. Quality classes according to the IBMWP

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 for Northern rivers of Portugal, integrates different metrics such as n^o of *taxa*, EPT, Pielou J' evenness, Shannon-Wiener H' diversity, IASPT and Sel. ETD, resulting in the following formula:

$$IPTI_N = N^{\circ} \text{ taxa} \times 0.25 + EPT \times 0.15 + \text{Evenness} \times 0.1 + (IASPT - 2) \times 0.3 + \text{Log (Sel. ETD+1)} \times 0.2$$

where:

- **EPT**: N^o families belonging to Ephemeroptera, Plecoptera, Trichoptera orders;
- **Evenness**: Defined as Pielou index or Evenness and calculated by the formula:

$$E = H' / \ln S \quad \text{where:}$$

H' - diversity of Shannon-Wiener

S - number of present *taxa*

Ln - natural or neper logarithm

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

$$H' = - \sum p_i \ln p_i \quad \text{where:}$$

$$p_i = n_i / N$$

n_i- n^o of individuals of each *taxon i*

N- total n^o 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)** - Log₁₀ of (1 + abundance of Heptageniidae, Ephemeridae, Brachycentridae, Odontoceridae, Limnephilidae, Goeridae, Polycentropodidae, Athericidae, Dixidae, Dolichopodidae, Empididae, Stratiomyidae);

Table 2.5 shows the reference values and boundary values for the quality classes, according to 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 Median dimension N1 > 100 km ²	1.00	≥0.88	[0.68-0.88[[0.44-0.68[[0.22-0.44[[0-0.22[

7) Functional Feeding Groups

The macroinvertebrate functional feeding group (FFG) method is based on the association between a set of feeding adaptations found in freshwater invertebrates and their basic nutritional resources categories (MERRITT & CUMMINS, 1996). To determine the FFG was used the software ASTERICS developed by the project **AQEM** - The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates. It was considered the general classification system for aquatic invertebrate trophic relations that can be observed in the following table (Table 2.6).

Table 2.6. Classification System for invertebrate trophic relations.

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

2.2.5. Biota: Fish Communities

The fish fauna is one of the biological quality indicator elements used in the classification of ecological status / potential for the category of lotic systems. According to Annex V of the WFD, three attributes are considered for these communities: abundance, composition, and age structure. The sampling of the fish community was based on the Manual for the Biological Assessment of Water Quality in River Systems, according to the WFD - Protocol for sampling and analysis for fish fauna (INAG, 2008b).

2.2.5.1. Sampling Procedures

In accordance with the procedures expressed there, the specimens were captured using electrofishing (**Figure 2.14**), which were subsequently identified to the species level. Captured fish was counted, measured and immediately released in the river (**Figure 2.15**). In all sampled sites, the same sampling effort (CPUE) was used, covering all habitats over a river extension of, at least, 100 m and a period of 30 minutes. The nomenclature used appears in the IUCN (International Union for Conservation of Nature) fee list, more specifically the Red List of Threatened Species (available at <http://www.iucnredlist.org/>) and was complemented by the list of AQUARIPORT Project book (OLIVEIRA et al., 2007), and the *Handbook of European Freshwater Fishes* (KOTTELAT & FREYHOF, 2007).



Figure 2.14. Sampling procedures of fish fauna using electrofishing (Spring 2020).



Figure 2.15. Biometric data collection and fish fauna identification (Spring 2020).

2.2.5.2. Metrics calculated for fish fauna

The Fish Biotic Integrity Index for wadable Rivers of Portugal (F-IBIP), developed by INAG & AFN (2012), consists of several metrics that try to reflect basic structural and functional characteristics of the fish communities in lotic systems of Portugal (OLIVEIRA et al., 2007, 2010). The metrics can decrease, or increase depending on the intensity of the anthropogenic disturbance and are included in two major groups: richness and specific composition (eg number of native species, percentage of exotic individuals) and ecological factors (related, for example, to food or reproduction). The F-IBIP index was determined using the software <http://www.isa.ulisboa.pt/wproj/fibip/>.

The F-IBIP score is obtained through the arithmetic mean of the metrics considered in each fish group. The final score of F-IBIP varies between 0 (zero), corresponding to bad quality, and 1 (one) corresponding to excellent quality (Table 2.7).

Table 2.7. Variation values of the F-IBIP quality classes

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

2.2.6. Data treatment

Data treatment was made through the univariate and multivariate analysis. The software PRIMER 7 & PERMANOVA + (CLARKE & GORLEY, 2006) was used to perform the *db*RDAs for environment conditions and *non-metric multi dimensional scaling* (NMDS) for the analysis of invertebrate and fish communities to discriminate local classified environmental quality of more disturbed systems. For this analysis the abundance data were transformed [Log (x + 1)] and applied the Bray-Curtis similarity coefficient. Furthermore, analyses of similarity were done through the calculation of ANOSIM tests.

2.3. RESULTS

The results are presented in terms of 1) water quality, 2) riparian and aquatic habitat characterization, and 3) biota, namely benthic invertebrates and fish communities. The most relevant aspects in terms of ecological evaluation of the River Fervença is presented below.

2.3.1. Physical and chemical water quality

The results obtained for the physical and chemical variables (i.e. Temperature, dissolved oxygen, electric conductivity, pH, total dissolved solids, total nitrogen and total phosphorus) of the water in seven sampling sites of River Fervença can be observed in **Table 2.8**.

Table 2.8. Physical and chemical variables of water quality – River Fervença (Spring 2020)

Variable	F1	F2	F3	F4	F5	F6	F7
Temperature (°C)	13.7	12.5	11.5	12.1	16.7	15.7	17.2
D.O. (mg/L)	8.54	7.74	9.21	8.97	6.36	6.94	9.45
pH	6.6	6.56	6.9	6.99	7.01	7.04	7.38
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	94	126	152	192	378	236	215
TDS (mg/L)	24	32	45	56	157	112	123
Total – N (mg/L)	0.01	0.1	0.15	0.59	3.34	2.35	2.4
Total – P (mg/L)	0.01	0.01	0.01	0.01	0.5	0.45	0.2
BDO5 (mg/L)	0.3	0.2	1.25	1.75	4.8	3.2	2.5

The results showed that the anthropic activities affected the physico-chemical variables. In fact, electric conductivity, total dissolved salts and particularly the most limitant nutrients in water ecosystems, i.e. nitrogen and phosphorus, increased substantially their concentration near and after Bragança city. Inclusively the agriculture activity made upstream from Bragança can promote the input of organic and inorganic compounds leading to an increase in primary production of the River Fervença. An increase of the BDO5 can also be observe till F5 with 4.8 mg/L indicating the presence of

large amount of organic matter especially downstream the city. A decrease of this value is observed from F6 coinciding also with the slight increase of the concentration of dissolved oxygen may be due to the effect of slope that promote the input of oxygen.

The dbRDA analyse showed that conductivity and TDS were the main variables justifying the discrimination between highly disturbed sampling sites (F5, F6, F7) and the remain sampling sites (**Figure 2.16**)

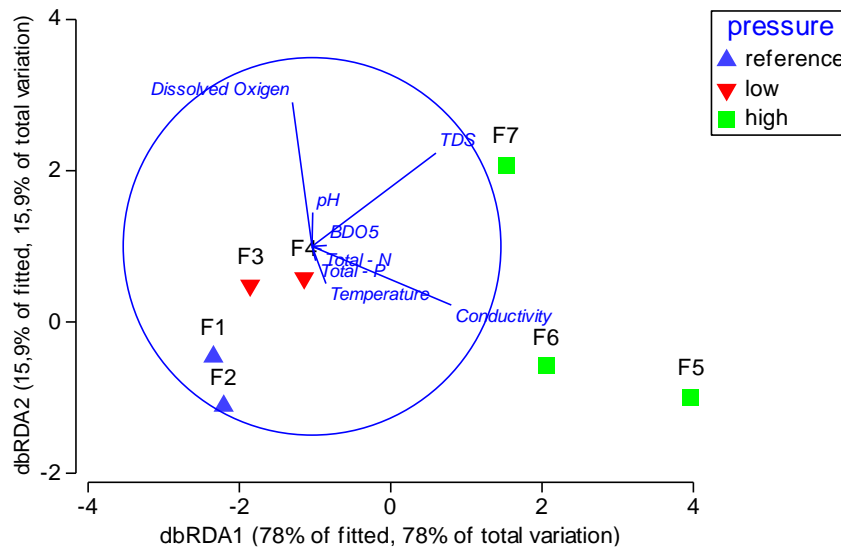


Figure 2.16. dbRDA of environmental conditions- water quality (Spring 2020).

2.3.2. Quality of aquatic and riparian habitats

The classification of both QBR and GQC indexes is presented in the **Table 2.8**. The scores ranged between Good (Class II) and Reasonable (Class III) classification. The riparian habitats are more disturbed due to the agriculture activity and the recent mortality detected in alder trees (**Table 2.9**).

Table 2.9. Scores of QBR and GQC indexes in River Fervença (Spring 2020).

Sampling site	Final Score		Habitat's index	
	QBR	GQC	QBR	GQC
F1	80	30	II	II
F2	70	29	III	II
F3	70	26	III	II
F4	65	29	III	II
F5	65	21	III	III
F6	70	22	III	III
F7	90	21	II	III

2.3.3. Macroinvertebrate Communities - Metrics

The total number of individuals captured and distributed by the 7 sampling sites can be visualized in Figure 2.17. A total of 4406 individuals of invertebrates were identified, belonging to 58 faunistic groups, mainly families (**Figure 2.17**).

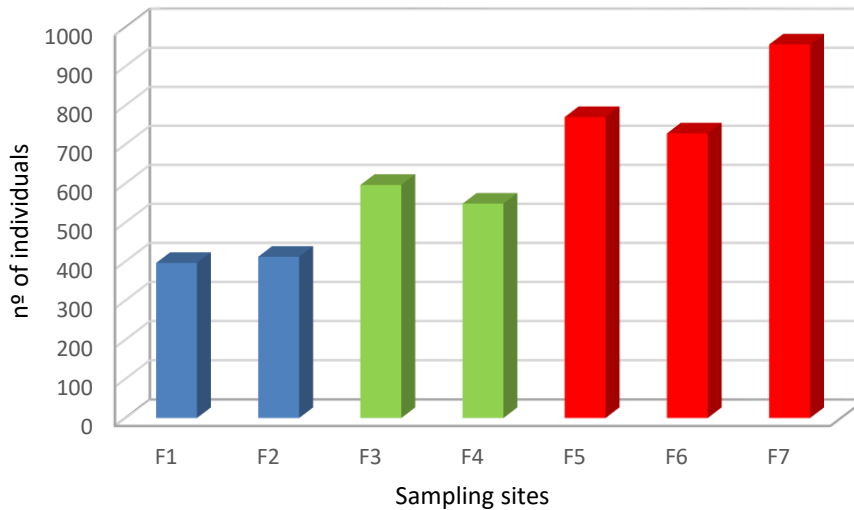


Figure 2.17. Number of individuals present in each sampling site (Spring 2020).

The disturbed sampling sites (i.e., F5, F6 and F7) presented a higher number of individuals, ranging between 728 to 956 individuals. However, in reference (i.e., F1 and F2) and low disturbed sampling (F3 and F4) sites were obtained representative assamblages of invertebrates, since the minimum value was 396 individuals (F1).

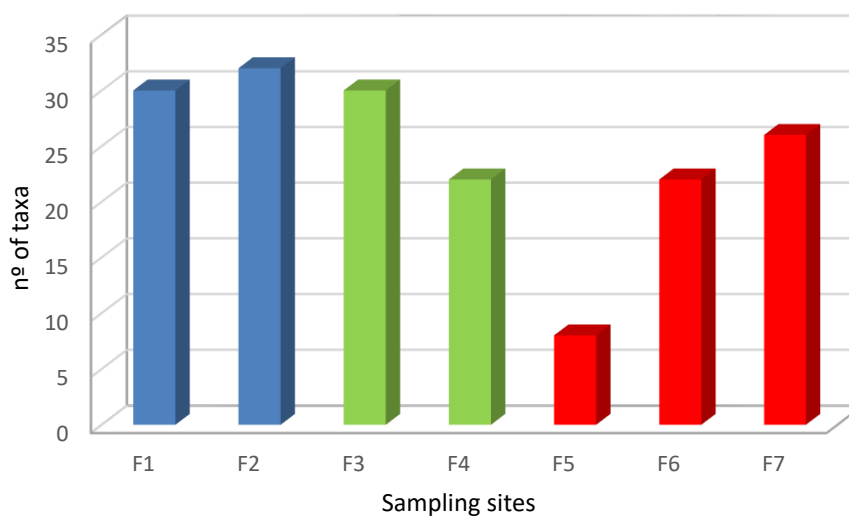


Figure 2.18. Total number of taxa present in the sampling sites (Spring 2020).

The taxa richness (e.g. families, with the exception of Oligochaeta) was superior in reference sites, reaching the highest value of 32 faunistic groups in F2. The lowest value was obtained in the most disturbed site (F5), just downstream from Bragança city, where only 8 taxa were identified (Figure 2.18).

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

The variation of Shannon-Wiener diversity (H') and Pielou Equitability (J') can be observed in Figure 2.19.

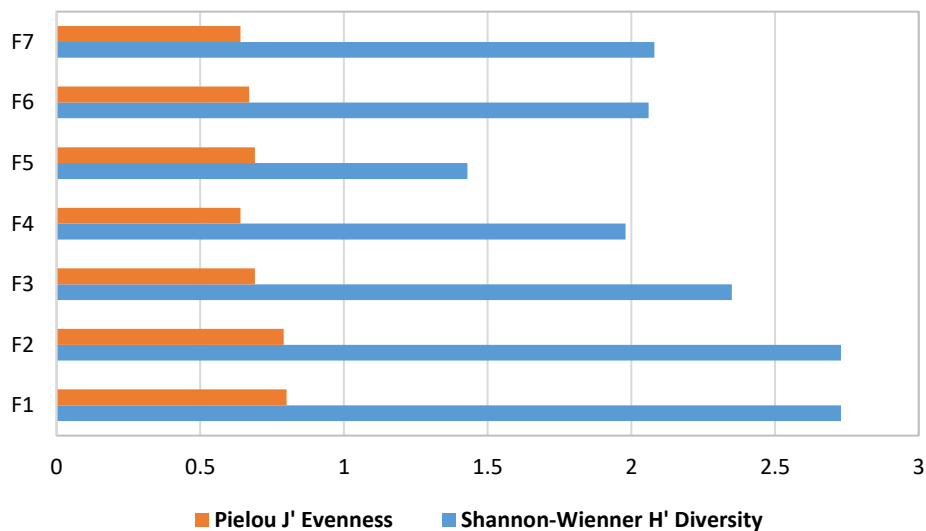


Figure 2.19. Variation of Shannon-Wiener diversity (H') and Pielou evenness (J') indexes (Spring 2020).

The highest diversity, i.e., Shannon-Wiener H' index was obtained the upstream zone (F1, F2), contrasting with the minimum value detected in F5. The same trend was observed for the Pielou J' evenness index among sampling sites, showing a more homogeneous distribution of individuals by each taxon in undisturbed ecosystems.

2.3.3.2. Faunal composition

The global proportion of each invertebrate taxon can be observed in the Figure 2.20. The main aspects that must be highlighted are:

- The evident dominance detected for Diptera (32%);
- EPT (Ephemeroptera, Plecoptera, Trichoptera) represented 41%;
- Important value registered for annelids (Hirudinea and Oligochaeta) (17%).

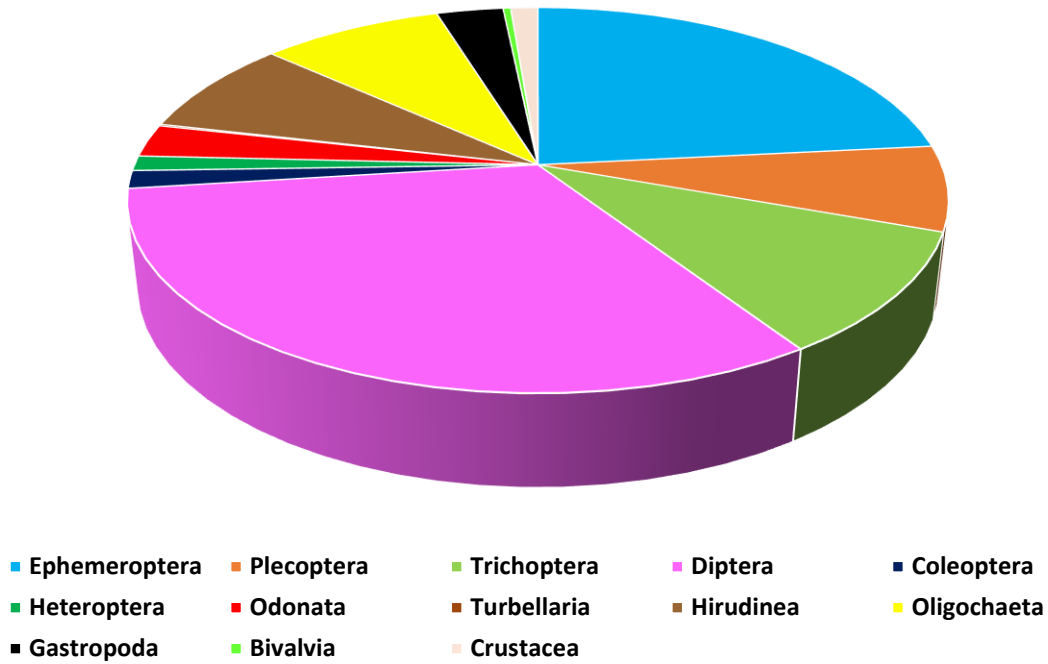


Figure 2.20. Faunal composition of invertebrates in all sampling sites (Spring 2020).

The intra and intervariation for the taxa richness, among sampling sites, can be consulted in the **Figure 2.21**, where the faunal composition changes between different sites are very easy to identify.

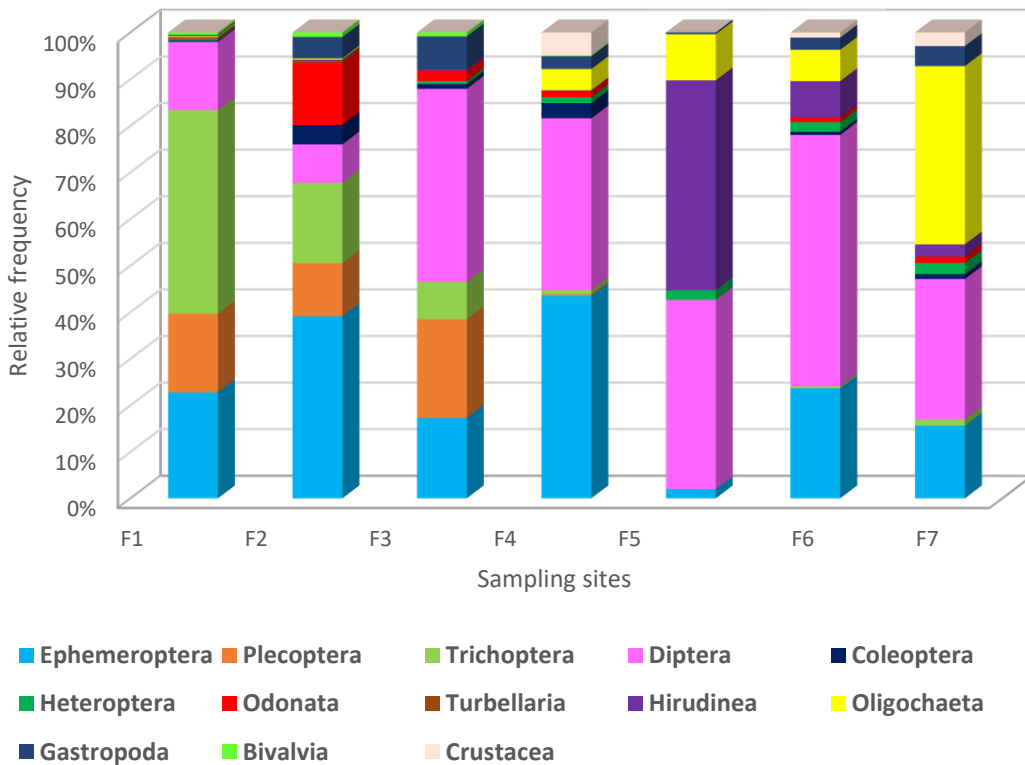


Figure 2.21. Faunal composition of invertebrates in river Fervença (Spring 2020).

In fact, EPT families, typically present in high abundance in stenobiont rivers, are more abundant in the reference sites (F1, F2), and almost absent in downstream zone, while Diptera, Hirudinea and Oligochaeta (euribiont organisms, adapted to polluted rivers) are dominating the disturbed zones of the River Fervença.

2.3.3.3. Functional Feeding Groups

The analysis of the variation in terms of functional feeding groups is shown in the following figure (Figure 2.22). It was detected an important presence of collectors (gathering and filter feeders) in almost all the sites. Shredders are present only in undisturbed sites, i.e., F1 and F2, while predators and parasites had an important increase in the most disturbed sampling site (i.e., F5).

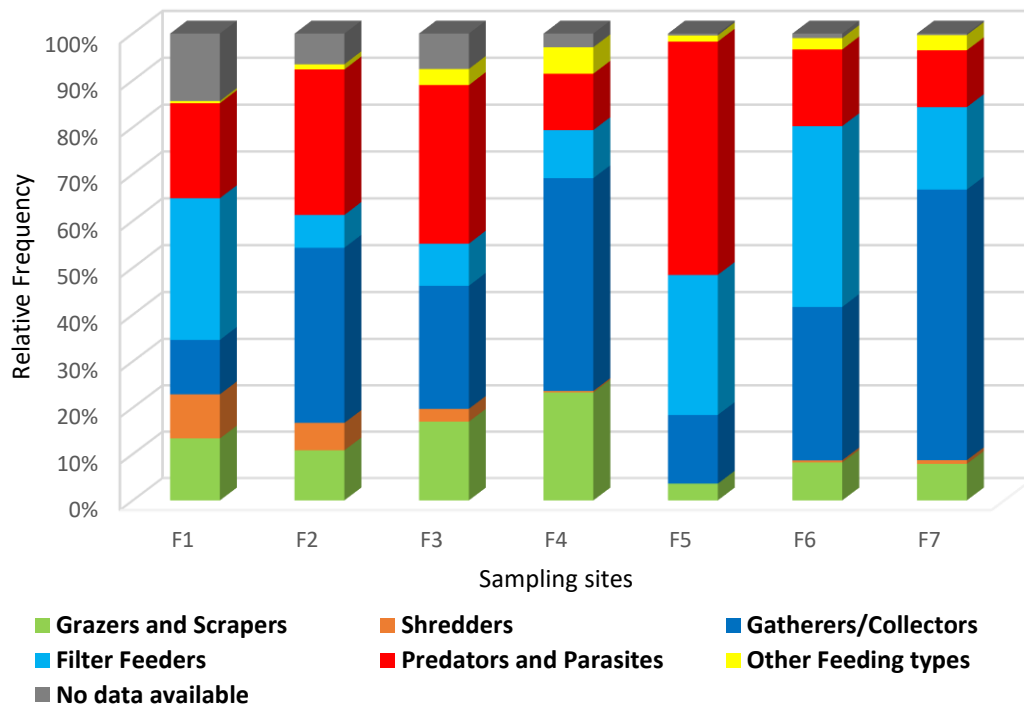


Figure 2.22. Functional feeding groups in River Fervença (Spring 2020).

2.3.3.5. IBMWP and IPT_N indexes

The results obtained for IBMWP and IPT_N indexes, based on macroinvertebrate assemblages, showed to be sensible to the environmental changes detected in the longitudinal gradient of River Fervença. Indeed, it was obtained an excellent biotic condition, for the headwater streams (i.e., F1 and F2, Table 2.10). In the more degraded downstream zones of river Fervença were obtained a poor (F5) and reasonable condition (F4, F6 and F7), sign of evident decrease in the biological quality. Significant

differences were found between the classification of unimetric (IBMWP) and multimetric (IPtI_N) indexes. The IPtI_N index shoed to be more rigorous than IBMWP. Several other metrics, calculated by amiib software (see annexes IV.1, IV.2 and IV.3), corroborate this tendency.

Table 2.10. Scores and classification of IBMWP and IPtIN for each sampling site (Spring 2020).

Sampling site	IBMWP		IPtI _N	
	Score	Classification	Score	Classification
F1	203	Excellent	1.02	Excellent
F2	201	Excellent	0.96	Excellent
F3	172	Excellent	0.84	Good
F4	113	Excellent	0.59	Reasonable
F5	24	Poor	0.24	Poor
F6	100	Excellent	0.65	Reasonable
F7	114	Excellent	0.66	Reasonable

2.3.3.6. Biotypology of macroinvertebrate communities

The non-metric MDS analysis (2D stress value of 0.02, an indicator of an excellent two-dimensional representation of data), based on the macroinvertebrate community, showed, clearly, the separation between reference sites, upstream from Bragança city, and highly disturbed sampling sites located downstream from urban area (**Figure 2.23**).

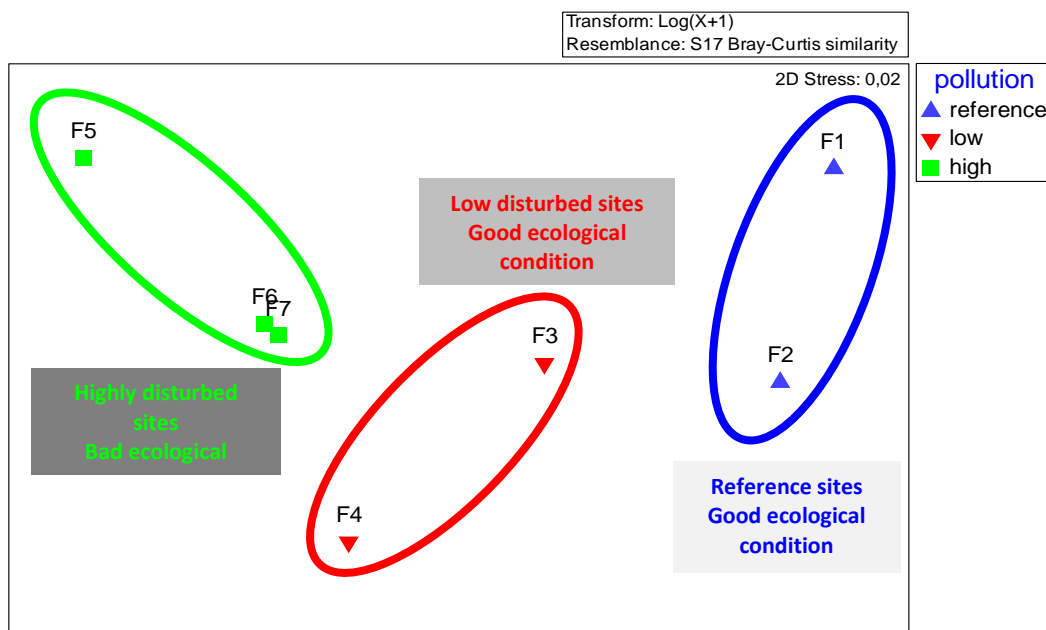


Figure 2.23. Non-MDS ordination of sampling sites, based on invertebrate communities: Blue triangles - reference; Red triangles - low disturbed; Green triangles – highly disturbed sites.

The ANOSIM similarity (one-way) tests confirmed the observed tendency of the ordination and significant differences ($P < 0.01$) were obtained just between highly disturbed sites (F5, F6, F7) and both reference (F1 and F2) and low disturbed sites (F3 and F4).

The Non-MDS ordination of the macroinvertebrate communities showed, typically, a separation between families more stenobiont, belonging to the orders Trichoptera (Philopotamidae, Sericostomatidae), Plecoptera (Perlodidae), Diptera (Blephariceridae) and families more euribiont, i.e. more adapted to disturbed sites, such as Hirudinea (Hirudidae, Erpobdellidae), Oligochaeta, Dipera (Simuliidae and Chironomidae) and also exotic crayfishes (*Pacifastacus leniusculus* and *Procambarus clarkii*) (Figure 2.24).

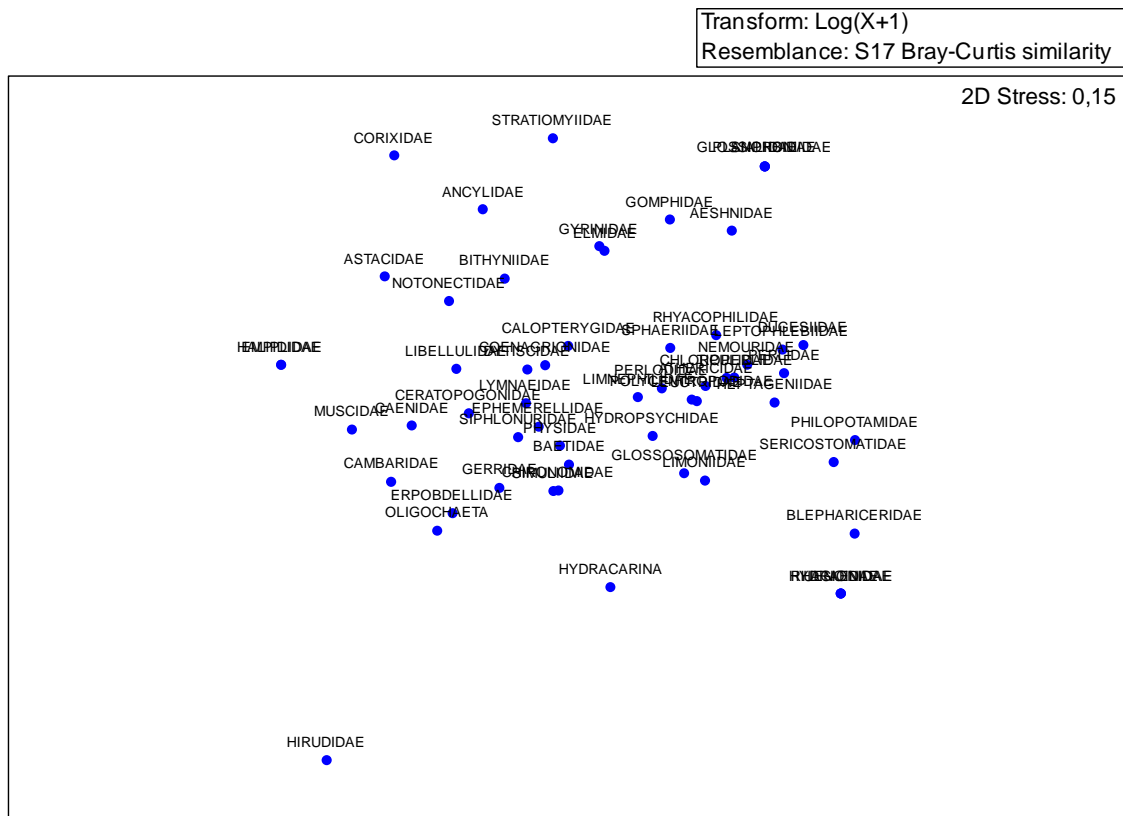


Figure 2.24. NMDS Ordination of invertebrate communities of River Fervença (Spring 2020).

2.3.4. Fish Communities - Metrics

2.3.4.1. Composition and Abundance

Seven fish species were captured in the sampling sites of the River Fervença, considering four native cyprinids (*Squalius carolitertii*, *Squalius alburnoides*, *Pseudochondrostoma duriense* and *Luciobarbus bocagei*) and three non-native species (*Lepomis gibbosus*, *Gobio lozanoi* and *Cobitis paludica*) (Figure 2.25).



Figure 2.25. Captured fish species in River Fervença (Spring 2020).

The native cyprinids are endemic species of Iberia Peninsula and *Squalius alburnoides* and *Pseudochondrostoma duriense* are classified by IUCN as vulnerable species (IUCN, 2020). *Lepomis gibbosus* is an exotic and invasive species, widely dispersed in Douro basin, showing an ecological plasticity to promote their adaptation to different river conditions. *Gobio lozanoi* is a small fish also widely dispersed, native from Bidasoa and Ebro basins. *Cobitis paludica* is a native species of Southern Iberia, introduced in northern basins as a bait for fishing activity, namely for piscivorous fish (*Esox Lucius*, *Micropterus salmoides*) in reservoir environments.

A total of 180 fishes were captured only in three sampling sites. In the upstream sampling sites, i.e. F1, F2, and also in the more polluted river zone – F5 it was not detected fish fauna. The distribution and abundance of the fish species in the remaining sites of the River Ferveça, i.e. F3, F4, F6 and F7 can be observed in the following figure (Figure 2.26).

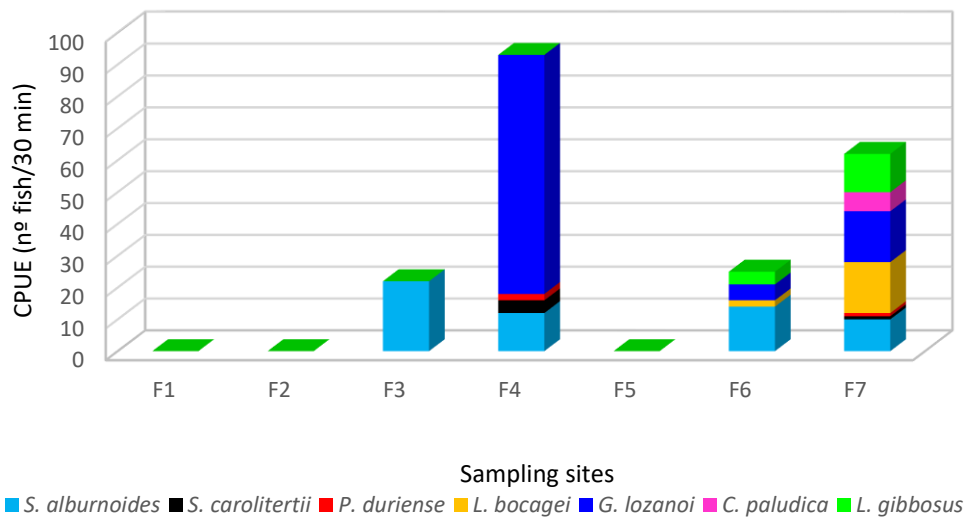


Figure 2.26. Absolute abundance (CPUE) and distribution of fish in River Ferveça (Spring 2020).

The relative abundance highlights the dominance of non-native species. In fact, modified aquatic ecosystems are «open doors» for the increasing density and biomass of exotic species, and in particular of invasive alien species, causing substantial changes in terms of the composition and structure of the aquatic ecosystem (Figure 2.27).

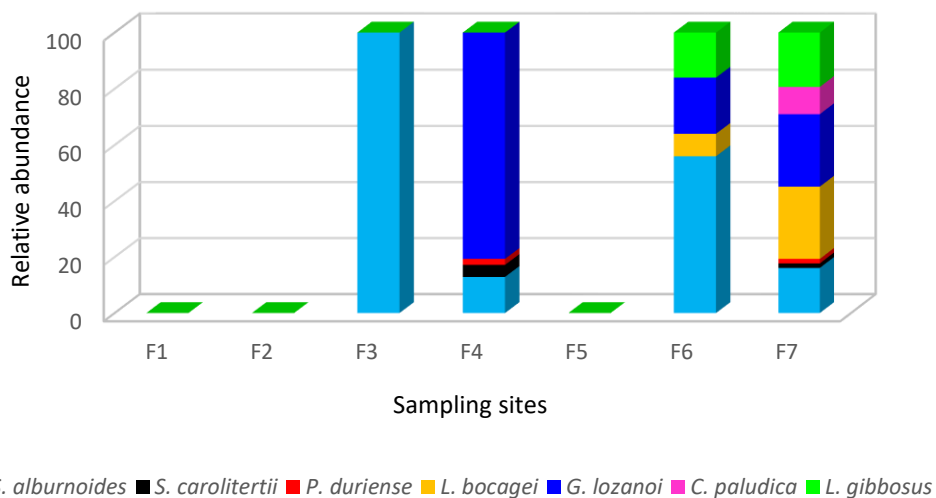


Figure 2.27. Relative abundance and distribution of fish in River Ferveça (Spring 2020).

2.3.4.2. F-IBIP index

The evaluation of the biological quality of the fish fauna, based on the Biotic Integrity Fish Index for Wadeable Rivers of Portugal (F-IBIP), can be shown in **Table 2.11**.

Table 2.11. Scores and classification of F-IBIP for sampling sites with fish (Spring 2020).

	F3	F4	F6	F7
River Type	Group 1	Group 2	Group 2	Group 2
F-IBIP	0.667	0.047	0.296	0.148
Biological Quality	REASONABLE	BAD	POOR	BAD

The Bad/Poor fish quality classification obtained through F-IBIP is strongly influenced by the presence and particularly the dominance of non-native species.

2.4. DISCUSSION

The ecological evaluation of a low order stream of Northeastern Portugal, i.e., River Fervença, showed synergic negative impacts on water quality and biota caused by water contamination and habitat degradation resulted, mainly, from agricultural and urban activities. During the spring season of 2020, abiotic and biotic characterization of 7 sampling sites, distributed from the source to the mouth (confluence with River Sabor), allowed to identify a decrease on the ecological integrity, namely after the urban zone. According to the results, significant differences were obtained among the three considered zones in the River Fervença defined as reference (F1, F2), low (F3, F4) and highly (F5, F6, F7) disturbed sites.

The reference sites located in River Fervença are typically influenced by mountain waters. It was found a good physical and chemical state of water, i.e. high oxygenation levels and low temperature, electric conductivity, and contents of dissolved solids and nutrients, namely nitrogen and phosphorus compounds, the most limitant substances for primary production. Relatively to the hydromorphological elements it was observed some signs of degradation (i.e., F2) in the riparian quality classification (QBR index), mainly due to the recent death linked to alder tree in Portugal. Relatively to the channel quality (GQC index), a good classification was obtained. These environmental conditions

are responsible for the high biodiversity (H' Shannon Wiener index) and evenness (J' Pielou Index) present in terms of macroinvertebrate assemblages. In fact, several metrics contribute to highlight the good ecological state, such as the number of EPT families (Ephemeroptera, Plecoptera and Trichoptera), which are particularly sensible to organic pollution. Fish species are not present in the headstream (F1) but a population of a vulnerable species, i.e. Calandino (*Squalius alburnoides*) was detected in this zone (F2). The good ecological integrity of Mountain Rivers of Northeastern Portugal has been reported by several studies (TEIXEIRA et al., 2006; SOUSA et al., 2015).

The degradation of downstream sites is strongly linked to the human activities. Agriculture and urban development promote substantial changes in water quality and a substantial increase in dissolved salts ($TDS > 100 \text{ mg.L}^{-1}$; conductivity $> 300 \mu\text{S.cm}^{-1}$) and organic matter ($BDO_5 > 10 \text{ mg O}_2.\text{L}^{-1}$) and also in total nitrogen ($> 3 \text{ mg.L}^{-1}$ Total N) and total phosphorous ($> 1 \text{ mg.L}^{-1}$ Total P). Furthermore, the clearcut of riparian zone and the sedimentation observed in the substrata led to the disappearance of stenobiont species, highly restricted by the good ecological conditions. Two biotic indexes (IBMWP and IPTIN) and particularly a new index developed for Portuguese rivers (IPTIN) showed to be more sensible to sign the lower biological quality, based on macroinvertebrate communities, detected in this zone. In fact, euribiont taxa composed by leeches (Hirudinea), worms (Oligochaeta) and mosquito flies (Diptera) are the dominant faunistic groups. Other metrics, such as the functional feeding groups, allowed to identify particular trends. The high dependance of low order streams from the riparian inputs was not verified in the disturbed zones, where the proportion of predators and parasites is abnormally high.

Comparing to previous studies in rivers from Northeastern Portugal, affected by different kind of stressors (e.g. belonging to the Sabor, Tâmega and Tua rivers, tributaries of Douro Basin), upper zones maintain the natural good ecological condition and downstream zones are more influenced by human activities (agriculture, removal of riparian vegetation, industries and urban wastes) affecting the normal functioning of river ecosystem (CLARO, 2010; NOGUEIRA, 2011; RAMOS, 2011; RODRIGUES, 2013).

Freshwater ecosystems provide a wide variety of ecosystem services including water consumption, irrigation, flood protection and biodiversity maintenance. These systems

are very fragile and affected by several stressors (NAIMAN et al., 2002; JANSSON et al., 2007). Throughout world, agriculture is the type of land use with the most significant impacts on freshwater ecosystems (e.g., DAVIES et al., 2009; POOLE et al., 2013). In fact, is common the identification of negative impacts in water quality due to pollution, eutrophication, and sedimentation. However, other stressors are also very important causing different kind of disturbances not only in water quality but also in the natural functioning of aquatic ecosystems.

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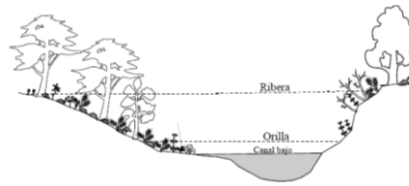
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ANNEXES

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	If connectivity between the riparian forest and the woodland is total
+5	If the connectivity is higher than 50%
-5	Connectivity between 25% and 50%
-10	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	At least 50% of the channel has helophytes or shrubs
+5	If 25–50% of the channel has helophytes or shrubs
+5	If trees and shrubs are in the same patches
-5	If trees are regularly distributed and shrubland is >50%
-5	If trees and shrubs are distributed in separate patches, without continuity
-10	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	If the tree community is continuous along the river and covers at least 75% of the edge riparian area			
+5	The tree community is nearly continuous and covers at least 50% of the riparian area			
+5	When the number of shrub species is	> 2	> 3	> 4
-5	If there are some man-made buildings in the riparian area			
-5	If there are some isolated species of non-native ^{b)} trees			
-10	Presence of communities of non-native ^{b)} trees			
-10	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	River bed with rigid structures (e.g., wells)
-10	Transverse structures into the channel (e.g., weirs)

Final score (sum of four section scores)	
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		Score	
		Left	Right
<p>^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.</p>			
Slope and form of the riparian zone			
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 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: <i>Populus deltoides</i> , <i>Populus x canadensis</i> , <i>Populus nigra</i> ssp. <i>italica</i> , <i>Salix babilonica</i> , <i>Ailanthus altissima</i> , <i>Celtis australis</i> , <i>Robinia pseudo-acacia</i> , <i>Platanus x hispanica</i> .			

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
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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 predominates 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: Siphonuridae, 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, Dugesidae	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, Hicrobiidae, Dytiscidae, Gyrinidae M: Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae, Bithyniidae, Bythinellidae, Sphaeriidae Hr: Glossiphoniidae, Hirudidae, Erpobdellidae C: Asellidae, Ostracoda	3
D: Chironomidae, Culicidae, Muscidae, Thaumaleidae, Ephydriidae	2
O: Oligochaeta (All Families) D: Syrphidae	1

Chapitre 3

CAN NATIVE AND NON-NATIVE SPECIES BE HOST FISHES FOR DUCK MUSSEL (*Anodonta anatina* L., 1758) IN NORTHERN RIVERS OF PORTUGAL (DOURO BASIN)?

ABSTRACT

The proportion of native and non-native fishes are dramatically changing in mediterranean rivers of Iberia. Several antropic factors such as pollution and eutrophication of water, physical sedimentation, habitat fragmentation and riparian degradation have led to severe alterations in the environmental conditions. These negative impacts are also highlighted by the climate change allowing, with the introduction of alien species, to diminish or disappearance of singular habitats and complex ecosystems, threatened native species and specific biotic interactions. Unionid species are freshwater bivalves (Mollusca) with a particular and complex life cycle since an obligatory parasitic phase on a limited group of host fish species must occur. This host-parasite interaction is essential for the management of wild fish and mussel populations, especially in watercourses with threatened native species. One of the major rivers of Iberia, Douro River, is severely impacted by big and small dams contributing to the recent dominance of non-native species. In this study, it was tested the ability of native and non-native fish species to be hosts of a bivalve, the duck mussel *Anodonta anatina*, living in sympatry in the Sabor River, a tributary of Douro Basin. Four native species (*Luciobarbus bocagei*, *Squalius alburnoides*, *Squalius carolitertii* and *Pseudochondrostoma duriense*) and one non-native species (*Alburnus alburnus*) were selected and successful results obtained, in laboratorial conditions, both in terms of infestation and production of viable juveniles of *A. anatina* for all fish species. No significant differences were detected between native and non-native fish species. *S. carolitertii* was the more effective host fish species, reaching a metamorphosis rate of 75.7% and viable 357 juveniles per fish. The results suggested that priority habitats must be identified and protected in the Sabor River basin in order to maintain the wild native fish communities and the ecological integrity of those ecosystems, as basic strategy action for the conservation of mussel populations.

Keywords: *Anodonta anatina*, host-parasite relationships, glochidia, native fish

3.1. INTRODUCTION

The worldwide extinction of species affects a large number of species and ecosystems, especially freshwater ecosystems. Freshwater mussels, globally spread organisms and sedentary benthic invertebrates are one of the most threatened species worldwide (STRAYER, 2008). Part of these threats are due to the habitat degradation generally caused by the:

- water pollution, sedimentation and toxic chemical that affect water quality;
- small and big dams that fragment river connections and form silt-laden impoundments;
- Water abstraction due of a growing demand for water by agricultural, industrial and recreational activities, especially in southern European countries (e.g. Portugal, Spain, Italy and Greece) (BENEJAM et al., 2010);
- habitat loss, alteration and modification by stream channelization, ditching, and dredging, wetland drainage and field tilling that leads to rapid water runoff, bank erosion, and streambed destabilization, amount others;
- Introduction of invasive alien species (IAS) which invade the living environment and affect negatively native species (e.g. the Zebra mussel, *Dreissena polymorpha*);
- Loss of fish hosts (most likely to occur for mussel species with restricted host fish spectra, such as *M. margaritifera* and *M. auricularia*);
- Climate change; and
- Overexploitation by commercial harvesting (e.g. for the pearls of freshwater pearl mussels *M. margaritifera*).

On the other hand, which is very important, their threat is strongly linked to some of their biological characteristics (FERREIRA-RODRÍGUEZ et al., 2019) and in particular the complex life-cycle based on their obligatory parasitic phase on host fish (**Figure 3.1**). This parasitic phase could be advantageous for nutrition and growth, but also for dispersal, by providing the possibility to disperse upstream (BARNHART et al., 2008). Nevertheless, it also represents a higher risk of co-extinction because the mussels are completely dependent upon their hosts to complete their life cycle (DIAS et al., 2020). Although the co-extinction rates and declines of highly specialized affiliate species (which directly depend on the presence of another species) have been well documented, the effects of

biotic homogenization on affiliate species that are considered to be generalists remain understudied (DOUDA et al., 2013).

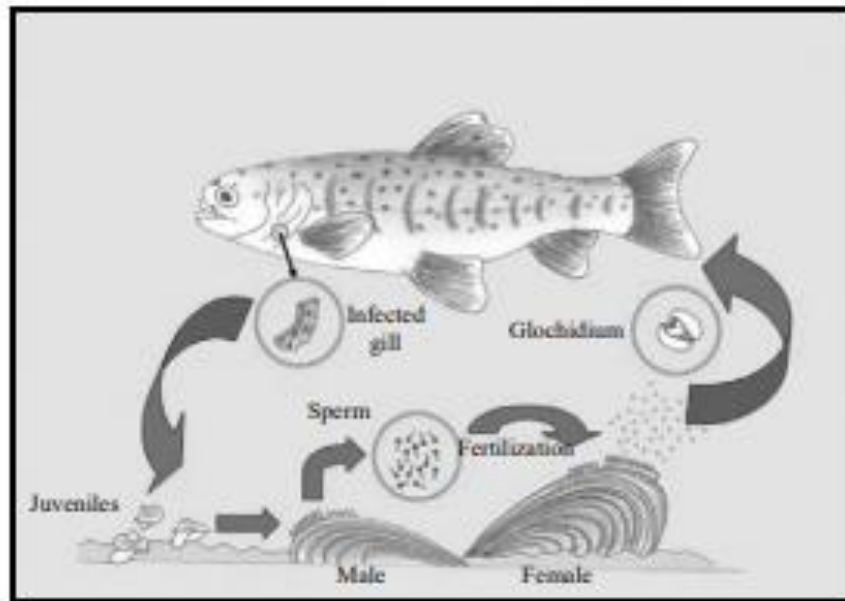


Figure 3.1. Basic life-cycle of a unionoid mussel (adapted from Reis 2006).

The knowledge of the host fish assemblage is essential for effective conservation of *Anodonta anatina* (HUBER & GEIST, 2019) a freshwater mussel that is widespread in Europe, from the Iberian Peninsula and Northern Africa to Scandinavia in the North and to Russia (Lake Baikal) in the East (GRAF, 2007) for which there have been recorded several population declines around Europe and which population decrease can potentially impact also other species and functions of aquatic habitats (DOUDA et al., 2013) (**Figure 3.2**).



Figure 3.2. Freshwater Duck mussel, *Anodonta anatina* (Source: Wikimedia Commons).

The forces that determine distributions of Unionida species can be best described by: (i) biogeographic history; (ii) host fish distribution; and (iii) local environment including

biotic and abiotic factors (VAUGHN & TAYLOR, 2000). At the local scale, habitat characteristics such as current velocity or water and sediment quality and biotic interactions such as competition, predation, parasitism and facilitation are usually considered key aspects determining Unionida species composition, density and distribution (for a review, see STRAYER, 2008).). *A. anatina* has a relatively high plasticity and tolerance to different abiotic conditions and can thus be found from fast-flowing streams to lentic habitats (ZIERITZ & ALDRIDGE, 2011)

By considering the host fish distribution to which mussel distributions are determined to a large extent, species such as *M. auricularia*, that specialize on host fishes with constrained habitat requirements, typically have more restricted distributions than generalist mussel species such as many of the unionines and anodontines (*A. anatina*), (LOPES-LIMA et al., 2017). The availability of experimental methods for studying host compatibilities has resulted in the use of unionid bivalves as a common model group for the study of host–affiliate relationships involving endangered species (SPOONER et al., 2011; DOUDA et al., 2012).

Anodonta anatina typically live for less than 30 years and reach the sexual maturity at 1-4 years (LOPES-LIMA et al., 2017). Favoring hermaphroditism in lentic habitats and gonochorism in lotic habitats (HINZMANN et al., 2013), they are long-term brooders (bradyticitic), keeping the larvae over winter and releasing them from late winter/early spring through the summer (LOPES-LIMA et al., 2017). This change in its sexual behavior can be influenced by environmental conditions, and can be due to the presence of low population densities where dicious individuals increase their reproductive success.

Females inhale the sperm that was released by males in the water, fertilizing the eggs that are located in brooding chambers formed by the gills; the eggs develop into parasitic larvae (glochidia or lasidia) which are released in the water by the female; the larvae attached to the epithelium of a fish, metamorphose into juvenile bivalves and then release themselves; they fall to the river substratum, grow to adult size and start another cycle (REIS, 2010). The larvae (glochidia) of *A. anatina* are large and hooked, released in mucous webs (WOOD, 1974; HAAG & WARREN, 1997) and like all anodontines, the species is considered a generalist using a wide fish hosts spectrum

(BAUER, 2001). The release of glochidia by *A. anatina* can be stimulated by the presence of the fish host (JOKELA & PALOKANGAS, 1993) (**Figure 3.3**).



Figure 3.3. *Anodonta anatina* releasing larvae (glochidia) in mucous webs

Some species as *Margaritifera margaritifera* individuals are known to be able to change from dioecious to hermaphrodite in response to decreasing population density, being able to self-fertilize (BAUER, 1987). And dioecious and hermaphrodite individuals are determined by hormone levels (KAT, 1983), that vary due to intrinsic factors (causing species-specific patterns and strategies with a selective value, like in *M. margaritifera*) and external influences (causing variations with no apparent selective value like occasional hermaphroditism) (REIS, 2010).

The parasitic life stage may benefit the mussel through dispersal and provides a source of nutrients (DENIC, TAEUBERT & GEIST, 2015). The brooding behavior and selectivity of hosts varies among mussel species. But information on host fishes is poorly known for most mussel species and local adaptations to specific host populations can occur (e.g. TAEUBERT et al., 2010, 2012; KARLSSON et al., 2014).

However, there is a risk that *A. anatina* and other species considered to be host generalists may be unable to exploit the majority of individuals in novel host communities that are the result of human-mediated biotic homogenization. In this case, biotic homogenization would have manifold implications for the persistence and conservation of these affiliate species (DOUDA et al., 2013).

In this study, we will evaluate based on laboratory experiment, the ability to exploit and the behavior of *Anodonta anatina* to the host community including native (*Squalius carolitertii*, *Squalius alburnoides*, *Pseudochondrostoma duriense*, *Luciobarbus bocagei*), and non-native species (*Alburnus alburnus*), evaluate their interaction and determine if these species can be host for *Anodonta anatina*.

3.2. METHODOLOGY

3.2.1. Host Fish determination for *Anodonta anatina*

The reproduction of the unionid species *Anodonta anatina* occur during winter season, normally between December and February. To develop the experiments, seven (7) pregnant females (99.4 ± 1.3 mm, mean \pm SD) of *Anodonta anatina* were captured and selected, between 15 and 28 of February, in the Sabor River, near Santulhão village (Figure 3.4).



Figure 3.4. Collection of pregnant females of *Anodonta anatina* and host fishes in River Sabor (near Santulhão village) (February 2020).

The identification of pregnant females was done after the observation of a swollen and grey aspect of outer gills, sign of the presence of glochidia in the gills. The selected *A. anatina* females were transported to the laboratory in aerated tanks and maintained in a small aquarium. For one week, it was checked the presence of glochidia conglomerates (Figure 3.5) and subsequently captured for the development of fish infestation procedures.

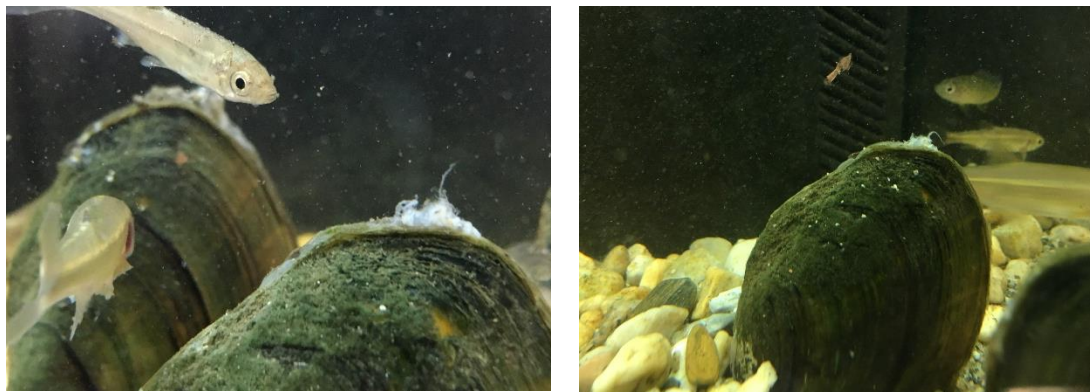


Figure 3.5. Glochidia conglomerates of *A. anatina* obtained in lab conditions (February 2020).

The fish species were captured just one week before the experiments (20 of January), using a portable electrofishing device, with direct (DC) and impulse (AC) current output (Hans Grassl ELT; 300-600V). It was followed the methodology described in the Manual for Biological Water Quality Assessment of Fluvial Systems according to the WFD: Sampling protocol and analysis for fish fauna (INAG 2008). All fishes captured were identified and measured with an ictiometer (precision 0.1 cm) and transported in aerated tanks to the Aquaculture Lab of Polytechnic Institute of Bragança, for an acclimatation period in captivity (**Figure 3.6**).

Five (5) fish species were used in this study: 1) four (4) native fish, such as Northern Iberian chub (*Squalius carolitertii*), Calandino roach (*Squalius alburnoides*), Douro Nase (*Pseudochondrostoma duriense*), Common Iberian barbel (*Luciobarbus bocagei*) all endemic cyprinids of Douro Basin and 2) one (1) non-native species, common bleak (*Alburnus alburnus*) (**Figure 3.6**).

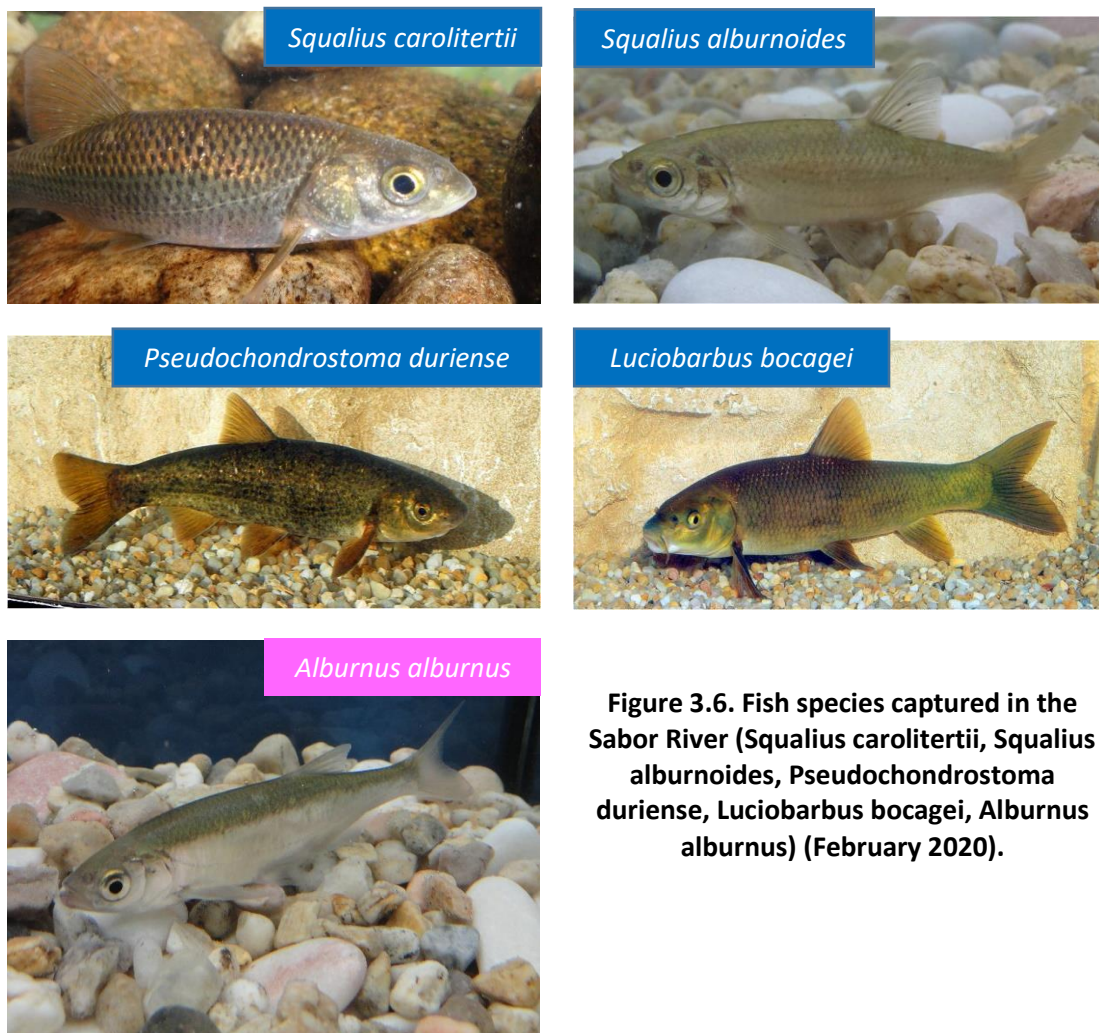


Figure 3.6. Fish species captured in the Sabor River (*Squalius carolitertii*, *Squalius alburnoides*, *Pseudochondrostoma duriense*, *Luciobarbus bocagei*, *Alburnus alburnus*) (February 2020).

The experiments were done in an Aquaneering Systems®, composed of 40 tanks of 16 L, with recycling water, temperature control system (maintained throughout the experiment a temperature between 18.0 – 20.0 °C), physical and activated carbon filters and UV light for maintenance of a good water quality (**Figure 3.7**).



Figure 3.7. Aquaculture laboratory: Aquaneering Systems (February 2020).

The experimental procedures in the Aquaculture Laboratory (IPB) began with the the infestation process on 20th of February. Previously, the viability of collected glochidia was checked, using a small portion of NaCl saturated solution to observe if mature glochidia presented fast opening/closing movements of the valves, as reported by DOUDA et al. (2013). After the glochidia collection, the seven wild duck mussel females were placed in the same river reach, where they had been previously captured.

All fishes were infested with a glochidia suspension of 2500 ± 128 glochidia/L. Previously, the fishes were placed for 30 minutes in a bucket, with aeration pumps promoting strong oxygenation and glochidia suspension, in order to increase the probability of fish infestation. After the infestation period, two fishes of each species were distributed in each tank of the Aquaneering System. Firstly, it was done a previous evaluation of the success of the infestation, through the observation of the fins and gills of a sacrificed fish sample on the 1st day of the experiment (**Figure 3.8**).



Figure 3.8. Infestation process and 1st evaluation of success procedures (February 2020).

The evaluation and registration of glochidia and juvenile counts was recorded on a videophotograph system, coupled with the stereomicroscope *Olympus SZX10*, with magnification 10-132x (Figures 3.8).



Figure 3.9. Identification of *Anodonta anatina*: glochidia (center) and juvenile (right).

During the experiments, it was used a protection (plastic net) on the bottom of each tank, to prevent the fish to feed juveniles of bivalves. Fishes were fed daily with a commercial flake fish food. Tanks were siphoned daily, using a net with a mesh size of 180 μm (Figure 3.9). The experiment was finished when, for each fish species, were not detected the production of juveniles after a period of 3 days without any registration.

3.3. RESULTS

The experiments showed that *Anodonta anatina* infested all fish species, considering both native (*Luciobarbus bocagei*, *Squalius alburnoides*, *Squalius carolitertii* and *Pseudochondrostoma duriense*) and non-native (*Alburnus alburnus*) cyprinids present in northeastern watercourses of Portugal, i.e. Sabor and Tua rivers (Douro Basin). From day 1 until day 9 it was observed the same decreasing tendency in the number of detached glochidia for each species, namely for endemic cyprinids (**Figures 3.10 to 3.13**).

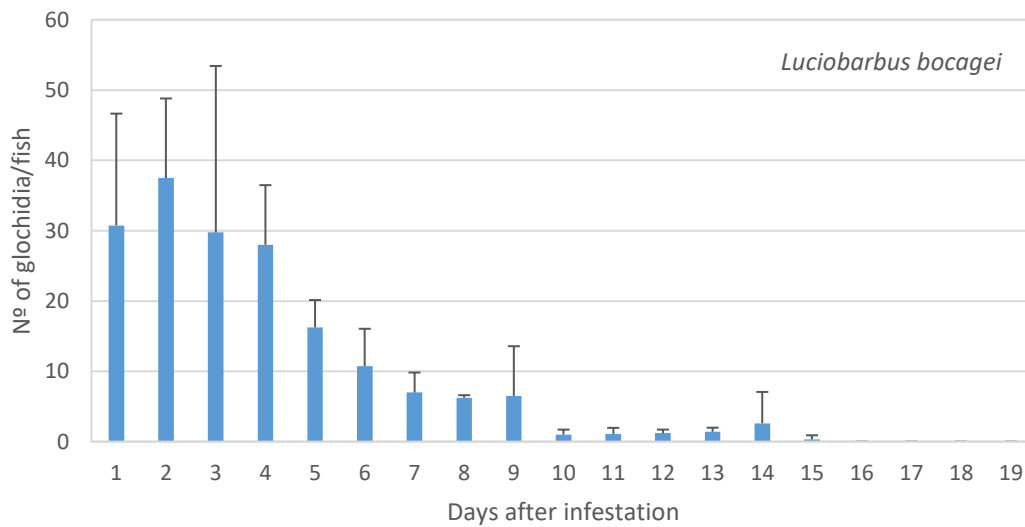


Figure 3.10. Variation of attached/detached glochidia number (Mean+SD) of *Anodonta anatina* on native host-fish *Luciobarbus bocagei* after infestation (February 2020).

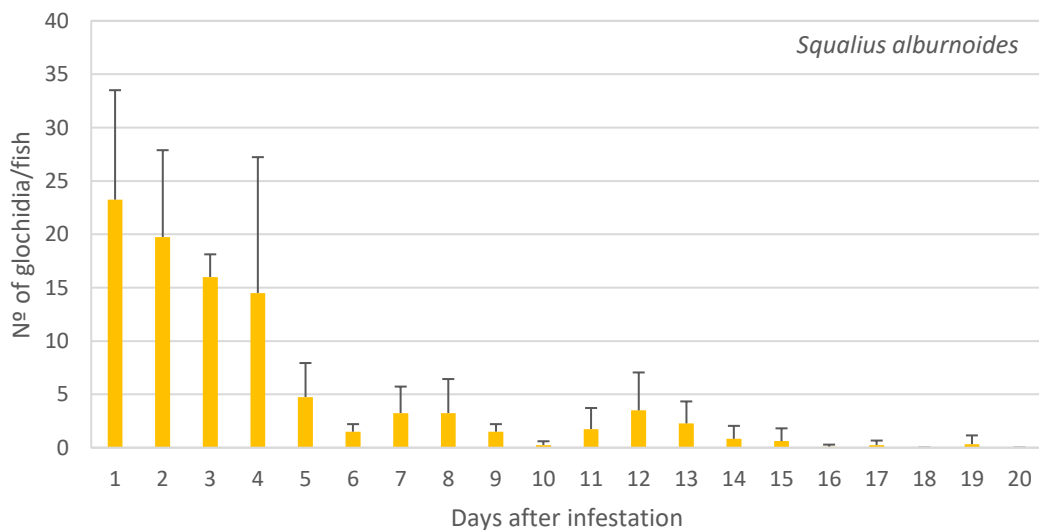


Figure 3.11. Variation of attached/detached glochidia number (Mean+SD) of *Anodonta anatina* on native host-fish *Squalius alburnoides* after infestation (February 2020).

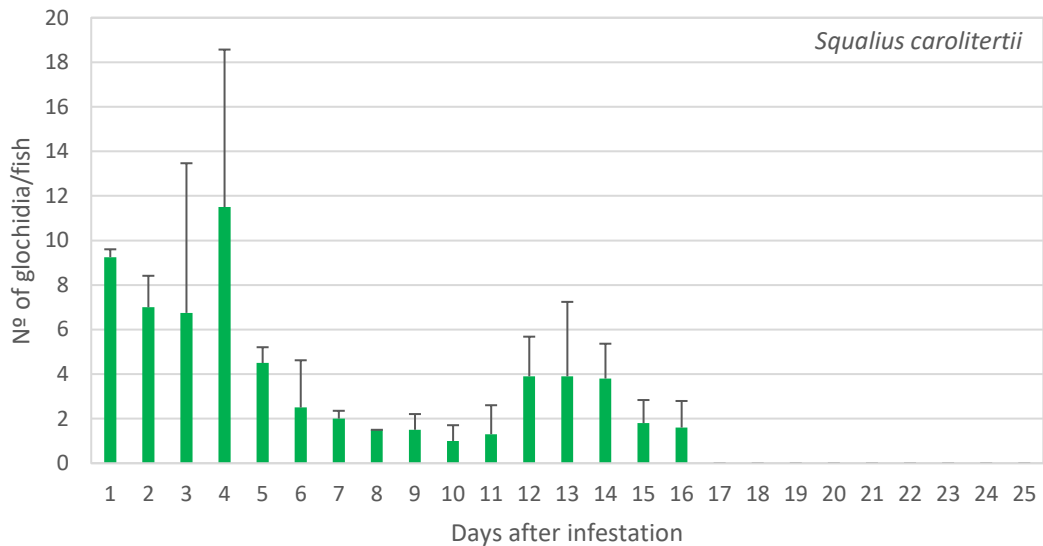


Figure 3.12. Variation of attached/detached glochidia number (Mean+SD) of *Anodonta anatina* on native host-fish *Squalius carolitertii* after infestation (February 2020).

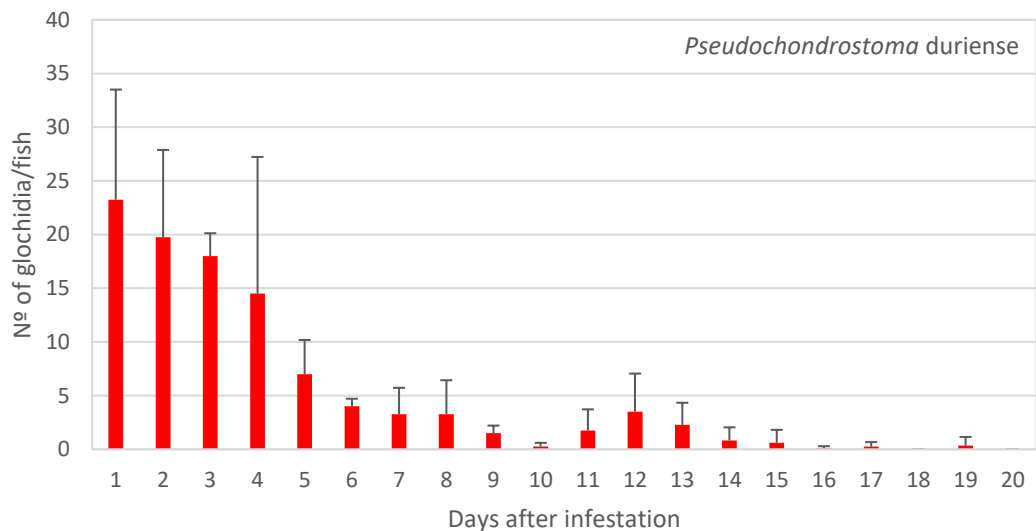


Figure 3.13. Variation of attached/detached glochidia number (Mean+SD) of *Anodonta anatina* on native host-fish *Pseudochondrostoma duriense* after infestation (February 2020).

In the infestation of the non-native species, *Alburnus alburnus*, it was detected a low number of detached glochidia, comparatively with most of native cyprinids (i.e. *L. bocagei*, *S. carolitertii*, *P. duriense*) (Figure 3.14).

From day 9 to day 17 it was also detected some open shells, identified as dead juveniles (not viable) for all species.

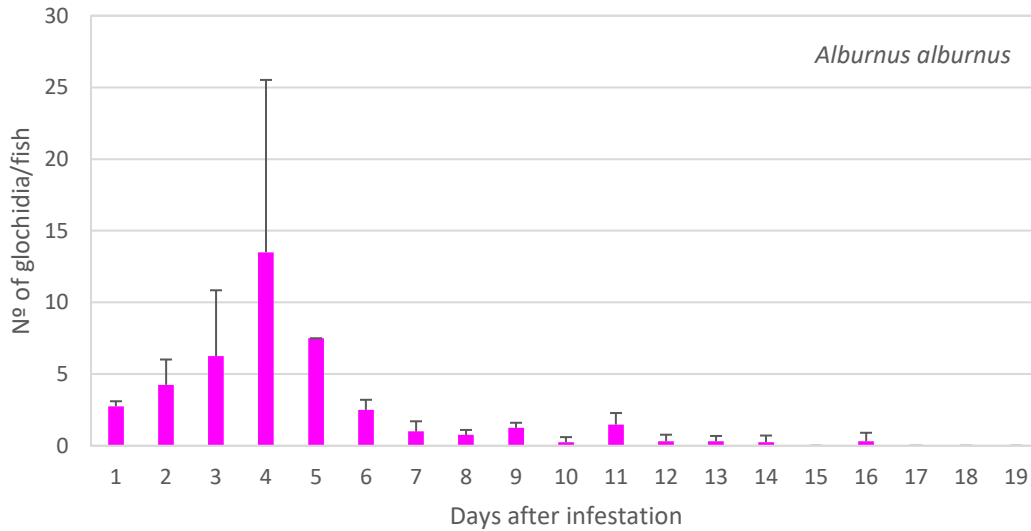


Figure 3.14. Variation of attached/detached glochidia number (Mean+SD) of *Anodonta anatina* on non-native host-fish *Alburnus alburnus* after infestation (February 2020).

A. anatina glochidia successfully transformed into viable juveniles for all fish species tested (100%). The complete metamorphosis started, for host native species, at day 9 and finished differently from day 17 (e.g. *Luciobarbus bocagei*) to day 25 (e.g. *Squalius carolitertii*) (Figures 3.15 to 3.18). In the case of the non-native species the period observed for the production of viable juveniles ranged between day 8 and day 17 (Figure 3.19).

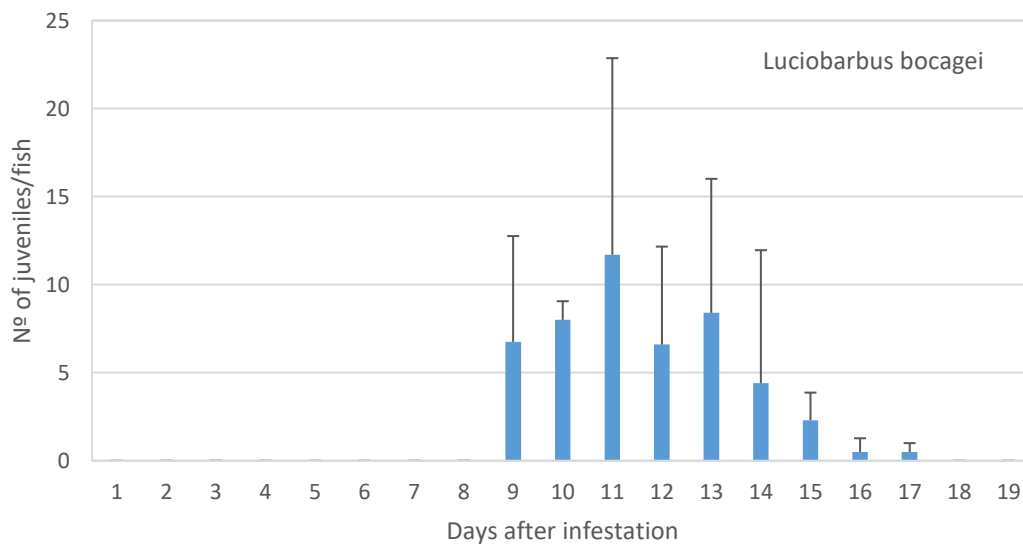


Figure 3.15. Variation (Mean+SD) of juveniles per fish of *Anodonta anatina* for the host fish species *Luciobarbus bocagei* in lab conditions (February 2020).

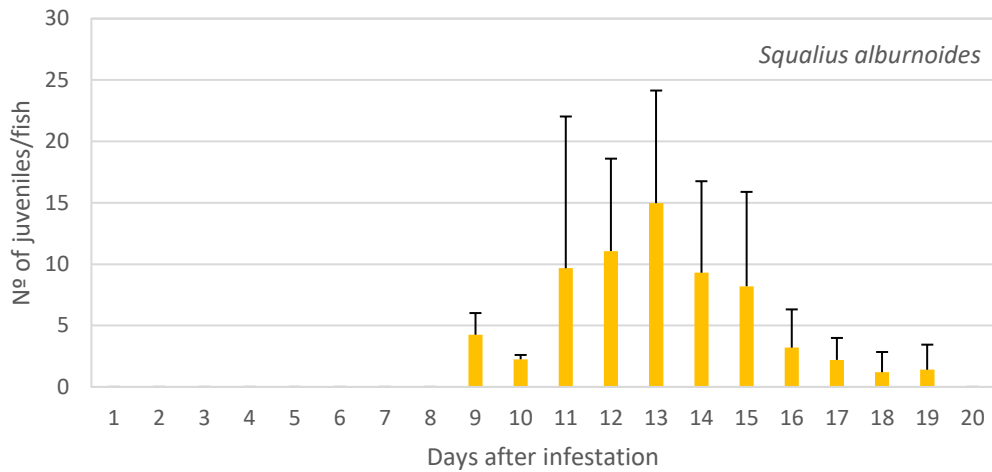


Figure 3.16. Variation (Mean+SD) of juveniles per fish of *Anodonta anatina* for the host fish species *Squalius alburnoides* in lab conditions (February 2020).

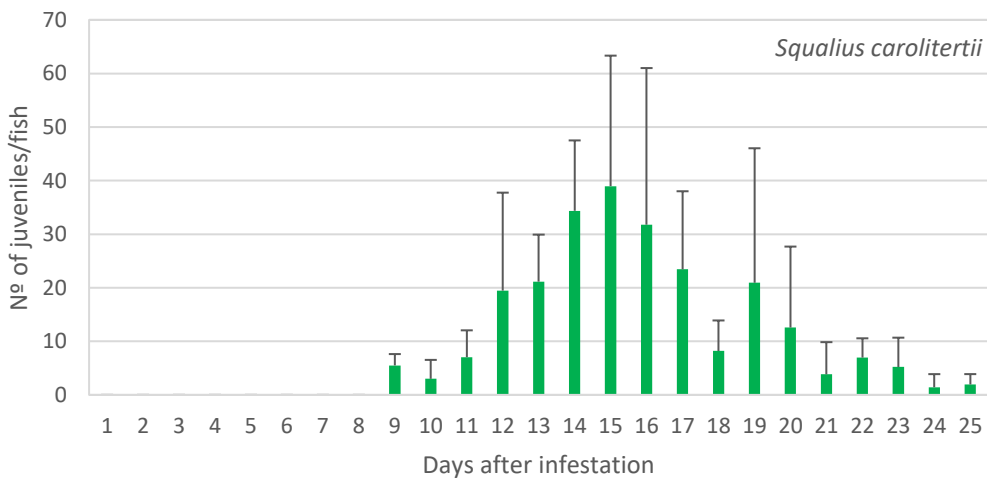


Figure 3.17. Variation (Mean+SD) of juveniles per fish of *Anodonta anatina* for the host fish species *Squalius carolitertii* in lab conditions (February 2020)

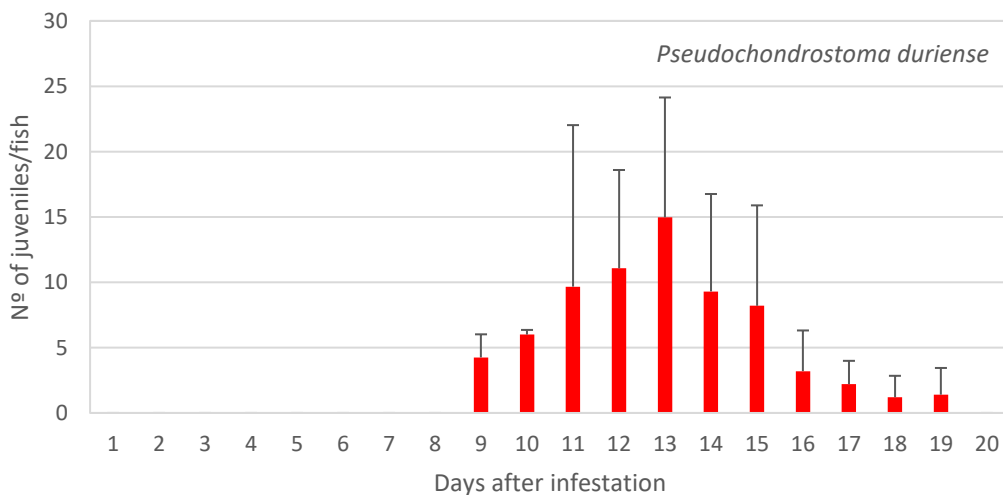


Figure 3.18. Variation (Mean+SD) of juveniles per fish of *Anodonta anatina* for the host fish species *Pseudochondrostoma duriense* in lab conditions (February 2020)

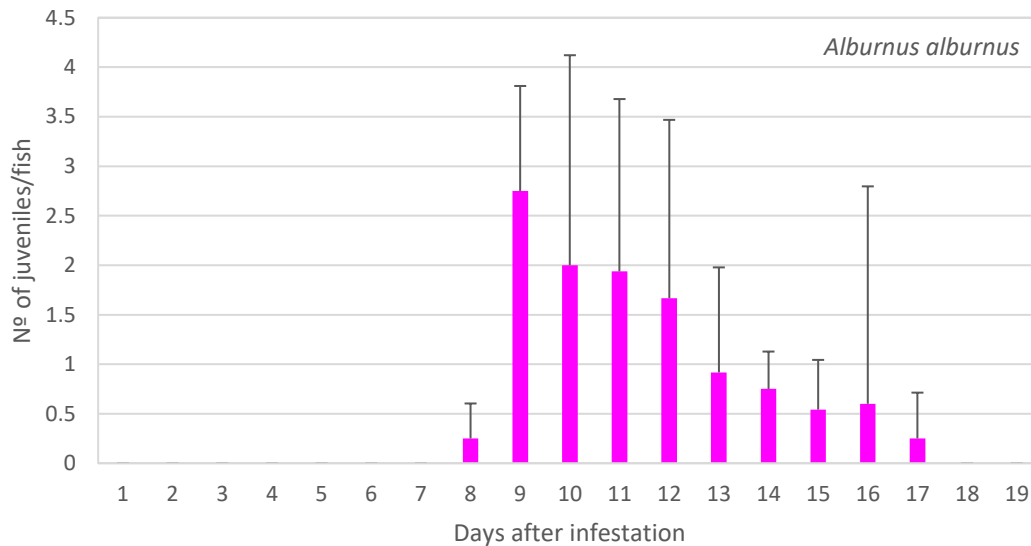


Figure 3.19. Variation (Mean+SD) of juveniles per fish of *Anodonta anatina* for the host fish species *Alburnus alburnus* in lab conditions (February 2020)

The transformation rate ranged between 13.1% detected for *Luciobarbus bocagei* and 75.7% for *Squalius carolitertii*. Fully developed juveniles were obtained between day 8, only for the non-native species *Alburnus alburnus* and day 25 for *Squalius carolitertii* (mean temperature of 20.0 °C) (Table 3.1, Figure 3.20).

Table 3.1. Compatibility test results, including the number and mean (\pm SD) length of fish per species, mean number of attached glochidia, mean number of viable juveniles produced and transformation rate. ‘Transformation rate’ indicates the proportion of *Anodonta*.

Fish species	Fish number/ (replicates)	Mean \pm SD Fish length (mm)	Mean number glochidia per fish	Mean number Juveniles/ fish	Transformation rate (%)/ Degree days
Native					
<i>L. bocagei</i>	14 (7)	9.0 \pm 2.5	231	35	13.1 (180 – 340)
<i>S. alburnoides</i>	18 (6)	7.2 \pm 1.2	31	12	27.1 (180 – 380)
<i>S. carolitertii</i>	12 (6)	8.2 \pm 1.8	114	357	75.7 (180 – 500)
<i>P. duriense</i>	16 (8)	10.2 \pm 2.4	125	53	29.8 (180 – 380)
Non-native					
<i>A. alburnus</i>	12 (6)	6.6 \pm 1.4	51	14	21.7 (160 – 340)

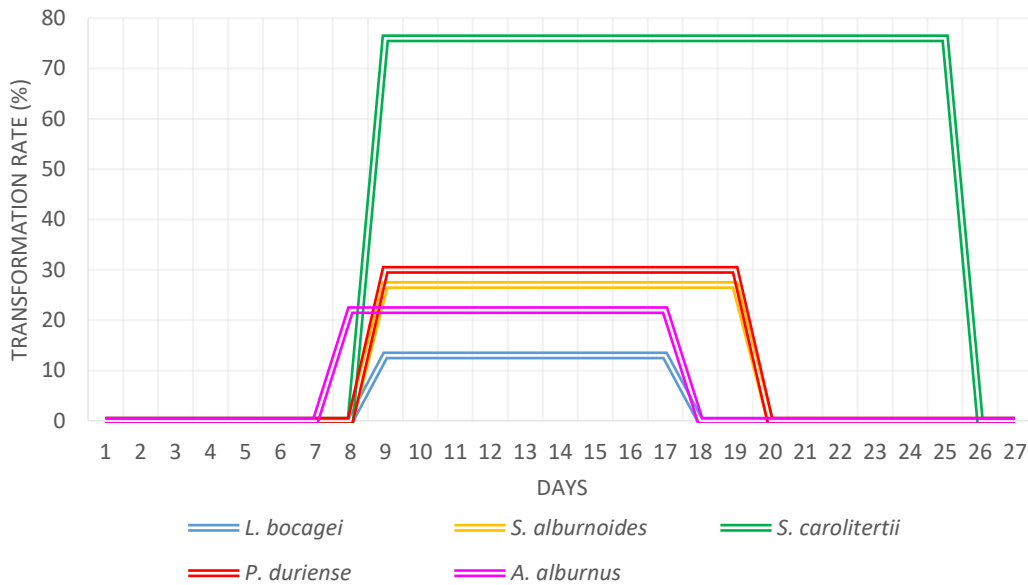


Figure 3.20. Glochidial transformation rate and attachment periods (bars) per fish host species

3.4. DISCUSSION

The complex life cycle of Unionoid mussels including an obligate parasitic phase is probably a major reason for their decline. Therefore, knowledge about basic ecological requirements such as host fish suitability is crucial when developing effective conservation strategies for this endangered freshwater mussel species (BARNHART et al., 2008; GEIST, 2010).

Through this study, we can confirm the status of *A. anatina* as a host generalist, showed in its performance to develop successfully on all five fish species tested, given by the transformation rate between 13.1% and 75.7%. These results are according with other studies (WEBER, 2005; DOUDA et al., 2013), that corroborated the ability of *A. anatina* to parasitize almost every native fish species that is exposed to its infective glochidia, but also non-native species (i.e. *Alburnus alburnus*) in Iberia.

Indeed the transformation rates were especially high in all fish species from the genus *Squalius*, followed by *Pseudochondrostoma*, and *Luciobarbus* species. This indicates a strong co-evolutionary relationship of *A. anatina* with native co-occurring fish and especially with *Squalius* species. Another important issue determining the co-evolutionary dynamics of host–affiliate relationship between particular taxonomical groups are the costs and benefits to both partners (SHELDON & VERHULST, 1996). When

the costs to hosts are low compared to the benefits to affiliates, the selection power for host defense may be relatively weak (SADD & SCHMID-HEMPEL, 2009). This is probably also the case of *A. anatina* and the other species of unionid bivalves, which cause only weak nutritional and fitness costs to their hosts (FISHER & DIMOCK, 2002; CRANE et al., 2011); nevertheless, these species cannot complete its development without hosts. As a result, selection for defense against parasitizing glochidia may be weak, leading to a rapid adaptation of mussels to their local fish species (DOUDA et al., 2013).

We also observe that the period of infestation, the duration of metamorphosis phase as well as the duration and rate of juvenile mussel differed significantly between all host species. For DIAS et al. (2020), the differences observed among host species may result from differences in behavior and microhabitat preferences between fish species. The example is given with *Pseudochondrostoma duriense* (northern straight-mouth nase) which prefers microhabitats with a higher current velocity, and usually occurs further away from the river banks, thus lowering the chance of being infested by *A. anatina* glochidia, comparatively with the *Squalius carolitertii*, which occupy a higher position in the water column, enabling feeding on drift.

Additionally, the ability of *A. anatina* to successfully develop on fish species depend on the whether the species are native or non-native. And the same result appear in numerous study of authors like DOUDA et al. (2013), who found an average transformation rate (\pm SD) of $33.6 \pm 20.3\%$ for native species and $6.0 \pm 15.4\%$ for non-native species tested. However, the authors attributed these differences to the geographical origin since the non-native fish species used in the experience were from a distant geographical region from the mussels. Although there are no clear rules governing host compatibility between host and affiliate species from distinct regions (POULIN, 2007). The fish community tested in our study were from same river and the result obtained didn't allow us to highlight this difference. Moreover, this result remain based on a laboratory experience, like most of the available data that also exclusively refer to artificial infestations in the laboratory which provide insights into the relative suitability of different fish hosts under standardized conditions (BEDNARCZUK, 1986; MAAß, 1987)

However, such approaches do not necessarily reflect the situation under natural conditions and are thus not reliable when the contribution of different fish species to the recruitment (HOCHWALD, 1997; TAEUBERT et al., 2012), of *A. anatina* needs to be evaluated. Further studies may be needed in natural ecosystems in order to find the functional hot-fish community for *A. anatina*.

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Chapitre 4

CONCLUSIONS AND FINAL CONSIDERATIONS

The **main conclusions** of the present work can be summarized in the following topics, highlighting the influence of abiotic and biotic factors in the conservation and management of lotic ecosystems in NE of Portugal:

- 1) In the River Fervença, a low-order watercourse, tributary of the Sabor river (River Douro basin, NE of Portugal), significant signs of disturbance were detected, namely in the vicinity of an urban area, the city of Bragança (~ 23,500 inhabitants). The metrics used were sufficiently sensitive to determine a decrease in the abiotic and biotic components reported by:
 - a. Lower physical-chemical quality of the water, based on the substantial increase in dissolved salts and nutrients (namely nitrogen and phosphorous compounds) and on the decrease in the oxygenation rate (needed for the decomposition of organic matter);
 - b. Superior degradation of aquatic habitats downstream of Bragança by the embebedeness of the interstices with organic and inorganic materials and consequent homogenization of the microhabitat substrate granulometry;
 - c. Modification of natural riparian galleries of alder, ash and willow trees. The recent detection of death of alder tree and an increase in the presence of hybrid poplar plantations will contribute in the immediate future to further degradation of the riverine environment;
 - d. Loss of biodiversity in invertebrate communities. In fact, water pollution and eutrophication led to the disappearance of stenobiont taxa - insects of the orders Ephemeroptera, Plecoptera and Trichoptera whose abundances are dominant in low-order watercourses and with good ecological integrity. In the case of the disturbance noted in the River Fervença, the dominant euribionts taxa belong to the Diptera, Hirudinea, Oligochaeta, Gastropoda and Crustacea faunistic groups;
 - e. Alteration in the functional feeding groups of the lotic system, reflected in the energy flow along the food chain, due to the role played by macroinvertebrates as a link between lower levels (plants) and upper levels (fish);
 - f. The degradation of lotic ecosystems is usually accompanied by the introduction of non-native species, some of them with invasive potential trend. This is the case of red swamp

crayfish of Louisiana (*Procambarus clarkii*) and signal crayfish (*Pacifastacus leniusculus*) present in the Sabor basin and in particular in the Fervença river;

g. In turn, at the level of the fish community, the size of the river and the pressures accumulated over time are at the base of the distribution pattern found. In this context, the presence of 2 non-native species, such as *Gobio lozanoi* and *Lepomis gibbosus*, reinforce human action in the introduction of species and consequent modification of the fish community.

2) The influence of biotic factors and in particular the parasitism/symbiosis relationship established between a bivalve and the fish community present in the Sabor River basin, warns of the high complexity existing in lotic ecosystems. Indeed, the co-evolutionary mechanisms established over extended time periods also reflect the integrity and vulnerability of these systems, namely to unpredictable disturbances. In the case of a study concerning laboratory tests developed for the bivalve species *Anodonta anatina* showed that:

a. For all the native and non-native species tested, it was possible to obtain viable juveniles of *A. anatina*, confirming the worldwide distribution of this species, contrary to other unionid species present in the same river (e.g. *Unio delphinus* and *Potomida littoralis*) dependent only of native fish for their reproduction success.

b. The Northern Iberian chub, *Squalius carolitertii*, was the species where the metamorphosis rate was higher probably because both species tend to colonize river zones near the banks increasing the specific relationship between both species.

In terms of **final considerations**, several measures for the conservation and rehabilitation of the lotic ecosystems of the NE of Portugal must be focused:

- 1) Elimination of the point source pollution and their adequate treatment in WWTP's and mitigate the effect of diffuse pollution increasing, for example, the riparian galleries and avoiding the excessive use of pesticides and fertilizers;
- 2) Establishment of aquatic and riparian rehabilitation programs for the recovery of ecosystem services;
- 3) Avoidance of the introduction of invasive alien species, recurring to prevention, mitigation and eradication in particular habitats with high conservation values;
- 4) Promotion of appropriate legislation and fiscalization oriented to the development of good practices in wild aquatic and riverine environments;

- 5) Education and environmental awareness with all stakeholders related to water policy and management of freshwater ecosystems;
- 6) Definition of a Global Action Plan for the management and conservation of priority habitats and native aquatic species in NE Portugal.