Czech University of Life Sciences Prague
Faculty of Environmental Sciences
Department of Applied Ecology

DIPLOMA THESIS

MSc. Nature Conservation

Occurrence and ecology of freshwater shrimp (*Gammarus fossarum*) in water springs of Lusatian Fault

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Advisor: Mgr. Michal Bílý, Ph.D.

Prague, 2018

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

Diego Sebastian Serrano Suarez

Nature Conservation

Thesis title

Occurrence and ecology of freshwater shrimp (Gammarus fossarum) in springs of Lusatian Break

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Objectives of thesis

Detect main factors affecting presence and abundance of G. fossarum in a set of 40 springs across the Lusatian break area

Methodology

1)Sampling benthic fauna in selected springs

2) Sorting the samples and selecting of G. fossarum specimens.

3) Determination of the other benthic species to main large taxonomic groups.

4) Counting of G. fossarum abundance (absolute numbers) in every sample and G. fossarum dominance (% of occurrence among the benthic community) in every sample

5) Analysis of factors affecting G. fossarum occurrence and dominance based on available physical and chemical and biological data including heavy metal analysis in sediment and biota.

The proposed extent of the thesis

70

Keywords

freshwater shrimp, Gammarus fossarum, springs, Lusatian Break

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Recommended information sources

Barquín, José, and Mike Scarsbrook. ”Management and conservation strategies for coldwater springs.” Aquatic Conservation: Marine and Freshwater Ecosystems 18.5 (2008): 580-591.

Lampert, Winfried, and Ulrich Sommer. Limnoecology: the ecology of lakes and streams. Oxford university press, 2007.

MacNeil, Calum, Jaimie TA Dick, and Robert W. Elwood. ”The trophic ecology of freshwater Gammarus spp.(Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept.” Biological Reviews 72.3 (1997): 349-364

Ticha, Kamila, et al. ”Detrital components in submontane organogenic springs in relation to their morphology, microhabitats and macroinvertebrates.” Pol. J. Ecol 60.1 (2012): 163-175

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Electronic approval: 15. 11. 2017

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Dean

Prague on 10. 04. 2018

DECLARATION

This report is intended to fulfill the graduation requirements of the author for the Master of Sciences in *Engineering Ecology - Nature Conservation* at the Czech University of Life Sciences, Prague. It has been written with the guidance of thesis supervisor, Dana Komínková and thesis advisor, Michal Bílý. All sources of external information are cited in the references chapter, according to scientific protocols.

Although the author and the research colaborators of the project have made every effort to ensure that the information in this thesis was correct at press time, the author and Pramení Spojují team do not assume and hereby disclaim any liability to any party for any disruption caused by errors or omissions.

ACKNOWLEDGEMENTS

I am very fortunate to have had so many people assist me in producing this research and in learning about the importance of helping the world address water scarcity. Listening to experts on different disciplines allowed me to see the complexity of the water springs science and inspired me to learn more and to include more variables for the analysis of springs' ecology.

While developing this project I had the chance to interact with specialists on hydrology, water chemistry, geology, ecology, cartography, landscape, conservation, law, education, journalism, and others working on water related topics. It makes me feel optimistic about science. Their generous conversations were inspiration to develop this research.

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When starting the project, it was my advisor Michal Bílý, the one who guided me to define the aim of the research. His expertise in zoology of aquatic invertebrates, sampling methods and laboratory skills allowed me learn a lot about ecology and comprehensive research techniques. I thank Michal for his dedication and unconditional help.

The Prameny Spojují team has been always helpful. Every person I have reached with questions about the project has been very supportive. I thank Jiří Šmida, the project leader, because the conversations since the first workshop helped me to understand Czech culture and the importance of maps. His communication skills lead the project in right direction.

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...Continued on next page

I thank to my travelling companion, Juan Camilo Gómez Ángel, for his unconditional support providing me design guidance and effective communication skills. His words and the trip to the rainiest region on Earth were crucial for the growth of my research.

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Jan Plesník from the Czech Nature Conservation Agency who taught me the importance of understanding well the basics, travelling and telling stories to educate about nature.

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Water springs connect, no doubt about it!

PROJECT FRAMEWORK

This Diploma Thesis was developed within the cooperation project *Prameny spojují krajiny a státy - environmentální vzdělávání a kooperace v regionu Liberec- Zittau*, (Springs connect countries and states - environmental education and cooperation in the region Liberec-Zittau), which was financed by the European Regional Development Fund, # 100249739, **2016 – 2019**.

The general source of information about the water springs characteristics belongs to the project database, developed by multidisciplinary team from three universities, with the participation of the author.

- Technical University of Liberec
- Czech University of Life Sciences Prague
- Technical University of Dresden

About the Project

The main objective of the project is to establish a co-operative network of academic and non-academic institutions operating directly (located in the territory) or indirectly (activities carried out with an impact on the territory) in the Liberec-Zittau border area. Its purpose is to joint environmental education, transfer of know-how, mobility of students and experts in the fields of geography, hydrology and ecology of springs in the field of this location and their economic use. The network will build the foundation for an accredited double-degree education program.

Occurrence and ecology of freshwater shrimp (Gammarus fossarum) in water springs of Lusatian Fault

Abstract:

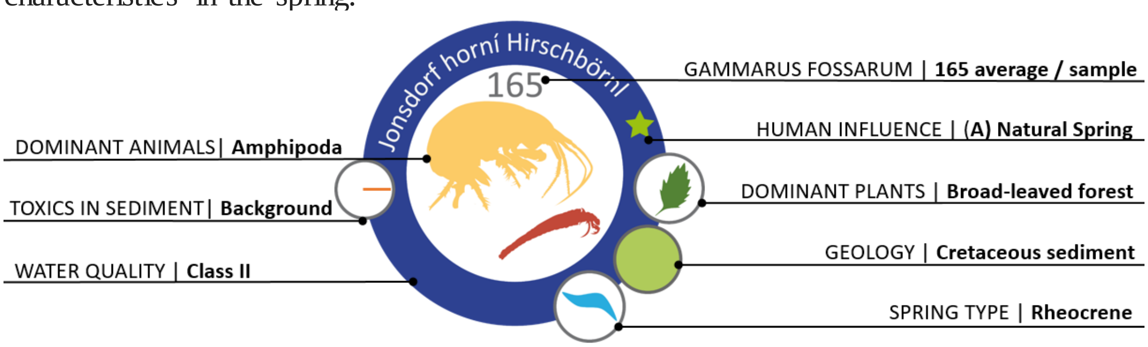
This research studied water springs in the Czech-German border to understand patterns of water quality and the distribution of small amphipods *Gammarus fossarum*. The rocks along the geological fault offer a diverse set of springs in this humid region, and *G. fossarum* is the most abundant aquatic invertebrate found among the analyzed springs. This widespread shrimp dwells in 27 of 40 springs and prefers aquatic habitats with at least 4.5mg/L of dissolved oxygen and 5.5 pH units for this region. Its adaptability was evaluated with several factors of influence related with their population number. The groups analyzed were dominant geology, plants, spring type, human influence, land cover, toxic metals in sediment, and water quality. For each group, some categories established its preference of habitat. It was found, that very strongly polluted water quality (Class V, according to Czech legislation) limits their distribution, *G. fossarum* prefers Class III and II. The most thriving populations are usually in waters with high dissolved oxygen, neutral pH and low toxic metals. They adapt to any type of geology across the fault. Neither spring type, nor dominant forest or human influence had a clear trend on their numbers, however, springs with higher toxic metals concentration in sediment (As, Cd, Cr, Cu, Pb, Ni, Zn) showed relation with less abundance of shrimps. It was confirmed that artificial bottom substrate in a spring prevents *Gammarus* presence and reduces general benthic diversity. In the time being, it is crucial to detect the main factors affecting presence and abundance of this species because it can help to assess the impact of land cover changes through time.

Keywords:

Water spring; G*ammarus fossarum* distribution; freshwater shrimp; aquatic ecology; habitat selection; Lusatian fault; water quality.

Graphic best habitat:

Gammarus fossarum adapts to a wide variety of springs habitats. However, the most abundant populations found in this study presented the following environmental characteristics in the spring.



Design: Diego Sebastián Serrano Suárez
Art: Juan Camilo Gómez Ángel

Výskyt a ekologie blešivce potočního (*Gammarus fossarum*) v pramenech Lužického zlomu

Abstrakt:

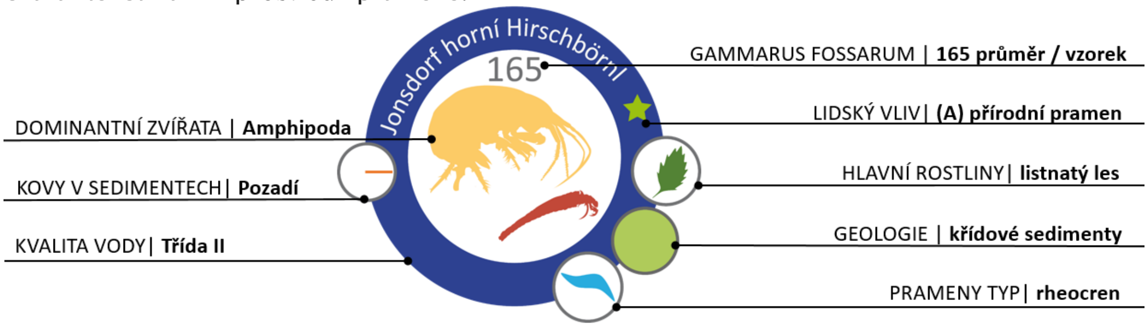
Práce se zaměřila na studium pramenů v oblasti Česko - Německého pohraničí s cílem porozumět výskytu drobných korýšů *Gammarus fossarum*. V této na srážky bohaté oblasti, horninové složení v oblasti geologického zlomu umožňuje vznik velkého množství pramenů. *Gammarus fossarum* je nejrozšířenějším druhem makrozoobentosu pramenů této oblasti. Tento široce rozšířený korýš se vyskytuje v 27 ze 40 sledovaných pramenů a preferuje v této oblasti vodní habitaty, které se vyznačují koncentrací rozpuštěného kyslíku větší než 4,5mg/L a pH vyšším než 5,5. Adaptační schopnost tohoto druhu byla hodnocena dle řady parametrů ve vztahu k početnosti populace. Mezi hodnocené parametry patří převládající geologické podloží, typ pramene, lidský vliv, půdní kryt, toxické kovy v sedimentech a kvalita vody. Pro každý parametr, bylo určeno několik kategorií, dle kterých byl stanoven vhodný habitat. Výsledky ukázaly, že velmi znečištěná voda (V. třída kvality dle ČSN 757221) není vhodná pro výskyt *G. fossarum*, který preferuje kvalitu vody odpovídající II. a III. třídě kvality. Nejlépe prosperuje *G. fossarum* ve vodách s vysokým obsahem kyslíku, neutrálním pH a nízkými koncentracemi toxických kovů. Druh se adaptoval na všechny horninová podloží v oblasti geologického zlomu. Typ pramene, dominantní vegetace nebo lidský vliv nemají jasný trend ovlivňující početnost populace. Naopak prameny vyznačující se zvýšeným obsahem toxických kovů (As, Cd, Cr, Cu, Pb, Ni, Zn) v sedimentech se vyznačují poklesem abundance *G. fossarum*. Prameny s umělým dnovým substrátem nepodporují výskyt *G. fossarum* a obecně vedou k poklesu diversity bentického společenstva. V současné době je důležité zjišťovat hlavní faktory ovlivňující přítomnost a hojnost tohoto druhu, protože může pomoci posoudit dopad změn půdního krytu v čase.

Klíčová slova:

Prameny; výskyt *Gammarus fossarum*; sladkovodní korýš; ekologie vodního prostředí; výběr habitatu; Lužický zlom; kvalita vody.

Grafický nejlepší habitat:

Gammarus fossarum se přizpůsobuje širokému spektru pramených stanovišť. Nicméně nejpočetnější populace nalezené v této studii jsou spojeny s následujícími charakteristikami prostředí pramene.



Návrh: Diego Sebastian Serrano Suárez
Umění: Juan Camilo Gómez Ángel

Table of contents

List of figures

List of tables

List of maps

Annex A: Statistical protocol

Annex B: Photos of the springs

CHAPTER 1

Introduction

This chapter presents an overview of water springs. Firstly, a general background related with springs formation and ecology is provided. After that, the motivation of studying the influence of habitat variation in *Gammarus fossarum* populations. Then, the formulation of the objectives and research questions, including the analyzed environmental factors. Last but not least, the innovation and practical value are explained. This is a fundamental chapter to illustrate the expectations of the research. Likewise, it is also a preparation for the detailed description of the ecological requirements of *G. fossarum* in the next Chapter.

1.1. Background

The springs across Lusatian fault offer special habitats for invertebrates with relatively constant temperature during the year, and no big predators like fish. The water chemistry in the aquifer that holds the water beneath the surface is bound to the soil composition. Also, the rain water composition, filtered by this soil during long periods of time can influence the spring characteristics. The existence of a spring depends on three main factors: the hydrologic conditions recharging the aquifers, the soil composition affecting the infiltration rate, and the vegetation regulating water retention. The topography and edaphic layers will determine where exactly the water appears coming out from the ground. The water characteristics are crucial to define the services that these ecosystems can provide like source of drinking water, agriculture, mining processes, energy generation and even aesthetic purposes. In addition, when the water runs naturally, without human intervention, it supports essential habitats for aquatic invertebrates that can influence the whole ecosystem structure, interconnecting other animals, and even plants in the surroundings.

Groundwater and surface water are fundamentally interconnected, they recharge each other and hence they also contaminate each other (Trček & Zojer, 2010). While clean water is source of life and support biodiversity, polluted water can spread toxic conditions with disastrous consequences, therefore the importance of monitoring water quality through time. These studies should include the whole contribution area (springshed) to detect sources of pollution and control the damages, however this area is hard to delimitate realistically, because springs are influenced by underground water dynamics. Springs pollution may be caused by wastewater discharges, urban or agricultural runoff, soil erosion, lixiviates from mines or garbage dumps and acid rain that also dissolves away metals and minerals in soil. Accelerating the extraction rate of water contributes to dry out the springs. Also destroying vegetation and adding paved surfaces decrease chance of recharge when rain comes. Every time the land use changes, the quality of a spring might be affected because increasing impervious surface coverage homogenizes the trait diversity of macroinvertebrate communities in streams (Barnum, Weller, & Williams, 2017). When the watershed has paved portions, less amount of water can percolate through the soil and therefore the springs recharge will be affected. Besides the runoff composition from impervious surfaces, higher flow could flush away benthic habitats.

The spring characteristics in central Europe can be assessed using *Gammarus fossarum*, a common spring dwelling species. The yellowish bodies of these small shrimps, can be seen jumping rapidly under water or attached to surfaces while eating detritus from other benthic organisms, fallen leaves, sticks, algae, plankton, dead animals or feces. Species like *Gammarus fossarum* abundantly inhabits mountain brooks of central Europe (Schmidlin, Fumetti, & Nagel, 2015b), and they could be used as bioindicators of water quality, knowing their preferred habitat conditions e.g. high dissolved oxygen and neutral pH (Pestana, Ré, Nogueira, & Soares, 2007). Also, *Gammarus* are useful to measure toxic metals accumulated in its tissue, giving a clue of chronic pollution in the area. This is convenient when analyzing the impacts of different landscapes and assessing the water spring health.

In general, the pollution of environment will decrease the benthos diversity when it is caused by excess of nutrients, organic matter or toxic chemicals, limiting the uses of water. Runoff from agriculture and paved zones is an important threat for water quality of springs in this area and also wastes from settlements can damage the ecosystem. However, there is a lack of knowledge about the actual infiltration areas that influence the aquifers. There is not enough data about connections of the underground water and therefore, it is hard to predict the sources of pollution in springs.

Each site has a unique combination of environmental factors and, thus, a unique composition of species (Liess & Von Der Ohe, 2005). It is important to assess together several factors of water quality in assays implemented with gammarids (Coulaud et al., 2011) because individual information can mislead the habitat components of our species of interest. In this case, the distribution of *G. fossarum* in the field will allow to understand their needs for survival in terms of different variables.

1.2. Objectives of thesis

1.2.1. General Objective

Detect main factors affecting the presence and abundance of *Gammarus fossarum* in a set of 40 springs across the Lusatian Fault area, in the Czech-German border.

1.2.2. Specific Objectives

- To compile information of *G. fossarum* populations and its ecological needs.
- To understand the implications of environmental changes on *G. fossarum* abundance, detected in water springs of central Europe.
- To design a comprehensive methodology that integrates water quality data with benthic animals, and other factors influencing the springs in the region.

1.2.3. Research questions

Evidencing ranges of survival in natural conditions of this area, can allow to synthetize the habitat preferences of *Gammarus fossarum*. This study will put together the knowledge about aquatic ecology analyzing different environmental factors that can influence the invertebrates' diversity. These factors from different disciplines will be evaluated to solve two main questions.

- Water chemistry
- Hydrological spring types
- Geological units
- Dominant plants
- Pollution of sediment by toxic metals
- Land use in the spring watershed

<u>Question 1</u>: Which of these conditions are related with the **absence** of *G. fossarum* in some water springs of the Lusatian Fault?

<u>Question 2</u>: What are the factors that matter the most to find **abundant** *G. fossarum* populations? Does the runoff watershed land cover have any influence?

1.2.4. Hypothesis

<u>Answer to question 1</u>: The **absence** of *G. fossarum* is related with acid water, and therefore granite geology (rich in ions) and conifer forest. Also, higher toxic metals concentration and lower dissolved oxygen reduce the fitness of this species.

<u>Answer to question 2</u>: The most **abundant** populations should be in springs with a higher percentage of forest cover in the spring watershed, with neutral pH and high dissolved oxygen.

1.3. Motivation

These 40 springs represent and important asset in the human landscape, as some of them are used by the community for drinking purposes and touristic point. Moreover, they support plant growth by adding moisture to the soil and improving the nutrient cycle, which is useful for farming and silviculture. Springs offer direct habitat for invertebrates, however, also birds and mammals are beneficiated by springs in the ecosystem.

Figure 1: Water spring in the project area, AS1043.

While individuals think in terms of months and years, water planners need to plot activity mostly in decades.

Seth M. Siegel
(Let there be water)

To understand the long-term consequences of modifying the water quality on nature, it is necessary to integrate different sciences, including ecology. This study follows the distribution of aquatic invertebrates in springs of central Europe, focusing on a common freshwater shrimp to analyze different environmental conditions that can influence the water dynamics, chemistry or hydrological connections, and therefore their success to colonize a spring. This comprehensive study compiles data about *Gammarus fossarum* ecology and their potential as bioindicators. Also, it opens the conversation about watershed management, to clarify the debated effect of landscape composition in the surroundings of each spring, due to expansion of agriculture.

1.4. Innovation and practical value

This study can offer high quality maps to explain the findings about *G. fossarum* distribution across the Lusatian fault. It will be a tool for reaching the local community and improve their knowledge about springs in a multidisciplinary arena. This map will allow scientists, decision makers and citizens to recognize the factors affecting their water quality in each watershed. **The challenge is to make it simple, clear and applicable, so it can have a real impact in shaping our environment in a positive way, even influencing water regional policies.**

CHAPTER 2

Literature Review

The purpose of this chapter is to understand the context of *Gammarus fossarum* ecology and to provide a clear spectrum of variables that could influence their habitat preference and adaptation. It links with the previous chapter giving more detail information. Here the most relevant studies in this topic are classified according to the environmental factors they are measuring and it is divided in 3 main parts: Their distribution in Europe, the natural components of their ecosystems and their response to toxic concentrations.

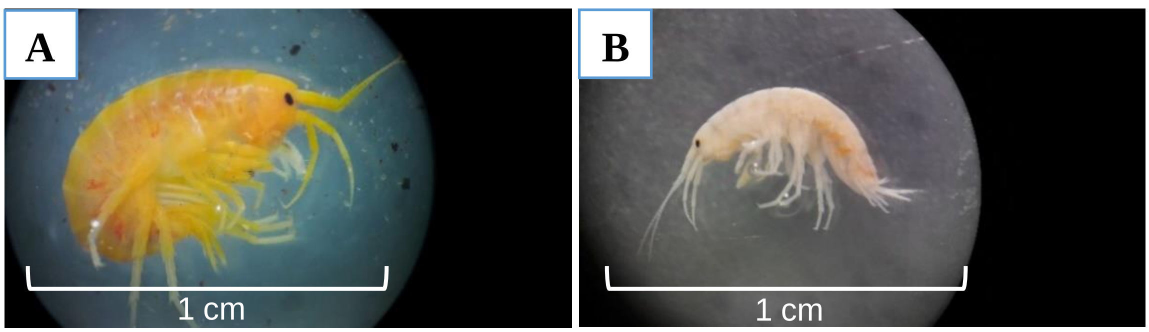


Figure 2: Individuals of freshwater shrimp Gammarus fossarum in stereoscope. A: Adult G. fossarum, B: juvenile G. fossarum. Source: Author

Gammarus sp. can be considered suitable organisms for assessing possible impacts of global change on species inhabiting springs (Noriega 2017). They are segmented invertebrates with 2 pairs of antenna and commonly 7 pairs of legs, as visible in Figure 2. *Gammarus* genus has more than 200 species, which survive under different conditions, being sensitive to changes in the aquatic environment. However different species or lineages within a species can respond differently to specific stressor, as well as the juvenile/adult stage. Closely related species in Europe such as *Gammarus lacustries* and the recently discovered *G. alpinus* (Alther, Fišer, & Altermatt, 2016) can be morphologically similar but occupy a different distribution range. While *G. lacustris* is widely distributed and not endangered at a global scale, *G. alpinus* is endemic to the Alps and its habitat is negatively affected by eutrophication, non-native species and possibly climate change (Alther, Fiser and Altermatt, 2016). Nevertheless, the most common species reported in Germany and the Czechia are *Gammarus fossarum*, *G. pulex* and *G. roeseli*. These three species were analyzed in the largest study about *Gammarus sp.* found so far: 1530 sites sampled on the German Fulda-Eder-basin during 20 years (1968–1988), and after considering relief, geology, and water quality in neighborhood of human settlements, the study concluded that the lack of oxygen (due to organic pollution) and low pH (souring) are the most important factors which alter natural distribution patterns of the three species, with stronger impact in *G. fossarum* populations that are more fragile than the others in terms of pollution and high temperatures resistance (Meertinus P. D. Meijering, 1991). The impact on *Gammarus* populations may be influenced also by invasive species like *Dikerogammarus haemobaphes* and *D.villosus*, which are extraordinarily successful invaders in Western Europe (Pöckl, 2007), having evidence of predation from them to the native *Gammarus fossarum*.

2.1. Distribution

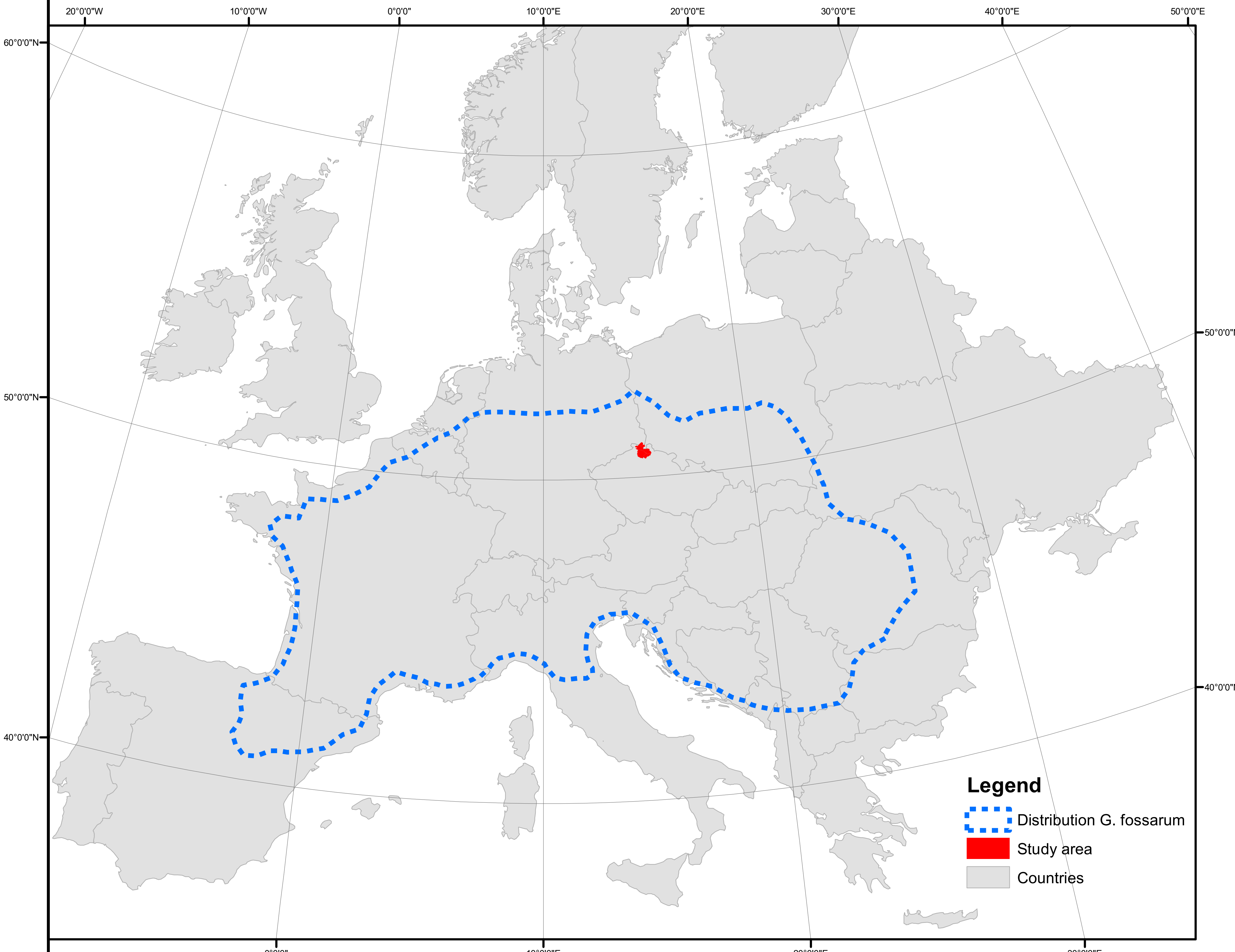
This crustacean contains several cryptic species (morphologically identical, but not genetically) (A. M. Westram, Jokela, & Keller, 2010), and it can share habitat with other freshwater amphipods. *Gammarus fossarum* individuals found in the zone of this study, belong to the Central and Western European clade according to the classification used by (Copilaş-Ciocianu, Rutová, Pařil, & Petrusek, 2017). Many water springs in Czechia hold healthy populations of *Gammarus fossarum*, however there are some that lack of these invertebrates, even being reachable in their distribution area. The intention of this study is to understand the characteristics that make a spring inhabitable for this species. According to Weiss and Leese (2016), the connectivity between *Gammarus fossarum* populations is influenced by water pollution and habitat fragmentation of freshwater ecosystems. It was analyzed the ranges of tolerance of salinity, temperature, acidity, oxygen, and chemical substances, in order to evaluate the environmental conditions. This species also play a fundamental role in aquatic ecology: a drastic decrease of their populations can have severe consequences for other trophic levels (Eisenring, Altermatt, Westram, & Jokela, 2016), they help to breakdown plant litter improving water quality and they are a food source for other aquatic invertebrates, different types of fish and birds.

The species *Gammarus fossarum* was described by Koch in 1835, and since then, it has been registered in streams from Switzerland (Anja Marie Westram, Jokela, Baumgartner, & Keller, 2011); France (Ciliberti et al., 2017), Netherlands, Luxemburg and Belgium (Nijssen, 1963); Spain (Alonso, De Lange, & Peeters, 2010); Germany (Weiss & Leese, 2016); Slovenia (Fišer et al., 2007); Austria (Pöckl, 2007); Czechia, Slovakia, Poland, Hungary, Serbia, Croatia, Romania (Copilaş-Ciocianu et al., 2017).

See in the next page:

<u>Map 1: Distribution of *G. fossarum*</u>

DISTRIBUTION OF GAMMARUS FOSSARUM IN EUROPE



PROJECT STUDY AREA

PRAMENY SPOJUJÍ
QUELLEN VERBINDEN

Author: Diego Sebastian Serrano Suarez
Data source: ESRI online

Czech University of Life Sciences
April, 2018

ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

European Union
European Regional Development Fund

Note: The freshwater shrimp Gammarus fossarum was first described by Koch in 1835 (Nijssen, 1963). This species has only been found in European water bodies.

References of location: Switzerland (Westram et al., 2011) (Alther, Fišer and Altermatt, 2016); France (Labaude, Rigaud and Cézilly, 2017) (Ciliberti et al., 2017); Netherlands, Luxemburg and Belgium (Peeters and Gardeniers, 1998) (Nijssen, 1963); Spain (Alonso, De Lange and Peeters, 2010); Germany (Weiss and Leese, 2016); Slovenia (Fišer et al., 2007); Austria (Pöckl, 2007); Czechia, Slovakia, Poland, Hungary, Serbia, Croatia, Romania (Copilaş-Ciocianu et al., 2017).

Transboundary cooperation 2016 – 2019
European Regional Development Fund

Map 1

2.2. Components of *G. fossarum* ecosystem

There are several stressing factors that contribute to the absence of *Gammarus sp*. in locations that they could reach. Once some habitat is colonized by random or induced processes, it is crucial that such conditions like temperature, oxygen, pH, altitude, shelter, food, water velocity and chemistry are tolerable, so the population can settle in the place. Once the site is inhabited, secondary factors like competition, predation and parasites will influence also the quality of the population and its long-term permanence in the environment. According to (Ciliberti et al., 2017), the assessment of the possible local degradation of gammarid populations is based on the definition of reference levels of gammarid abundance, i.e. abundance expected in the different investigated watercourses when undisturbed.

2.2.1. Water flow and velocity

G. fossarum generally avoid the fastest flowing stream sections with large stones and gravel (Eisenring et al., 2016). The strong turbulence creates a hostile environment for their attachment to food sources and the large stones surface usually carry less nutritious components, compared with sand surfaces. In comparison with other *Gammarus* species, *G. fossarum* inhabits the faster running parts of the stream while *G. pulex gallicus* is restricted to the calmer, more slowly running waters near the bank (Karaman & Pinkster, 1977). Both *G. roeseli* and *G. pulex* are known to react to the current at about $0.05 ms^{-1}$ flow rate (Perkin, Franz, & Heller, 2014).

In order to preserve *G. fossarum* populations it requires stable hydrological conditions (mainly stream flow) turned out to have the strongest influence on the abundance and structure of macroinvertebrate communities (Gli, Astel, Pomorska, River, & Poland, 2016). They require a stream that does not get totally dry or flooded eventually, but also a stream with enough sources of food and shelter. That is why it is crucial to protect the diversity of springs and not only one type of them, so the species can find their place in the ecosystem, their niche. Even individuals categorized as *Gammarus fossarum*, can belong to different cryptic species and be associated to different watershed, river size and, ecomorphology, e.g. in (Eisenring et al., 2016) research, *G. fossarum* types A and B had opposite occurrence pattern in a region of Switzerland. Whereas type B was mainly found in less forested areas with higher human impact, type A occupied forested zones with larger gravel, larger stones and less macrophytes than habitats occupied by type B.

2.2.2. Altitude

Altitude can be determinant to analyze the distribution of crustaceans. For *G. fossarum*, sites above about 1600 m.a.s.l. seem not habitable (Eisenring et al., 2016). *G. pulex* instead, is confined to regions under 450 m.a.s.l. The availability of food is affected by the altitude since the high mountain peaks may not have enough leaves and organic matter for the individuals to feed themselves.

The abundance of *Gammarus* was evaluated in 365 stream sites in France. A clear trend showing more individuals in hard water at high altitude (about 1300 m.a.s.l.) than in soft water at lower altitudes (Ciliberti et al., 2017). High elevation sites however, are also characterized by less agricultural use (Eisenring et al., 2016), so it would be hard to assure a direct relationship between agricultural practices and *Gammarus* populations.

2.2.3. pH

G. pulex tolerates better conditions with low pH than *G. fossarum*, however both species avoid acid waters (Peeters & Gardeniers, 1998). The acidity level is crucial for any aquatic organism survival. Mortality of *G. pulex*, for example increases dramatically at pH < 6.0, causing drop of the Na concentration in the invertebrate body and affecting osmoregulation (Andrén & Eriksson Wiklund, 2013). A French study also measured in laboratory the survival rate of *G. pulex* exposed to acid conditions, with resulting survival in control water at pH 7.9 of 98%, and after 38 h of exposure, the survival rates of organisms exposed to pH 6.0, 5.1 and 4.1 were 88%, 72%, 29% respectively (Felten et al., 2008). That way, a neutral pH is ideal for this *Gammarus* species and levels below 6.0 would represent an inhabiting factor, reducing slowly the population.

Besides reducing the survival rate, an inadequate pH can affect the locomotion activity, measured by counting the distance crossed during 1 min by one *G. pulex* in a 5-mL test tube positioned horizontally (length: 7 cm, Ø: 1.3 cm) (Felten et al., 2008). Freshwater acidification make the animals slower and significantly alters the action of shredders processing leaf litter in the acidic stream, including species like *G. fossarum* (O. J. Dangles & Guérold, 2000).

Acidity is also crucial on leave breakdown process, being 10 times slower in the most acidified streams (pH < 5.0) compared with the sites of pH 7.0 (O. Dangles, Gessner, Guerold, & Chauvet, 2004). Nevertheless, a neutral pH is only one factor for the ideal conditions of the amphipod. In a complex headwater streams the water pH and calcium concentrations were similar in each of them, however there were several springs with absence of them (Kobayashi, S., et al, 2013).

2.2.4. Dissolved Oxygen

Gammarus fossarum is a typical inhabitant of running waters rich in oxygen and it abundantly inhabits springs and spring brooks in mountainous regions of Central Europe (Schmidlin et al., 2015b). However, it is difficult to specify the lowest oxygen concentration that they can bear. Henry and Danielopol (1998) observed a 24 h LC50 values of 0.4–3.0 mg/L for some *Gammarus* species. Other species like *Gammarus lacustris* was found with a LC50 survival at 0.2mg/L O_2 during a 7 days experiment, 7-d LC50 (Nebeker 1992). Nebeker (1992) also proved that the resistance to low oxygen decreases if animals do not have access to surface of water.

Low oxygen can persist due to high biomass decomposing in a shallow stream, and it can influence the locomotion of these invertebrates towards regions with better conditions. *Gammarus roeseli* for example, displays a positive rheotaxis (moving to upstream) in well-oxygenated waters, but under hypoxic stress it will travel toward locations with higher oxygen concentrations in open aquatic systems, independent of the direction of water flow in an open channel (Henry & Danielopol, 1998). Different conditions in the water might affect the resistance of some *Gammarus* species to low oxygen. It is believed that activities of the animals like locomotion, feeding and specific dynamic action contribute significantly to the oxygen consumption of *G. fossarum* when it is analyzed in situ (Tatjana Simčič, Lukančič, & Brancelj, 2005) i.e. if the animal moves more and struggle to find food, partner, shelter, etc. it will need more oxygen for their metabolism. In the case of sensitive amphipods like *Atyaephyra desmarestii*, the reaction to low oxygen concentrations is shown by the reduction in the feeding rates (Pestana et al., 2007).

The dissolved oxygen can also be related to the resistance of some toxic metals in the species. It was found that the higher tolerance to cadmium may be related to a wider tolerance range of low dissolved oxygen (Alonso et al., 2010).

2.2.5. Temperature

Galic and Forbes (2017) found that the optimal temperature was between 17 and 24°C and somatic growth rates slowed down at lower temperatures between 7 and 11°C. However, it can be influenced by other factors, such as elevation and geographical location. Different times of the year in Europe will generate challenge for survival to *Gammarus* populations. The differences among seasons show that different life stages of each species prefer different microhabitat and different hydrological conditions (Komínková, Nábelková, & Vitvar, 2017). When the summer is coming, more food is available from leaves falling and the aquatic plants bloom.

In laboratory experiments, precopula time of *G. pulex* is dependent on water temperature. As temperature increases, precopula will be faster, e.g. at 10°C is 8.8 days and at 18.5°C is 4.5 days (Sutcliffe, 1992), unfortunately this precise information is not available for *G. fossarum*. Schmidlin, Fumetti and Nagel, (2015b) were able to prove that the ideal temperature for optimal reproduction of *G. fossarum* is 12°C and increasing water temperatures increased feeding activity significantly, however, Galic and Forbes (2017) have shown that survival under starvation was significantly lower in warmer treatments, maybe because warming is expected to increase metabolic costs and turnover in organisms, leading to faster mortality, even under no starvation conditions. Even though, warmer environment supports the reproductive cycle and the most active scenario of *Gammarus*. It accelerates also the time required for eggs incubation and emergence from the brood pouch. The durations of both incubation and post-hatch periods are chiefly dependent on water temperature, although other environmental factors such as salinity and oxygen content may also be important, as is the size of the eggs. For example, *G. pulex* need 20-23 days for embryo hatching at 11°C, but at colder temperatures (4°C) it can take over 100 days (Sutcliffe, 1992). Pöckl, (2007) reported 40 days in *G. fossarum* and 44 days in *G. roeseli*; at 4°C it was 1.8 and 3.5 times longer in *G. fossarum* and *G. roeseli*. The number of embryos that hatched into live young is greatest at 8 to 12°C, for *G. fossarum*, where some 70-80% of the embryos survived, and at 10 to 16°C for *G. roeseli*, where some 40-50% survived, with 100% mortality for both species at 26°C (Sutcliffe, 1992).

Warming has been shown to generally increase leaf litter processing rates (Moghadam & Zimmer, 2015), allowing better quality of feeding. Once leaf litter is depleted in the system, organism survival could be jeopardized due to higher costs of metabolism under global warming. It is expected, however, that a rise in water temperature will increase metabolic activity of *Gammarus pseudolimnaeus* and other leaf litter processing organisms (Galic & Forbes, 2017).

Other stressors of *Gammarus* populations might be also activated with temperature changes in the field. For example, the presence of parasites or predators, and also direct human influence as runoff from agriculture fields. Thus, the ideal range of temperature should be studied for each population in situ, in relationship with the real influences, otherwise the result will be just theoretical values of population survival.

2.2.6. Food

There is a wide range of food for *Gammarus*. They are described as opportunistic feeding consumers, predators and shredders (Sroda & Cossu-Leguille, 2011). The main sources of food for *Gammarus* are plant litter from riparian vegetation (leaves and wood), dead aquatic plants, algae and phytoplankton, dead zooplankton from other invertebrates and vertebrates and the feces of living animals. *Gammarus* show preferences for certain leaf types like elm, oak, sycamore and alder (Hutchinson, L. 2005). Often they are able to exploit additional food sources such as fresh aquatic plant material (Eisenring et al., 2016). It counts not only the type of leaves but also their stage of decomposition in case of consumption of leaves previously colonized and conditioned by fungal and bacterial communities (Galic & Forbes, 2017).

These crustaceans can survive without food for several days. According to (Hervant et al. 1997), the lethal time of starvation to lose 50% of the population (LT50), as estimated by the Trimmed Spearman-Karber method (12), for starved *G. fossarum* was 35.2 days. Mortality was 0% prior to 7 days and 100% at 70 days. When it comes to reproductive process, feeding inhibition of females reduces the number of oocytes but not their size (Coulaud et al., 2015).

High nutrient levels of the stream sections can be found also in human settlement surroundings. It will improve the food supply and it will help the shrimp's population unless it reaches eutrophication. A high amount of submerged aquatic vegetation, positively correlates with aquatic invertebrate abundance (Eisenring et al., 2016). Macroinvertebrates, such as the northern spring amphipod, *Gammarus pseudolimnaeus* (Bousfield, 1958) are major contributors to leaf litter breakdown. Leaf litter decomposition constitutes a central ecosystem process driven by macroinvertebrate shredders, fungi and bacteria (Hiebber & Gessner, 2002).

Organisms used for field ecotoxicity allow to see the effect of their food in their survival rate or tissue composition. The feeding rate of Crustacean species is sensitive also to temperature, hardness and alkalinity (Pestana et al., 2007). Other factors like copper ions have a tendency to decrease the feeding activity and significantly decrease ETS activity (Schmidlin et al., 2015b).

Digestive enzymes have been studied to analyze how environmental factors affect assimilation of the products resulting from food digestion. In experiments developed in France, calibrated male organisms from the same population of *Gammarus fossarum* were caged and used at 23 sites, assessing the influence of two abiotic factors: temperature and conductivity. They found significant effect of temperature on reduction of digestive enzyme activity at the lowest temperature (7 °C) when analyzing cellulase (which breaks the crystalline structure of cellulose into polysaccharide fragments) (Charron et al., 2013).

2.2.7. Substrate rocks or vegetation

Adaptations of locomotion and diet have shaped the habitat selection of amphipods in freshwater streams. In studies with two different types of *Gammarus fossarum*, it has been shown that cryptic species type A prefers stony habitats with leaves, while type B prefers plants and muddy substrate (Anja Marie Westram et al., 2011).

The preference of certain substrate or roaming activities within the same population can differ or not, depending on the age stage of the amphipod. For example *G. roeseli* adults and juveniles prefer always aquatic weeds, but *Dikerogammarus villosus* adults usually stay in gravel (Kley et al., 2009). Also, the quality of the gravel substrate can influence their substrate choice, the porosity in the surface can hold different food resources, the structure of plants, shape of leaves, etc.

Fissured substrate offers more refuge, more possibilities for attachment and may also host more prey organisms than substrate with smooth surface (Kley et al., 2009). Substrate such as vegetation and coarse detritus (Hutchinson, L. 2006) can benefit the ecosystem of *Gammarus*.

2.2.8. Light

Gammarids are generally more active under dark than under light conditions and in higher density populations (Augusiak & Van den Brink, 2015; T Simčič & Brancelj, 2007), however there is no specific observation that can prove how light conditions can influence directly the survival of *Gammarus*. Indirectly, light is important for the plant growth and therefore the organic matter available as food supply for detritivores and shredders in the water source. The effect of light on the individual's activity can include changes in the direction of locomotion (from upstream to downstream or vice versa). An experiment held with a flume that allowed *Gammarus* to travel found no evidence that gammarid night drift rate was inhibited by artificial light at night (Perkin et al., 2014). Opposite to a hypothesis pointing negatives of light pollution. Nevertheless, when studying the reaction of animals to light, special precaution must be taken to avoid any influence of external variables in the experiments. For example, a Laboratory in Netherlands found that the tagging procedure increased drastically the resting times of the animals (Augusiak & Van den Brink, 2015), while analyzing the movement of *Gammarus pulex* tagging individual animals with little rectangular pieces of a fluorescent material in their back and returning them into fresh water aquarium.

The light can have important influence in the consumption of oxygen of crustacean species like the hypogean (living under earth's surface) *Niphargus stygius*. However, for *Gammarus fossarum*, an epigean species, only a slight increase of oxygen consumption could be present with artificial light, probably explained as a stress reaction to light due to its dark preference. To prove it, they were exposed to light intensities of 720 and 4700lx at 10°C. Oxygen consumption increased significantly in *N. stygius* exposed to both low and high intensities of light, but no significant increase was observed in *G. fossarum* at either intensity (T Simčič & Brancelj, 2007).

2.2.9. Competition

Being shredders and detritivores, most of the natural environments inhabited by *Gammarus sp*. have plenty of food options, however its quality is variable. The stage of the leaves and the morphology of the stream can affect the competition between species.

Competition can affect substratum choice shifts, swimming activities and mortality between native and invasive species. In a laboratory of Netherlands, the more recent invaders *Gammarus tigrinus* and *Dikerogammarus villosus* were more likely to prefer stone substratum, whereas the native *Gammarus pulex* and an earlier invader *Gammarus roeseli* were found more frequently in the water layer (van Riel, Healy, van der Velde, & bij de Vaate, 2007). It was also noted that the greatest shifts in substratum preference arose when one species had occupied a substratum before the other one was introduced, especially when *D. villosus* was already present before *G. pulex* was introduced, possibly indicating preemptive competition. However in another experiment in Germany with *G. roeseli*, adults and juveniles clearly preferred aquatic weeds independent of the presence or absence of the invader *Dikerogammarus villosus* (Kley et al., 2009).

The microhabitat dynamics are very difficult to predict when competition is and influential factor in the niche requirements, thus it is important to evaluate not only the presence of the invader, but also the morphology of the habitat. In other scenarios of Europe, *Gammarus* species of central Europe can be considered as invader, e.g. in Irish rivers, the introduced *Gammarus pulex* replaces the native *Gammarus duebeni celticus* (Kelly, Bailey, MacNeil, Dick, & McDonald, 2006).

A big asset in competition is the number of fertile eggs laid per clutch. Pöckl (2007) described a relationship between the body weight of the female carrying eggs and its number of eggs as following: *G. fossarum* average held 15 eggs with 13 mg body weight, and maximum held 59 eggs and 49 mg bodyweight. *G. roeseli* average held 32 eggs with 27mg body weight, and maximum held 87 eggs and 61mg bodyweight.

2.2.10. Predation

Their main predator is fish. (van Riel et al., 2007) observed *G. pulex* seeking shelter in the presence of a predator, and (Kley et al., 2009) described that hiding under stones and reduced activity may be an effective antipredator strategy. It was found that high density populations in the wild rely on aggregation as an antipredator behavior (Labaude, Rigaud, & Cézilly, 2017). Also, a fast reproduction process will enhance their survival options. Other factors can make it vulnerable. For example, *G. pulex*, even in presence of a predator (gudgeon fish), reduced its hiding behavior when food was available in the form of finely ground leaves. In the absence of food, gammarids were clearly able to distinguish and hide in the presence of kairomones (Szokoli, Winkelmann, Berendonk, & Worischka, 2015).

Kairomones are chemical signals that the fish produce and can be detected by *Gammarus* in the water, as a warning of predators, just as pheromones are a signal for mating.

On the other side, amphipods including *G. pulex* can be highly predatory, interfere with feeding, and induce drift behavior in a range of invertebrates that includes ephemeropterans, plecopterans, and trichopterans (Kelly et al., 2006).

2.2.11. Parasites

Gammarids can hold *acanthocephalan* parasites that are known to induce effects like modifications on their functional role (Labaude et al., 2017). There could be an interesting link between temperature, parasitism and competition, which make difficult to generalize if a temperature shift might affect negatively or not some population. While higher temperatures might induce an increase in the shredding efficiency of gammarids, infection by *acanthocephalan* parasites can also impact negatively the shredding role of gammarids (Labaude et al., 2017). It is necessary to explore in detail other ways of parasitism that can affect negatively this populations.

2.3. Toxicity and human impact

The design of modern cities makes the citizens unaware of changes in the natural environment. The water source is located usually far away from the tap, and can be influenced by pollution from industry, agriculture, tourism or other settlement. Measuring toxic substances in the water sediments or in tissue of *Gammarus* is useful to determine the influence of these pollutants that can be diluted in the water and undetectable in standard physicochemical studies.

2.3.1. Toxic Metals

The accumulation of toxic metals in macroinvertebrates make them very useful for determining long term contamination in water sources, while a water test can ignore the toxic concentration it in punctual samplings. Amphipods are very often used in ecotoxicology because of their role in detritus breakdown, their place in food chain, their abundance and sensitivity to a wide range of toxicants (Alonso et al., 2010). Some species of *Gammarus* present in European freshwater ecosystems could develop different adaptations to environmental stressors, (e.g. *G. fossarum*, *G. pulex*, *and G. roeseli*). As an example of sensibility comparison, a Dutch research found that *G. fossarum* was more sensitive to cadmium than *G. pulex*, and the opposite sensibility resulted using the antibiotic ivermectin as stressor. Cadmium was more toxic to juveniles and ivermectin showed the same toxicity for both stages, according to the mode of action of toxicants (Alonso et al., 2010). Additionally, some linages inside each species can also differ in their resistance ranges for presence of toxics. Members of the *G. fossarum* cryptic species complex (linages) are regularly used in ecotoxicological studies. They are sensitive to anthropogenic acidification and pollution, which may cause local extinctions with potentially serious impacts on ecosystem functioning (Anja Marie Westram et al., 2011).

Measuring the impact of wastewater discharges in water springs with Amphipods can be useful (Wigh et al., 2017), however, different populations can be more sensitive to specific insecticides and fungicides than others When it is about copper pollution, it was possible to show this divergent sensitivity measuring antioxidant enzymes in two amphipod species exposed to Cu: *Dikerogammarus villosus* probably has specific physiological properties compare to *Gammarus roeseli* that enable it to resist copper toxicity and thus become the best competitor (Sroda & Cossu-Leguille, 2011).

Experiments about Cd and Zn tolerance were performed with amphipods like *Atyaephyra desmarestii*, and *Echinogammarus meridionalis*, in Portuguese freshwaters. The results presented that they appear to be promising test organisms, however an increase in metal concentrations at sub-lethal levels resulted in significant reductions of the feeding rate of both species (Pestana et al., 2007). In an experiment, Dedourge-Geffard *et al.* (2009) also found the inhibition of five digestive enzymes occurring concurrently with the decrease of feeding activity in the organisms transplanted into a metallic contaminated site. Thus, a question arises: if feeding rate decreases and less mass is consumed in presence of the toxic, could it limit the studies of animal tissue, when a big amount of the contaminant is clearly present in the water? We cannot assure that yet.

Human impact has a strong influence on hydrological cycle and climate change, therefore it will affect at some extent ecomorphology and *Gammarus* populations in water springs (Eisenring et al., 2016). Influence of specific chemicals has been found in *Gammarus* laboratory and field experiments using copper sulphate, which reduces the metabolic activity (Schmidlin, Fumetti, & Nagel, 2015a), nevertheless, the most accurate methods to measure the influence of chemicals should be the in situ bioassays, where some individuals contained in small cages are placed in the natural stream, to evaluate their survival rate or the bioaccumulation of metals.

Some researches use *Gammarus* as sensible bioindicator for performing water toxicity assessments. Caged *G. fossarum* in situ is a robust and useful tool to monitor bioavailable contamination trends of metals according to Besse *et al.* (2013). These French researchers designed cylinder cages (10cm long and 5.5cm diameter) that allow the water running through when they are submerged. Placing 20 individuals, males and morphologically similar in each cage, it was possible to determine toxic metals and pesticides present in the water, collecting the animals and making tissue analysis. They also got the first study to investigate the implementation of contaminants threshold values in the context of active biomonitoring, however only 7 days of exposure to the environment, having for further analysis longer periods to identify chronic consequences.

Short-term exposure (one week) of *G. pulex* may be used for acute toxic disturbances (Marmonier et al., 2013). But it is recommended to use other species if it is needed to analyze chronic disturbances.

Levels of Cd, Hg, Ni and Pb contamination assessed by active biomonitoring with caged *Gammarus fossarum* were compared to abundances of on-site gammarids on 94 sites in France. Concentrations measured in caged *G. fossarum* indicated significant bioavailable contamination. The sites were studied by watercourse size, geology, urban or agricultural land use, hydrobiological quality (Ciliberti et al., 2017).

It is interesting to note that even some lethal doses of toxics described in literature can vary, according to the conditions in the place. As described by Lebrun, Uher and Fechner (2017), that the mixture of several toxics can have different effects (synergy) than isolated concentration of one toxic.

2.3.2. Other substances

It is important to identify the influence of watershed, land use and the runoff direction, in order to analyze all possible toxic stressors affecting the water quality. For example, landscape information is crucial to predict negative effect of pesticides (Liess & Von Der Ohe, 2005). Areas of agriculture surrounding the stream without a forest belt in between, are more likely to allow pollution by pesticides or fertilizers than those with better resilience capacity. *G. fossarum* is a sensitive tool to quick prediction of a pesticide exposure. Following in vitro exposure to atrazine and imidacloprid, were measured by a combination of electron transport system (ETS) activity and respiration. Laboratory tests confirmed that *G. fossarum* is more sensitive to short-term pesticide exposure than *Asellus aquaticus* (Lukančič, et al. 2010).

The selection of male/female or juvenile/mature individuals for caged assessment can determine different results in the study. For example, a bioassay with ivermectin (a chemical used in medicine as antiparasitic agent, which can pollute the streams after excretion), preferred the use of juveniles because they show the same or lower tolerance than adults (Alonso et al., 2010).

Alive *G. fossarum* are also useful assessing the removal of micropollutants in wastewater treatment plants. A tertiary treatment process in Germany found significantly reduced overall ecotoxicity of municipal wastewaters treated separately by with three techniques: ozone concentration, TiO_2 or activated carbon, using *G. fossarum* survival for testing before and after treatment (Bundschuh, Zubrod, Seitz, Stang, & Schulz, 2011). Also *G. fossarum* was found as model organism to study silver nanoparticles effects (Mehennaoui et al., 2016) and micro plastics (PA fibers) (Blarer & Burkhardt-Holm, 2016).

CHAPTER 3

Study area

This section explains the characteristics of the analyzed ecosystem and its importance for the region. Here is clarified the location of the project, the relevance of a geological fault and why these 40 springs were selected. This area is very rich in freshwater springs that complement the basin of Elbe and Oder River and therefore it deserves special attention.

3.1. Location

The project includes a multidisciplinary analysis of 40 springs in the surroundings of Liberec and Zittau, (Czech-German border) across the Lusatian fault. This humid region holds many springs in a rich variety of geology and habitats. The average annual rainfall of 604 mm in Liberec (http://climate-data.org), the range of temperature in the analyzed springs goes from 6.9 to 14.7 °C and the altitude from 836 m.a.s.l in Ještěd Mountain to 280 m.a.s.l. in the north of Zittau. Transboundary cooperation is very important for this comprehensive study of water sources because the water does not follow political limits; some zones in Czechia usually influence the German streams.

3.2. Geological fault

The Lusatian Fault is one of the most prominent products of the latest Cretaceous to Paleogene, separating crystalline units from Paleozoic in the northeast and Cretaceous units in the southwest in the northern Bohemian Massif (Coubal, Adamovič, Málek, & Prouza, 2014). It seems obvious the influence of rock formations on the water infiltration and accumulation of aquifers. Different porosity and chemistry in the soil changes the underground water flow. However, there is not enough scientific data to prove this. According to Coubal *et al.*, (2015), the Bohemian Massif remains one of the insufficiently known regions.

3.3. Selection of springs

These places were rigorously selected by a multidisciplinary team (Vitvar et al., 2017), taking into account a representative sample of different flow types, geological units, landscape composition, and human disturbance. Some of the springs have clear signs of intervention from human (as shown in Figure 3), such as advertisements, fences, sitting areas, and even cups to drink from them, which symbolizes the cultural importance of springs in the area of study. Others are naturally hidden in the landscape, mostly surrounded by secondary forest for wood production, extensive agricultural areas, urban zones and roads of asphalt or unpaved. See **Annex B: Pictures of the springs**.

Figure 3: Human intervention in spring surroundings Leutesdorf-Ziegenborn, AN1037.

3.4. Hydrological connections

The area of study was divided into 4 hydro-geographical regions (catchments NORTH, EAST, SOUTH, and WEST), according to the connections of surface water, which allows to analyze the accessibility of *Gammarus* from the streams to the spring sites. The Figure 4 shows how the water that raises in the hydro-geographical regions (catchments) of this study, can reach the Ocean in German coast (Elbe River) or in Polish coast (Oder River).

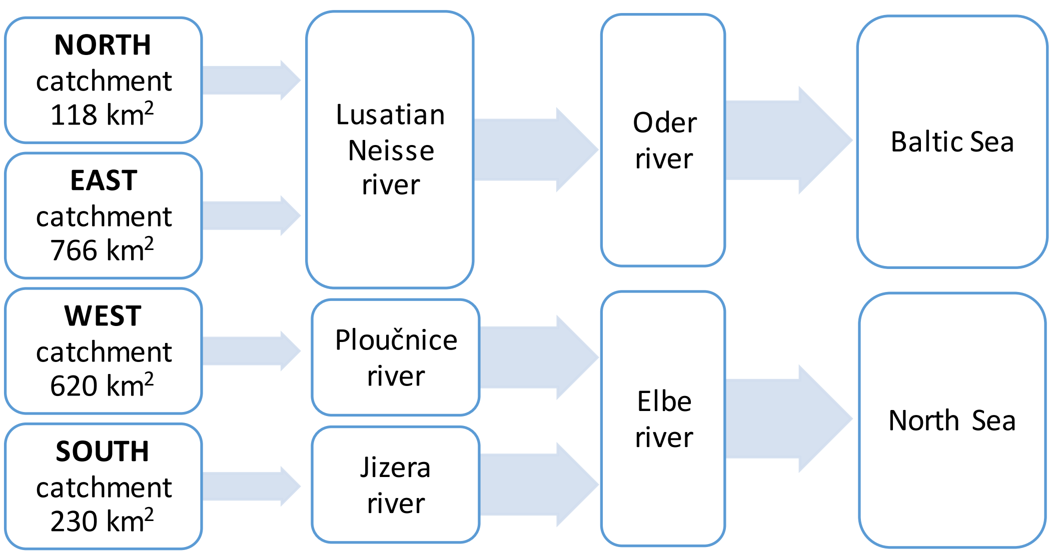


Figure 4: Diagram of Hydrological tributaries and discharge water bodies of this study.

See in the next pages:

Map 2: Hydro-geographical regions

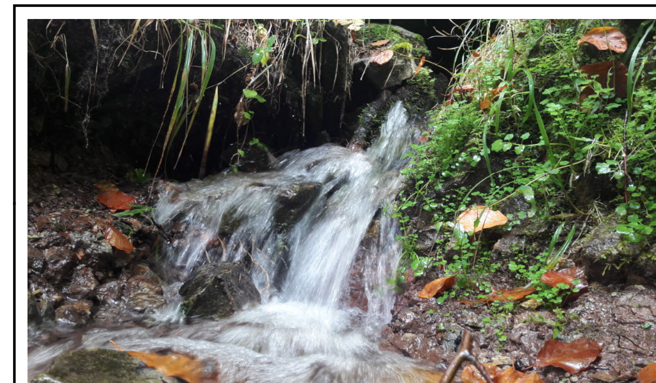
Map 3: Topography of the terrain

PRAMENY SPOJUJÍ
QUELLEN VERBINDEN

HYDRO-GEOGRAPHICAL REGIONS (CATCHMENTS)

Legend

- Springs
- Streams in Germany
- Streams in Czechia
- Lusatian Fault
- Country borders
- Cities
- Catchments



Author: Diego Sebastian Serrano Suarez
Data source: ESRI online
Coordinate System: S-JTSK Krovak East North
Projection: Krovak
Datum: S JTSK

Czech University of Life Sciences
Date: April, 2018

ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

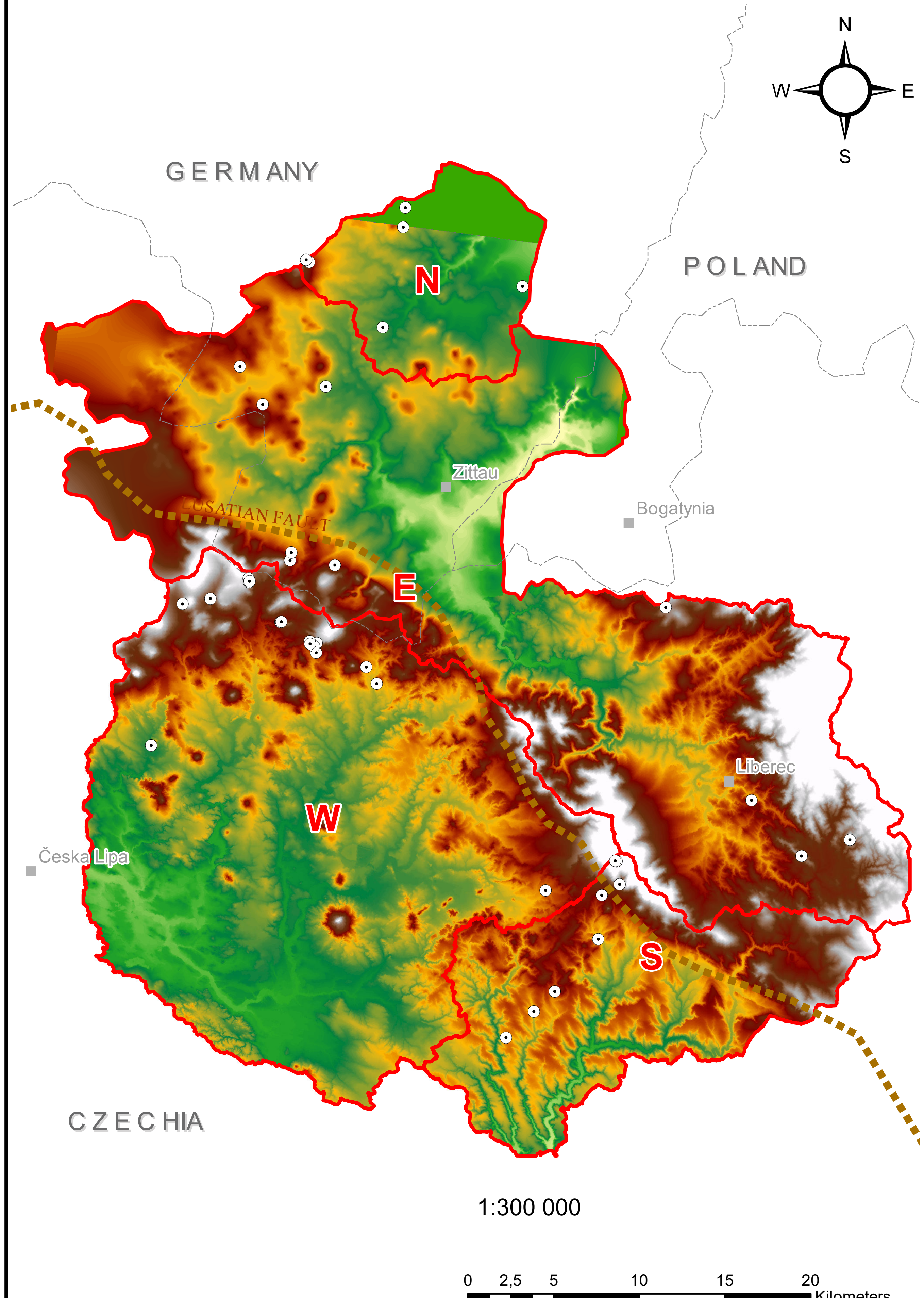
European Union
European Regional Development Fund

Transboundary cooperation 2016 – 2019
European Regional Development Fund

Map 2

TOPOGRAPHY OF THE TERRAIN

PRAMENY SPOJUJÍ
QUELLEN VERBINDEN



Legend

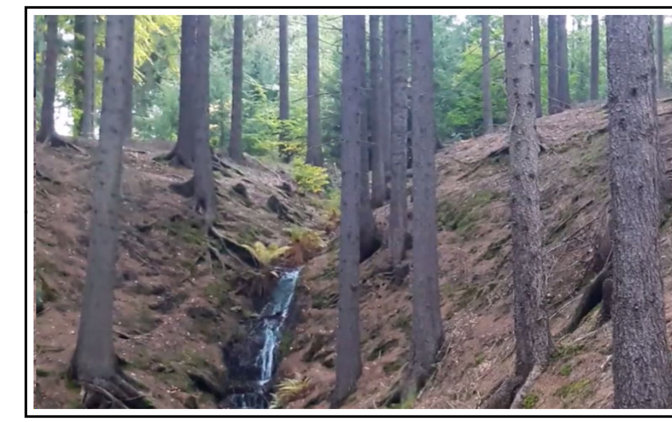
- Springs
- Cities
- Lusatian
- Country borders
- Catchments

Elevation (m.a.s.l.)

Value

High : 1011

Low : 141



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European Union
European Regional Development Fund

Transboundary cooperation 2016 – 2019
European Regional Development Fund

Map 3

CHAPTER 4

Methodology

The aim of this chapter is to describe the procedure for the data collection on this project as well as the steps followed to analyze the information, so the reader can understand, replicate or improve the techniques as needed. The work included field sampling, taxonomy of aquatic species, assessment of water chemistry and toxic metals occurrence, creation of maps in GIS to compile data and the use of R Studio for generating statistical analysis.

The study was divided in 4 general stages to facilitate explanation:

1) **Finding *Gammarus*,** identifying presence and abundance of *Gammarus sp*.
2) **Classifying habitat information,** compiling the variables for analysis.
3) **Measuring toxic metals,** detecting elements in animals, sediment and water.
4) **Analyzing relationships,** correlating environmental factors with the abundance of *Gammarus fossarum*.

4.1. Stage 1: finding *Gammarus*

This section covers the methods used to sample the biota in springs in 2017. Composed circular sampling, and artificial substrate cylinder. Also, the techniques used in laboratory for sorting samples and analyzing abundance of benthic animals. Each of the springs sampled are listed in the Table 1, specifying the country where they belong, the hydro-geographical region (catchment) and the water flow when the information is available.

4.1.1. Sampling benthic fauna

The first sampling was performed in the summer, at the end of June, and the second sampling was in fall, at the beginning of October, 2017.

The main method used to find the macroinvertebrates in selected locations (composed circular sampling) consisted in a method with 6 subsamples per spring. Collecting each sub-sample requires a plastic pipe piece of diameter 12cm and length 20cm. As shown in Figure 5, the pipe piece is placed in vertical position and pressing strongly into bottom allows to isolate a portion of the spring biota, with less disturbance to the spring than other methods like *kick-sampling*. The sediment and water enclosed by the pipe walls is collected in a plastic tray using a stainless-steel ladle, up to a soil depth of 3 to 4 cm. This way, a representative sample of the biota is obtained by choosing 6 points randomly in the spring bed, trying to cover the different types of substrate occurring in the bottom (sand, gravel, mud, etc.) and also variety of distances from the shore, very close to the point where the water gets in the surface.

It was allowed only one sampling per season in each water spring to minimize the disturbance on benthos habitat. The author shared sampling duties with Karel Koudela and Kateřina Bubaničková (2017). Details of the method according to Michal Bílý (6/2017, personal communication)

Figure 5: Procedure of composed circular sampling with ladle.

The substrates from the 6 subsamples were mixed in the plastic tray and then filtered using a bronze sieve (0.5mm) to remove fine sediments, while washing the sample with clear water from the spring. The sediment and biota inside the bronze sieve are put in a plastic 1L bottle using a funnel, and then fixed with 70% ethanol for preservation. It is very important to shake gently the sample several times, so the alcohol can penetrate inside the sediment and avoid decomposition of biota. Finally, a label must be verified in the bottle and put in transportation box.

To get a realistic register of the invertebrate community, some springs required an alternative sampling method (artificial substrate cylinder), complementing the composed circular sampling. This method used one-month traps, with simulated habitat of rocks (as shown in Figure 6), useful to find turbellaria specimens and to reach *Gammarus* individuals where the springs were too deep for sampling with the plastic pipe piece (in three cases: ES1014, ES2220, BS1204). This method was implemented by Lucie Heřmanova and Evžen Nesrovnal (2017) in parallel research.

Figure 6: Artificial substrate cylinder installed for sampling biota as alternative method.

Table 1: List of springs and flow values measured when a clear outflow was available. GE: Germany, CZ: Czechia, N: North, S: South, E: East, W: West.

N	Spring ID	Location name	Country	Catchment	~Flow liters/min
1	AN1029	Kottmar Bushwiesen	GE	N	5.79
2	AN1030	SchieSborn	GE	N	6.45
3	AN1036	Leutersdorf - Kirschbaumplantage	GE	E	23.83
4	AN1037	Leutersdorf - Ziegenborn	GE	E	9.52
5	AS1043	Mařenice - třízdrojový	CZ	W	140.46
6	BN1024	Jonsdorf horní Hirschbörnl	GE	E	10.03
7	BN1025	Jonsdorf dolní Buchberg unten	GE	E	16.55
8	BN1033	Bornwaldchen	GE	N	-
9	BN1034	Unterh. Spitzberg	GE	E	12.15
10	BN1041	Jonsberg u sportoviště	GE	E	-
11	BN2242	Jungfrauenquelle	GE	N	-
12	BN2243	Steinbogen	GE	N	-
13	BN2244	Birke	GE	N	-
14	BS1016	Radvanec	CZ	W	-
15	BS1019	Krompach	CZ	W	-
16	BS1027	Vodopád u Heřmanic	CZ	W	189.55
17	BS1040	tzv. "sirný pramen"	CZ	W	39.55
18	BS1200	Heřmanice - Nad Borůvčím	CZ	W	57.58
19	BS1203	Heřmanice - 4. propustek	CZ	W	36.10
20	BS1204	Heřmanice - Babiččin odpočinek	CZ	W	6.22
21	BS2228	Petrovice - ve skalce	CZ	W	-
22	BS2229	Myslivny - U smrku	CZ	W	38.47
23	BS2230	Krompach - Pod Kulichem	CZ	W	-
24	BS2232	Myslivny - Pod Buky	CZ	W	13.90
25	BS2233	Myslivny - Nad Pasekou	CZ	W	-
26	BS2235	Heřmanice - U Oplocenky	CZ	W	3.62
27	CS2227	Kněžice	CZ	W	-
28	DN1012	Začátek Vítkovského potoka	CZ	E	28.84
29	EN1005	Ještěd Frantina stud	CZ	S	18.35
30	EN1006	Ještěd Pramen lesních panen	CZ	S	56.05
31	EN1011	Vesec-Jiříčkov	CZ	S	16.21
32	EN2238	Mšeno nad Nisou (ul. Arbesova)	CZ	E	-
33	EN2239	Rýnovice - věznice	CZ	E	-
34	EN2241	Starý Harcov - Temná ul-Hrubínova	CZ	E	-
35	ES1008	Vápno	CZ	S	4.88
36	ES1009	Lesnovek	CZ	S	12.79
37	ES1014	Prameny Ploučnice	CZ	W	-
38	ES1104	Všelibice	CZ	S	-
39	ES1108	V Moskových dolech-Modlibohov	CZ	S	71.02
40	ES2220	Světá pod Ještědem Rozstání GOLF	CZ	S	-

4.1.2. Preparation of samples and sorting

G. fossarum specimens are selected carefully in laboratory and other benthic animals identified in main taxonomic groups. The sediment less than 0.5mm is washed off again to reduce turbidity of the sample and the animals are collected with tweezers to put them in separate bottles with ethanol. Checking morphology of biota with a stereoscope, it was determined the taxonomical Order of every specimen (Amphipoda, Trichoptera, Plecoptera, Diptera, etc). The only Amphipod species found in the springs was *Gammarus fossarum* and according to Copilaş-Ciocianu *et al.*, (2017) *Gammarus fossarum* individuals found in the zone of this study, belong to the Central and Western European clade.

4.1.3. Analyzing abundance

Counting absolute numbers and *G. fossarum* dominance (% of occurrence among the benthic community) it was evaluated their ecological importance in the ecosystem communities. Individuals were counted in approximate numbers regardless of their size, gender or juvenile stage. The quantity of Gammarids gives a clue of the bloom of their population, however, the stability depends not only on how many shrimps are alive, but how efficiently they colonize the niches on the spring in presence of other species and depending on food sources. The relevance of the species at the community level is represented by the number of *Gammarus* specimens found divided by the total of alive individuals in the sample.

4.2. Stage 2: classifying habitat information

This section clarifies how the environmental parameters were collected and categorized. The correlation of different variables with *G. fossarum* abundance is important to understand its habitat preference, therefore an interactive map was created to condense all categories (**See map 8: LEGEND for springs details**). The analyzed environmental factors, were classified as explained below.

4.2.1. Water quality

Basic physic-chemical measurements in situ were registered with multi-meter devices (HACH) and (WTW Multi 3430) before performing the biota sample collection. It was registered temperature, dissolved oxygen, conductivity and Redox potential.

Water samples were analyzed in the TUD-IHI laboratory in Zittau for major ions (NO_3^-, SO_4^{2-}, Cl, and F) by Ion Chromatograph (Dionex ICS-1100). For details about measurement of toxic metal concentrations see below the section 474.3.1.
Flow discharge was measured with "Bucket and Stopwatch". Not every location obtained data because of different reasons: some springs have too large discharge, too many flow

paths, too flat area or is difficult to build a dam (Matthias Kaendler, 4/2018, personal communication)

The classification of surface water quality followed the Czech normative for running water ČSN 75 7221. After comparing all measured values with the parameters in the law, the lowest class obtained is assigned as the overall Class for the spring, e.g. if every value in a spring corresponds to Class II except one in Class V, the spring will be Class V.

Light blue Class I - unpolluted water: a state of surface water not significantly affected by human activity where water quality indicators do not exceed those corresponding to the normal natural background in the flows.

Dark Blue Class II - slightly polluted water: the state of surface water that has been affected by human activity so that water quality indicators reach values that allow a rich, balanced and sustainable ecosystem to exist.

Green Class III - Polluted water: the state of surface water that has been affected by human activity so that water quality indicators reach values that do not need to create the conditions for a rich, balanced and sustainable ecosystem.

Yellow Class IV - heavily polluted water: the state of surface water that has been affected by human activity so that water quality indicators reach values that create conditions that allow the existence of only an unbalanced ecosystem.

Red Class V - very heavily polluted water: the state of surface water that has been affected by human activity, so that the water quality indicators reach values that create conditions allowing the existence of only a highly unbalanced ecosystem.

4.2.2. Hydrological spring types

The springs were classified in three main types of natural springs (rheocrene, limnocrene and helocrene) as shown in Table 2, described by Bornhauser (1913) and Hynes (1970), according to the Springs Stewardship Institute of Arizona. Also, it was included the category 'artificial' for those springs heavily modified or when the water is brought to the surface using a pipeline as shown in picture D of Figure 7.

This categorization depends on the amount of information about the spring and the way it reaches the surface from the underground. Several observations throughout the year are sometimes necessaries to evidence changes in spring's structure. According to Simon (2017), it is recommended to analyze the spring type during winter, when there is more visibility without vegetation.

Table 2: Spring types in the study area

SPRING TYPE	DESCRIPTION
Rheocrene	The water reaches the ground in slope, forming a stream channel
Limnocrene	The water reaches the ground forming a pool or pond
Helocrene	Water emerges as a wet surface (wetland) without punctual source
Artificial	A pipe or man-structure carries the water to reach the surface

Figure 7: Types of springs in the studied area.
A=Rheocrene spring (AS1043), B=Limnocrene spring (AN1030), C=Helocrene spring (BN1033), D=Artificial spring (ES1108). Source: Author and Lucie Heřmanová.

4.2.3. Geological units

The study area is located across the Lusatian fault, one important geological feature that separates crystalline rocks from Paleozoic and sandstones from Cretaceous (Coubal et al., 2014) as shown in Figure 8: Type of rocks in the geology categories.Figure 8. The cretaceous background is responsible for more Ca^+ and SO_4^{2-} ions, being less acidic; in the other hand, the granitic (crystalline) background has more Na^+, K^+ and Cl^-, so it could reduce pH in general conditions (Kamil Zágoršek, 1/2018, personal communication). Besides, rock porosity changes from one location to another, changing water dynamics for infiltration, formation of aquifers and springs.

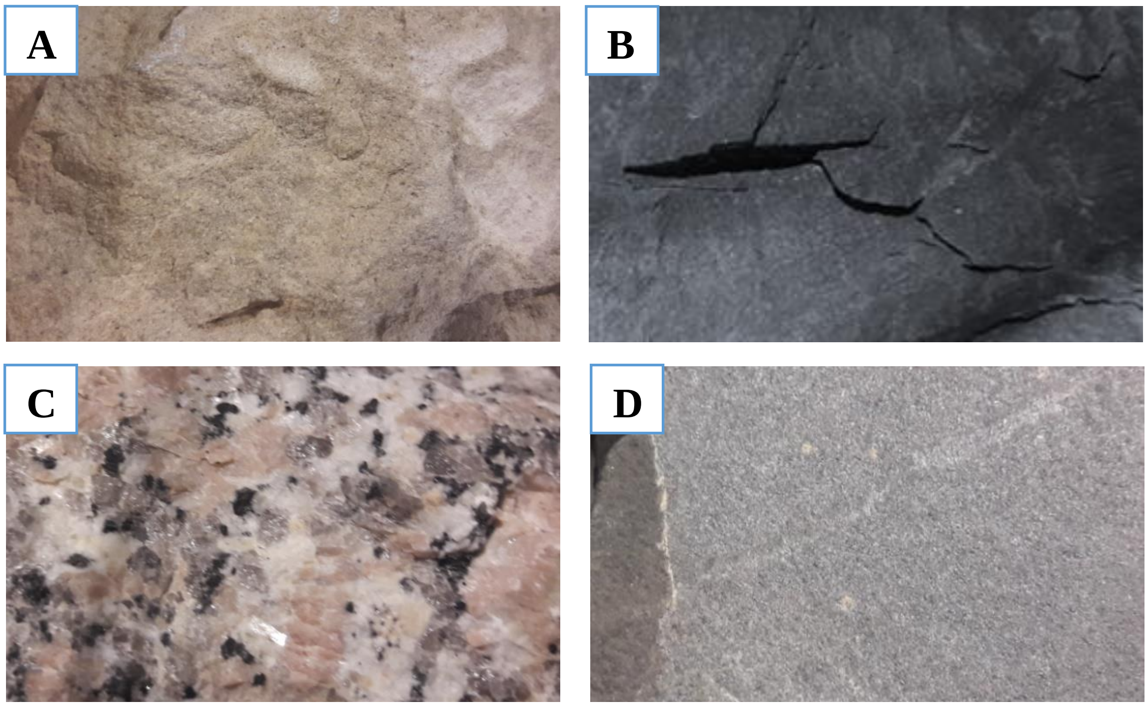

Figure 8: Type of rocks in the geology categories.
A=sandstone, cretaceous sediment; B=basalt, volcanic sediment; C=granite, palleozoic chrystalline; D=limestone, palleozoic sediment. Source: Author in rocks exhibition

Table 3: Dominant Geology classification in the study area

GEOLOGY TYPE	DESCRIPTION
(CS) Cretaceous sediment	Primary rock type is sandstone
(TV) Tertiary vulcanite	Mainly basalt volcanic rocks, solidified from lava, dark color and small pores
(PC) Palaeozoic crystalline	Mainly granite rocks from minerals crystallized under pressure
(PS) Palaeozoic sediment	Primary rock type is limestone, rich in calcium carbonate and secondary sandstone

Details according to Kamil Zágoršek (3/2018, personal communication)

See in the next page, **Map 4: Dominant geology**

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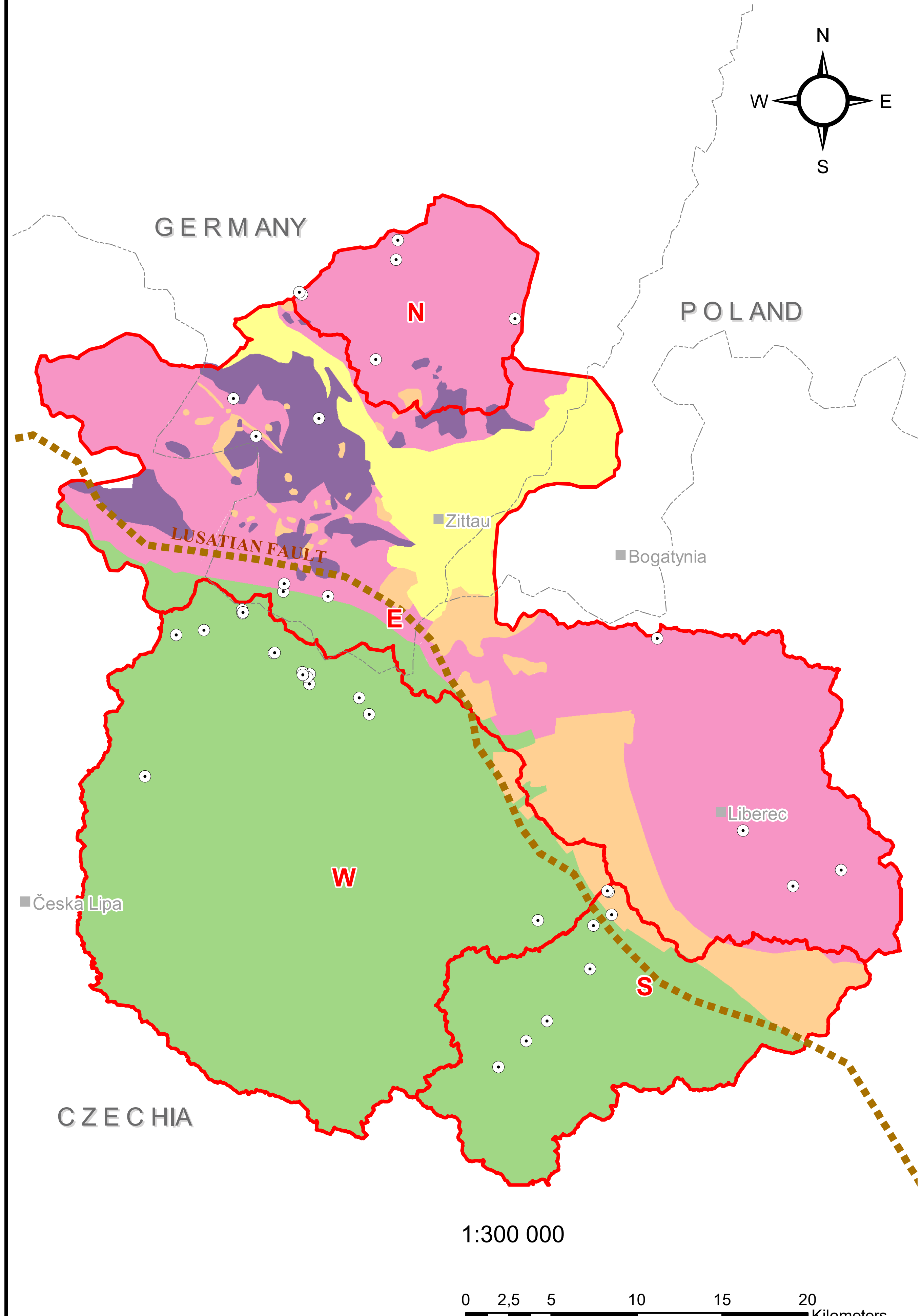
DOMINANT GEOLOGY



Legend

- Springs
- Lusatian fault
- Country borders
- Cities
- Catchments

Geology

- Cretaceous sediment
- Palleozoic crystalline
- Palleozoic sediment
- Tertiary sediment
- Tertiary vulcanite

Note:
This is a simplified map representing dominant geology in the study area. The rock type of springs was verified in situ by Kamil Zágoršek.

Author: Diego Sebastian Serrano Suarez
Data source: ESRI online
Coordinate System: S-JTSK Krovak East North
Projection: Krovak
Datum: S JTSK

Czech University of Life Sciences
Date: April, 2018

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Map 4

4.2.4. Dominant plants

The associated vegetation has an important role in the water spring characteristics. According to the forest composition, the spring is able to keep its water, because the plants allows the creation of soft permeable soil that retains the moisture from the precipitation. The plants influence water chemistry, and also define the habitat available for aquatic organisms. The shelter of *Gammarus* includes dry leaves in the water and sticks. Several species of coniferous trees like *Pinus sylvestris* and *Pinus nigra* are known for decreasing pH over the years, due to the low calcium in their foliage (Thompson, 2014).

The classification to describe the plant composition in Table 4 and was identified according to the perception of the author in each locality. No survey was conducted to classify the forest at this stage. Figure 9 clarifies differentiation of the selected categories.

Table 4: Dominant plants classification
Details according to Kateřina Berchová (3/2018, personal communication)

DOMINANT PLANTS	DESCRIPTION
Coniferous forest	Mainly needle trees (e.g. *Picea abies*) are above or around the spring point
Broad-leaved forest	Mainly trees with flat leaves (e.g. *Fagus sylvatica*) are above or around the spring point
Mixed forest	Both types of trees influence the spring
Non-forest	There is meadow or open pasture area around

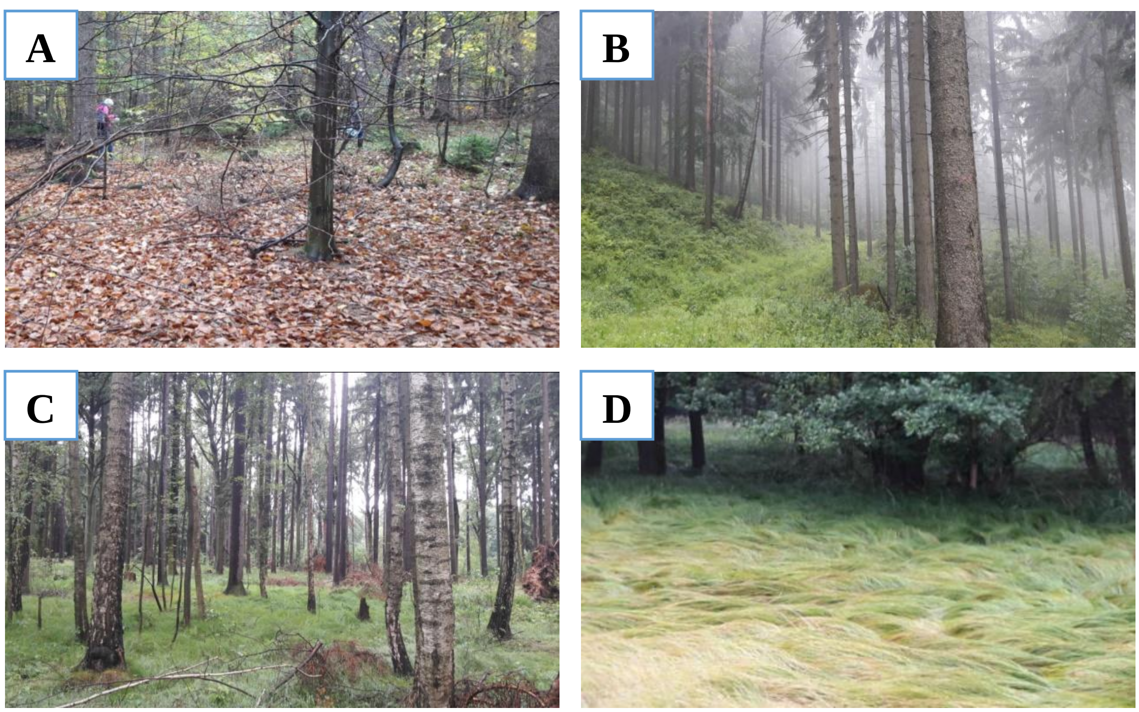


Figure 9: Comparison of forest type categories for this project.
A=broad-leaved forest around BN1024; B=coniferous forest around BN1025; C=mixed forest around BN1034; D=non-forest vegetation around CS2227

4.2.5. Human influence

The springs' characteristics are influenced by the substrate that supports habitat of the surrounding plants and the dwelling invertebrates. The intake of many springs is usually ensured by installing pipes underground that bring the water to the surface using gravitational pressure. Others, build structures to keep the water in a pond, however natural substrate usually cover these structures. Others, for aesthetic purposes receive more attention, adding plastic substrate or gardening around the springs, which is considered as heavily modified by the author, as shown in Table 5.

Table 5: Human influence categories in the study area

HUMAN INFLUENCE	DESCRIPTION
Class A	Natural spring, there is no sign of human impact
Class B	Artificial intake, there is only a pipe taking water from the soil
Class C	Artificial structure, the shore has moderate modification
Class D	Heavily modified, the bottom substrate is no natural

4.2.6. Land cover proportion in watershed

The run-off area influencing the spring was identified using ArcGIS 10.4.1. The method to determine the watershed area and the percentage of each land use type are explained in the table below, however other methods could reach similar result. Before start, it is necessary to count on high resolution elevation raster DEM (maximum size of pixels 10x10 meters), land cover shapefile (Corine) and the spring coordinates.

See map 5: Land cover in study area

The Table 6 shows the detailed process to build the watershed regions and the required tool set for this analysis. Some polygons resulting for this technique needed some adjustments, thus it is recommended to check every polygon with the contour lines after finished.

Table 6: Description of ArcGIS method for hydrology studies

ACTIVITY	TOOL	INPUTS
1. Add DEM and springs	**Add Data**	DEM, Spring points
2. Cut the area of interest	**Clip** (spatial analyst)	DEM raster
3. Smooth spaces of Surface	**Fill** (spatial analyst)	DEM raster

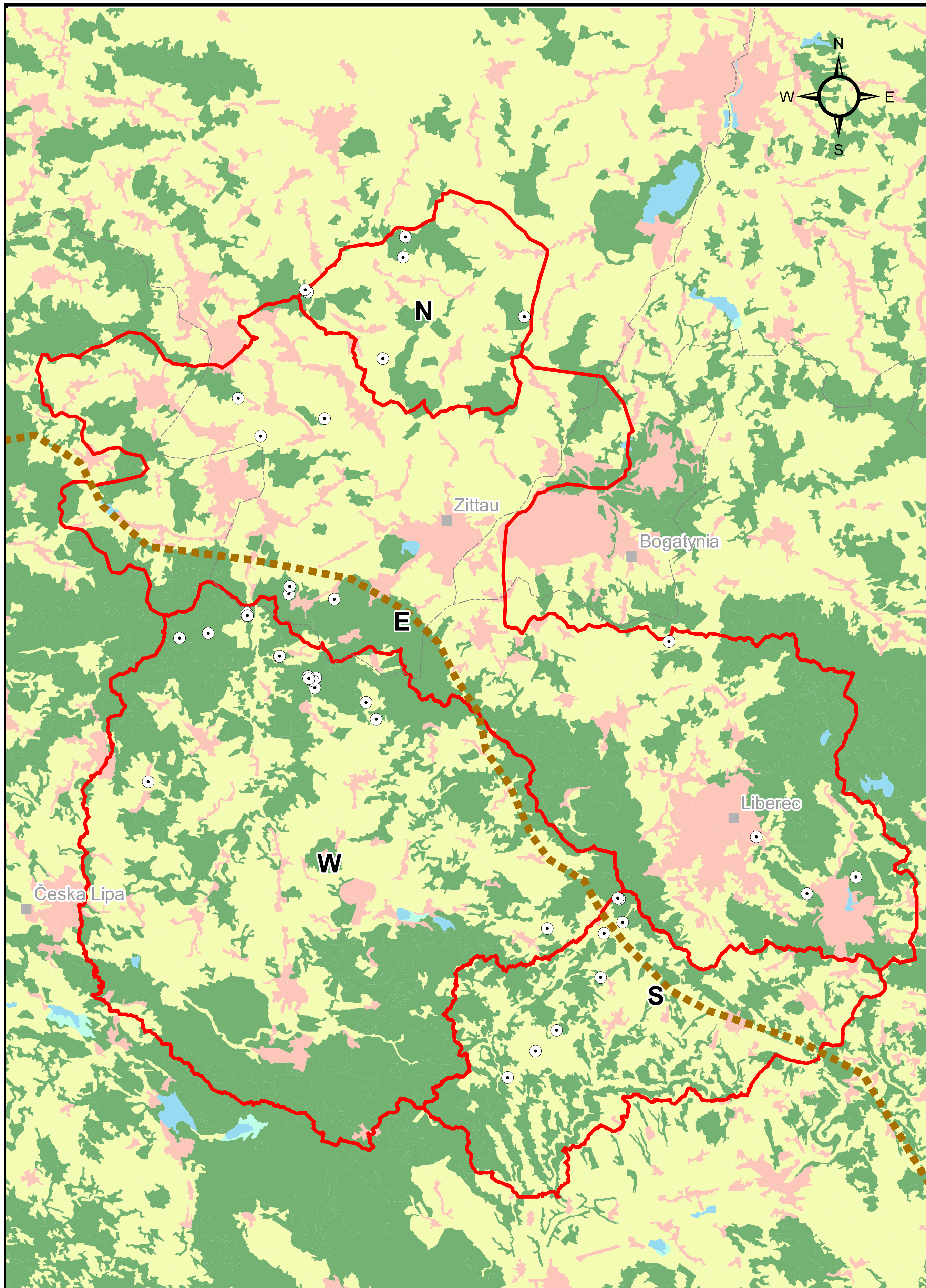
4. Define the direction of Flow in each pixel	**Flow direction** (spatial analyst)	Filled raster
5. Find areas where water flows	**Flow Accumulation** (spatial analyst)	Flow direction raster
6. Allow the program to count the 50m surrounding	**Snap Pour Point** (Spatial Analyst)	Spring points, Flow Accum, 50 meters,
7. Build the watershed raster	**Watershed** (Spatial Analyst)	Flow direction, snapped points
8. Convert to polygons	**Raster to Polygon**	Watershed raster
9. Join all polygon shapefiles in one file	**Merge**	Watershed polygons, Contours, Main catchments (basin)
10. Compare land use that belongs to each polygon.	**Intersect**	Corine Land-use 2006, watersheds
11. Merge all polygons belonging to the same cover category in each spring to facilitate area calculation	**Dissolve**	Intersection of land cover and watersheds
12. Show the percentage information for landcover	**Calculate Geometry (area), calculate field (% formula), add labels**	Watersheds shapefile (attribute table)

After obtaining the specific values of percentage in each locality, the classification followed Table 7.

Table 7: Classification according to forest proportion in spring watershed (run-off)

DOMINANT LAND COVER	DESCRIPTION
Forest	The highest % of watershed corresponds to trees
Agriculture	The highest % of watershed corresponds to planted monocrops
Impervious	The highest % of watershed is artificial cover or urbanized.

To check general surface composition, see in the next page:
<u>Map 5: Land cover in study area</u>



1:300 000

0 2,5 5 10 15 20 Kilometers

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LAND COVER IN STUDY AREA

Legend

- Springs
- Cities
- Lusatian
- Country borders
- Catchments

Landuse

surface cover

- Agricultural areas
- Artificial surfaces
- Forest and semi natural areas
- Water bodies
- Wetlands

Author: Diego Sebastian Serrano Suarez
Data source: ESRI online, Corine land cover, 2006
Coordinate System: S-JTSK Krovak East North
Projection: Krovak
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Map 5

4.3. Stage 3: measuring toxic metals

This section explains the methods and equipment used in laboratory to measure the toxic chemicals in water, in sediment and in dwelling biota of the spring. The main portion of measurements was developed in external laboratories. The author effort was focused on gathering this data, performing the index calculations and examining the legal guidelines of water quality. The formulas and details of methods are also covered below.

4.3.1. Detection of metals concentration

The metal content was analyzed in water, sediment and macrozoobenthos.

The methods for analyzing the water parameters in Zittau laboratory by IHI Dresden Technical University. The selected elements concentration was detected by inductively coupled plasma with optical emission spectrometry (ICP-OES, PerkinElmer) according to DIN EN ISO 11885 (www.iso.org), and inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer) according to DIN EN ISO 17294-2 (www.iso.org) depending on their concentration (Vitvar et al., 2017). The following elements were measured:

V	Cr	Mn	Co	Ni	Cu	Zn
As	Ag	Cd	Pb	Fe	Mg	Ca

The sediment was collected in plastic boxes, dried and sieved. The content of metals in benthic organism were measured when enough tissue was available with freeze-dried organisms. Prior to metals analyses the solid samples (sediment and biota) has to be microwave digested to transfer them to liquid form. The acid digestion was prepared in Czech University of Life Sciences laboratory by Lucie Heřmanova and Evžen Nesrovnal (4/2018, laboratory work) with the support of the author, following US EPA method 3052. The metal concentration was quantified with flame 55 Atomic Absorption Spectrometer (F55AAS) and graphite furnace atomizer AA (GTA 120) (both Agilent technologies), described in previous publication of the project by Noriega (2017).

The results on metal content and distribution are expressed on a dry weight basis. For the analysis of hazard from toxics metals concentration in water, sediments and *Gammarus* tissue, the information can be simplified using following factors:

- Distribution or partition coefficient
- Hazard Quotient
- Mobility Factor
- Biota Sediment Accumulation Factor

Note: For this study, only are calculated distribution coefficient (Kd) and hazard quotient (HQ), which relate water quality and sediment quality, as explained in the next subchapter. The other factors were excluded due to insufficient data to get reliable statistics about concentration of toxic metals in *Gammarus* tissue.

4.3.2. Calculation of metal accumulation

Distribution (or partition) coefficient

This coefficient represents the relation of metal that has been attached to the bottom of the spring through sedimentation, over the presence of metal dissolved in water. That proportion gives an idea about the different forms of the metals available in the spring. Log Kd values of 3 or less identify metals occurring mostly in dissolved form, while Log Kd values above 4 identify metals preferably bound to sediment (Borovec et al. 1993 ex. (Komínková, Nábělková, & Vitvar, 2015)).

$$Kd = \frac{Cs}{Cw} \quad = \quad \frac{Metal\ concentration\ in\ sediment\ \ (mg/kg)}{Metal\ concentration\ in\ water\ (mg/L)}$$

Hazard quotient

This quotient reveals the level of hazard for aquatic biota according to metal concentration in the sediment, related with a quality standard from literature (Barnthouse et al., 1982 in Komínková et al., 2015). The equation allows to classify field sites according to metal pollution in four categories: background, low, medium, and high (Clements et al. 2000 ex. ex. Komínková et al, 2015) Based on the value of HQ, it is possible to predict changes in benthic community composition. The Table 8 below, shows the values that will be considered for classification.

$$HQ = \frac{Cs}{EQS} = \quad \frac{Metal\ concetration\ in\ sediment}{Environmental\ quality\ standard\ (treshold)}$$

Table 8: Hazard quotient cumulative criteria (Clements et al, 2000 ex. Komínková et al, 2015)

Sediment Hazard Quotient	Description
Background < 1	Unpolluted locality with no effect on aquatic organisms
Low 1 - 2	Low pollutant load with no acute danger for organisms
Medium 2 - 10	Intermediate load with fatal effect to sensitive species
High > 10	Significant decrease on macroinvertebrate diversity

The hazard quotient estimation does not give a probability of a health effect in a exposed population, however, it indicates the risk level due to pollutant exposure (Storelli, 2008). It allows the classification of sediment quality with standardized values that are proven through extensive research to influence (USEPA) the habitat of aquatic invertebrates in springs. *Gammarus fossarum* are considered as non-sensitive species, thus some range of tolerance is expected.

Noriega (2017) recalls absence of suitable criteria in Czech national legislation and recommends to use US Environmental Protection Agency (USEPA) benchmark Threshold Effect Concentration (TEC) and Probable Effect Concentration (PEC). The Table 9 was extracted from consensus-based freshwater sediment quality guidelines (EPA, 2000), about metals of interest of this study.

Table 9: Sediment guidelines that reflect threshold (TEC) and effect concentration. (PEC, above which harmful effects are observed) (MacDonald et al. 2000 ex.EPA,2000)

Metals (in mg/kg DW)	**Toxic Effect Threshold TEC**	**Consensus-based PEC**
Arsenic	17	33
Cadmium	3	4.98
Chromium	100	111
Copper	86	149
Lead	170	128
Nickel	61	48.6
Zinc	540	459

4.4 Stage 4: analyzing relationships

The relationships will enable the researchers to understand why this species is absent in some locations, and whether or not the number of gammarids gives a clue about the conditions of the environment.

4.4.1 Statistical methods

The relationship of *Gammarus* abundance with the different environmental variables was analyzed using the statistical software R Studio. The sample is considered with 40 values, each corresponding to the average counting of *Gammarus* between summer and fall samples. However, it was found one extreme number from BN1024 with 244 individuals in fall and 86 in summer (165 average), so it was excluded from the list to allow a clearer statistical analysis. The data of water quality was obtained in average values per spring. The variables considered in the data analysis spreadsheet were: spring ID, season, spring type, geology, human influence, dominant plants, water quality (according Czech legislation), acidity, dissolved oxygen, toxic metals in sediment, dominant land cover and of course, *Gammarus* number.

We generated one plot per variable to visualize the number of *G. fossarum* in each category, and to identify the dominant factors influencing their abundance.

The data showed overdispersion, hence a logarithmic conversion was required. The results give an idea of the importance of each category for the adaptation of these widespread freshwater shrimps, however, it is recommended to develop further research in different localities to validate the results with the same sampling method.

One model adding all variables allowed to detect the most relevant categories, which are statistically significant. This model (general linear model, GLM) used Poisson regression and ANOVA (chi). The linearity should show a *p value* higher than 0.05 to validate the hypothesis of dependency. To test data on non-parametric model, it was implemented also ANOVA (Kruskal-Wallis) test.

Following this step, it is necessary to choose the significant categories and develop a new model that will show more refined results.

According to literature, the most critic values for adaptation of this species are acidity and dissolved oxygen, thus it was implemented a linear regression to evaluate that hypothesis.

For details of the model in R Studio, see **Annex A: Statistical protocol**.

CHAPTER 5

Results

During this chapter, the reader can observe the findings according to the methodology proposed in the previous section. In total, 80 samples were analyzed, corresponding to 40 springs during summer sampling and the same 40 springs during fall sampling. This section is organized following the 4 stages proposed in the methodology:

1) **Finding *Gammarus*,** identifying presence and abundance of *Gammarus sp*.
2) **Classifying habitat information,** compiling the variables for analysis.
3) **Measuring toxic metals,** extracting data from tissue, sediment and water.
4) **Analyzing relationships,** correlating environmental factors with the abundance of *Gammarus fossarum*.

5.1. Stage 1: finding *Gammarus*

Gammarus fossarum is the most common species in the selected water springs. A total of 25 over 40 springs presented *Gammarus* presence in both seasons.

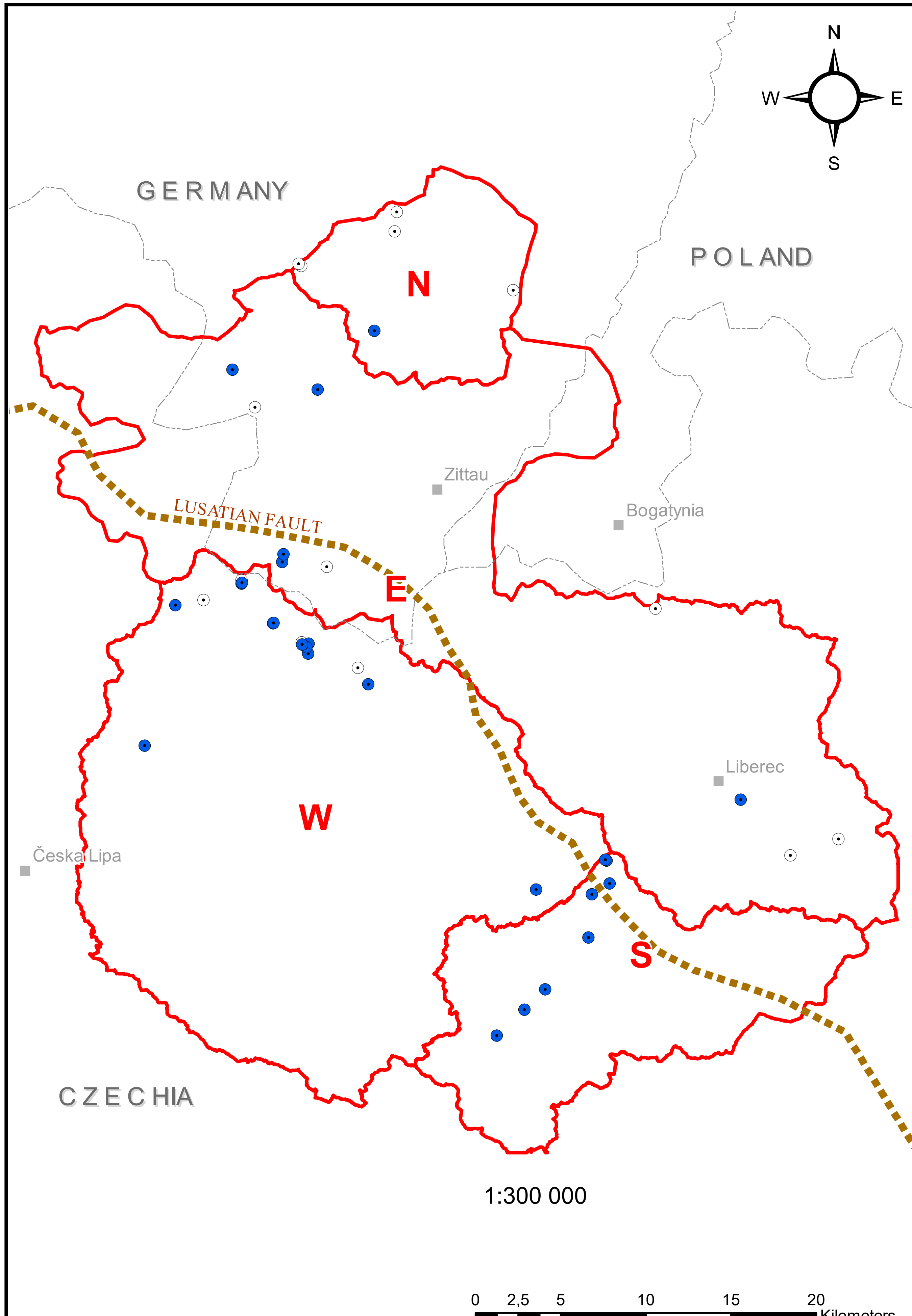
The most abundant population of *Gammarus fossarum* found with the composed circular method (see Methodology chapter) was the spring Jonsdorf horní Hirschbörnl BN1024. This spring reached 86 specimens in summer and 244 during fall. This special case was excluded from calculation in all statistical to improve the behavior of the analysis.

About spring BN1024 - Jonsdorf horní Hirschbörnl

This is a rheocrene spring within a broad-leaved forest having *Fagus sylvatica* trees as dominant species. The main geology of this natural spring is cretaceous sediment. During the sampling time, the spring bottom was covered by sand and a generous quantity of dried leaves. The average flow was 10 liters per minute and it is located in a steep slope ~1:1. Temperature in fall sampling (when the most abundant population was found) was 8.4°C, dissolved oxygen 10.59 mg/L, pH 6.88, and conductivity 196.5µS/cm.

See in the next page:

<u>Map 6: Presence of *G. fossarum* in study area</u>



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PRESENCE OF GAMMARUS FOSSARUM IN STUDY AREA

Legend

- Springs with G. fossarum
- Springs without G. fossarum
- Cities
- Lusatian
- Country borders
- Catchments

Author: Diego Sebastian Serrano Suarez
Data source: ESRI online
Coordinate System: S-JTSK Krovak East North
Projection: Krovak
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Map 6

Figure 10: Sample with abundant G. fossarum in stereoscope. BN1024

The constant accessibility of habitat for *G. fossarum* cannot be proved with this study of only 2 samplings during one-year period, because some springs can dry temporally. However, the presence of *G. fossarum* in at least one of the springs from each hydro-geographical region (North, East, West, South), confirms that the species distribution expands to the whole study area.

The results from different seasons: summer and fall (July and October), are presented in the Figure 11. The season with most abundant populations of *G. fossarum* was summer, however the highest peak was presented in fall. This peak means 244 individuals found in BN1024 (shown in Figure 10), which was excluded from this graphic.

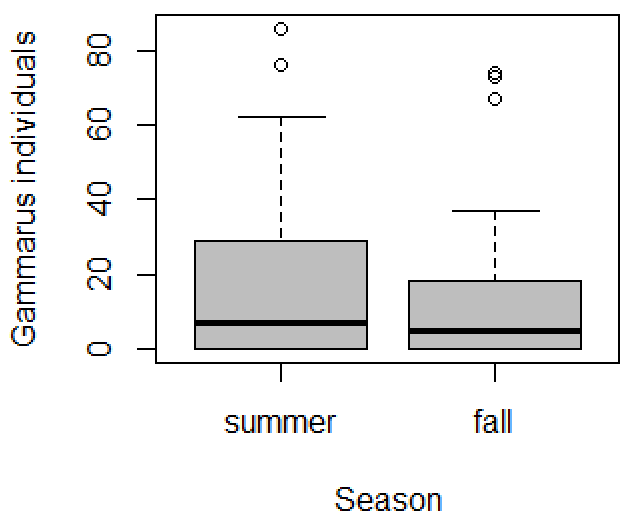


Figure 11: Boxplot of G. fossarum abundance in different sampling seasons

5.2. Stage 2: classifying habitat information

The results of habitat information are displayed in 3 tables for this section.

Table 10 gives an idea of the water quality measured in situ with the multi-meter, accordingly, the reader can check the abundance of *G. fossarum* in each season and compare this number with the conditions in each spring. The data used in the statistical analysis (section 5.4), contains average values including these and more measurements of water quality and also average values of *Gammarus* population. This will improve the reliability of the study, even considering that the equipment was calibrated and the measurements were taken rigorously.

Table 11 is the compilation of findings according classification of different environmental factors established for this study. All details regarding to each category, selection method and abbreviations is explained above in the methodology section 4.2. The values for water quality respond to the Czech legislation analysis, including the average values of NO_3^-, SO_4^{2-}, Cl, F, V, Cr, Mn, Co, Ni, Cu, Zn, As, Ag, Cd, Pb, Fe, Mg, and Ca. The measuring methods are specified above in subsection 4.2.1 for ions in water and subsection 4.3.1 for metals in water. The classification of spring type, geology, human influence and dominant plants are expressed according to the criteria of the author and his collaborators.

Table 12 is the result from the GIS analysis of land cover, which is explained above in section 4.2.6. The area obtained for each cover category (forest, agriculture and urban) was divided by the total area of the watershed to identify their percentage of importance in the contribution run-off.

Table 10: Water measurements in situ on sampling days

N	Spring ID	mgO2/L		pH		µS/cm		°C		**Gammarus number**	
		Summer	**Fall**	**Summer**	**Fall**	**Summer**	**Fall**	**Summer**	**Fall**	**Summer**	**Fall**
1	AN1029	9.84	9.89	6.03	6.15	175.5	190.1	14.7	9.8	0	0
2	AN1030	7.1	7.22	6.39	6.41	185.2	199.4	10.5	9.1	0	0
3	AN1036	7.13	5.32	6.52	6.14	348	418	11.9	10.2	0	0
4	AN1037	3.65	4.15	6.78	6.56	109.3	119.1	9.5	9.1	10	15
5	AS1043	11.18	10.67	7.28	6.34	91.5	92.2	6.9	7.1	13	23
6	BN1024	10.55	10.59	7.37	6.88	175.6	196.5	9.1	8.4	86	244
7	BN1025	8.39	8.63	6.18	6.07	229	255	9.3	8.8	55	25
8	BN1033	7.35	5.6	7.45	7.66	488	501	13.5	14.6	27	37
9	BN1034	4.9	4.97	7.23	7.59	326	394	10.6	10.5	12	74
10	BN1041	9.18	9.11	5.77	5.46	100.5	110.3	9.1	8.8	0	0
11	BN2242	4.53	7.92	5.86	5.37	391	288	11.6	8.6	0	0
12	BN2243	9.3	7.64	5.64	5.93	253	215	9.15	9.4	0	0
13	BN2244	8.27	6.14	6.87	5.65	237	154.8	17.2	9	0	0
14	BS1016	8.75	8.67	6.56	5.85	106.6	117.3	10.1	9.6	33	15
15	BS1019	2.27	2.52	7.28	6.99	198.4	201.6	12.1	9.9	50	28
16	BS1027	9.31	9.33	6.98	6.57	115.8	116.2	7.9	7.6	36	18
17	BS1040	0.22	1.02	8.02	5.84	210.9	205.7	7.7	9.6	0	0
18	BS1200	10.17	9.74	6.49	4.27	107.8	108.7	9.1	9.4	4	15
19	BS1203	10.21	10.06	7.71	7.69	1106	73.3	13.4	9.5	27	14
20	BS1204	9.55	9.15	7.21	6.49	115.1	111.5	8.5	8.3	0	0
21	BS2228	5.18	5.56	5.59	5.58	146.7	164.6	10.3	9.3	0	0
22	BS2229	9.78	10.11	6.21	5.83	80.6	88.2	10.2	7.9	0	0
23	BS2230	9.46	9.19	7.79	7.33	158.9	163.5	10.7	9.9	30	73
24	BS2232	8.9	9.07	6.4	5.74	83.5	96.8	9	7.3	6	19
25	BS2233	9.08	8.71	6.22	6.19	97.9	86.9	7.4	8.3	18	5
26	BS2235	9.31	9.28	6.68	6.05	83.2	91.1	10.2	8.9	3	7
27	CS2227	5.67	5.59	6.98	6.65	108.1	122.3	10.1	9.3	10	15
28	DN1012	7.99	7.88	4.86	5	83.3	83.9	10.5	10.4	0	0
29	EN1005	7.32	7.21	7.99	7.6	275	308	8	7.2	28	16
30	EN1006	10.69	10.87	7.72	7.57	191.9	208.4	7.4	6.9	30	67
31	EN1011	9.95	10.58	7.63	7.66	360	395	10.4	9.8	8	5
32	EN2238	-	10.63	-	4.85	-	66.6	-	9.3	0	0
33	EN2239	-	10.21	-	5.61	-	88.1	-	9.5	0	0
34	EN2241	-	9.18	-	6.27	-	358	-	10.9	1	0
35	ES1008	9.16	9.72	7.91	7.48	605	668	10.5	9.6	30	1
36	ES1009	9.26	9.83	7.43	7.38	516	566	9.7	9.3	62	0
37	ES1014	8.11	8.04	7.68	7.16	223	240	9.4	9	46	50
38	ES1104	9.68	9.18	7.33	7.45	444	485	9.2	9.1	76	37
39	ES1108	10.5	10.66	9.85	7.83	406	438	9.4	9.5	2	4
40	ES2220	9.92	9.82	7.64	7.3	365	494	10.1	10.9	15	19

Table 11: Results of springs categorization and Gammarus abundance per season

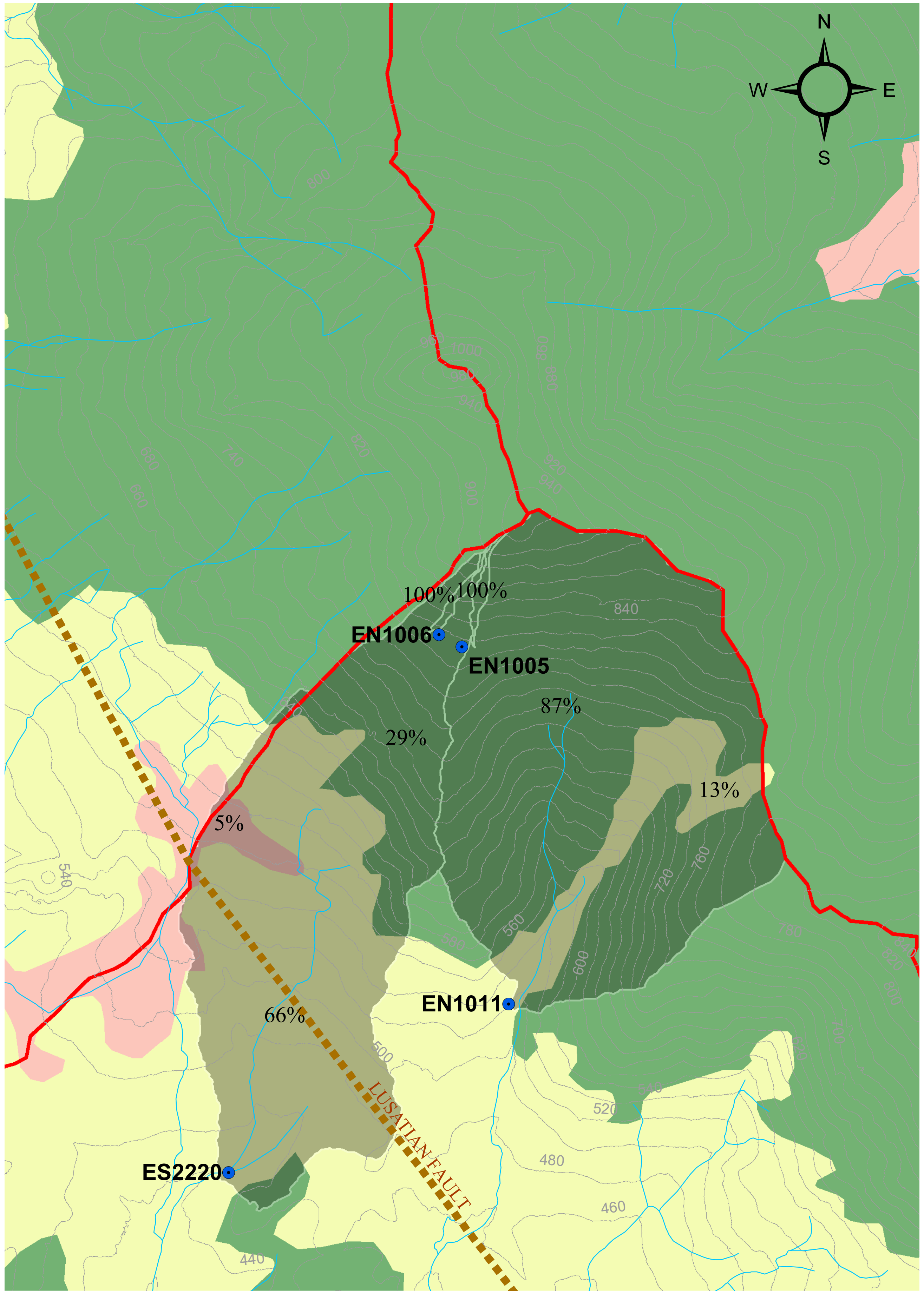
N	spring ID	location	type	water quality	dominant geology	human influence	dominant plants	Gammarus average
1	AN1029	Kottmar Bushwiesen	Artificial	Class V	PC	Class D	Non-forest	0
2	AN1030	SchieSborn	Limnocrene	Class IV	PC	Class C	Broad-leaved	0
3	AN1036	Leutersdorf - Kirschbaumplantage	Limnocrene	Class IV	TV	Class C	Coniferous	0
4	AN1037	Leutersdorf - Ziegenborn	Limnocrene	Class IV	PC	Class C	Mixed forest	13
5	AS1043	Mařenice - třízdrojový	Rheocrene	Class II	CS	Class A	Broad-leaved	18
6	BN1024	Jonsdorf horní Hirschböml	Rheocrene	Class II	CS	Class A	Broad-leaved	165
7	BN1025	Jonsdorf dolní Buchberg unten	Rheocrene	Class IV	PC	Class A	Coniferous	40
8	BN1033	Bornwaldchen	Helocrene	Class III	PC	Class A	Broad-leaved	32
9	BN1034	Unterh. Spitzberg	Limnocrene	Class III	TV	Class A	Mixed forest	43
10	BN1041	Jonsberg u sportoviště	Rheocrene	Class V	PC	Class A	Broad-leaved	0
11	BN2242	Jungfrauenquelle	Helocrene	Class V	PC	Class A	Coniferous	0
12	BN2243	Steinbogen	Limnocrene	Class IV	PC	Class C	Broad-leaved	0
13	BN2244	Birke	Rheocrene	Class II	PC	Class A	Coniferous	0
14	BS1016	Radvanec	Artificial	Class IV	CS	Class B	Non-forest	24
15	BS1019	Krompach	Artificial	-	CS	Class C	Broad-leaved	39
16	BS1027	Vodopád u Heřmanic	Limnocrene	Class II	CS	Class A	Mixed forest	27
17	BS1040	tzv. "sirný pramen"	Limnocrene	Class V	CS	Class A	Coniferous	0
18	BS1200	Heřmanice - Nad Borůvčím	Rheocrene	Class IV	CS	Class A	Coniferous	10
19	BS1203	Heřmanice - 4. propustek	Rheocrene	Class II	CS	Class A	Broad-leaved	21
20	BS1204	Heřmanice - Babiččin odpočinek	Rheocrene	Class IV	CS	Class A	Broad-leaved	0
21	BS2228	Petrovice - ve skalce	Artificial	Class V	CS	Class C	Broad-leaved	0
22	BS2229	Myslivny - U smrku	Rheocrene	Class V	TV	Class A	Mixed forest	0
23	BS2230	Krompach - Pod Kulichem	Rheocrene	-	CS	Class A	Broad-leaved	52
24	BS2232	Myslivny - Pod Buky	Rheocrene	Class IV	CS	Class A	Broad-leaved	13
25	BS2233	Myslivny - Nad Pasekou	Rheocrene	Class IV	CS	Class A	Broad-leaved	12
26	BS2235	Heřmanice - U Oplocenky	Rheocrene	Class IV	CS	Class A	Non-forest	5
27	CS2227	Kněžice	Rheocrene	Class III	CS	Class A	Non-forest	13
28	DN1012	začátek Vítkovského potoka	Helocrene	Class V	PC	Class A	Broad-leaved	0
29	EN1005	Ještěd Frantina stud	Rheocrene	Class II	PC	Class B	Broad-leaved	22
30	EN1006	Ještěd Pramen lesních panen	Rheocrene	Class II	PC	Class B	Broad-leaved	49
31	EN1011	Vesec-Jiříčkov	Rheocrene	Class II	PS	Class B	Broad-leaved	7
32	EN2238	Mšeno nad Nisou (ul. Arbesova)	Helocrene	Class V	PC	Class A	Coniferous	0
33	EN2239	Rýnovice - věznice	Rheocrene	Class V	PC	Class A	Coniferous	0
34	EN2241	Starý Harcov - Temná ul-Hrubínova	Rheocrene	Class V	PC	Class A	Broad-leaved	1
35	ES1008	Vápno	Limnocrene	Class V	CS	Class B	Non-forest	16
36	ES1009	Lesnovek	Artificial	Class IV	CS	Class B	Non-forest	31
37	ES1014	Prameny Ploučnice	Limnocrene	Class II	CS	Class A	Broad-leaved	48
38	ES1104	Všelibice	Rheocrene	-	CS	Class C	Non-forest	57
39	ES1108	V Moskových dolech-Modlibohov	Artificial	Class II	CS	Class C	Broad-leaved	3
40	ES2220	Světá pod Ještědem Rozstání GOLF	Limnocrene	Class II	CS	Class A	Broad-leaved	17

Table 12: Percentage of land cover in watersheds (area of influence of spring by runoff)

N	Spring_ID	AREA (Ha)	% forest	% agriculture	% urban	Dominant land cover
1	AN1029	2.47	100			Forest
2	AN1030	1.15	97	3		Forest
3	AN1036	1.55		100		Agro
4	AN1037	74.88	39	61	0	Agro
5	AS1043	1.33	100			Forest
6	BN1024	1.01	100			Forest
7	BN1025	8.02	100			Forest
8	BN1033	48.57		100		Agro
9	BN1034	32.08	5	95		Agro
10	BN1041	7.85	100			Forest
11	BN2242	32.96	74	26		Forest
12	BN2243	2.08	94	6		Forest
13	BN2244	28.89	55	45		Forest
14	BS1016	1266.85	73	25	2	Forest
15	BS1019	0.49	78	22		Forest
16	BS1027	0.49	100			Forest
17	BS1040	527.68	100			Forest
18	BS1200	0.24	100			Forest
19	BS1203	2.98	100			Forest
20	BS1204	4.05	100			Forest
21	BS2228	2.12	20	80		Agro
22	BS2229	2.74	100			Forest
23	BS2230	324.76	41	47	12	Agro
24	BS2232	10.59	92	8		Forest
25	BS2233	0.01	100			Forest
26	BS2235	3.06	100			Forest
27	CS2227	790.27	78	22		Forest
28	DN1012	13.42	1	99		Agro
29	EN1005	1.09	100			Forest
30	EN1006	0.90	100			Forest
31	EN1011	161.12	87	13		Forest
32	EN2238	6.79	100			Forest
33	EN2239	4.61	100			Forest
34	EN2241	4.56			100	Urban
35	ES1008	13.72	4	96		Agro
36	ES1009	140.08	12	88		Agro
37	ES1014	106.15	53	47		Forest
38	ES1104	197.57	50	50		Forest
39	ES1108	77.85	71	29		Forest
40	ES2220	151.20	29	66	5	Agro

To check an example of the result layout in ArcGIS, see in the next page:

Map 7: Land cover proportion in watershed.



1:20 000

0 0,25 0,5 1 1,5 2 Kilometers

Legend

- Springs with G. fossarum
- Cities
- Lusatian
- Country borders
- contours elevation
- Catchments
- Watersheds

Land use

Surface cover

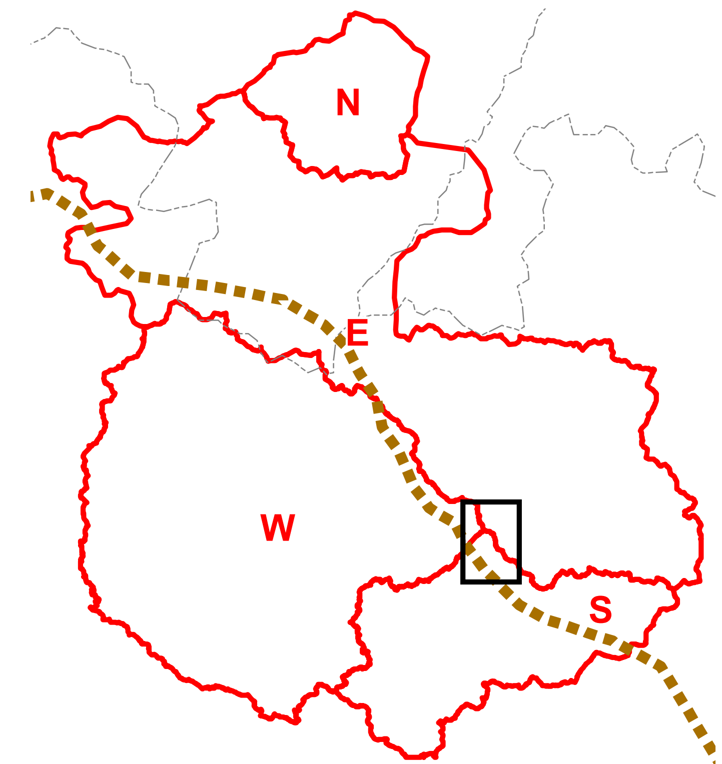
- Agricultural areas
- Artificial surfaces
- Forest and semi natural areas
- Water bodies
- Wetlands

Author: Diego Sebastian Serrano Suarez
Data source: ESRI online
Coordinate System: S-JTSK Krovak East North
Projection: Krovak
Datum: S JTSK

Czech University of Life Sciences
Date: April, 2018

PRAMENY SPOJUJÍ
QUELLEN VERBINDEN

LAND COVER PROPORTION IN WATERSHED



Springs displayed:

EN1005: Ještěd Frantina stud
EN1006: Ještěd Pramen lesních panen
EN1011: Vesec-Jiříčkov
ES2220: Světá pod Ještědem Rozstání GOLF

ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

European Union
European Regional Development Fund

Transboundary cooperation 2016 – 2019
European Regional Development Fund

Map 7

5.3. Stage 3: measuring metal content

This section elucidates the toxicology findings of this research. This is presented as a guide to detect the springs with more pollution traits, whether it is allocated in the water or the sediment of the spring. Only metals which are present on the EPA (2000) regulation were included, according to the methodology explained above in the section 4.3.

Two tables are showing the results of the factors of toxic metals identification.

Table 13 displays the distribution coefficient (Kd), which means the proportion of metals found in sediment over the proportion of metals found in water. This will clarify if the main concentration of metal is available in the water flow or attached to the substrate, which is important for understanding mobility of metals in the trophic chain. Overall, lead (Pb) was present mainly in solid phase, in contrast with niquel (Ni) mainly in liquid phase.

Table 14 containd the hayard quotient (HQ), which evaluates only the metals in sediments and its relationship with the values recommended by EPA (2000). It gives a clue about the effect that the metal concentration could have on the benthic community in general. Ideally, these values would correspond to the effect on *Gammarus fossarum* only, but that information does not exist yet from a reliable source.

The measurement of metals revealed that the most common metal in the water of the springs is iron, which gives an orange color to the bottom substrate and prevent the existence of diverse biota as it reduces the available dissolved oxygen. Three springs were found to be ferric: BN2242, BN1033 and BS1040, with concentrations 3.8, 1.1 and 0.7 mg/L respectively. Surprisingly, there were *Gammarus* in some sections of the spring BN1033, Bornwaldchen. The lowest dissolved oxygen was reached in BS1040, average 1.2 mg/L.

Other metal indicating concentration higher than normal in some springs was nickel. Springs AN1029, AN1030, BN2242 and BS2228 contained average of 34, 16, 17 and 18 Ni µg/L respectively, which means these are polluted environments, either for natural causes or human impact.

Some springs presented a slightly polluted water by cadmium: AN1029, BN1041, BS2228, BS2229, DN1012, EN2238, and EN224, between 0.1 and 0.5 Cd µg/L.

Table 13: Relationship of toxics between liquid and solid phase in the springs

N	Spring ID	Metals in water (µg/L)							Metals in sediment (mg/kg)							Log Kd						
		As	Cd	Cr	Cu	Pb	Ni	Zn	As	Cd	Cr	Cu	Pb	Ni	Zn	As	Cd	Cr	Cu	Pb	Ni	Zn
1	AN1029	0,3	0,5	1,0	1,4	0,2	34,4	43,2	12,0	0,8	26,8	13,3	36,2	38,1	44,6	4,6	3,3	4,4	4,0	5,2	3,0	3,0
2	AN1030	0,7	0,0	0,3	0,2	0,0	15,9	2,7	40,3		40,3	14,5	55,1	41,8	75,3	4,7		5,2	4,8	6,2	3,4	4,4
3	AN1036	0,5	0,0	0,6	0,5	0,1	1,1	18,4	23,2	0,3	32,4	10,6	34,3	7,8	94,5	4,7	3,8	4,7	4,3	5,7	3,9	3,7
4	AN1037	0,1	0,0	0,3	0,6	0,0	1,5	2,1	12,3	0,3	20,2	9,0	34,1	18,1	37,9	5,0	4,4	4,8	4,2	6,1	4,1	4,3
5	AS1043	0,1	0,0	0,1	0,1	0,0	0,3	0,2														
6	BN1024	0,3	0,0	0,6	0,3	0,1	4,8	3,1	6,5		3,0		6,5	7,2	12,2	4,3		3,7		4,8	3,2	3,6
7	BN1025	0,2	0,0	0,5	0,6	0,1	2,7	5,4	6,8		4,5	3,2	14,1	3,8	17,1	4,6		3,9	3,8	5,4	3,1	3,5
8	BN1033	0,3	0,0	0,7	0,3	0,1	3,8	0,7	20,4		15,2	2,8	21,8	10,2	53,3	4,8		4,3	3,9	5,3	3,4	4,9
9	BN1034	0,4	0,0	2,4	0,2	0,1	1,2	1,0			14,8	2,2	8,6	3,6	14,2			3,8	4,0	5,1	3,5	4,1
10	BN1041	0,1	0,2	0,3	0,4	0,0	4,8	13,2														
11	BN2242	0,4	0,0	0,3	0,2	0,0	16,8	8,0	16,6		16,8	11,5	47,7	20,0	33,8	4,7		4,7	4,8	6,5	3,1	3,6
12	BN2243	0,1	0,0	0,4	0,5	0,0	4,4	1,9	7,3		20,8	4,9	25,9	13,0	48,0	4,7		4,7	4,0	6,2	3,5	4,4
13	BN2244	0,4	0,0	0,4	0,3	0,1	3,5	1,0	11,3		18,7	6,6	41,3	11,6	31,5	4,4		4,7	4,4	5,8	3,5	4,5
14	BS1016	0,4	0,0	0,5	0,3	0,0	5,2	3,3			2,0		25,5	2,1	3,4			3,6		6,0	2,6	3,0
15	BS1019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	BS1027	0,1	0,0	0,4	0,4	0,0	2,9	3,1			2,3		3,5	3,8	6,2			3,8		5,1	3,1	3,3
17	BS1040	0,5	0,0	0,2	0,1	0,0	1,4	0,9	8,7		6,7	1,5	17,2	8,1	36,5	4,3		4,5	4,0	6,0	3,8	4,6
18	BS1200	0,1	0,0	0,3	0,1	0,0	6,0	7,9														
19	BS1203	0,3	0,0	0,2	0,2	0,0	3,3	3,3														
20	BS1204	0,6	0,1	0,7	0,3	0,5	6,9	8,9														
21	BS2228	0,1	0,1	0,3	0,4	0,0	17,6	22,8			3,6	3,6	7,9	1,6	4,7			4,1	3,9	5,2	2,0	2,3
22	BS2229	0,1	0,2	0,2	0,5	0,1	6,4	18,9														
23	BS2230	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	BS2232	0,2	0,0	0,2	0,2	0,0	1,0	2,0	11,3		8,2	17,1	20,2	7,6	63,3	4,8		4,7	4,9	5,7	3,9	4,5
25	BS2233	0,2	0,0	0,1	0,3	0,0	1,7	4,0	7,7		5,9	2,1	20,5	3,9	17,4	4,6		4,7	3,9	6,0	3,4	3,6
26	BS2235	0,1	0,1	0,1	0,1	0,0	8,9	10,6			4,1	1,2	6,9	8,1	7,8			4,6	4,0	5,4	3,0	2,9
27	CS2227	0,6	0,0	0,2	1,4	0,1	2,1	3,6	9,6	0,3	9,2	33,0	22,0	7,1	21,0	4,2	4,4	4,8	4,4	5,6	3,5	3,8
28	DN1012	1,6	0,1	0,3	0,3	0,0	4,1	3,9	16,7	0,5	19,4	22,9	33,3	22,9	49,9	4,0	3,7	4,7	4,8	6,2	3,7	4,1
29	EN1005	0,1	0,0	0,1	0,4	0,1	1,8	1,2	10,3		11,0	4,5	12,6	16,3	58,8	4,9		4,9	4,1	5,0	4,0	4,7
30	EN1006	0,4	0,1	0,3	0,2	0,2	1,4	2,9	13,2	0,6	5,4	4,3	25,9	10,1	70,5	4,5	3,8	4,3	4,3	5,1	3,8	4,4
31	EN1011	0,4	0,0	0,5	0,1	0,0	2,4	0,3	25,2	0,3	19,8	7,8	25,9	22,6	42,7	4,8	5,2	4,6	5,0	6,1	4,0	5,2
32	EN2238	0,1	0,4	0,2	0,7	0,1	0,9	3,2	14,1		16,2	3,0	23,5	4,8	25,3	5,1		4,9	3,6	5,5	3,7	3,9
33	EN2239	0,1	0,1	0,2	0,9	0,1	0,8	2,8			9,8	2,8	16,5	8,0	36,6			4,6	3,5	5,2	4,0	4,1
34	EN2241	7,2	0,2	0,8	1,8	0,1	6,8	3,0	8,5		14,0	4,9	24,6	6,8	63,0	3,1		4,2	3,4	5,4	3,0	4,3
35	ES1008	9,8	0,0	0,3	2,1	0,2	4,1	0,7	10,9		3,0	1,4	4,1	2,3	6,3	3,0		4,0	2,8	4,4	2,8	4,0
36	ES1009	0,9	0,0	0,6	0,5	0,0	4,0	0,2	13,0		18,4	8,2	8,2	48,5	73,6	4,1		4,5	4,2	5,7	4,1	5,6
37	ES1014	0,1	0,0	0,4	0,2	0,0	1,4	0,6	9,5		12,7	5,1	17,6	6,1	29,4	4,9		4,5	4,3	5,9	3,6	4,7
38	ES1104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	ES1108	0,6	0,0	0,4	0,2	0,0	2,7	3,3	14,7		3,6		3,0	5,5	4,9	4,4		4,0		5,2	3,3	3,2
40	ES2220	0,3	0,0	0,3	0,3	0,0	2,8	0,3	7,0		2,8	2,7	5,5	3,2	7,4	4,3		3,9	3,9	5,4	3,1	4,3

below detection limit   metals bound to water   metals bound to sediment

Table 14: Hazard Quotients = toxic metals in sediment divided by threshold (TEC)

N	Spring	As-HQ	Cd-HQ	Cr-HQ	Pb-HQ	Cu-HQ	Ni-HQ	Zn-HQ	SUM	∑ HQ
1	AN1029	0,71	0,27	0,27	0,16	0,21	0,62	0,08	2,33	Medium
2	AN1030	2,37	0,00	0,40	0,17	0,32	0,68	0,14	4,09	Medium
3	AN1036	1,37	0,08	0,32	0,12	0,20	0,13	0,17	2,40	Medium
4	AN1037	0,72	0,08	0,20	0,10	0,20	0,30	0,07	1,68	Low
5	AS1043	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Background
6	BN1024	0,38	0,00	0,03	0,00	0,04	0,12	0,02	0,59	Background
7	BN1025	0,40	0,00	0,05	0,04	0,08	0,06	0,03	0,66	Background
8	BN1033	1,20	0,00	0,15	0,03	0,13	0,17	0,10	1,78	Low
9	BN1034	0,00	0,00	0,15	0,03	0,05	0,06	0,03	0,31	Background
10	BN1041	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Background
11	BN2242	0,97	0,00	0,17	0,13	0,28	0,33	0,06	1,95	Low
12	BN2243	0,43	0,00	0,21	0,06	0,15	0,21	0,09	1,15	Low
13	BN2244	0,67	0,00	0,19	0,08	0,24	0,19	0,06	1,42	Low
14	BS1016	0,00	0,00	0,02	0,00	0,15	0,03	0,01	0,21	Background
15	BS1019	0,00	0,00	0,01	0,00	0,06	0,01	0,01	0,10	Background
16	BS1027	0,00	0,00	0,02	0,00	0,02	0,06	0,01	0,12	Background
17	BS1040	0,51	0,00	0,07	0,02	0,10	0,13	0,07	0,90	Background
18	BS1200	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Background
19	BS1203	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Background
20	BS1204	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Background
21	BS2228	0,00	0,00	0,04	0,04	0,05	0,03	0,01	0,16	Background
22	BS2229	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	Background
23	BS2230	0,38	0,00	0,02	0,00	0,02	0,02	0,02	0,47	Background
24	BS2232	0,67	0,00	0,08	0,20	0,12	0,12	0,12	1,31	Low
25	BS2233	0,45	0,00	0,06	0,02	0,12	0,06	0,03	0,75	Background
26	BS2235	0,00	0,00	0,04	0,01	0,04	0,13	0,01	0,24	Background
27	CS2227	0,56	0,09	0,09	0,38	0,13	0,12	0,04	1,41	Low
28	DN1012	0,98	0,15	0,19	0,27	0,20	0,38	0,09	2,26	Medium
29	EN1005	0,61	0,00	0,11	0,05	0,07	0,27	0,11	1,22	Low
30	EN1006	0,78	0,18	0,05	0,05	0,15	0,17	0,13	1,51	Low
31	EN1011	1,48	0,11	0,20	0,09	0,15	0,37	0,08	2,48	Medium
32	EN2238	0,83	0,00	0,16	0,03	0,14	0,08	0,05	1,29	Low
33	EN2239	0,00	0,00	0,10	0,03	0,10	0,13	0,07	0,43	Background
34	EN2241	0,50	0,00	0,14	0,06	0,14	0,11	0,12	1,07	Low
35	ES1008	0,64	0,00	0,03	0,02	0,02	0,04	0,01	0,76	Background
36	ES1009	0,77	0,00	0,18	0,10	0,05	0,80	0,14	2,03	Medium
37	ES1014	0,56	0,00	0,13	0,06	0,10	0,10	0,05	1,00	Low
38	ES1104	0,00	0,00	0,06	0,04	0,03	0,18	0,01	0,32	Background
39	ES1108	0,87	0,00	0,04	0,00	0,02	0,09	0,01	1,02	Low
40	ES2220	0,41	0,00	0,03	0,03	0,03	0,05	0,01	0,57	Background

5.4. Stage 4: analyzing relationships

The information about *Gammarus* abundance related with the different variables is shown in plots to facilitate the readability of some findings. The criteria for the selection of each category is explained in detail in the methodology section of this report. The relationship of each variable was analyzed with average numbers of *Gammarus* together from summer and fall. At the end of this section, the reader will find a set of interactive maps that integrate all the collected data about each spring, including representative biota and environmental parameters from different disciplines. See legend in page 67.

5.4.1. Water quality

According to the classification of surface water quality, following the Czech normative for running water ČSN 75 7221, it was proved that in the study area there are no springs that belong to Class I (excellent and unpolluted water). *G. fossarum* showed presence in Class II, III and IV, however in Class V, there was practically no presence of *Gammarus*, as it is shown in Figure 12. The abundance presents the highest general values in Class III and the maximum peaks of individuals per sample, belongs to Class II.

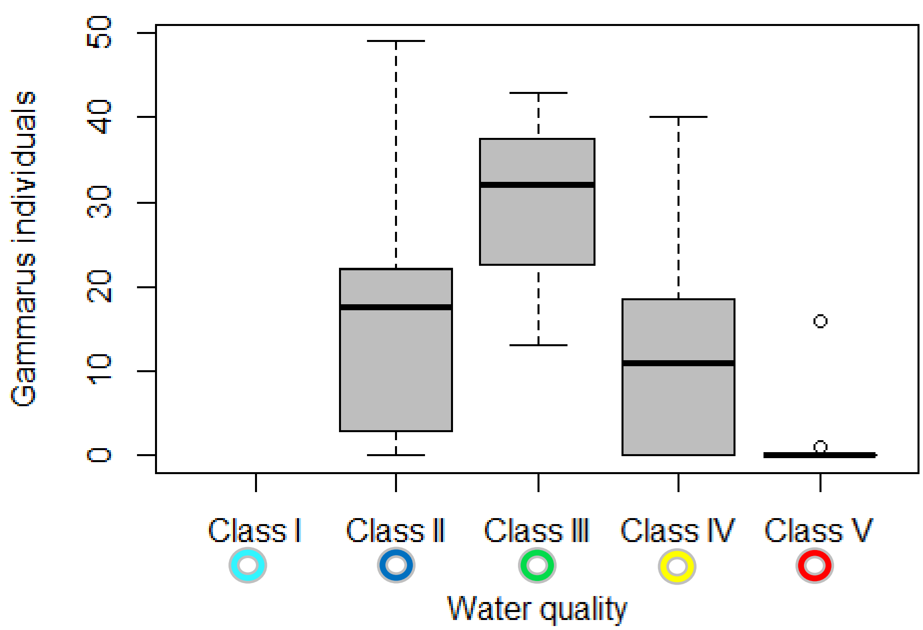


Figure 12: Boxplot of G. fossarum abundance in different water quality.

As shown in Figure 13, the dissolved oxygen concentration does not represent a linear factor determining the quantity of *Gammarus fossarum* individuals. That is the reason why, no regression line was displayed for this plot.

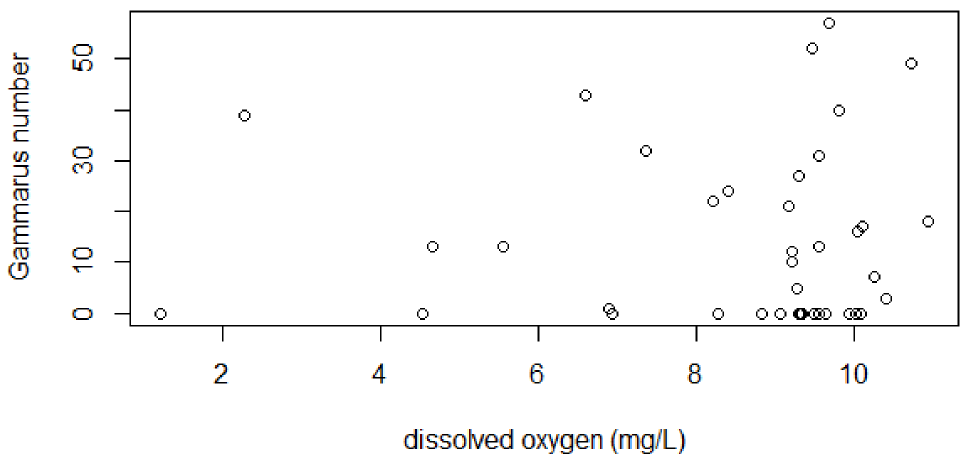


Figure 13: G. fossarum abundance in different dissolved oxygen concentration

In contrast, pH did show a representative trend, which is expressed in the equation below on Figure 14, which means that higher pH presents higher number of *G. fossarum*.

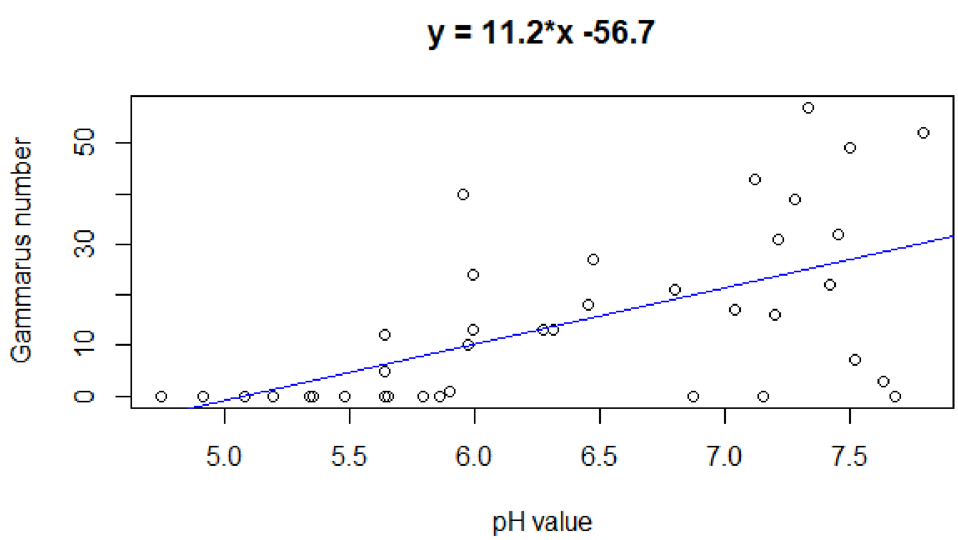


Figure 14: G. fossarum abundance in different acidity conditions

5.4.2. Other environmental factors

According to Figure 15, *G. fossarum* is present indifferently in all hydrological types of spring, which means this is not a limiting factor to detect the presence of this species. The dominant plants according to the analyzed categories was also not representative, although statistically the box with highest number is mixed forest in Figure 16.

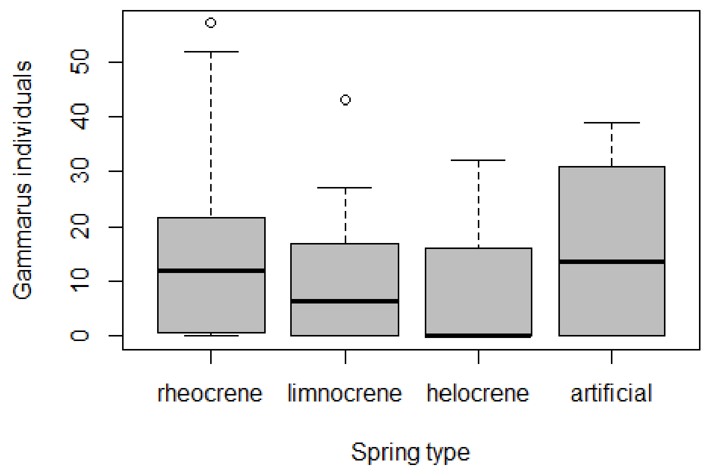


Figure 15: Boxplot of G. fossarum abundance in different spring type.

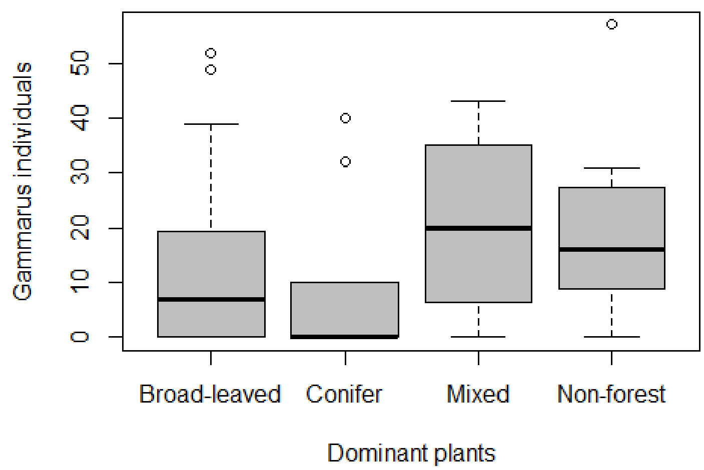


Figure 16: Boxplot of G. fossarum abundance in different dominant vegetation.

In Figure 17 it is visible that all geology types can hold *G. fossarum* populations, however it is hard to determine the real influence of the rocks on their abundance, since only few samples corresponded to Paleozoic sediment and Tertiary vulcanite types. About land cover influencing the watershed (Figure 18), also the results were negative, a specific dominant type does not influence directly the abundance of *Gammarus*.

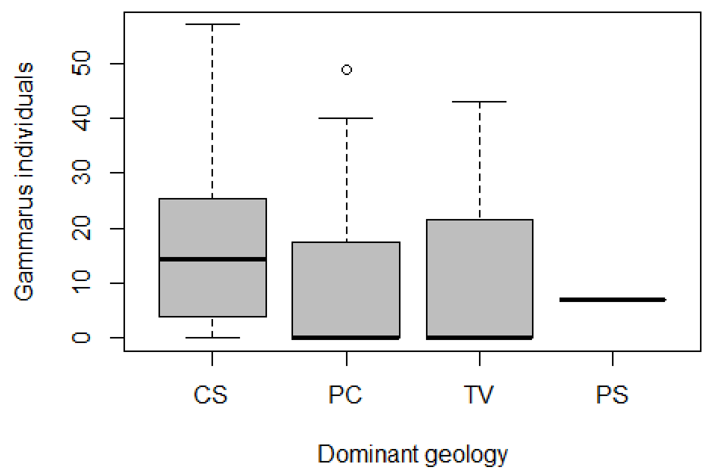


Figure 17: Boxplot of G. fossarum abundance in different dominant geology

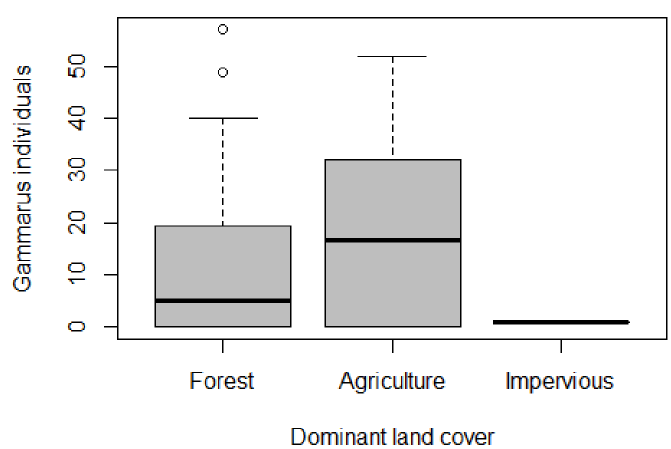


Figure 18: Boxplot of G. fossarum abundance when the watershed cover changes

However, for human influence, the model expressed dependency between the type of influence and the *G. fossarum* number (Figure 19). Those springs Class B, with natural substrate and an artificial intake are the preferred ones. Class D, the ones with artificial substrate show absence. Also, for toxicity in the sediment is visible some expected trend, and the linear model confirmed the hypothesis. As the Figure 20 shows, when concentration of metals increases in sediment, less abundance of *Gammarus* is identified.

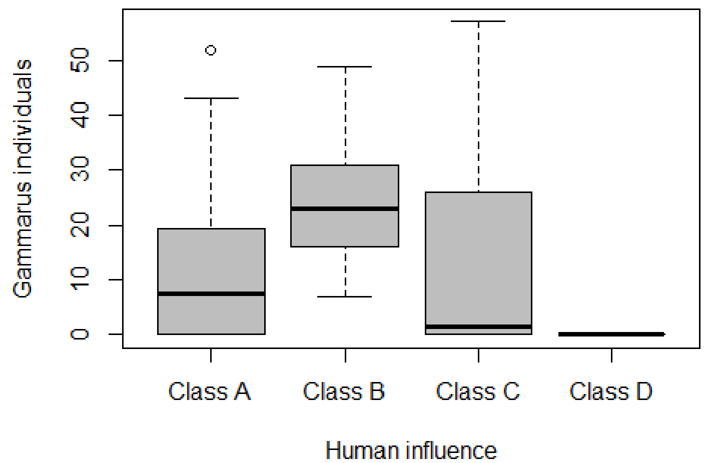


Figure 19: Boxplot of G. fossarum abundance in different human influence level

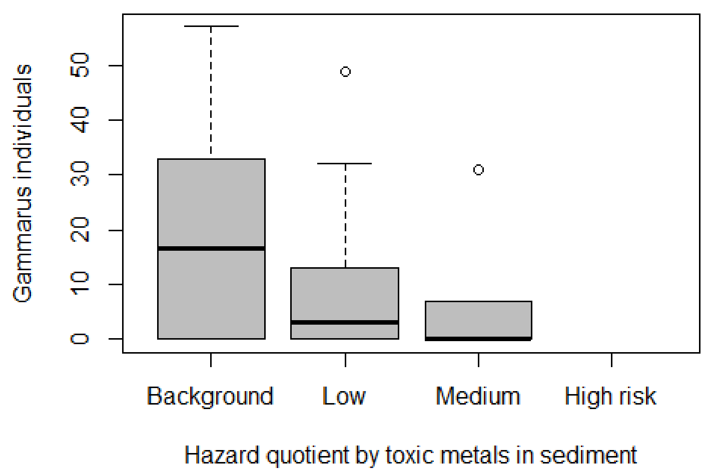
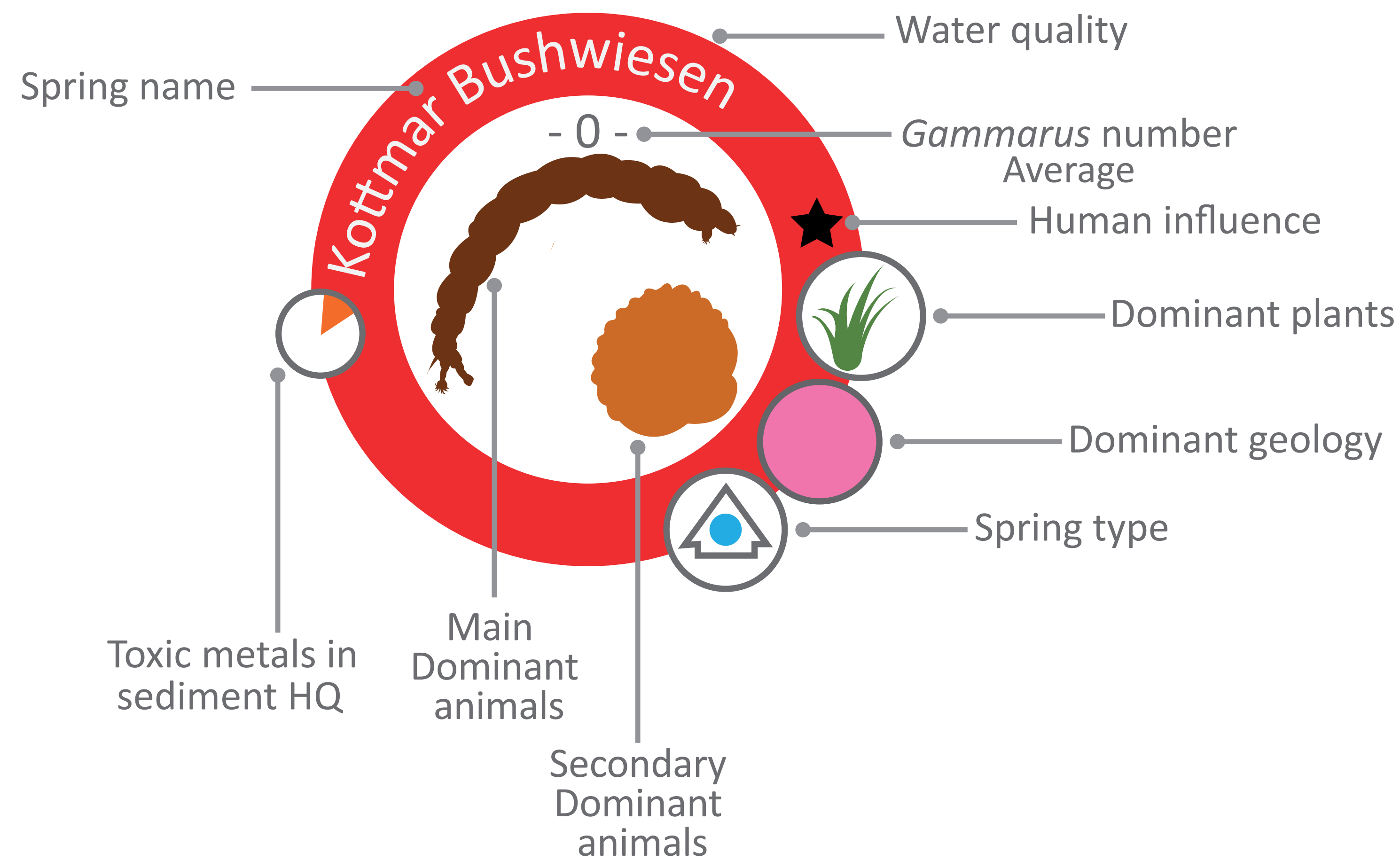


Figure 20: G. fossarum abundance in different toxicity in sediment
HQ = (As +Cd + Cr + Cu + Pb + Ni + Zn)

For checking *G. fossarum* average abundance among different environmental variables at the same time, see in the next pages: **Maps 8 – 12: Spring details**

LEGEND



DOMINANT ANIMALS

WATER QUALITY

- Not polluted
- Slightly polluted
- Polluted
- Strongly polluted
- Very strongly polluted

SPRING TYPE

- Rheocrene
- Helocrene
- Limnocrene
- Artificial

DOMINANT GEOLOGY

- Cretaceous sediment
- Palaeozoic crystalline
- Tertiary vulcanite
- Palaeozoic sediment

DOMINANT PLANTS

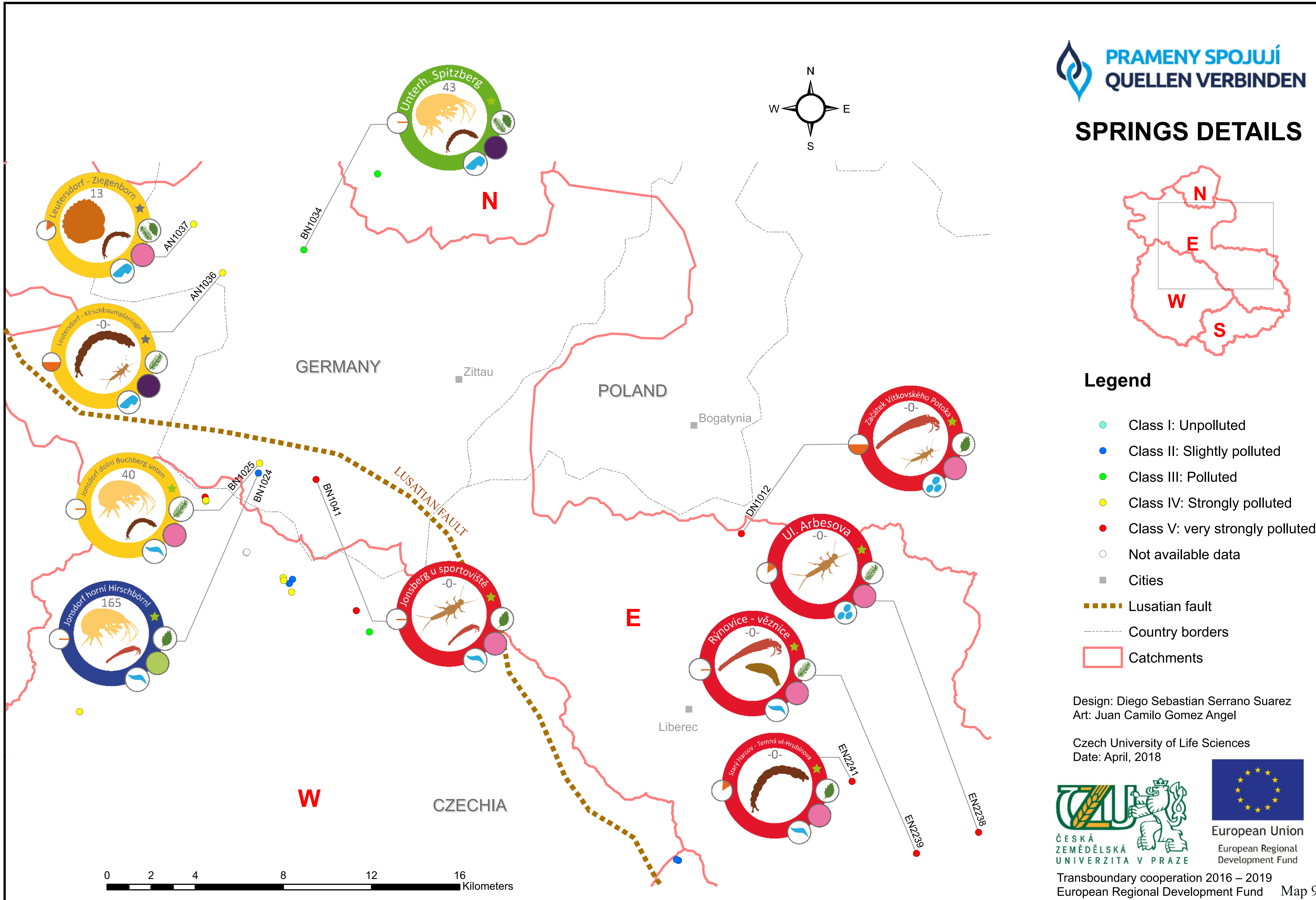
- Coniferous forest
- Broad-leaved forest
- Mixed forest
- Non-forest

TOXIC METALS IN SEDIMENT HQ

- Background < 1
- Low 1 - 2
- Medium 2 - 10
- High > 10

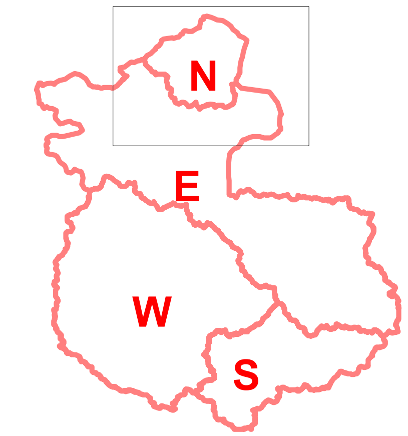
HUMAN INFLUENCE

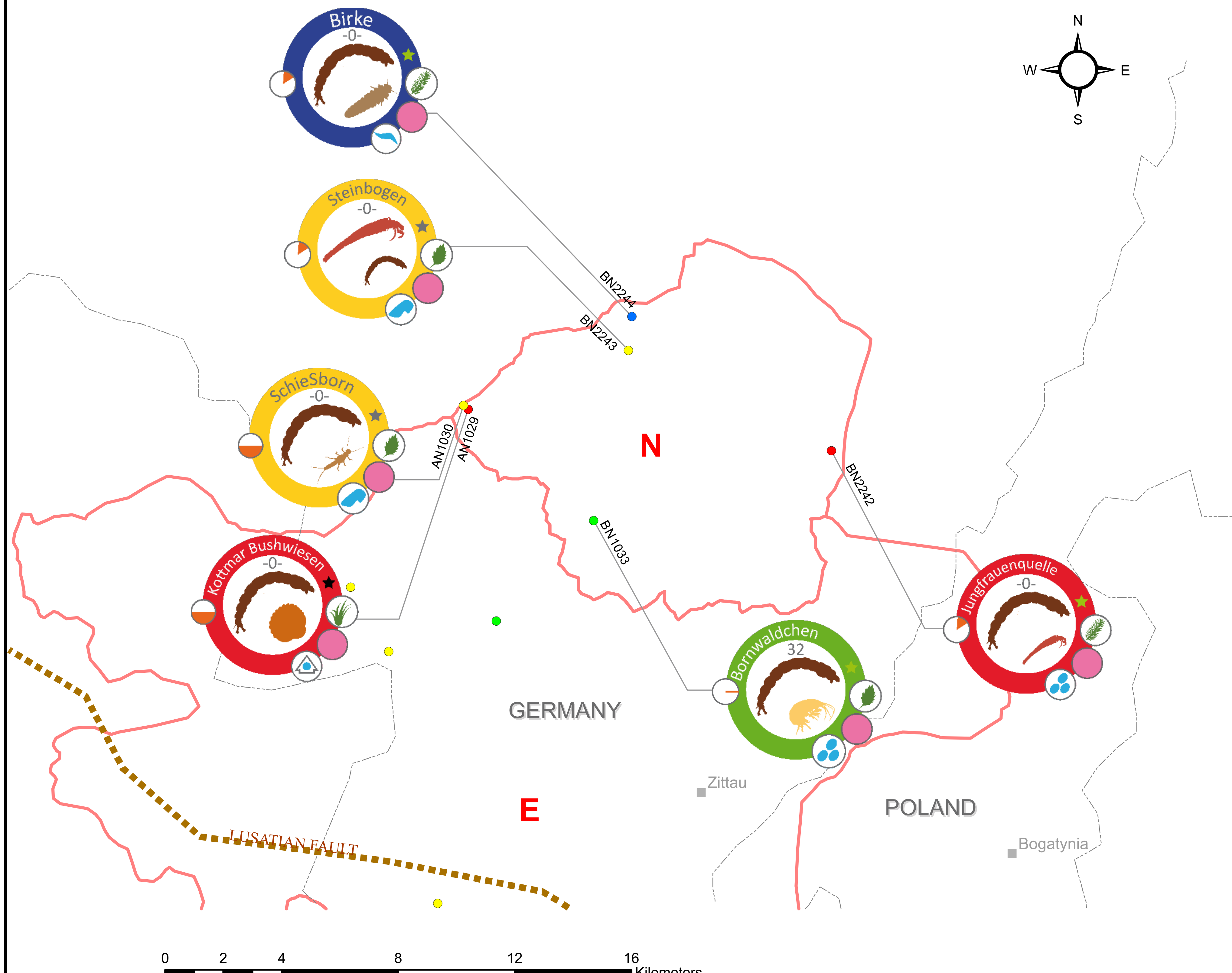
- Natural spring
- Artificial intake
- Artificial structure
- Heavily modified


PRAMENY SPOJUJÍ
QUELLEN VERBINDEN
SPRINGS DETAILS
N
E
W
S
Legend
Class I: Unpolluted
Class II: Slightly polluted
Class III: Polluted
Class IV: Strongly polluted
Class V: very strongly polluted
Not available data
Cities
Lusatian fault
Country borders
Catchments
Design: Diego Sebastian Serrano Suarez
Art: Juan Camilo Gomez Angel
Czech University of Life Sciences
Date: April, 2018
ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
European Union
European Regional Development Fund
Transboundary cooperation 2016 – 2019
European Regional Development Fund
Map 9
GERMANY
POLAND
CZECHIA
Zittau
Bogatynia
Liberec
LUSATIAN FAULT
Unterh. Spitzberg
43
Leutersdorf - Ziegenborn
13
Leutersdorf - Kirschbaumplantage
-0-
Jonsdorf dolní Buchberg unten
40
Jonsdorf horní Hirschbörnl
165
Jonsberg u sportoviště
-0-
Začátek Vítkovského Potoka
-0-
Ul. Arbesova
-0-
Rýnovice - věznice
-0-
Starý Harcov - Temná ul.-Hrubínova
-0-
BN1034
AN1037
AN1036
BN1025
BN1024
BN1041
DN1012
EN2241
EN2239
EN2238
0
2
4
8
12
16
Kilometers

PRAMENY SPOJUJÍ
QUELLEN VERBINDEN

SPRINGS DETAILS





Legend

Water springs quality

- Class I: Unpolluted
- Class II: Slightly polluted
- Class III: Polluted
- Class IV: Strongly polluted
- Class V: very strongly polluted
- Not available data
- Cities
- Lusatian fault
- Country borders
- Catchments

Design: Diego Sebastian Serrano Suarez
Art: Juan Camilo Gomez Angel

Czech University of Life Sciences
Date: April, 2018

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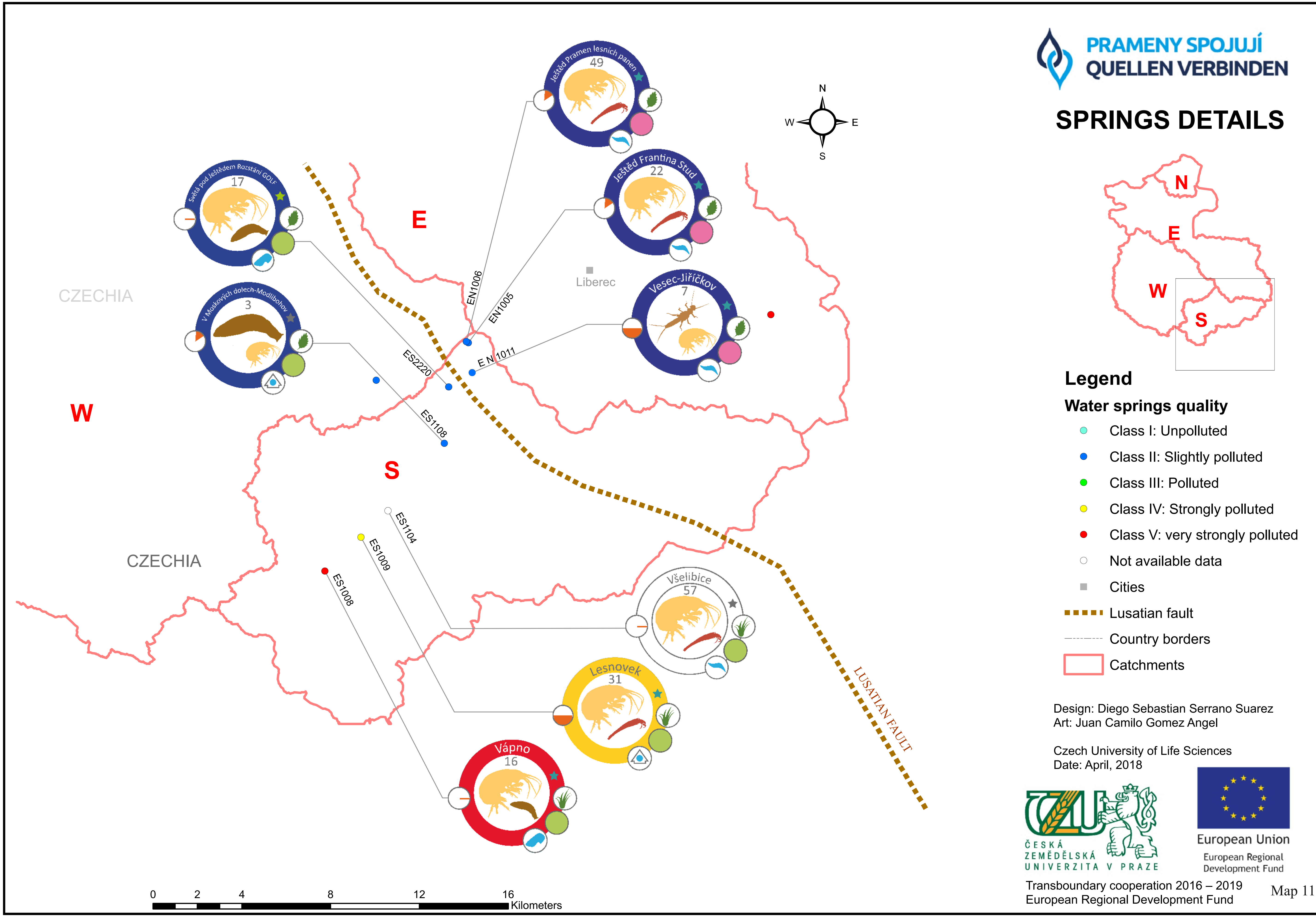
European Regional Development Fund

Transboundary cooperation 2016 – 2019
European Regional Development Fund

Map 10



PRAMENY SPOJUJÍ
QUELLEN VERBINDEN
SPRINGS DETAILS
N
E
W
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CZECHIA
CZECHIA
Liberec
LUSATIAN FAULT
49
22
17
3
7
57
31
16
Vesec-Jiříčkov
Všelibice
Lesnovek
Vápno
EN1006
EN1005
E N 1011
ES2220
ES1108
ES1104
ES1009
ES1008
Legend
Water springs quality
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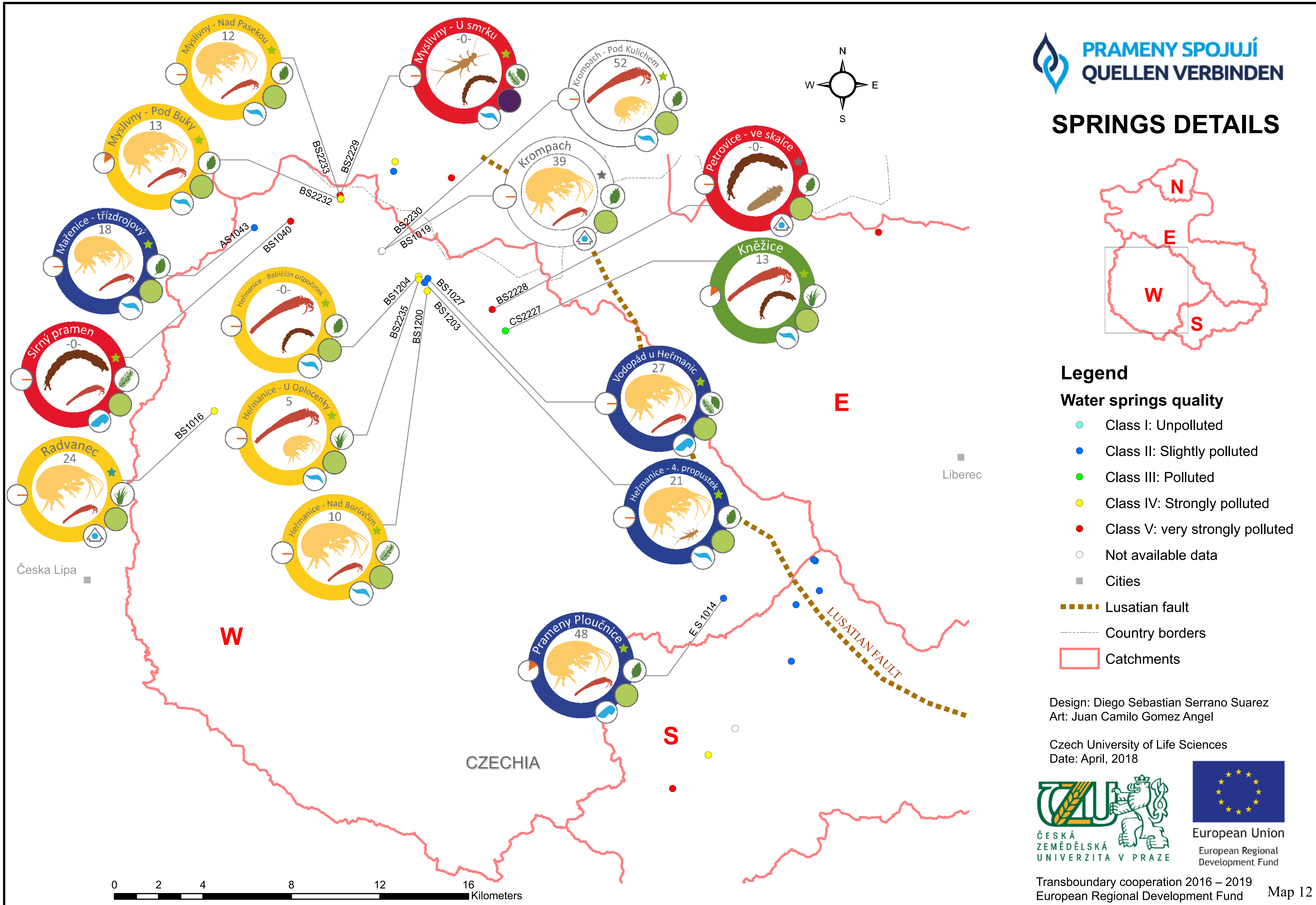
European Union
European Regional Development Fund
Transboundary cooperation 2016 – 2019
European Regional Development Fund
0 2 4 8 12 16 Kilometers
Map 11



PRAMENY SPOJUJÍ
QUELLEN VERBINDEN
SPRINGS DETAILS
Legend
Water springs quality
Class I: Unpolluted
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ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
European Union
European Regional Development Fund
Transboundary cooperation 2016 – 2019
European Regional Development Fund
Map 12
N
W
E
S
Myslivny - Nad Pasekou
12
Myslivny - U smrku
-0-
Krompach - Pod Kulichem
52
Myslivny - Pod Buky
13
Krompach
39
Petrovice - ve skalce
-0-
Mařenice - třízdrojový
18
Heřmanice - Babiččin odpočinek
-0-
Kněžice
13
Sirný pramen
-0-
Heřmanice - U Oplocenky
5
Vodopád u Heřmanic
27
Radvanec
24
Heřmanice - Nad Borůvčím
10
Heřmanice - 4. propustek
21
Prameny Ploučnice
48
BS2233
BS2229
BS2232
AS1043
BS1040
BS2230
BS1019
BS1204
BS1027
BS2235
BS1200
BS1203
BS2228
CS2227
BS1016
E S 1014
LUSATIAN FAULT
Liberec
Česka Lipa
CZECHIA
0 2 4 8 12 16 Kilometers

CHAPTER 6

Discussion

This chapter explains the significance of the findings and their applicability to similar research. The results from the last chapter are examined here to elucidate if there is a realistic dependency between the analyzed environmental factors and abundance of the studied species.

Gammarus fossarum is very successful colonizer of water springs in central Europe. The highest number of individuals found in this research belonged to that species, confirming that amphipods in springs are an ideal model system to study the biological effects of water quality (Glazier, 1998). It is relatively easy way to collect them, and its permanence in aquatic environment during the whole life cycle allows to make reliable studies to measure chronic changes in the water from the springs.

Nevertheless, it is complex to relate their numbers in nature with other factors besides water parameters, like features in the landscape, rocks, trees or man-made structures that will slowly also influence the water quality. It is complex and yet possible if researchers keep trying multidisciplinary approach and improving techniques.

Our study proved that low pH reduces the number of *G. fossarum* (Andrén & Eriksson Wiklund, 2013; Felten et al., 2008; Kobayashi, S., et al, 2013; O. J. Dangles & Guérold, 2000; Peeters & Gardeniers, 1998). Laboratory experiments have potential to determine threshold values and biological effects of critical parameters like pH, however the real response of *G. fossarum* to the stressor in nature is not the same as in aquariums and it is necessary to study it in detail. For example, a 38h experiment exposing caged *Gammarus pulex* to low pH conditions (pH<6.0) showed significant reduction of locomotor activity (Felten et al., 2008), however it is hard to predict the response of *Gammarus* in nature.

Causes of pH decrease may be some types of rock like Paleozoic crystalline (granitic), and also leaf litter decomposition (Ferreira and Guérold, 2017). Assuming that these factors influence *Gammarus* population, it was tested if animals number is dependent on dominant geology and dominant plants around the springs. Clear link was not detected in the model, although forest type was more likely to influence the abundance than rocks. The most abundant population was broad-leaved forest dominated by *Fagus sylvatica* and cretaceous sediment geology.

If more detailed results are desired, it is necessary to study the forest cover depending on the species of trees, since there is effect from leaves, shadow, wind, and water retention. For example, an influence from the roots was evidenced in the spring BN1025 during both sampling moments. A thin layer of pine oil was floating in the surface with a very strong odor of resin. The oil apparently comes from the roots of a *Picea abies* tree situated above the spring and it does not seem to affect the *Gammarus* individuals that were swimming actively in the spring.

Some studies demonstrate active selection of regions with more favorable oxygen concentrations (Henry & Danielopol, 1998), i.e. in theory, *Gammarus* would travel towards springs looking for more oxygenated water. For this study, it is questionable that oxygen is the main driver that influence their shift because the presence of *Gammarus* was confirmed in relatively low concentration of dissolved oxygen (<5mg/L). However, it is fair to consider fluctuations in the dissolved oxygen of the spring, for example short terms of low concentration.

The adaptation of the animals when they reach a spring, is dependent on water quality and also on morphology of the place. In this sense, it was analyzed if the classification of springs type (rheocrene, helocrene, limnocrene and artificial) could have an effect. Unexpectedly, many populations of Gammarus thrive on springs with artificial intake with natural substrate, however, there is not reliable dependency according to the model since they can live in any type of spring. Maybe substrate heterogeneity is more relevant than substrate type or spring type (Kubíková et al., 2012).

Springs of human impact Class B, which were influenced by a pipeline or structure to get the water (without modifying the natural bottom substrate), were in general the most abundant for *G. fossarum*. It is assumed that the water may increase their oxygen content while coming out from the ground with more pressure. The negative influence of plastic bottom is clearly visible, limiting the habitat for macroinvertebrate species in AN1029, which was almost null. No *Gammarus* were found.

According to the Czech legislation parameters (Mičaník et al. 2017), this study area does not have any unpolluted spring (Class I). *Gammarus* prefer polluted or slightly polluted water. During the project it was found that they are not present in unpolluted springs very strongly polluted environments in general (Class V - red). Some toxic metals have been also responsible of decreasing locomotion of *Gammarus* in laboratory conditions, for example, lead, copper, zinc, nickel and cadmium (Lebrun et al. 2017). Although these animals are resistant to some pollution factors, the toxicity of sediment seemed to have a trend where higher hazard quotient categories were reflecting less abundant gammarids. The metals analyzed were only the ones with available threshold values from literature (As, Cr, Cu, Cd, Pb, Ni and Zn), and the hazard quotient analyzed represents the sum of those metals.

Land cover and runoff from urban settlements or agriculture can be responsible for higher pollution and toxic metals concentration. However, the watershed analysis is limited by the unknown real area of infiltration. Having only data of runoff, the statistics found no dependency for this land cover and *Gammarus* abundance. The water quality in the springs is directly affected by land use, e.g. the spring with highest concentration of NO_3^- was ES1008, and 96% of its watershed is covered by agricultural fields. However, it does not seem to vanish population of shrimps, which are present even there at 60 mg NO_3^-/L.

It is important to find links between land cover and water quality. The high quality of the aquatic habitat is crucial to increase diversity, even before protection of terrestrial habitats, so conservation management of freshwater insects should be prioritized in freshwater habitats (Harabiš, 2017).

There is a limitation to study the watershed influence because the zone resulting from Digital Elevation Model analysis represents only to runoff influence. The actual infiltration areas cannot be predicted with the current data, which would need complementary information about underground water dynamics. It can be suggested that areas with more vegetation and lower slope can serve as infiltration zone in the valleys, increasing the chance of water to percolate. Nevertheless, soil porosity plays a crucial role to determine whether or not the aquifers can be held for certain time.

Interestingly, the spring EN2241 Starý Harcov, obtained the highest value of chlorine ions: average 493 mg Cl / L. This water source located inside Liberec, is the only spring of this study with 100% of urban land cover in its watershed. An assumption points to salt from the roads as suspicious source of Cl^-.

Some species exclusively inhabiting springs are considered crenobionts (Schmidlin et al., 2015b). This species has proved to be able to live in habitats different from springs, so they are not considered crenobionts, however the reasons of their success in these ecosystems can respond to different causes, e.g. the high reproduction rate and absence of predators play an important role in their relative abundance. Also, temporary range shifts can depend on hydrologic conditions. In the study area, two springs presented active populations during the summer and total absence during fall: Starý Harcov EN2241 and Lesnovek ES1009.

Important efforts are being made to document effects of land use on hydrologic recharge of aquifers (Batalha et al. 2018), others even analyze reactive transport models to understand mobilization of specific chemicals, like arsenic from coal seam gas co-produced water that is injected in the soil for disposal (Rathi et al. 2017). Benthic animals in springs can be the key to understand transport of toxics from underground.

It is crucial to invest more efforts deciphering the connections between different springs. **Maps 8 – 12** are an attempt to integrate information of different factors that might be overlooked using statistics. Adding more springs to this kind of study could enrich the interactive maps to find relationships between zones. Converting this information to a digital atlas will certainly facilitate the strategies to share information about water springs health with other scientists and with locals.

CHAPTER 7

Conclusions and recommendations

This chapter summarizes the results answering the two-research question marked in the introduction chapter. Additionally, it has recommendations including valuable lessons learned during the project.

Gammarus presence does not contribute to increase the water spring value, however it is an important tool to evaluate the environmental conditions influencing the watershed. Ecological analysis and observation in nature can bring valuable knowledge to study springs health.

Question 1: Which of the conditions are related with the **absence** of *G. fossarum* in some water springs of the Lusatian Fault?

> Answer: It was found that freshwater shrimps are usually absent in very strongly polluted water, Class V, and also in a heavily modified environment, where the bottom substrate of the spring is replaced by plastic.

Question 2: What are the factors that matter the most to find **abundant** *G. fossarum* populations? Does the runoff watershed land cover have any influence?

> Answer: The project analyzed the main factors that can influence the abundance of biota and detected that the widely distributed *G. fossarum* is highly dependent on pH. The minimum average pH registered was 5.5 and as it increases, also the population number does. In the other hand, land cover in watershed does not influence their abundance with a clear trend. It was proved there is no potential for bioindication with this species so far, due to the broad range of their populations.

Other factors beyond the scope of this research can be considered to explain the absence of *G. fossarum* in further studies. For example, influence of light, water velocity, and other specific chemicals in the water individually like ammonia, phosphorus or mercury.

The methodology designed for the interactive maps that explain water quality have a notable positive impression. This comprehensive methodology integrates water quality data with benthic animals, and other factors influencing the springs in the region

We know water is essential for life, but most people do not know about the water quality of their local river, where our water comes from and what is the impact of our settlements and how to help river recovery. For this region, it is worth to focus attention on pollution by Cl, As, Ni, Cd and NO_3^- and find effective strategies to share the knowledge, making it easier for scientists to explain what they do, for decision makers to be wise, and for citizens to understand what is going on around them.

CHAPTER 8

Future research

This is the last chapter! As science is always in construction, this section highlights interesting approaches to continue research, to understand better water springs dynamics and its ecology.

Integrating disciplines: Innovation and creativity does not usually come from studying the individual elements, but from connecting variables, therefore, a multidisciplinary approach is strongly recommended. More researchers should be encouraged to join with different disciplines. What is the most important combination of sciences for water springs? How could Social Sciences support conservation of water springs in this communities of central Europe?

Springs have constant temperature: *Gammarus fossarum* not only inhabits springs, however, it is clearly successful colonizing some of these habitats. It would be interesting to check how the range of temperature change impacts their populations, as springs are known to have almost constant temperature throughout the year. Is this species affected by climate change or direct discharges on streams?

Distribution and genetics: Springs might be isolated habitats depending on hydrologic events, hence, it can influence the migration dynamics of species populations that travel upstream looking for water with better conditions, with more oxygen and stable temperature. Accordingly, a molecular genetic analysis could reveal biogeographical links, and it might expose ecological evolutionary effects of water quality in the watersheds. Can *G. fossarum* specimens resist conditions of non-permanent springs?

Springshed: A clear, accessible and reliable method to determine the area of influence for every water spring does not exist yet. Little is known also about interconnections between springs. However, we can monitor these water bodies constantly to find evidence about its quality along time and check specific changes on water, sediment and biota composition. What mechanisms could be implemented in the region Liberec-Zittau to improve monitoring of waters springs connectivity?

Communication: Science is valuable for the impact that it can bring to people. It is important to keep combining research with useful ways to share the information. How could a digital atlas provide information to draw attention and make people willing to know? Science is not meant to be boring or confusing. It is a powerful tool to improve our lifestyle!

Policies: Current policies like the Bern Convention, support initiatives to encourage the public understanding of the environmental cost of invertebrate decline. When the community learn more, the conversation will open for new questions and the existing regulations can evolve to conserve watersheds and its ecological connections more efficiently.

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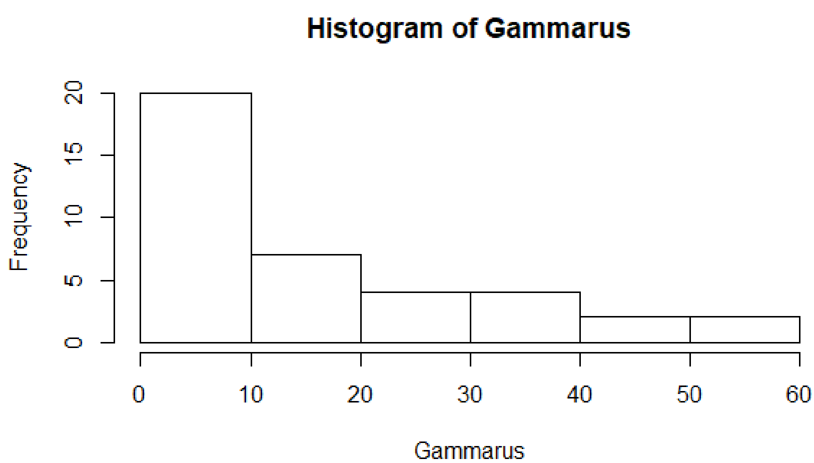
Annex A: Statistical protocol

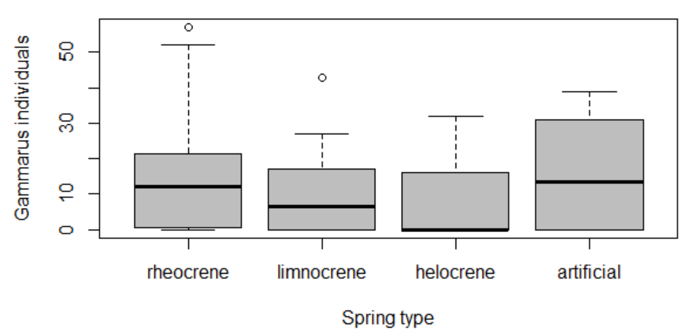
Results from R studio models

```
> #Set filepath
> getwd()
[1] "D:/Results_Prameny/R Studio/Gammarus_final"

> #uplooad the table info
> read.table("data_gammarus8.txt",header=TRUE,sep = "\t")
    N      ID type Geology Human Plants Water      HQ   PH    DO   Land  Gammarus

 1 AN1029     A       PC      4     NO     5     Medium 5.08  9.48 Forest        0
 2 AN1030     L       PC      3     BO     4     Medium 5.86  9.64 Forest        0
 3 AN1036     L       TV      3     CO     4     Medium 5.79  9.32   Agro        0
 4 AN1037     L       PC      3     MI     4        Low 6.31  4.65   Agro       13
 5 AS1043     R       CS      1     BO     2 Background 6.45 10.93 Forest       18
 6 BN1025     R       PC      1     CO     4 Background 5.95  9.80 Forest       40
 7 BN1033     H       PC      1     CO     3        Low 7.45  7.35   Agro       32
 8 BN1034     L       TV      1     MI     3 Background 7.12  6.60   Agro       43
 9 BN1041     R       PC      1     BO     5 Background 5.34  9.33 Forest        0
10 BN2242     H       PC      1     CO     5        Low 5.86  4.53 Forest        0
11 BN2243     L       PC      3     BO     4        Low 5.64  9.30 Forest        0
12 BN2244     R       PC      1     CO     2        Low 6.87  8.27 Forest        0
13 BS1016     A       CS      2     NO     4 Background 5.99  8.39 Forest       24
14 BS1019     A       CS      3     BO    NA Background 7.28  2.27 Forest       39
15 BS1027     L       CS      1     MI     2 Background 6.47  9.29 Forest       27
16 BS1040     L       CS      1     CO     5 Background 7.68  1.22 Forest        0
17 BS1200     R       CS      1     CO     4 Background 5.97  9.21 Forest       10
18 BS1203     R       CS      1     BO     2 Background 6.80  9.16 Forest       21
19 BS1204     R       CS      1     BO     4 Background 5.65  9.55 Forest        0
20 BS2228     A       CS      3     BO     5 Background 4.91  6.94   Agro        0
21 BS2229     R       TV      1     MI     5 Background 5.35 10.09 Forest        0
22 BS2230     R       CS      1     BO    NA Background 7.79  9.46   Agro       52
23 BS2232     R       CS      1     BO     4        Low 5.99  9.54 Forest       13
24 BS2233     R       CS      1     BO     4 Background 5.64  9.20 Forest       12
25 BS2235     R       CS      1     NO     4 Background 5.64  9.27 Forest        5
26 CS2227     R       CS      1     NO     3        Low 6.27  5.56 Forest       13
27 DN1012     H       PC      1     BO     5     Medium 5.19  9.05   Agro        0
28 EN1005     R       PC      2     BO     2        Low 7.42  8.20 Forest       22
29 EN1006     R       PC      2     BO     2        Low 7.50 10.71 Forest       49
30 EN1011     R       PS      2     BO     2     Medium 7.52 10.26 Forest        7
31 EN2238     H       PC      1     CO     5        Low 4.75 10.02 Forest        0
32 EN2239     R       PC      1     CO     5 Background 5.48  9.93 Forest        0
33 EN2241     R       PC      1     BO     5        Low 5.90  6.90  Urban        1
34 ES1008     L       CS      2     NO     5 Background 7.20 10.03   Agro       16
35 ES1009     A       CS      2     NO     4     Medium 7.21  9.55   Agro       31
36 ES1014     L       CS      1     BO     2        Low 7.15  8.82 Forest        0
37 ES1104     R       CS      3     NO    NA Background 7.33  9.68 Forest       57
38 ES1108     A       CS      3     BO     2        Low 7.63 10.41 Forest        3
39 ES2220     L       CS      1     BO     2 Background 7.04 10.11   Agro       17

Excluded value
40 BN1024     R         CS      1      BO      2 Background 6.36  9.96 Forest      165
```

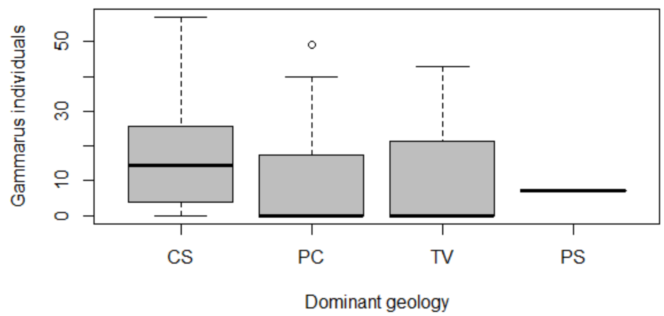
```
> #create vector from the table
> zoo<-read.table("data_gammarus8.txt",header=TRUE,sep = "\t")

> #check info in the vector
> summary(zoo)
       ID        type    Geology      Human         Plants      Water
 AN1029 : 1   A: 6    CS:20   Min.   :1.000   BO:19   Min.   :2.000
 AN1030 : 1   H: 4    PC:15   1st Qu.:1.000   CO: 9   1st Qu.:2.000
 AN1036 : 1   L:10    PS: 1   Median :1.000   MI: 4   Median :4.000
 AN1037 : 1   R:19    TV: 3   Mean   :1.641   NO: 7   Mean   :3.667
 AS1043 : 1                   3rd Qu.:2.000           3rd Qu.:5.000
 BN1025 : 1                   Max.   :4.000           Max.   :5.000
          (Other):33                                          NA's   :3
          HQ              PH               DO            Land        Gammarus
 Background:20   Min.   :4.750   Min.   : 1.220   Agro  :10   Min.   : 0.00
 Low       :13   1st Qu.:5.645   1st Qu.: 8.235   Forest:27   1st Qu.: 0.00
 Medium    : 6   Median :6.270   Median : 9.300   Urban : 1   Median :10.00
                 Mean   :6.371   Mean   : 8.513               Mean   :14.49
                 3rd Qu.:7.205   3rd Qu.: 9.740               3rd Qu.:23.00
                 Max.   :7.790   Max.   :10.930               Max.   :57.00

#check distribution of data
> hist(Gammarus)
```



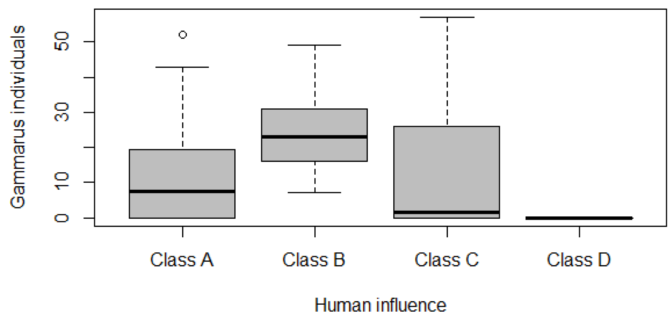

```
> #Build one boxplot with each variable

> #type
> zoo$type=as.factor(zoo$type)
> Rheocrene=zoo$Gammarus[zoo$type=="R"]
> Limnocrene=zoo$Gammarus[zoo$type=="L"]
> Helocrene=zoo$Gammarus[zoo$type=="H"]
> Artificial=zoo$Gammarus[zoo$type=="A"]
> boxplot(Rheocrene,Limnocrene,Helocrene
,Artificial,names=c("rheocrene","limnocr
ene","helocrene","artificial"), xlab="Sp
ring type",ylab="Gammarus individuals",c
ol="gray")
```



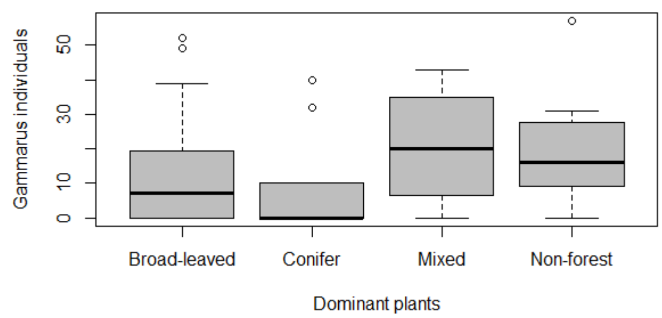
```
> #geology
> zoo$Geology=as.factor(zoo$Geology)
> tv=zoo$Gammarus[zoo$Geology=="TV"]
> cs=zoo$Gammarus[zoo$Geology=="CS"]
> pc=zoo$Gammarus[zoo$Geology=="PC"]
> ps=zoo$Gammarus[zoo$Geology=="PS"]
> boxplot(cs,pc,tv,ps,names=c("CS","PC"
,"TV","PS"), xlab="Dominant geology",yl
ab="Gammarus individuals",col="gray")
```



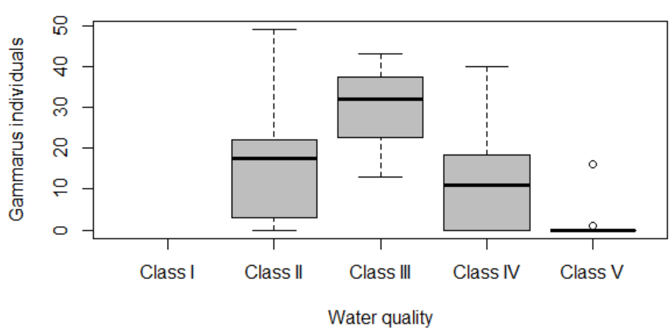

```
> #human influence
> zoo$Human=as.factor(zoo$Human)
> natural=zoo$Gammarus[zoo$Human=="1"]
> intake=zoo$Gammarus[zoo$Human=="2"]
> structure=zoo$Gammarus[zoo$Human=="3"
]
> modified=zoo$Gammarus[zoo$Human=="4"]
> boxplot(natural,intake,structure,modi
fied,names=c("Class A","Class B","Class
C","Class D"),xlab="Human influence",yl
ab="Gammarus individuals",col="gray")
```



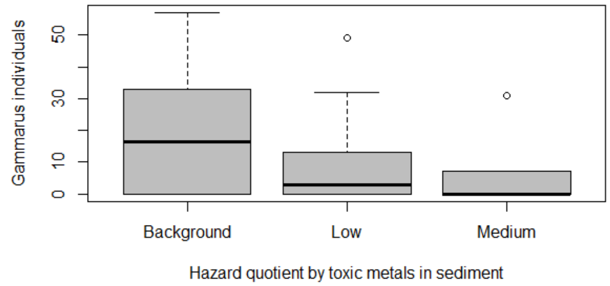

```
> #dominant plants
> zoo$Plants=as.factor(zoo$Plants)
> broadleaved=zoo$Gammarus[zoo$Plants=="
BO"]
> conifer=zoo$Gammarus[zoo$Plants=="CO"]
> mixed=zoo$Gammarus[zoo$Plants=="MI"]
> non=zoo$Gammarus[zoo$Plants=="NO"]
> boxplot(broadleaved,conifer,mixed,non,
names=c("Broad-leaved","Conifer","Mixed"
,"Non-forest"), xlab="Dominant plants",y
lab="Gammarus individuals",col="gray")
```



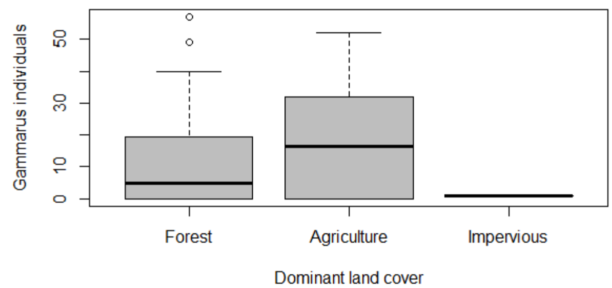

```
> #water quality
> zoo$Water=as.factor(zoo$Water)
> I=zoo$Gammarus[zoo$Water=="1"]
> II=zoo$Gammarus[zoo$Water=="2"]
> III=zoo$Gammarus[zoo$Water=="3"]
> IV=zoo$Gammarus[zoo$Water=="4"]
> V=zoo$Gammarus[zoo$Water=="5"]
> boxplot(I,II,III,IV,V,names=c("Class
I","Class II","Class III","Class IV","C
lass V"),xlab="Water quality",ylab="Gam
marus individuals",col="gray")
```



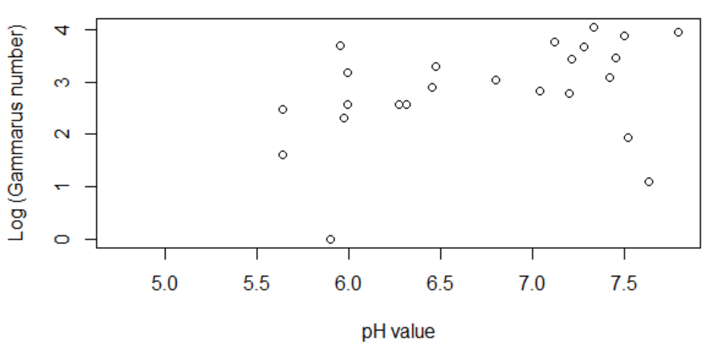
```
> #toxic metals in sediment HQ
> zoo$HQ=as.factor(zoo$HQ)
> Background=zoo$Gammarus[zoo$HQ=="Backgro
und"]
> Low=zoo$Gammarus[zoo$HQ=="Low"]
> Medium=zoo$Gammarus[zoo$HQ=="Medium"]
> #the results did not show springs "high
risk"
> boxplot(Background, Low, Medium, names=c
("Background","Low","Medium"),xlab="Hazard
quotient by toxic metals in sediment",ylab
="Gammarus individuals",col="gray")
```

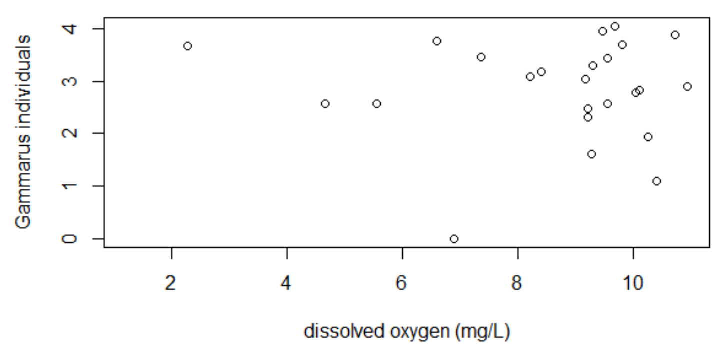



```
> #dominant landcover
> zoo$Land=as.factor(zoo$Land)
> Forest=zoo$Gammarus[zoo$HQ=="Forest"]
> Agro=zoo$Gammarus[zoo$HQ=="Agro"]
> Urban=zoo$Gammarus[zoo$HQ=="Urban"]
> boxplot(Forest, Agro, Urban, names=c("For
est","Agriculture","Impervious"),xlab="Domi
nant land cover",ylab="Gammarus individuals
",col="gray")
```

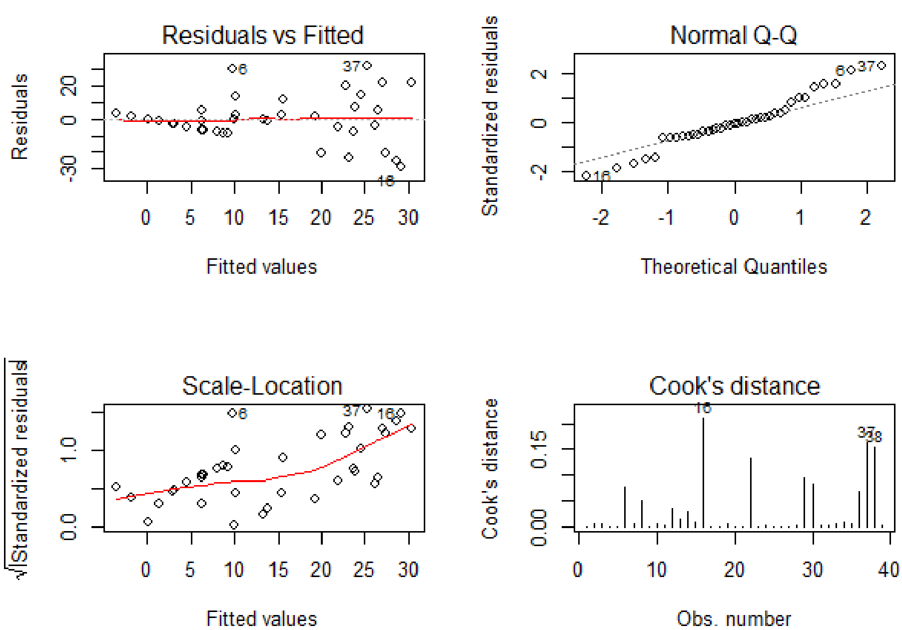



```
> #create one vector for each category
> Gammarus=zoo$Gammarus
> type=zoo$type
> Geology=zoo$Geology
> Human=zoo$Human
> Plants=zoo$Plants
> Water=zoo$Water
> HQ=zoo$HQ
> Land=zoo$Land

> # check relationship between Gammarus number and pH, DO
> abundance=(zoo$Gammarus)
> PH=zoo$PH
> DO=zoo$DO
> plot(PH,abundance,xlab="pH value",ylab="Log (Gammarus number)")
> plot(DO,abundance,xlab="dissolved oxygen (mg/L)",ylab="Gammarus individuals")
```

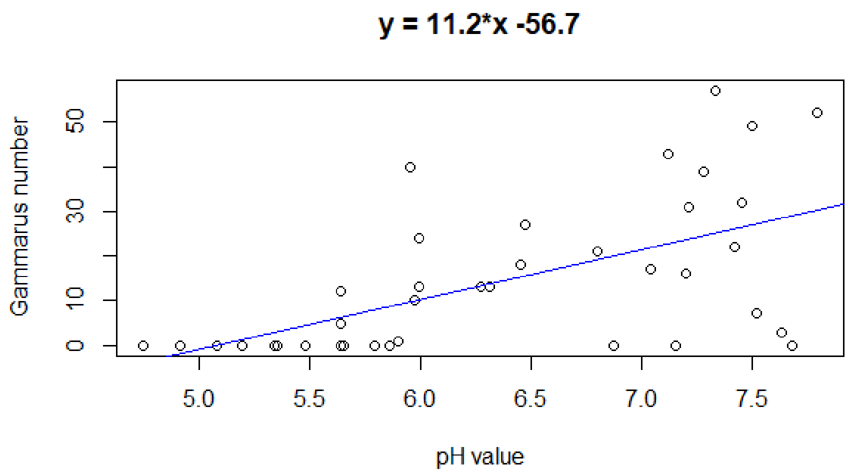




```
Kruskal-Wallis rank sum test

data:  Gammarus by PH
Kruskal-Wallis chi-squared = 37.044, df = 34, p-value = 0.3303

> #linear regression for pH

> model_PH = lm (abundance ~ PH)
> anova(model_PH,test = "Chi")
Analysis of Variance Table
Response: abundance
          Df Sum Sq Mean Sq F value    Pr(>F)
PH         1 3739.9  3739.9   18.91 0.0001032 ***
Residuals 37 7317.8   197.8
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary (model_PH)
Call:
lm(formula = abundance ~ PH)
Residuals:
    Min      1Q  Median      3Q     Max
-29.116  -7.090  -0.805   5.568  31.795

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   -56.72      16.53  -3.431 0.001492 **
PH             11.18       2.57   4.349 0.000103 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Residual standard error: 14.06 on 37 degrees of freedom
Multiple R-squared:  0.3382,     Adjusted R-squared:  0.3203
F-statistic: 18.91 on 1 and 37 DF,  p-value: 0.0001032
```

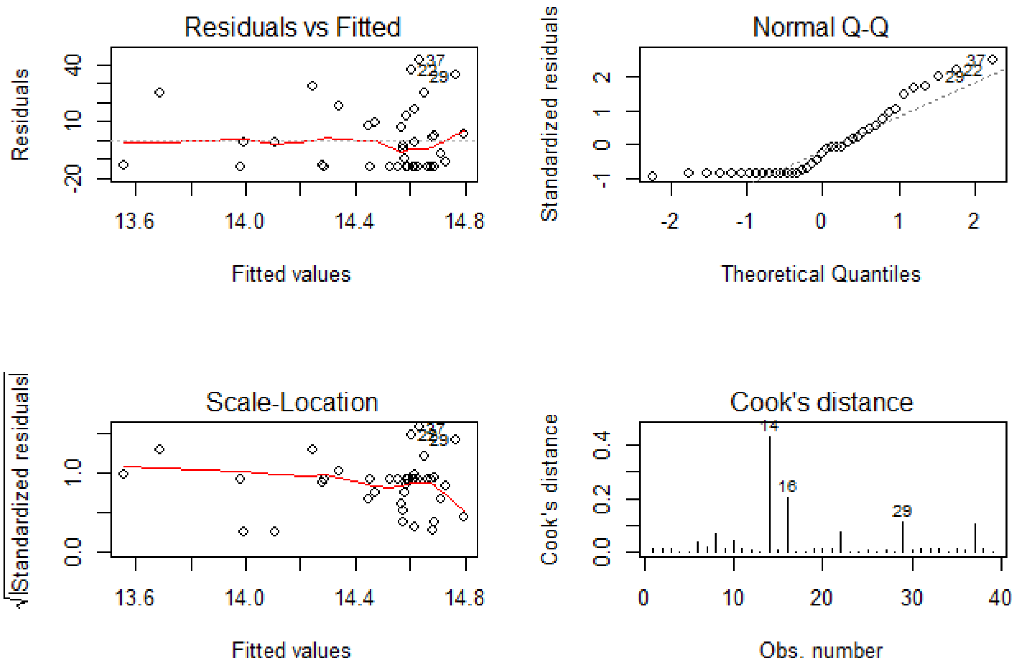


```
> coeff=coefficients(model_PH)
> # equation of the line :
> eq = paste0("y = ", round(coeff[2],1), "*x ", round(coeff[1],1))
> # plot
> par(mfrow=c(1,1))
> plot(PH,abundance,xlab="pH value",ylab="Gammarus number", main=eq)
> abline(model_PH, col="blue")
```



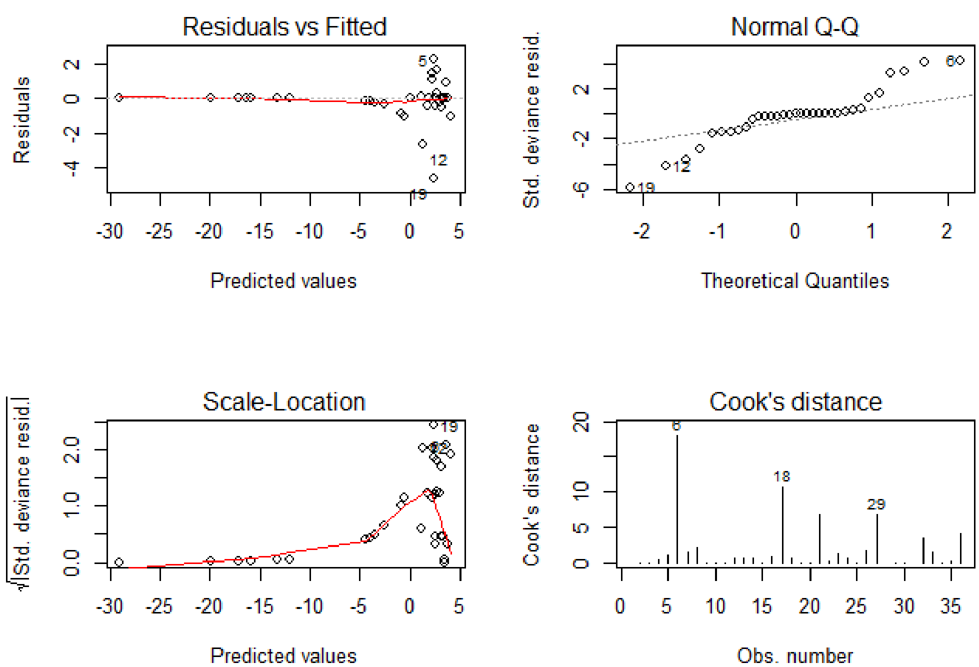

```
#nonparametric analysis for DO
> kruskal.test(Gammarus~DO)

        Kruskal-Wallis rank sum test

data:  Gammarus by DO
Kruskal-Wallis chi-squared = 35.65, df = 37, p-value = 0.5323

#linear regression for DO
> model_DO = lm (abundance ~ DO)
> anova(model_DO,test = "Chi")
Analysis of Variance Table
Response: abundance
          Df Sum Sq Mean Sq F value Pr(>F)
DO         1      3   3.011  0.0101 0.9206
Residuals 37  11055 298.777
> summary (model_DO)
Call:
lm(formula = abundance ~ DO)
Residuals:
    Min      1Q  Median      3Q     Max
-14.688 -14.541  -4.576   8.541  42.364
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  13.4012    11.1655     1.2    0.238
DO            0.1276     1.2706     0.1    0.921
Residual standard error: 17.29 on 37 degrees of freedom
Multiple R-squared:  0.0002723,    Adjusted R-squared:  -0.02675
F-statistic: 0.01008 on 1 and 37 DF,  p-value: 0.9206
```

```
> par(mfrow=c(2,2))
> plot(model_DO)
```



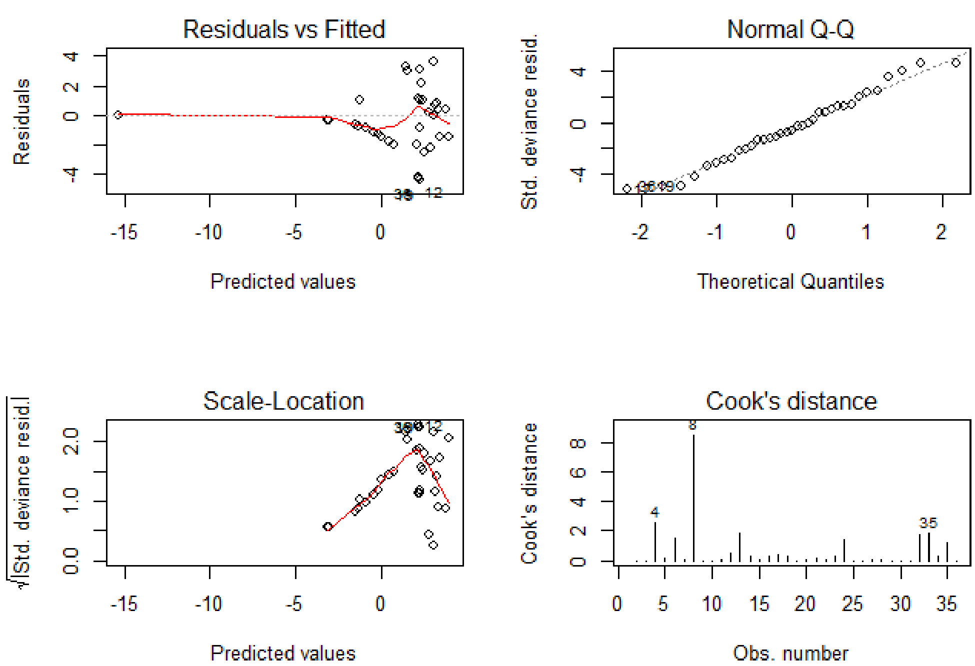

```
#linear regression for DO
model_DO = lm (abundance ~ DO)
anova(model_DO,test = "Chi")
summary (model_DO)
par(mfrow=c(2,2))
plot(model_DO)
plot(model_DO,which=1:4)
coeff=coefficients(model_DO)

# equation of the line :
eq = paste0("y = ", round(coeff[2],1), "*x ", round(coeff[1],1))

# plot
par(mfrow=c(1,1))
plot(DO,abundance,xlab="pH value",ylab="Gammarus number", main=eq)
abline(model_DO, col="blue")


#general linear model for all variables

> model_all = glm (abundance ~ DO+PH+Water+Geology+Human+Plants+Land+type+HQ,family = p
oisson)
> anova(model_all,test = "Chi")
Analysis of Deviance Table

Model: poisson, link: log

Response: abundance
Terms added sequentially (first to last)
```

```
        Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                       35     630.93
DO       1    7.021        34     623.91 0.0080574 **
PH       1  154.558        33     469.36 < 2.2e-16 ***
Water    3  137.015        30     332.34 < 2.2e-16 ***
Geology  3   18.963        27     313.38 0.0002782 ***
Human    3   94.937        24     218.44 < 2.2e-16 ***
Plants   3   56.039        21     162.40 4.121e-12 ***
Land     2    0.581        19     161.82 0.7479172
type     3   25.448        16     136.37 1.245e-05 ***
HQ       2   92.989        14      43.38 < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary (model_all)
Call:
glm(formula = abundance ~ DO + PH + Water + Geology + Human +
    Plants + Land + type + HQ, family = poisson)
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.5804  -0.2792  -0.0012   0.0004   2.1823

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)  -22.32057    4.25601  -5.244 1.57e-07 ***
DO             0.38717    0.09005   4.300 1.71e-05 ***
PH             4.31465    0.73959   5.834 5.42e-09 ***
Water3         5.16889    0.82060   6.299 3.00e-10 ***
Water4         4.00859    0.72912   5.498 3.85e-08 ***
Water5         1.56546    0.78344   1.998  0.04570 *
GeologyPC      0.94809    0.32312   2.934  0.00334 **
GeologyPS      7.91683    1.95304   4.054 5.04e-05 ***
GeologyTV    -11.65005    1.89826  -6.137 8.40e-10 ***
Human2        -2.01039    0.73076  -2.751  0.00594 **
Human3        -6.70766    1.40619  -4.770 1.84e-06 ***
Human4        -5.84886 2103.36349  -0.003  0.99778
PlantsCO      -1.14954    0.40561  -2.834  0.00460 **
PlantsMI       8.36476    1.50465   5.559 2.71e-08 ***
PlantsNO      -0.40408    0.42660  -0.947  0.34353
LandForest    -5.18576    1.22490  -4.234 2.30e-05 ***
LandUrban     -4.42273    1.83605  -2.409  0.01600 *
typeH        -12.49988    2.44639  -5.110 3.23e-07 ***
typeL         -9.07771    1.99533  -4.549 5.38e-06 ***
typeR         -2.22707    0.69929  -3.185  0.00145 **
HQLow         -1.67108    0.33221  -5.030 4.90e-07 ***
HQMedium     -10.64565    1.88613  -5.644 1.66e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 630.935  on 35  degrees of freedom
Residual deviance:  43.384  on 14  degrees of freedom
  (3 observations deleted due to missingness)
AIC: 182.89

Number of Fisher Scoring iterations: 14
```




```
> #general linear model for relevant variables
> model3 = glm (abundance ~ DO+PH+Water+Human+Plants+HQ,family=poisson)
> anova(model3,test = "Chi")

Analysis of Deviance Table

Model: poisson, link: log

Response: abundance
Terms added sequentially (first to last)
       Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                      35     630.93
DO      1    7.021        34     623.91  0.008057 **
PH      1  154.558        33     469.36 < 2.2e-16 ***
Water   3  137.015        30     332.34 < 2.2e-16 ***
Human   3   75.993        27     256.35 < 2.2e-16 ***
Plants  3   45.426        24     210.92 7.510e-10 ***
HQ      2   65.722        22     145.20 5.353e-15 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary (model3)
Call:
glm(formula = abundance ~ DO + PH + Water + Human + Plants +
    HQ, family = poisson)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.3569  -1.4751  -0.4917   0.8219   3.5707

Coefficients:
              Estimate Std. Error z value Pr(>|z|)
```

```
(Intercept)    -7.76309     1.43463  -5        .411 6.26e-08 ***
DO              0.25608     0.05774   4.435 9.21e-06 ***
PH              1.13213     0.19701   5.747 9.10e-09 ***
Water3          0.78245     0.24328   3.216 0.001299 **
Water4          1.17132     0.25864   4.529 5.93e-06 ***
Water5         -1.58281     0.30592  -5.174 2.29e-07 ***
Human2          0.72318     0.20170   3.585 0.000336 ***
Human3         -1.10133     0.31279  -3.521 0.000430 ***
Human4        -12.69011 1275.75398  -0.010 0.992063
PlantsCO        0.41176     0.17947   2.294 0.021775 *
PlantsMI        1.21017     0.22114   5.472 4.44e-08 ***
PlantsNO        0.40230     0.21333   1.886 0.059328 .
HQLow          -0.36040     0.17359  -2.076 0.037882 *
HQMedium       -1.84776     0.25845  -7.149 8.72e-13 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for poisson family taken to be 1)

    Null deviance: 630.93  on 35  degrees of freedom
Residual deviance: 145.20  on 22  degrees of freedom
  (3 observations deleted due to missingness)
AIC: 268.7

Number of Fisher Scoring iterations: 13
```



Annex B: Photos of the springs

Photo 1: Spring AN1029, Kottmar Bushwiesen

Photo 2: Spring AN1030, SchieSborn

Photo 3: Spring AN1036, Leutersddorf – Kirschbaumplantage

Photo 4: Spring AN1037, Leutesdorf – Ziegenborn

Photo 5: Spring AS1043, Mařenice – třízdrojový

Photo 6: Spring BN1024, Jonsdorf horní Hirschbörnl

Photo 7: Spring BN1025, Jonsdorf dolní Buchberg unten

Photo 8: Spring BN1033, Bornwaldchen

Photo 9: Spring BN1034, Unterh. Spitzberg

Photo 10: Spring BN1041, Jonsberg u sportoviště

Photo 11: Spring BN2242, Jungfrauenquelle

Photo 12: Spring BN2243, Steinbogen

Photo 13: Spring BN2244, Birke

Photo 14: Spring BS1016¸Radvanec

Photo 15: Spring BS1019, Krompach

Photo 16: Spring BS1027, Vodopád u Heřmanic

Photo 17: Spring BS1040, tzv. "sirný pramen",

Photo 18: Spring ES1200, Heřmanice - Nad Borůvčím,

Photo 19: Spring BS1203, Heřmanice - 4. propustek

Photo 20: Spring BS1204, Heřmanice - Babiččin odpočinek

Photo 21: Spring BS2228, Petrovice - ve skalce

Photo *22: Spring BS2229, Myslivny - U smrku*

Photo 23: Spring BS2230, Krompach - Pod Kulichem

Photo 24: Spring BS2232, Myslivny - Pod Buky

Photo 25: Spring BS2233, Myslivny - Nad Pasekou

Photo 26: Spring BS2235, Heřmanice - U Oplocenky

Photo 27: Spring CS2227, Kněžice

Photo 28: Spring DN1012, Začátek Vítkovského potoka

Photo 29: Spring EN1005, Ještěd - Frantina studánka

Photo 30: Spring EN1006, Ještěd - Pramen Lesních panen

Photo 31: Spring EN1011, Vesec-Jiříčkov

Photo 32: Spring EN2238, Mšeno nad Nisou (ul. Arbesova)

Photo 33: Spring EN 2239, Rýnovice - věznice

Photo 34: Spring EN2241, Starý Harcov - Temná ul-Hrubínova

Photo 35: Spring EN1008, Vápno

Photo 36: Spring EN1009, Lesnovek

Photo 37: Spring EN1014, Janův důl - Prameny Ploučnice

Photo 38: Spring EN1104, Všelibice

Photo 39: Spring ES1108, V Moskových dolech-Modlibohov

Photo 40: Spring ES2220, Světá pod Ještědem Rozstání GOLF