DNA Metabarcoding of Macroinvertebrates in Alpine Freshwater Ecosystems: Implication for Stream Zonation

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Abstract

Alpine spring ecosystems constitute highly specialized and sensitive habitats, whose biological communities are strongly governed by thermal and hydrological conditions. The classical concept of longitudinal zonation of running waters proposed by Illies (1961) distinguishes eucrenal, hypocrenal and epirhithral sections, the delineation of which has traditionally been based on morphological bioindication. The aim of this study was to evaluate the potential of eDNA metabarcoding for assessing biological complexity and longitudinal zonation in alpine spring systems.

Water samples were collected from nine alpine spring systems located within the UNESCO Biosphere Reserve Engiadina Val Müstair along predefined longitudinal sections. Methodologically, the study was based on the filtration of environmental DNA (eDNA) from water samples, followed by DNA extraction and PCR amplification of a fragment of the CO1 gene. Bioinformatic processing and subsequent statistical analyses using Detrended Correspondence Analysis (DCA) were applied to visualize biological dissimilarities among the investigated stream sections.

From an initial pool of 1123 detected taxa, a total of 228 aquatic species were identified. The highest diversity was observed within the class Insecta, particularly among Diptera of the family Chironomidae. Species richness increased with increasing distance from the spring outlet, from 265 species in the eucrenal to 540 species in the hypocrenal and 677 species in the epirhithral. In several systems, DCA revealed a clear sequential differentiation of biological communities, corresponding well with the classical concept of longitudinal zonation of running waters as described by Illies (1961).

Overall, this study demonstrates the high potential of eDNA metabarcoding as a non-invasive and taxonomically high-resolution approach for investigating alpine spring ecosystems. At the same time, the results emphasize the necessity of context sensitive interpretation and the integrative consideration of hydrological and abiotic parameters. In the long term, eDNA metabarcoding may provide an important tool for standardized monitoring and the conservation of highly sensitive alpine spring habitats in the context of ongoing climate change.

Table of Contents

List of Figures

List of Tables

1. Introduction

The zonation of running fresh water ecosystems has gained increasing importance for the description of specific ecosystems since the 20th century. With Joachim Illies' 1961 attempt at a biocenotic classification of running waters, a systematics was developed that is applicable to limnology (Von Fumetti et al., 2007). Running fresh water ecosystems can be divided into three main sub-ecosystems the crenal, the rhithral the section following the emergence of the spring, which is typically fast flowing and characterized by stable physicochemical parameters, and the potamal, which follows the rhithral and is characterized by lower current velocity and predominantly laminar flow and fluctuating physicochemical parameters (Illies, 1961). Based on this subdivision, the further delineation of river sections was carried out. The delimitation of the spring as an independent ecosystem can be distinguished from the rhithral by a maximum annual temperature amplitude of 5 °C (Illies and Botosaneanu, 1963; Von Fumetti et al., 2007). The crenal consists of two sections, the eucrenal, the spring head which can be distinguished by a difference in water temperature compared to the hypocrenal, and the hypocrenal, the actual spring brook (Erman and Erman, 1995; Myers et al., n.d.; Von Fumetti et al., 2007). As a more general definition, the formation of a spring is described as the intersection of an aquifer, a groundwater bearing layer, with the land surface (Bahlburg and Breitkreuz, 2017). This is followed by the outflow of the surface water and with it, the course of a running water body.

The composition of alpine freshwater ecosystems and in particular that of alpine springs, is described as unique habitats in spatially restricted and island like ecotones (Blattner et al., 2021; Cantonati et al., 2006a). Alpine spring ecosystems show very stable abiotic environmental factors, which are characterized by a highly specialized and diverse species composition consisting of spring specialists, crenobionts, crenophilous species, organisms not exclusively bound to springs groundwater specialists and rhithrobiont taxa adapted to fast flowing waters (Blattner et al., 2021; Cantonati and Bonettini, 1996; Von Fumetti and Blattner, 2016). Species composition changes within the crenal during the transition to the hypocrenal and subsequently into the next section of the rhithral, the epirhithral, where rhithrobiont organisms dominate (Illies, 1961). The change in species composition within each section is strongly determined by the respective expression of abiotic and biotic factors, which are manifested in the chemical composition of the spring water, the physical conditions of the environment, the substrate composition and changes in the flow regime and biotic interactions (Cantonati et al., 2006a; Gerecke et al., 1997; Van der Kamp, 1995).

Environmental conditions play a central role in the species composition of alpine ecosystems, therefore they are highly sensitive to environmental changes caused by global climate change and anthropogenic influences (Blattner et al., 2021; Cantonati et al., 2020; Reiss et al., 2016; Von Fumetti et al., 2017). In the course of these changes, biomonitoring programs characterized by surveillance and assessment are becoming increasingly indispensable (Gerecke et al., 2011; Harvey et al., 2020; Küry et al., 2017; Reiss et al., 2016). Current bioindication of aquatic ecosystems is implemented through the recording of the presence or absence of species that are crucial for environmental integrity (Blattner et al., 2021). The bioindication methods used for fresh water habitats have proven to be reliable and reproducible in both national and international biomonitoring programs (Elbrecht et al., 2017; Gerecke et al., 2011; Hering et al., 2004; Kuefner et al., 2020; Küry et al., 2017; Morinière et al., 2017; Robinson, 2009). Nevertheless, the conventional method based on morphological identification of organisms has disadvantages in its application. In addition to the high cost and time requirements, the accuracy of identification is strongly dependent on the individual expertise, which may lead to misidentifications and insufficient taxonomic resolution. (Elbrecht et al., 2017; Sweeney et al., 2011).

The integration of a method that meets the requirements of conventional bioindication while at the same time overcoming its associated disadvantages is being pursued through the application of molecular genetic approaches (Beng and Corlett, 2020; Elbrecht et al., 2017; Liu et al., 2020; Weigand et al., 2019; Zizka et al., 2020).

DNA metabarcoding as a method that was also applied in this study meets these requirements and offers concrete advantages in its application compared to the conventional method. Environmental DNA obtained from filtered water is amplified by PCR, sequenced on short-read sequencing systems, and the resulting sequences are matched against reference sequence databases, which enables highly resolved taxonomic identification. (Liu et al., 2020). This application shows advantages in several aspects compared to conventional bioindicator sampling. Sampling is non-invasive and therefore minimizes disturbance of habitats, which in conventional long term sampling can lead to habitat destruction. Instead eDNA is obtained from the water body by filtration and avoids the removal of living organisms (Beng and Corlett, 2020; Bossley and Smiley, 2019; Goldberg et al., 2016; Hernandez et al., 2020). Species identification occurs in parallel and is independent of different life stages, such as larval stages, as well as living individuals, and is therefore less dependent on precisely timed sampling (Elbrecht et al., 2017). Nevertheless, this application entails other challenges which relate to the detection of entire macroinvertebrate communities, false detection of non-target taxa additional bias caused by organic matter or incomplete sequence databases (Elbrecht et al., 2017; Leese et al., 2021; Weigand et al., 2019)

Given the importance of standardized biomonitoring programs at both national and international levels, the integration of the advantages of eDNA metabarcoding is of particular relevance. Therefore, the aim of this thesis is to evaluate the applicability of eDNA metabarcoding to investigate running water zonation and to assess the advantages of this method in comparison with conventional bioindication methods.

The aim of this study is found on detecting section-specific species within the eucrenal, hypocrenal and epirhithral along the spring and the spring brook. Within this framework, three central hypotheses are tested using empirical data:

1. In accordance with the classical longitudinal zonation of Illies (1961), it is expected that spring specialists can be detected mainly in the eucrenal and occur much less frequently in the hypocrenal.
2. Non-invasive eDNA metabarcoding is expected to allow the detection of spring specialists in the epirhithral.
3. The changes in species communities detectable by eDNA in the eucrenal, hypocrenal and epirhithral correspond to the patterns of longitudinal zonation described by conventional bioindication methods.

The use of eDNA metabarcoding in this study provides new opportunities to characterize the longitudinal zonation of alpine spring systems and to more clearly distinguish spring habitats from downstream water sections.

This molecular genetic approach enables a high-resolution and non-invasive assessment of species communities and thus contributes substantially to freshwater ecology and the conservation of spring specific biological communities. As a result, ecological connectivity and spatial extent of freshwater habitats can be quantified more precisely, allowing more targeted nature conservation and management strategies to be developed.

2. Methods and Materials

2.1 Study Area

The study was carried out in two areas in the southeastern part of Switzerland in the canton of Grisons. The investigated systems in both areas are located within the UNESCO-Biosphere Reserve Engiadina Val Müstair, comprising sites within the Swiss National Park (SNP) and the Val Müstair

The SNP (Figure 1: light blue area) was established in 1914 and is therefore one of the oldest national parks in the Alps. It extends from 1400 m above sea level at its lowest point to 3174 m above sea level at its highest point. Covering an area of 170.3 km^2 the park protects plants animals and ecosystems from human disturbance and is classified by the International Union for Conservation of Nature IUCN as category 1a which represents the highest level of protection in wilderness areas (Haller et al., 2013). The vegetation of the SNP consists of 29 % forest cover of which 99.5 % is coniferous. Dominant tree species include mountain pine (*Pinus mugo Turra*) Swiss stone pine (*Pinus cembra Linné*) and larch (*Larix spp.*) (Von Fumetti and Blattner, 2016). Alpine meadows account for 21 % of the vegetation while 51 % consists of sparsely or non-vegetated areas (Haller et al., 2013).

The Biosfera Val Müstair Nature Park, established in 2011, forms part of the UNESCO Biosphere Reserve Engiadina Val Müstair together with the SNP and parts of the municipality of

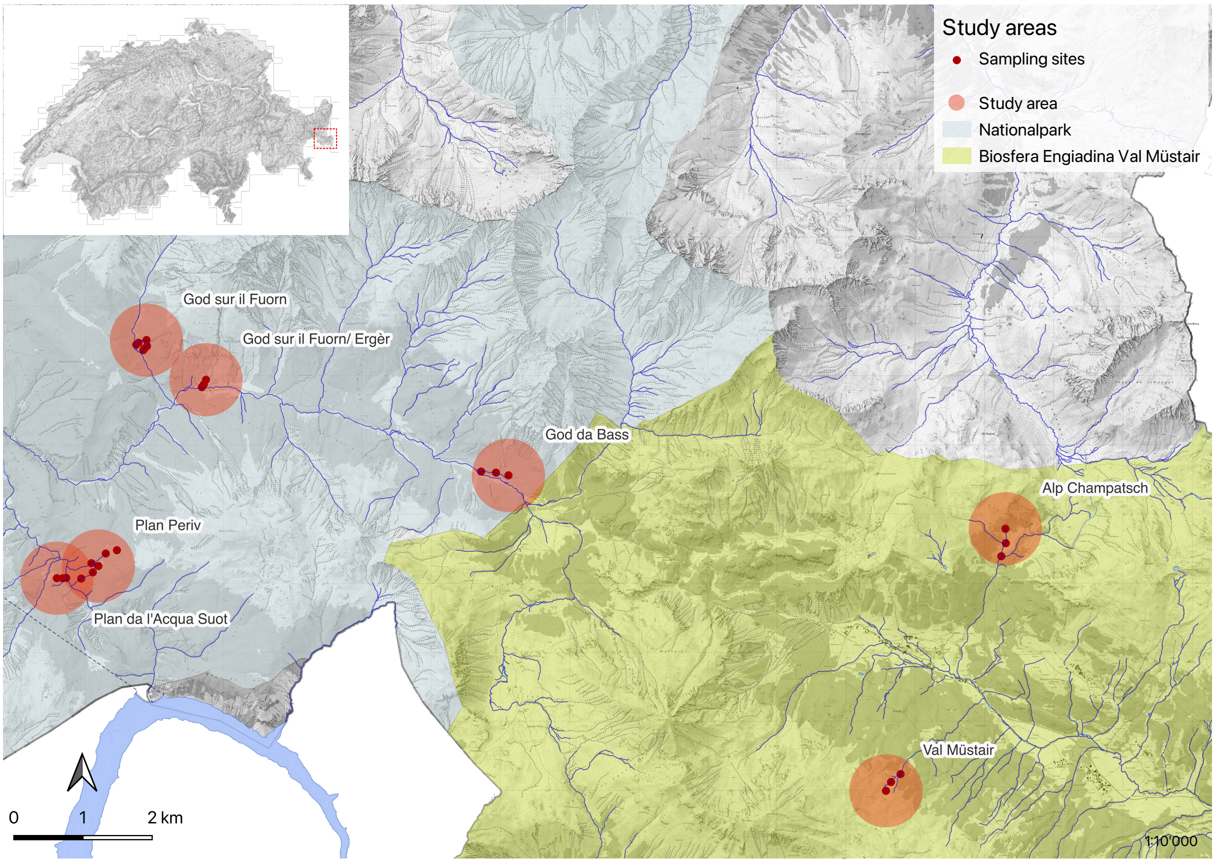


Figure 1. Map of the study areas within the National Park (SNP) and the Biosfera Engiadina Val Müstair Nature Park.

Scuol since 2017 (“Das UNESCO-Biosphärenreservat Engiadina Val Müstair,” 2026; “Schweizer Pärke,” 2025). The park covers an area of 199 km^2 and extends from the Ofenpass (at 2149 m a.s.l.) and the highest point at 2764 m a.s.l. (Piz Chavalatsch) to the border with South Tyrol (IT) with the lowest point in Glurns (lowest point in Glurns at 914 m a.s.l.) (“Schweizer Pärke,” 2025; Spinnler et al., 2020). The Rombach flowing through Val Müstair supports floodplain landscapes of national importance. In addition to these floodplain habitats the vegetation of the nature park forms a mosaic of forests wooded pastures dry grasslands and mountain nutrient poor grasslands which is reflected in high biodiversity with a large number of rare species (Spinnler et al., 2020).

In the UNESCO Biosphere Reserve Engiadina Val Müstair, a total of nine study areas were defined. The systems within the study areas are currently faunistically sampled in monitoring programmes or were previously investigated in other studies (Von Fumetti and Blattner, 2016).

Of the nine systems, seven are located in the Swiss National Park (SNP) in Plan Periv, Plan da l’Acqua Suot, God sur il Fuorn/ Ergèr and God da Bass (Figure 1: light red areas). These systems drain into different running waters including the Spöl (Plan Periv Plan da l’Acqua Suot), the Ova da Val Ftur/ Ova dal Fuorn (God sur il Fuorn/ Ergèr) and the Ova dals Pluogls (God da Bass) which then flows into the Ova dal Fuorn.

In the Biosfera Engiadina Val Müstair, two systems in the study areas Val Müstair were sampled (Figure 1: light red areas).

The study areas are situated in a complex geological setting shaped by a major tectonic fault expressed through vertical and lateral displacements. This fault zone known as the Engadinerline separates two geologically distinct units. South of the Engadiner Line the Engadine Dolomites occur while north of it the Upper Austroalpine Silvretta Nappe is present. The Silvretta Nappe is composed mainly of crystalline rocks including amphibolites mica schists paragneiss and orthogneiss which overthrust the Cretaceous and Malm limestones of the Penninic Tasna Nappe (BAFU, 2017).

South of the Engadiner Line sedimentary units composed of Triassic rocks dominate. These are characterized by a massive main dolomite formation derived from sediments of the ancient Tethys Ocean (Tethys) deposited around 220 million years ago. The vegetation of both areas is therefore strongly influenced by dolomite and silicate bedrock (BAFU, 2017).

Both protected areas are currently ice free but were shaped by postglacial processes. As a result periglacial landforms such as lakes frost patterned ground glacial striations and moraines are widespread (BAFU, 2017).

Local habitats in the study areas are mainly controlled by the combination of a continental inter alpine dry climate and the geological substrate particularly nutrient poor dolomitic soils. These conditions are further modified by hydrological processes and topographic variability. In contrast silicate substrates provide more vegetation friendly conditions which are especially evident south of the Engadiner Line (BAFU, 2017).

2.2 Sampling design

The study areas were selected to identify systems that allow a representative sampling of longitudinal zonation while ensuring comparability. Sampling was conducted in three predefined sections of each system. These sections comprised the eucrenal (spring), which is characterized by water emerging from the ground surface and extends approximately 0 to 5 m downstream, the hypocrenal (spring brook), which follows the lower boundary of the eucrenal and extends up to a maximum of 100 m downstream, and the epirhithral (upper trout zone), which includes the section from 100 m to 500 m downstream from the spring outlet (Lubini et al., 2016; Schuhmacher et al., 2001; Schwoerbel and Brendelberger, 2021). Although these distances represent a standardized operational definition, the actual spatial extent of these zones may vary among systems depending on local topographical and geomorphological conditions.

Prior to field sampling, the sampling locations were defined using Swisstopo and the distances between sites within each system were estimated. Within each study area, three sections were sampled and labeled using the following notation: “Springname_1_1_1/2”, “Springname_1_2_1/2” and “Springname_1_3_1/2” (Figure 2). In this notation, the first number indicates the number of sampled systems sharing the same spring name. The second number refers to the sampling section within the system, with 1 representing the eucrenal, 2 the

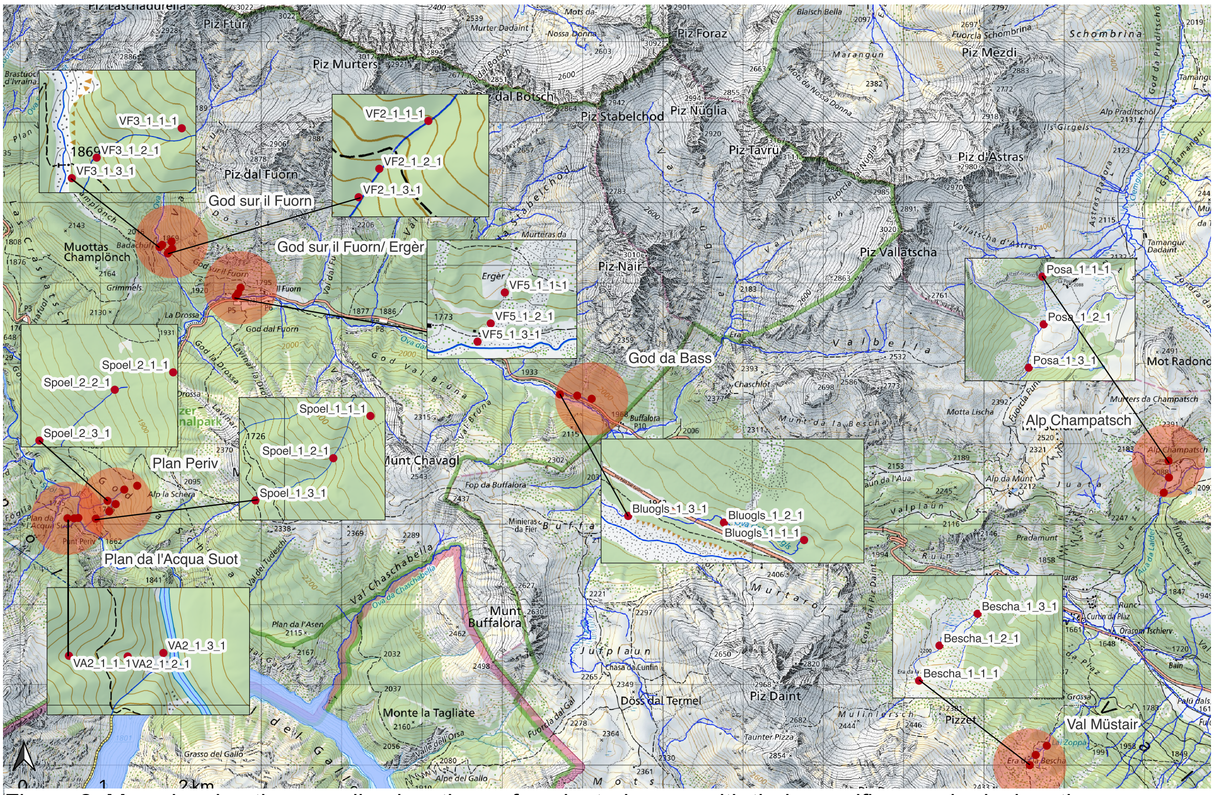


Figure 2. Map showing the sampling locations of each study area with their specific sample designation..

hypocrenal and 3 the epirhithral. The third number denotes the biological replicate, as two samples were collected per section.

Sampling was carried out in early June 2025. Environmental DNA (eDNA) was collected using a filtration based approach, in which DNA accumulates on a filter membrane (Pawlowski et al., 2020). The applied sampling protocol followed the filtration method described by Pawlowski et al. (2020), particularly regarding the use of a vacuum pump (Vampir sampler, Bürkle), the processed water volume, and the application of Sterivex® filters (Sterivex, Merck). The overall material setup was further adapted following Blattner et al. (2024) and included the use of Parafilm, disposable syringes, and ethanol for sample fixation (Blattner et al., 2024; Pawlowski et al., 2020).

At each sampling site, two eDNA samples (primary and secondary) were collected using the vacuum pump to account for spatial and analytical variability (Figure 3). Prior to sampling, all laboratory bottles (250 ml wide neck bottles with screw caps) were rinsed with spring or stream water to minimize the risk of cross contamination. In addition, the silicone tubing of the vacuum pump was flushed with 0.5 l of site water before each sampling event.

Figure 3. Sampling was conducted using a vacuum pump

Filtration was performed using a sterile Sterivex® filter (Sterivex, Merck) with a pore size of 0.22 µm and a flow capacity of 2000 ml, which was connected to the silicone tubing. A total of 1.5 l of surface water from the spring or stream was filtered in several pumping steps using the laboratory bottles. Following filtration, ambient air was pumped through the filter to remove residual water.

Subsequently, the lower outlet of the Sterivex filter was sealed with Parafilm (BRAND™ Seal R Film™). Using a 2 ml disposable syringe (OMNIFIX), 2 ml of 100% ethanol was drawn from a prefilled 5 ml tube (Eppendorf) and injected into the upper opening of the Sterivex filter, which was then sealed with Parafilm.

Disposable syringes and ethanol were replaced at each site to prevent cross contamination. Ethanol served to fix and preserve DNA on the filter. Both samples from each site were stored in zip lock bags (Rapid Grip), labeled with the corresponding site identifier, kept in a cooling bag in the field, and subsequently stored at −20 °C until further laboratory processing.

Sampling within each system started at the most downstream site to minimize the risk of cross contamination of upstream samples and to avoid disturbance by resuspended substrate.

Coordinates, elevation, and distance to the spring outlet were recorded for each site using the Swisstopo Application (https://www.swisstopo.admin.ch/de/swisstopo-app). Additionally, two photographs were taken at each site, oriented upstream and downstream. Distances between sampling sites were estimated by step counting. Detailed information on sampling locations is provided in Table 1, with additional visual documentation included in the Appendix (Table 3).

On each field day, a negative field control was collected at one spring outlet to assess potential false positive detections caused by cross contamination during fieldwork. The negative control was obtained using the vacuum pump by passing ambient air through a Sterivex filter, which was subsequently sealed with Parafilm and labeled as “Springname_1_N”.

Table 1. Site descriptions, study areas, coordinates (EPSG:2056, CH1903+/LV95), elevation (m a.s.l.) and distance to the spring (m).

Sample ID	**Study area**	**Latitude**	**Longitude**	**Elevation (m)**	**Distance to spring (m)**
Spoel_1_1_1	Plan Periv	2'810'185.00	1'169'246.00	1825.9	0
Spoel_1_2_1	Plan Periv	2'810'105.00	1'169'155.00	1769.3	220
Spoel_1_3_1	Plan Periv	2'809'937.00	1'169'064.00	1709.6	440
Spoel_2_1_1	Plan Periv	2'810'453.00	1'169'475.00	1937.8	0
Spoel_2_2_1	Plan Periv	2'810'292.00	1'169'427.00	1890.3	210
Spoel_2_3_1	Plan Periv	2'810'084.00	1'169'287.00	1809.3	440
Bluogls_1_1_1	God da Bass/ Ova dals Pluogls	2'816'116.00	1'170'558.00	1953	0
Bluogls_1_2_1	God da Bass/ Ova dals Pluogls	2'815'936.00	1'170'597.00	1947.1	240
Bluogls_1_3_1	God da Bass/ Ova dals Pluogls	2'815'721.00	1'170'612.00	1936.5	490
VF3_1_1_1	God sur il Fuorn	2'810'881.00	1'172'515.00	1938.4	0
VF3_1_2_1	God sur il Fuorn	2'810'765.00	1'172'475.00	1884.9	220
VF3_1_3_1	God sur il Fuorn	2'810'731.00	1'172'447.00	1870.2	310
Posa_1_1_1	Alp Champatsch/ La Posa	2'823'303.00	1'169'785.00	2133.5	0
Posa_1_2_1	Alp Champatsch/ La Posa	2'823'309.00	1'169'577.00	2056.1	270
Posa_1_3_1	Alp Champatsch/ La Posa	2'823'244.00	1'169'388.00	2003.3	510
VF5_1_1_1	God sur il Fuorn/ Ergèr	2'811'741.00	1'171'941.00	1804.9	0
VF5_1_2_1	God sur il Fuorn/ Ergèr	2'811'710.00	1'171'873.00	1782.3	100
VF5_1_3_1	God sur il Fuorn/ Ergèr	2'811'681.00	1'171'833.00	1774.8	160
VF2_1_1_1	God sur il Fuorn	2'810'888.00	1'172'427.00	1907.9	0
VF2_1_2_1	God sur il Fuorn	2'810'850.00	1'172'390.00	1886.3	70
VF2_1_3_1	God sur il Fuorn	2'810'834.00	1'172'368.00	1872.7	120
VA2_1_1_1	Plan da l'Acqua Suot	2'809'585.00	1'169'070.00	1713.5	0
VA2_1_2_1	Plan da l'Acqua Suot	2'809'669.00	1'169'069.00	1685	100
VA2_1_3_1	Plan da l'Acqua Suot	2'809'720.00	1'169'074.00	1658.4	160
Bescha_1_1_1	Era de la Bescha/ Val Müstair	2'821'575.00	1'165'995.00	2225.8	0
Bescha_1_2_1	Era de la Bescha/ Val Müstair	2'821'649.00	1'166'122.00	2173.4	274
Bescha_1_3_1	Era de la Bescha/ Val Müstair	2'821'787.00	1'166'237.00	2136	480

2.3 Molecular Analysis

Molecular analyses of the samples collected in the Swiss National Park and the Val Müstair were conducted in the molecular laboratory of the Geoecology research group at the Department of Environmental Sciences, University of Basel. All work with molecular samples followed strict cleanliness and contamination control procedures to prevent contamination with external eDNA. Working surfaces and tools such as tweezers and pliers were cleaned using DNA-ExitusPlus ™ and 100 % ethanol and were heat sterilised prior to use. Pipette tips and tubes were supplied sterile by the manufacturers.

2.3.1 DNA Extraction

The DNA extraction procedure followed the purification approach of the method optimized and adapted by L. Blattner. This protocol is suitable for both environmental DNA (eDNA) and sediment DNA (sedDNA) due to its high DNA yield and was therefore preferred over alternative extraction methods based on previous experience (Blattner et al., 2024).

Parafilm was removed and the ethanol contained in the Sterivex filter was extracted using a 10 ml disposable syringe (Pikdare S.PA). Disposable syringes were replaced after each sample duplicate to avoid cross contamination.

The plastic housing of the Sterivex filters was opened along the seams using pliers to expose the filter. The membrane filter was then cut open along the seams using a scalpel (Swann Morton) and tweezers (Bernstein). Tools were cleaned with 100 % ethanol and flame sterilized after each step. The filter membrane was cut into four small pieces and transferred into a previously centrifuged PowerBead Pro Tube (Qiagen Germany).

DNA was extracted using the DNeasy® PowerSoil® Pro Kit (Quick-Start Protocol; DNeasy® PowerSoil® Pro Kit, May 2019) following the manufacturer instructions with minor modifications. During the lysis step 20 µl Proteinase K (Qiagen Germany) was added to each sample together with 800 µl CD1 buffer (Step 3). Samples were incubated at 56 °C and 300 rpm for approximately 24 hours in a thermomixer (Thermomixer compact, Eppendorf). In the final elution step (Step 16) 110 µl C6 solution was used.

The extracted eluate was stored at - 4 ° Cuntil further PCR amplification (Blattner et al., 2021).

2.3.2 Marker Choice

The CO1 marker (cytochrome-c-oxidase subunit 1) was selected because of its widespread and successful use in metabarcoding studies and its specific advantages. CO1 enables high-resolution species identification (Blattner et al., 2024). Amplification of degraded environmental DNA (eDNA) and sediment DNA (sedDNA) is facilitated by the relatively short length of CO1 fragments, which are more likely to persist in fragmented DNA samples (Blattner et al., 2024; Brantschen et al., 2022).

Selection of an appropriate primer pair represents a key challenge in metabarcoding studies because primer specificity can introduce bias in taxonomic coverage (Blattner et al., 2024). In this study the primer pair FWH (fwhF2/fwhR2) was chosen. It is highly suitable for freshwater invertebrates due to its broad taxonomic coverage while minimizing amplification of non-target taxa such as bacteria fungi and phytoplankton (Blattner et al., 2024; Leese et al., 2021).

However, the use of universal primers in eDNA based studies also involves limitations that need to be considered. In particular CO1 markers amplified with universal primers show a higher probability of amplifying non-target taxa, which may result in underrepresentation of certain taxonomic groups (Brantschen et al., 2022; Leese et al., 2021). Primer bias can also occur with specifically designed primer sets and may affect the detection probability of individual taxa (Brantschen et al., 2022). This incomplete coverage of some taxonomic groups even when using specific primer sets was demonstrated by Brantschen et al. (2022) through the low detection rate of Trichoptera species (Brantschen et al., 2022).

In this study the universal primer set fwhF2/fwhR2 was used: (5'→ 3': fwhF2 (forward primer): GGDACWGGWTGAACWGTWTAYCCHCC; fwhR2 (reverse primer): GTRATWGCHCCDGCTARWACWGG).This primer set was selected because the aim of the study was not to target only specific taxa but to achieve a broad taxonomic coverage of freshwater invertebrates (Leese et al., 2021). The primer pair amplifies a CO1 fragment of approximately 254 bp (Blattner et al., 2024).

2.3.3 PCR-Amplification

PCR amplification was performed following the cleanliness and contamination control standards described in Chapter 2.3. All procedures were additionally carried out under a laminar flow cabinet, and samples were kept on an ice block during pipetting (PCR Cooler, Eppendorf).

The UCP® Multiplex PCR Kit (Qiagen, Germany) was used for amplification. This kit is designed to strongly reduce background DNA contamination and shows high tolerance to PCR inhibitors (Qiagen Group, 2018).

PCR amplification followed the manufacturer protocol (Quick Start Protocol, UCP® Multiplex PCR Kit, March 2018) with minor modifications. Each reaction had a total volume of 20 µl. The PCR mix consisted of:

- 5 µl DNA template
- 3.2 µl primer mix with 1.6 µl forward primer (0.8 µM) and 1.6 µl reverse primer (0.8 µM)
- 5 µl master mix (UCP Multiplex PCR Master Mix) (1x final concentration)
- 6.8 µl ultrapure water

A negative control was included in each run, in which the DNA template was replaced by 5 µl ultrapure water to detect potential contamination.

PCR reactions were carried out in a thermocycler (Labcycler 48, Sensoquest, Germany) using 40 cycles. The cycling conditions were modified from the manufacturer settings as follows:

Step 1: Polymerase activation at 95 °C for 2 minutes

Step 2: 40 cycles consisting of denaturation at 95 °C for 10 seconds, annealing at 48 °C for 30 seconds and elongation at 72 °C for 1 minute

Step 3: Final extension at 72 °C for 5 minutes

PCR products were validated by gel electrophoresis prior to pooling of PCR triplicates. Agarose gel preparation and electrophoresis followed the Solis BioDyne protocol. The gel was prepared using 1.8 g agarose 150 ml TAE buffer (1.2 % w/v) and 5 µl RedSafe (RedSafe Nucleic Acid Staining Solution, Germany). Electrophoresis was performed at 120 V for 35 minutes. Gel images were acquired using an imaging system (Azure Biosystem, USA).

2.3.3.1 Optimization Strategy of Improving PCR Products

Initial PCR amplification produced very weak products for some samples, as revealed by validation using gel electrophoresis. The amplifications rounds were therefore repeated with increased DNA template volumes. The amount of DNA template was increased from 5 µl to 10 µl while the volume of ultrapure water was reduced from 6.8 µl to 1.8 µl. This adjustment substantially improved PCR product yield, allowing the samples to be used for sequencing.

2.3.3.2 Purification of PCR-Products and Sequencing

To reduce potential biases in PCR products caused by PCR-inhibitors and stochastic differences in the amplification of individual DNA fragments all PCR reactions were carried out in independent triplicates. The triplicates were then pooled and purified using AMPure XP Beads (Beckman Coulter Life Sciences, USA) and eluted in 40 µl TE-buffer. This purification step removes buffers primers and DNA fragments shorter than 100 base pairs (Blattner et al., 2024).

The purified PCR products including the negative field samples were submitted for further processing to an external laboratory (Genomics Technology Facility, Lausanne). There library preparation and indexing of the samples were performed using a standardized Nextera XT two- step-PCR-process. Sequencing was subsequently carried out as paired-end sequencing 150 bp reads on an AVITI sequencing platform (Element Biosciences) (Blattner et al., 2024).

2.4 Bioinformatics

Raw sequencing data were processed through several bioinformatic steps including quality control filtering and taxonomic annotation following the workflow described by Blattner et al., (2024). Quality control of raw reads from each sample was performed using FastQC to evaluate sequence quality base composition and potential sequencing artifacts (Andrews, 2010; Blattner et al., 2024). The resulting quality reports were summarized and visualized with MultiQC (Blattner et al., 2024; Ewels et al., 2016). Forward and reverse reads were then merged into full length sequences using fastq_mergepairs in vsearch and primer sequences were removed using cutadapt (Blattner et al., 2024; Martin, 2011; Rognes et al., 2016). To capture biological diversity sequences were dereplicated and clustered into exact sequence variants (ESVs) using fastx_uniques in vsearch and the swarm algorithm (Blattner et al., 2024; Mächler et al., 2021; Mahé et al., 2021).

Taxonomic assignment of ESVs was performed using a curated CO1 reference database compiled by L. Blattner in November 2025 which includes freshwater invertebrate sequences from NCBI GenBank and BOLD (Blattner et al., 2024). Reference sequences were filtered dereplicated and taxonomically annotated. Species level assignment required a minimum sequence identity of 95 % with the reference sequence otherwise the assignment was downgraded to genus level. When multiple matches were obtained the lowest common ancestor (LCA) (last common ancestor) approach was applied to assign taxonomy (Blattner et al., 2024).

2.5 Data Analysis and Processing

The raw dataset compiled by L. Blattner containing 1123 identified taxa was filtered at the species level in accordance with the stated hypotheses and prepared for further analysis. Taxa identified only to genus level and those with less than 95 % sequence similarity to the reference database were removed (Blattner et al., 2024).

Species that were identified multiple times under different species names were excluded for reasons of reliability. In cases of ambiguous assignments, such as *Hygrobates norvegicus* and *Hygrobates cf. norvegicus* A LB 2019, either the confirmed species name (*Hygrobates cf. norvegicus* A LB 2019) was retained, or the identification was labelled using the designation “cf.” to indicate taxonomic uncertainty.

Read abundances were transformed into binary presence (1) and absence (0) data. The dataset was subsequently filtered for aquatic species based on taxonomic identification and zonation literature and reduced to a total of 228 species (Gerecke et al., 2010; Moog et al., 2002; Rozkosny and František, 2004; Schmedtje and Colling, 1996; Schuhmacher et al., 2001). Using the same sources, EPT taxa (Ephemeroptera, Plecoptera, Trichoptera; 56 species) and crenobionts (12 species) were identified and compiled into separate datasets (Moog et al., 2002; Rozkosny and František, 2004, 2004; Schmedtje and Colling, 1996; Schuhmacher et al., 2001).

The three resulting datasets were initially explored using detrended correspondence analysis (DCA). All analyses were conducted in the open-source software RStudio (v. 2024.12.1, PBC) using the vegan package (v. 2.7-2). Multivariate ordination techniques were applied to analyse species composition and the spatial structuring of aquatic communities along the investigated stream systems.

The objective of the statistical analysis was to identify major gradients in the eDNA based datasets and to visualise biological differentiation between the sampled sections (eucrenal, hypocrenal and epirhithral). Ordination plots derived from detrended correspondence analysis provide a descriptive representation of community structure, where distances between sampling points reflect their compositional dissimilarity and species turnover (Leyer and Wesche, 2008).

To detect potential zonation patterns at different levels of taxonomic resolution the analyses were conducted separately for three datasets comprising all aquatic taxa, EPT taxa and crenobionts.

3. Results

Analysis of the raw data reduced the initial set of 1123 identified taxa to a total of 228 aquatic species. Across the nine investigated systems, the epirhithral sections exhibited the highest cumulative number of species detections (677), followed by the hypocrenal sections with 540 detections and the eucrenal sections with 265 detections. These values represent the summed number of species occurrences across all systems, allowing multiple detections of the same species in different sites.

The class Insecta accounted for the highest species richness with 196 taxa, including 128 species of the order Diptera. The family Chironomidae was the most diverse group with 82 identified species. Arachnida represented the second most diverse class with 26 species within the order Trombidiformes. In contrast, Branchiopoda, Malacostraca, Ostracoda and Bivalvia exhibited very low diversity, with only one species each. Only the family Gammaridae contained two species.

Among the investigated systems, Posa and VF5 showed the highest diversity with 351 and 235 identified species, respectively (Figure 4). The lowest numbers of species were recorded in Spoel 1 with 29 species and in Spoel 2 with 89 species. The systems Bescha, VF2, VF3, Bluogls and VA2 showed comparable species richness, with 183 species in Bescha, 161 in VF2, 156 in VF3, 152 in Bluogls and 132 in VA2.

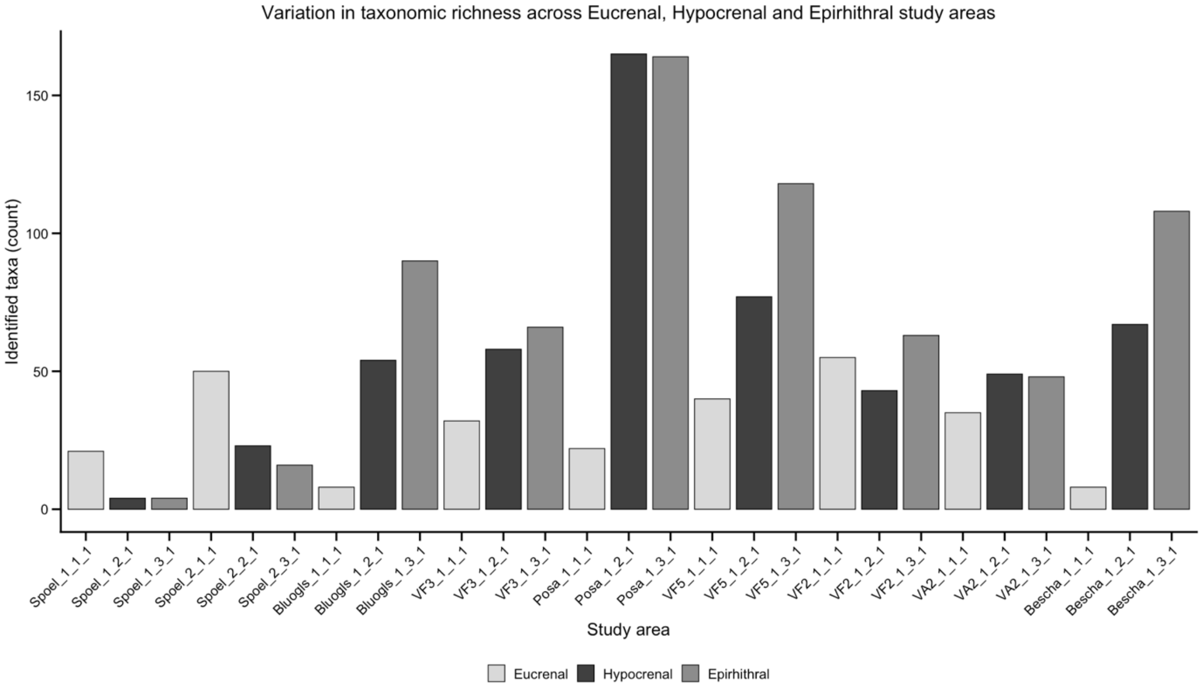


Figure 4. Number of taxa (identified taxa) in the eucrenal, hypocrenal and epirhithral sections of the studied sites.

In the eucrenal sections of VF2, Spoel 2 and VF5, the highest species richness was recorded, with 55 species in VF2, 50 in Spoel 2 and 40 in VF5. VA2, VF3, Posa and Spoel 1 showed comparable eucrenal richness ranging from 35 to 21 species, whereas Bluogls and Bescha contained only 8 species in their eucrenal sections.

Species richness differed substantially between the hypocrenal and epirhithral sections, but consistent patterns in the relative ranking of systems were evident across both zones.

In the hypocrenal, species richness ranged from only 4 species in Spoel 1 to a maximum of 165 species in Posa. High richness was also observed in VF5 with 77 species, Bescha with 67 species and VF3 with 58 species. Intermediate values occurred in Bluogls with 54 species, VA2 with 49 species and VF2 with 43 species, whereas Spoel 2, with 23 species, represented one of the species poor hypocrenal systems.

A similar ranking pattern was observed in the epirhithral. Spoel 1 again showed the lowest richness with 4 species, while Posa exhibited the highest richness with 164 species. High numbers were also found in VF5 with 118 species and in Bescha with 108 species. Intermediate richness was recorded in Bluogls with 90 species, VF3 with 66 species and VF2 with 63 species, whereas VA2 with 48 species and Spoel 2 with 16 species showed lower richness.

Systems that were species rich in the hypocrenal were also species rich in the epirhithral, whereas systems with low richness in the hypocrenal remained species poor in the epirhithral, indicating a strong concordance in species richness patterns across longitudinal zones

Trombidiformes

Within Trombidiformes nine families comprising 27 species were identified. Halacaridae was the most diverse family with 13 species and was detected in all systems except Bluogls and Bescha. Nine of the 27 species were classified as crenobiontic (Blattner et al., 2022, 2021; Gerecke et al., 2018, 2010).

The crenobiontic species *Partnunia cf. steinmanni* A LB 2019, *Partnunia cf. steinmanni* B LB 2019 and *Partnunia cf. steinmanni* were detected mainly in the eucrenal and hypocrenal, with few occurrences in VA2 and Posa. The species *Sperchon mutilus, Sperchon squamosus, Sperchon thienemanni* and *Sperchon violaceus* showed high detection frequencies in VF3, VF5 and Posa and are described as dominant alpine species with a preference for spring habitats (Gerecke et al., 2018). Crenobiontic species of the family Lebertiidae were detected only in Spoel 2, VF3, Posa, VF5, VF2 and VA2, mostly in the hypocrenal and epirhithral.

Diptera

Diptera represented the most species rich order across the investigated systems. Eight families comprising a total of 128 species were detected. Chironomidae was by far the most diverse family with 96 species and was detected in all nine systems. The species *Parametriocnemus stylatus*, *Tvetenia bavarica*, *Tvetenia calvescens*, *Thienemanniella cf. clavicornis* and

Corynoneura lobata showed high detection frequencies and were mainly detected in Spoel 2, Bluogls, VF3, Posa, VF5, VF2 and VA2.

Ephemeroptera

Within Ephemeroptera two families, Baetidae and Heptageniidae, were detected. The heptageniid species *Ecdyonurus helveticus* and *Epeorus alpicola* were detected only in a limited number of systems (VA2, Spoel 1 and Posa). The baetid species *Baetis alpinus*, *Baetis rhodani* and *Baetis cf. rhodani* were mainly detected in Bluogls, VF3, Posa, VF5 and VA2 across all three longitudinal sections. These species are widespread in Switzerland, with *Baetis alpinus* preferring cold stenothermal habitats (Lubini et al., 2010; Studemann et al., 1992).

Plecoptera

Plecoptera were recorded in all nine systems. Four families (Leuctridae, Nemouridae, Perlodidae and Taeniopterygidae) comprising 23 species were identifed. Perlodidae were absent from Spoel 1, Spoel 2 and VF2. The species *Leuctra armata*, *Nemoura sinuata* and *Protonemura lateralis* were detected in all three longitudinal sections. *Rhabdiopteryx neglecta* was recorded only in Posa.

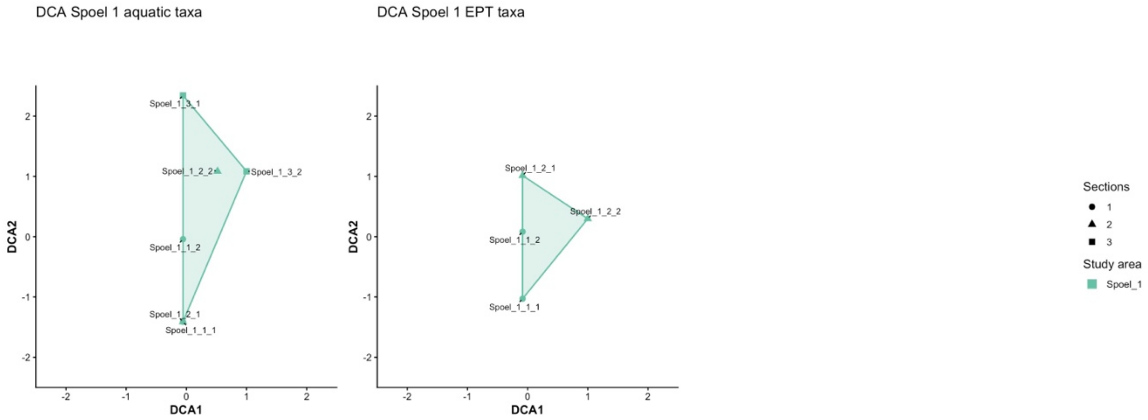
Two crenobiontic species were detected within Plecoptera, *Protonemura lateralis* and *Dictyogenus fontium* (Blattner et al., 2021). *Protonemura lateralis* was detected in Bluogls (eucrenal and epirhithral), in VF3, Posa and VF5 in all three sections, in VF2 and VA2 in the eucrenal and hypocrenal, and in Bescha exclusively in the eucrenal. *Dictyogenus fontium* was detected predominantly in the hypocrenal and epirhithral sections, with eucrenal detection limited to a single system (Posa).

Trichoptera

Nine trichopteran families comprising 27 species were detected. Limnephilidae was the most species rich family with 13 species and was detected in most systems. Rhyacophilidae were detected only in Posa, VF5 and VA2. *Lithax niger* was the only species classified as crenobiontic and was detected exclusively in the eucrenal section of VF3, whereas in Posa and VF5 it was detected only in the hypocrenal and epirhithral sections (Blattner et al., 2021).

3.1 Statistical Analysis

3.1.1 DCA Ordination of System Spoel 1



DCA Spoel 1 aquatic taxa

DCA Spoel 1 EPT taxa

Figure 5. DCA plots of the Spoel 1 system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

Analysis of aquatic taxa in Spoel 1 reveals a clear vertical separation of the three sections along the second ordination axis (DCA2). Eucrenal samples are located at a similar horizontal position along DCA1 but differ vertically along DCA2. Hypocrenal samples occupy an intermediate position, with Spoel_1_2_1 located very close to the eucrenal sample Spoel_1_1_1. Epirhithral samples cluster in the positive region of both axes, with the replicate Spoel_1_3_2 shifted to the right along DCA1 and downward along DCA2 (Figure 5).

For EPT taxa a broadly similar ordination pattern is observed for the first section, although overall differentiation along DCA2 is weaker (Figure 5). Epirhithral samples were not detected for EPT taxa. This is also reflected in the species list, where the few detected species were mainly recorded in the eucrenal or hypocrenal (Appendix Table 2).

In the crenobiont ordination, no samples from Spoel 1 are displayed because no crenobiontic species were detected in this system (Appendix Table 2).

3.1.2 DCA Ordination of System Spoel 2

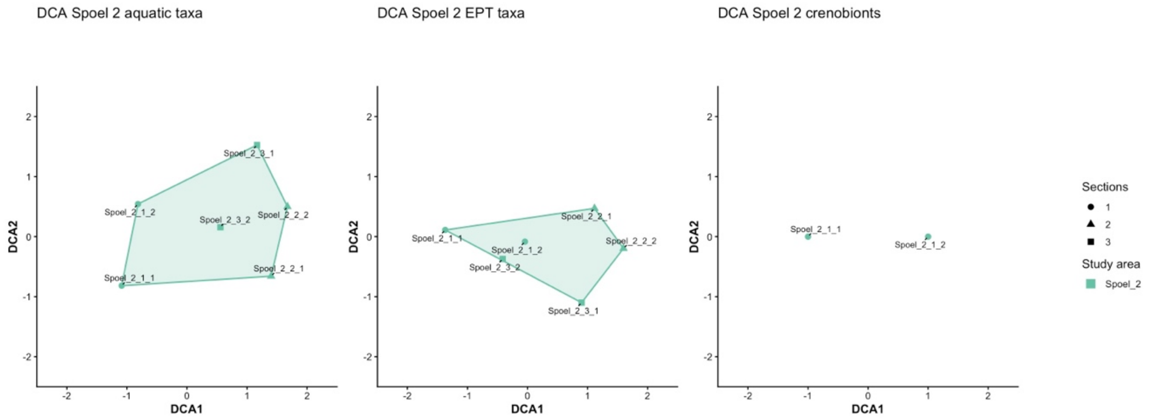


Figure 6. DCA plots of the Spoel 2 system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

Along the DCA1 axis, the aquatic taxa samples span a gradient from negative to positive values (Figure 6). Eucrenal samples are located at the negative end of this axis, whereas hypocrenal samples occupy the most positive positions. Epirhithral samples show an intermediate position, being closer to the hypocrenal than to the eucrenal. Replicates of each section are clearly separated along the DCA2 axis.

The ordination pattern for EPT taxa closely resembles that of all aquatic taxa. The eucrenal sample Spoel_2_1_1 is located at the extreme left, hypocrenal samples at the right, and epirhithral samples in between (Figure 6). Replicates show less vertical dispersion, with epirhithral replicates positioned closer to those of both the first and second sections. The only species recorded in all sections was *Wormaldia occipitalis* (Appendix Table 2).

In the crenobiont ordination, only eucrenal and hypocrenal samples are present. Across all samples, two species were detected, of which *Partnunia cf. steinmanni* B LB 2019 and *Sperchon mutilus* occurred exclusively in the eucrenal (Appendix Table 2).

3.1.3 DCA Ordination of System Bluogls

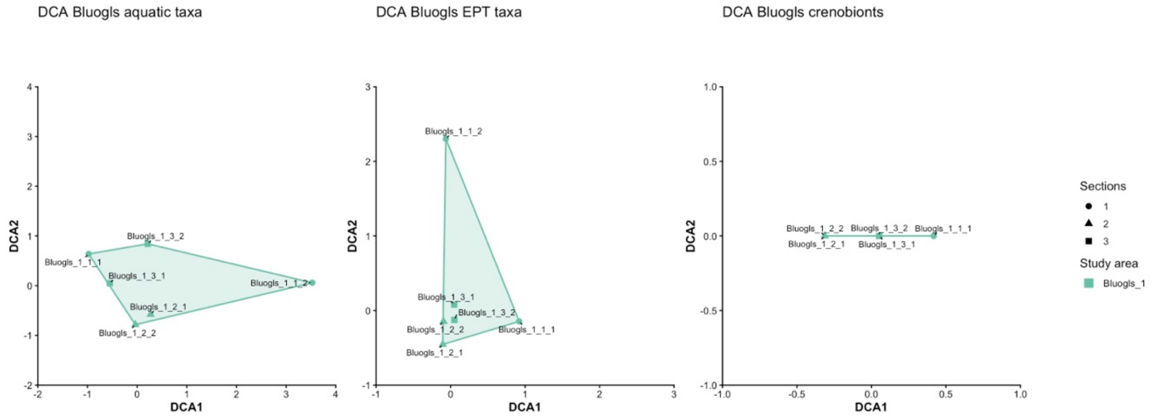


Figure 7. DCA plots of the Bluogls system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

DCA of aquatic taxa in Bluogls reveals pronounced variability within the eucrenal. Replicate samples are positioned both at the negative end of the DCA1 axis (Bluogls_1_1_1) and at the strongly positive end (Bluogls_1_1_2) (Figure 7). In contrast, hypocrenal and epirhithral samples cluster near the center of the ordination. Hypocrenal replicates show less vertical dispersion than those from the epirhithral.

For EPT taxa, eucrenal samples also display strong dispersion, mainly along the DCA2 axis. The second and third sections again group closely together and form a joint cluster in the lower part of the ordination space (Figure 7). Several Baetidae and Leuctridae species were detected in the hypocrenal and epirhithral. The three Nemouridae species *Nemoura mortoni*, *Nemurella pictetii* and *Protonemura brevistyla* were either present in all three sections or only in the hypocrenal and epirhithral (Appendix Table 2).

Crenobiont assemblages show very low variance, with all samples from the three sections clustering near the center of the ordination (Figure 7). The sample Bluogls_1_1_1 is slightly shifted to the right along DCA1. The crenobiont eDNA composition shows a high degree of similarity across all sections, which is also reflected in the species list. The crenobiont species *Protonemura lateralis* was detected in both the eucrenal and epirhithral (Blattner et al., 2021) (Appendix Table 2).

3.1.4 DCA Ordination of System VF3

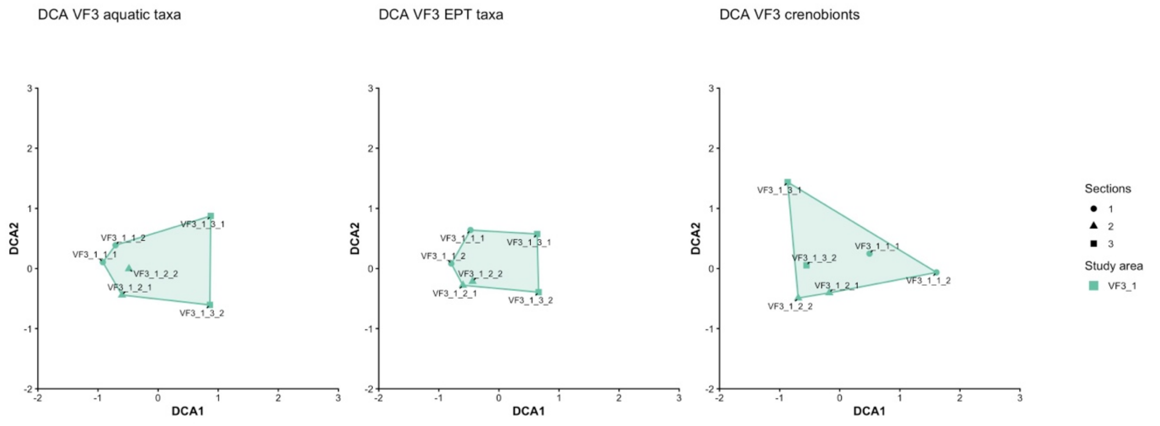


Figure 8. DCA plots of the VF3 system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

A clear separation between the upper reach and the spring proximal sections is evident along the DCA1 axis (Figure 8). Samples from the third section cluster in the positive range of DCA1, whereas samples from the first and second sections group together in the negative range. This indicates strong ecological overlap between the eucrenal and hypocrenal, while the epirhithral forms a distinct cluster.

The ordination of EPT taxa closely follows the pattern of the full aquatic community, although replicate dispersion is lower (Figure 8). Samples from the first and second sections again cluster tightly in the negative range of DCA1, whereas third section samples are clearly separated in the positive range. This is also reflected in the EPT species list (Appendix Table 2), where *Baetis alpinus* was detected in all three sections, while *Baetis cf. rhodani* occurred only in the two lower sections.

Crenobionts show a pronounced longitudinal gradient along DCA1. Samples from the first section occupy the most positive positions, whereas replicates from the hypocrenal and epirhithral are positioned closer to the center or further to the negative range of the ordination space (Figure 8). A marked vertical dispersion of the third section replicates is also evident along DCA2. The species *Protonemura lateralis* was detected in all sections (Appendix Table 2).

3.1.5 DCA Ordination of System Posa

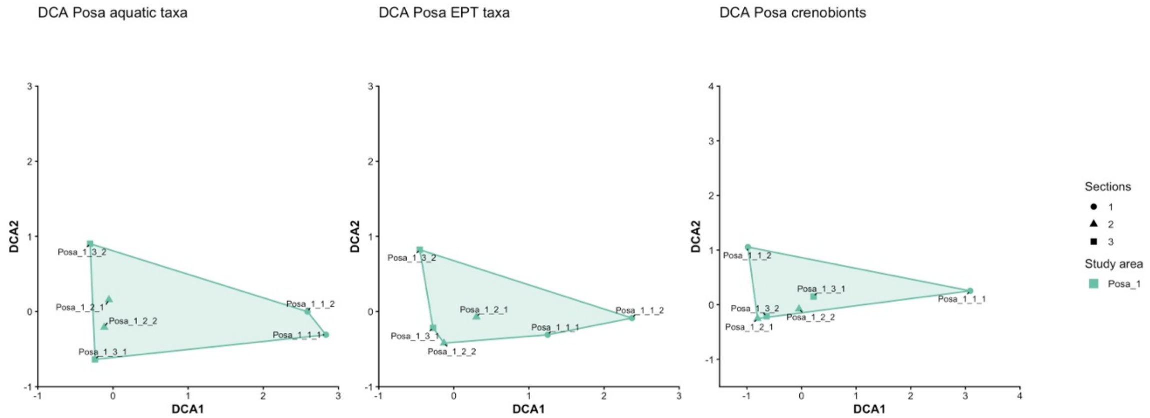


Figure 9. DCA plots of the Posa system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

In Posa, a strong sequential separation of the three sections is evident along the DCA1 axis (Figure 9). Eucrenal samples are located at the strongly positive end of the axis, whereas epirhithral samples occupy the most negative positions. Hypocrenal samples lie in between, close to the center of the ordination space. Replicate dispersion is greatest for the epirhithral samples, while samples within each section are tightly clustered along the first axis.

The same longitudinal gradient is also apparent for EPT taxa, with the three sections clearly separated from right to left along DCA1 (Figure 9). Eucrenal replicates show slightly higher dispersion along this axis than the other sections, which is mainly related to the occurrence of Nemouridae species that were predominantly detected in the hypocrenal and epirhithral (Appendix Table 2).

Crenobiont assemblages exhibit a distinct zonation pattern. The eucrenal sample (Posa_1_1_1) is isolated at the positive end of DCA1, whereas hypocrenal and epirhithral samples cluster in the negative range (Figure 9). This reflects that most crenobiontic species were detected in the two downstream sections. *Partnunia cf. steinmanni* A LB 2019, *Protonemura lateralis* and *Dictyogenus fontium* were the only species recorded in the eucrenal, with the latter two also occurring in the hypocrenal and epirhithral (Blattner et al., 2021) (Appendix Table 2).

3.1.6 DCA Ordination of System VF5

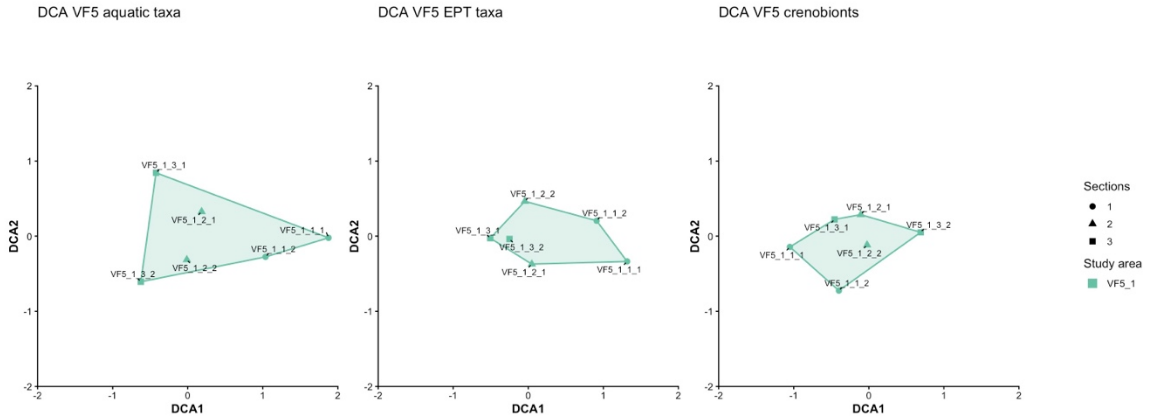


Figure 10. DCA plots of the VF5 system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

The VF5 system shows a clear longitudinal gradient along the DCA1 axis. Eucrenal samples occupy the most positive positions, whereas epirhithral samples lie in the negative range (Figure 10). Hypocrenal samples are positioned in between, close to the center of the ordination space. A pronounced vertical dispersion of epirhithral samples along DCA2 indicates higher community variability in this section.

EPT taxa display the same zonation pattern, with all three sections forming a distinct sequence along DCA1, indicating considerable species turnover along the longitudinal gradient. Some taxa still occur across multiple zones, which is particularly evident for Baetidae and Nemouridae species (Appendix Table 2).

Crenobiont assemblages show eucrenal samples at the negative end of DCA1, while the epirhithral sample VF5_1_3_2 is shifted towards positive values. This reflects a higher number of crenobiontic species detected in the epirhithral, especially in VF5_1_3_2. *Sperchon mutilus* and *Protonemura lateralis* were present in all sections (Appendix Table 2).

3.1.7 DCA Ordination of System VF2

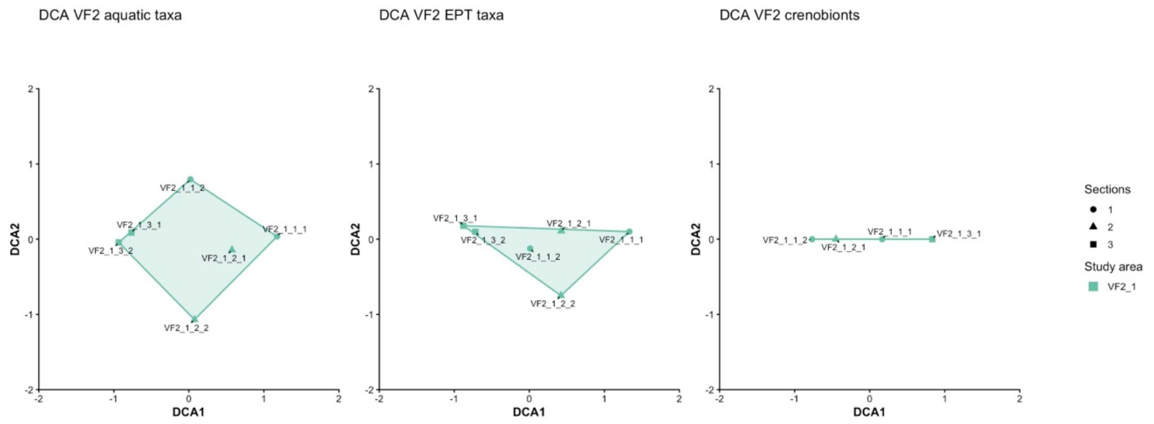


Figure 11. DCA plots of the VF2 system for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

Along the DCA1 axis, an ordered longitudinal sequence is observed (Figure 11). Samples from the eucrenal section are located in the positive range, whereas samples from the epirhithral are located in the negative range. Samples from the hypocrenal section occupy an intermediate position around the center of the ordination space. The gradient along the first axis indicates a clear species turnover between the sections.

The DCA pattern of EPT taxa confirms this zonation (Figure 11). The replicate VF2_1_2_1 shows a distinct position along the DCA1 axis compared to its replicate. The three sections form a clear sequence along the first axis from right to left. Species from the families Leuctridae and Nemouridae were detected in all sections, while most of the remaining species were mainly recorded in the epirhithral (Appendix Table 2).

Crenobiont samples are arranged along a horizontal line, with biological differentiation occurring primarily along the first axis (Figure 11). The sample from the first section (VF2_1_1_2) occupies the most negative position, followed by hypocrenal samples, whereas epirhithral samples are positioned in the positive range. *Partnunia cf. steinmanni* B LB 2019, *Partnunia cf. steinmanni* and *Protonemura lateralis* were detected in the eucrenal, with the first two also occurring in the hypocrenal. *Sperchon mutilus* was the only species also detected in the epirhithral (Appendix Table 2).

3.1.8 DCA Ordinations of System VA2

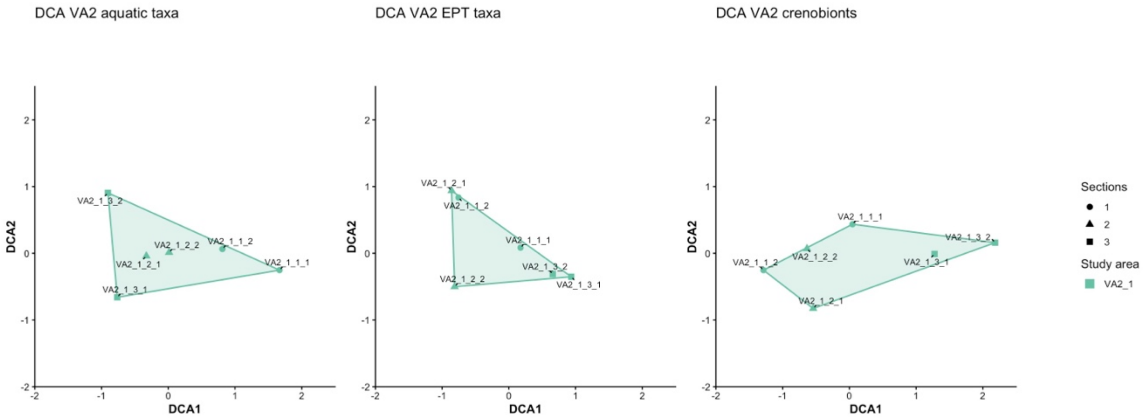


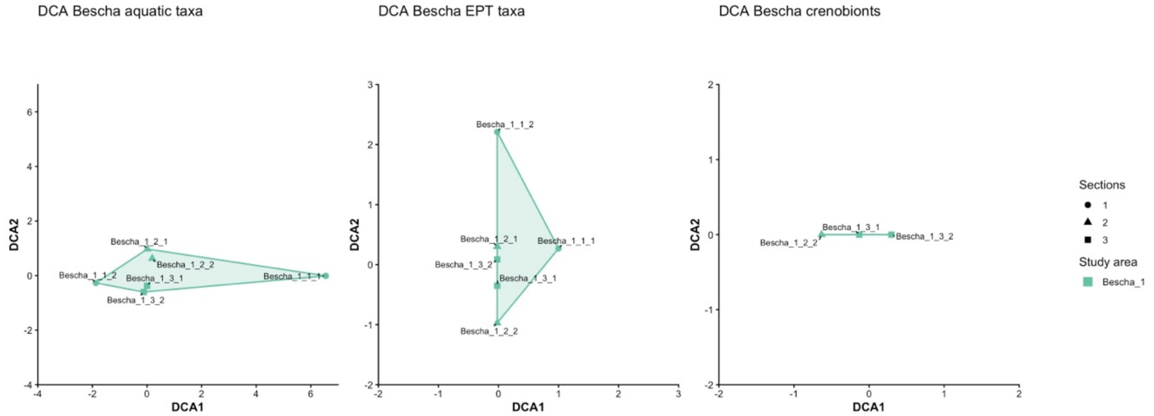
Figure 12. DCA plots of the system VA2 for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal, epirhithral).

The DCA analysis of the system VA2 shows a clear longitudinal sequence along the DCA1 axis (Figure 12). Eucrenal samples occupy the positive range of the first axis, hypocrenal samples are positioned intermediately near the center of the ordination space, and epirhithral samples cluster in the negative range.

For EPT taxa, the pattern is less linear. Hypocrenal samples are located at one extreme of the DCA1 axis, whereas epirhithral samples are positioned at the opposite extreme. Eucrenal samples occupy an intermediate position (Figure 12). Replicates from the eucrenal and epirhithral sections show greater dispersion along the DCA2 axis, while hypocrenal replicates cluster closely together. *Baetis alpinus* was the only species detected in all three sections (Appendix Table 2).

Crenobionts samples show pronounced dispersion along both DCA axes. Species detected in both the eucrenal and hypocrenal are *Partnunia cf. steinmanni* B LB 2019, *Partnunia cf. steinmanni*, *Sperchon mutilus* and *Protonemura lateralis*. *Bandakia cf. concreta* A LB 2019 was the only species detected in all systems (Gerecke et al., 2018, 2010) (Appendix Table 2).

3.1.9 DCA Ordinations of System Bescha


DCA Bescha aquatic taxa

DCA Bescha EPT taxa

DCA Bescha crenobionts

Figure 13. DCA plots of the system Bescha for aquatic taxa, EPT taxa and crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal, epirhithral).

The DCA analysis of the system shows high biological heterogeneity within the first section. The two replicates Bescha_1_1_2 and Bescha_1_1_1 are located at extreme positions in both the positive and negative ranges of the DCA1 axis (Figure 13). The resulting gradient length on the first axis is approximately 4.5 standard deviations. Since values above 4 standard deviations indicate an almost complete turnover in species composition this suggests that the two spring samples share almost no species, which is also reflected in the species list (Appendix Table 2). None of the identified species were detected in both Bescha_1_1_2 and Bescha_1_1_1. In contrast samples from the hypocrenal and epirhithral cluster compactly around the center of the diagram near the zero point.

A similar pattern is observed for EPT taxa (Figure 13). Hypocrenal and epirhithral samples form a compact cluster in the lower part of the ordination space, and most species were detected either in the hypocrenal or in the epirhithral, but rarely in both sections (Appendix Table 2).

Two crenobiontic species were detected, *Protonemura lateralis* and *Dictyogenus fontium*. Both taxa occurred mainly in the hypocrenal and epirhithral sections, while *Protonemura lateralis* was also detected in the eucrenal (Appendix Table 2).

3.2 Comparative analysis of DCA ordinations across all systems

The following figures present all systems within a common DCA framework for the datasets defined above. This allows direct comparison of ordination patterns among stream systems and enables similarities and differences in the spatial arrangement of samples across systems and sections to be visualised.

3.2.1 Cross system DCA ordination of aquatic taxa

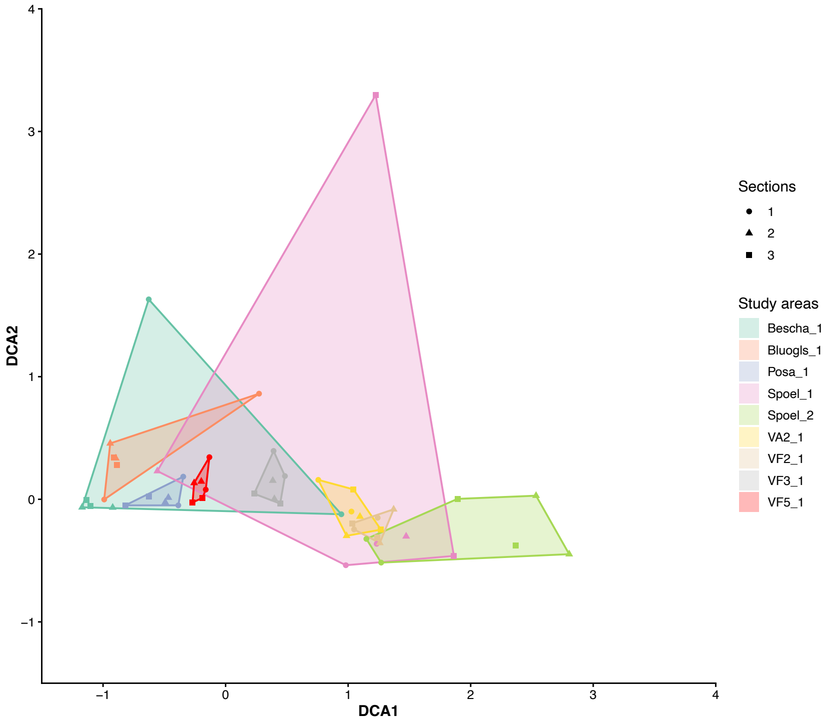


Figure 14. DCA plot of all systems for aquatic taxa. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral).

In the combined ordination, the DCA1 axis captures the main variation in species composition across the complete dataset. Samples from different running water systems (Bescha, Bluogls, Posa, VA, VF and Spoel) are primarily separated along this axis (Figure 14). In many cases, dissimilarity between systems is greater than variability within individual systems.

The DCA2 axis reflects the longitudinal gradient within systems (Leyer and Wesche, 2008). In several systems (Bescha, Posa, VF5, VF3 and VA2), samples from different sections show a clear spatial sequence along this axis. Replicates of individual samples are either located close together or show marked dispersion in ordination space, as already observed in Sections 3.1.1-3.1.9. The extent of most systems along the ordination axes remains below four standard deviations.

The species composition underlying these patterns is reflected in the taxonomic dataset. Taxa belonging to the families Chironomidae, Baetidae, Leuctridae, Nemouridae and Limnephilidae occur frequently in the systems Bluogls, VF3, Posa, VF5 and Bescha, particularly in the hypocrenal and epirhithral sections. Similar but less pronounced patterns are observed in VA2 and VF2, where these taxa are mainly detected in the eucrenal and epirhithral. Due to low species detection in Spoel 1 and Spoel 2, comparable patterns could only be assed to a limited extent for these (Appendix Table 2).

3.2.2 Cross system DCA ordination of EPT taxa

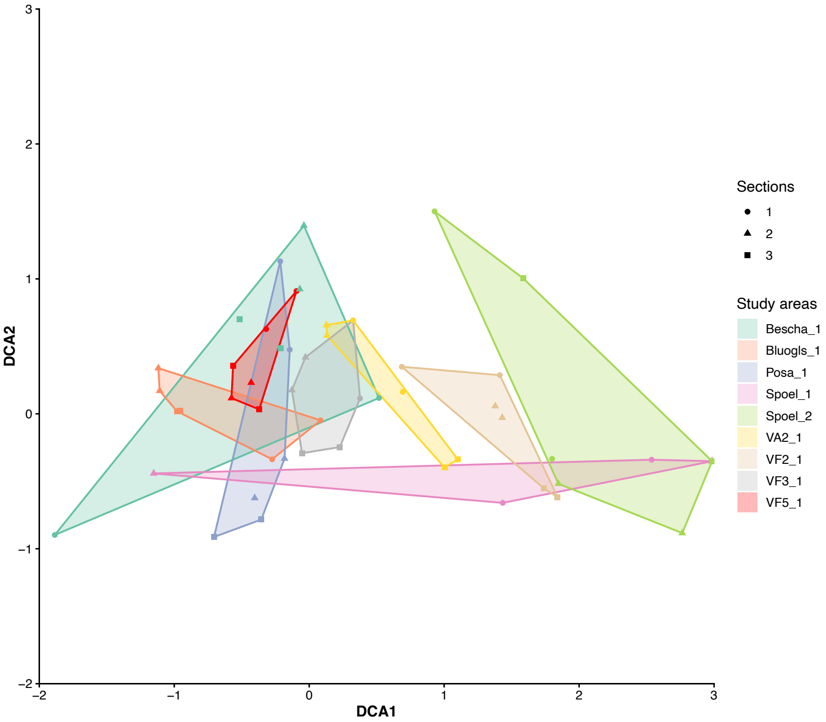


Figure 15. DCA plot of all systems for EPT taxa. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral)

The sites Posa, VF5, VF2, VF3 and VA2 form distinct clusters along the DCA1 axis. Replicate dispersion shows a similar pattern to that observed in Figure 14. In the systems Posa, VF5, VF2, VF3 and VA2, a directed sequence of the sampled sections (eucrenal, hypocrenal and epirhithral) is evident along the first ordination axis (Figure 15).

Species of the families Leuctridae and Nemouridae occur across the systems Posa, VF5, VF2, VF3 and VA2, with *Leuctra alpina*, *Nemoura obtusa* and *Nemoura sinuata* detected in most sections. In the systems Bescha and Bluogls, the detected species were mainly recorded in the hypocrenal and epirhithral sections. Due to low detection rates in Spoel 1 and Spoel 2, no consistent ordination patterns could be described for these systems (Appendix Table 2).

3.2.3 Cross system DCA ordination of crenobionts

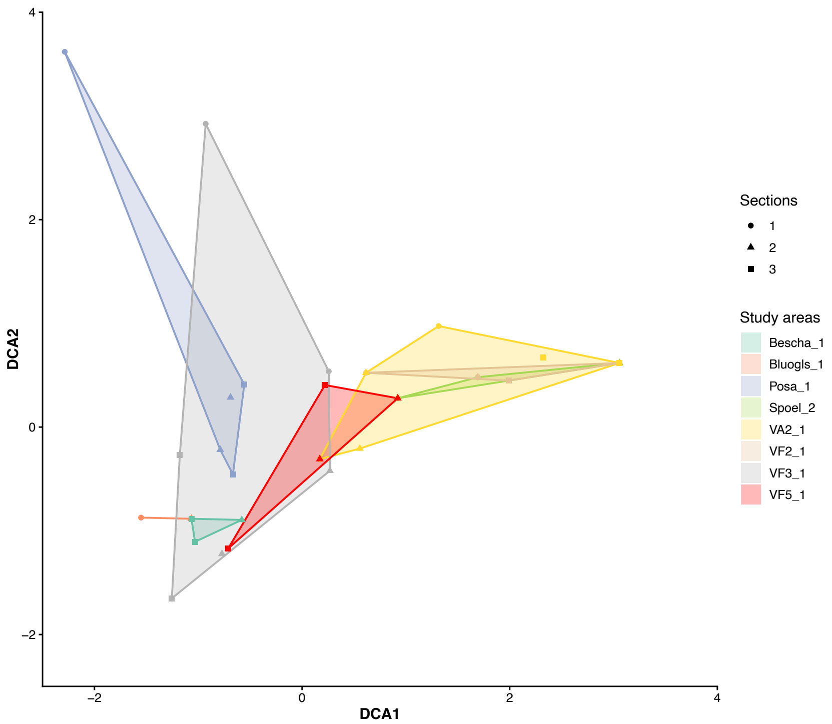


Figure 16. DCA plot of all systems for crenobionts. Sample points represent the three investigated stream sections (eucrenal, hypocrenal and epirhithral)

In this dataset, samples from different running water systems are separated along the DCA1 axis. The systems Posa and VF3 are clearly separated from Bescha and Bluogls in ordination space. Along the DCA2 axis, crenobiont samples show stronger clustering, with samples from the individual sections of Bescha and Bluogls located close to each other (Figure 16). In both systems, the species *Protonemura lateralis* was detected. This species was also recorded mainly in the hypocrenal and epirhithral sections of Posa, VF3, VF5 and VA2.

Spoel 2 and VF2 share the detection of *Partnunia cf. steinmanni* B LB 2019 and *Sperchon mutilus* in the eucrenal. The species *Bandakia cf. concreta* A LB 2019, *Lebertia cuneifera* and *Lebertia sefvei* were each detected in two systems only, namely in (VF3, VA2), (VF3, VF5) and (VF3, Posa), predominantly in the hypocrenal and epirhithral sections. The species *Lebertia lativentris* and *Hygrobates cf. norvegicus* were detected only once, in VF5 and Posa, respectively (Blattner et al., 2021; Gerecke et al., 2010). Overall, most crenobiont detections occurred in the hypocrenal and epirhithral sections, whereas in Spoel 2 and VF2 several species were also detected in the eucrenal (Appendix Table 2).

4. Discussion

4.1 Methodological discussion

The application of eDNA metabarcoding to define the longitudinal zonation of alpine spring systems proved to be a sensitive methodological approach that goes beyond the possibilities of conventional morphological identification. Nevertheless, the interpretation of eDNA signals requires critical evaluation, as they are influenced by complex methodological, hydrological and biotic factors (Curtis et al., 2021; Mächler et al., 2021).

Field sampling was conducted in early June 2025, when snowmelt strongly increases discharge in alpine springs (Cantonati et al., 2022; Curtis et al., 2021). Increased discharge leads to higher flow and dilution of eDNA concentrations, which can increase the probability of false negative results where taxa are present but not detected (Curtis et al., 2021). In addition, higher flow can enhance wash-in effects, introducing terrestrial DNA into the system and increasing apparent biological heterogeneity (Curtis et al., 2021; Mächler et al., 2021). Mächler et al. (2021) therefore recommend habitat specific sampling during hydrologically stable periods such as late summer to minimise dilution effects (Curtis et al., 2021).

In agreement with the first and third hypotheses, the results of the DCA analyses of the individual systems (Posa, VF5, and VA2) reveal a sequential separation of spring and downstream sections along the first ordination axis (Figure 9, 10 and 12). These patterns generally correspond to the conventional longitudinal zonation of running water systems. At the same time, analyses across all systems indicate that the spatial resolution of sampling may influence the interpretability of this zonation. From a methodological perspective, the selected distances between sections (Table 1) might therefore affect the sharpness of zonal boundaries and the delineation of specific species assemblages. Von Fumetti et al. (2007) describe that the faunistic boundary between eucrenal and hypocrenal is often reached already at a distance of about 5 m from the spring outlet or at the point where water temperature deviates by more than 1 °C from that of the spring outlet. In this study, sections were defined by fixed distances to ensure comparability. However, this approach may underestimate ecological separation between sections under local thermal deviations, as observed in VF3 (Von Fumetti et al., 2007). In the DCA of VF3, a strong overlap of the two downstream sections is evident, which may be attributed to both thermal and biological gradients. Continuous measurement of abiotic parameters such as temperature and electrical conductivity would have allowed more precise linkage and delineation of zones (Cantonati et al., 2022; Küry et al., 2017). In agreement with Küry et al. (2017), it can be stated that zonal classification without measurement of temperature amplitude introduces some methodological uncertainty. This is particularly relevant given the strong temperature gradient of approximately 0.37 °C per 100 m elevation in alpine spring systems, which strongly influences species composition (Küry et al., 2017).

Confirmation of the second hypothesis is a central result, showing that crenobionts such as *Bandakia cf. concreta* A LB 2019 and *Lithax niger* were detected in the epirhithral. Methodologically, it is important to distinguish between true occurrence of these taxa and downstream transport of eDNA. In running waters, eDNA behaves like particulate material that is subject to retention and resuspension, so detection in the epirhithral does not necessarily indicate a resident population (Shogren et al., 2017). The presence absence approach used here should therefore be critically evaluated against analyses based on ESV-read abundances. In this study, read abundances were transformed into binary data (0/1) to increase robustness to stochastic effects and to match the hypotheses. However, studies on fish eDNA suggest that eDNA concentration can be used as a proxy for organism density (Mächler et al., 2021; Shogren et al., 2017; Takahara et al., 2012). True presence of crenobionts in the epirhithral could thus be better supported if ESV-read abundances were significantly higher in the eucrenal than in the epirhithral (Shogren et al., 2017). In addition more frequent sampling at shorter intervals would reduce the influence of local retention in the substrate and allow a more precise delineation of faunal transitions (Shogren et al., 2017; Von Fumetti et al., 2007).

Handling of singletons sequences occurring only once in the entire dataset represents a critical step in data processing. In this study, replicates were not pooled and singletons were retained because of low detection rates in some systems. Singletons are usually removed to reduce technical artefacts arising from PCR or sequencing errors (Laroche et al., 2017). However, this filtering risks removing rare taxa, particularly highly specialised crenobionts of alpine spring systems, thereby underrepresenting key components of eucrenal assemblages (Laroche et al., 2017). At the same time, retaining singleton sequences may result in the inclusion of isolated detections of species whose occurrence does not correspond to their documented distribution in the investigated area based on records from Info Fauna, as observed for *Ecdyonurus insignis*, *Leuctra fusca* and *Leuctra geniculate* (“Info fauna Carto,” 2026). In the present datasets, singletons, together with replicate effects, contributed to outliers in the DCA analyses in systems such as Bescha and Bluogls, which could have been reduced by filtering.

4.2 Results discussion

The investigated alpine spring systems exhibited exceptionally high biological diversity. The 228 aquatic species retained from an initial pool of 1123 detected taxa illustrate the ecological richness of these isolated island like habitats (Cantonati et al., 2006a).

Patterns of EPT taxa largely followed classical spring and headwater zonation. Their presence and absence provide a strong biological signal of longitudinal structuring driven by gradients in thermal stability and flow regime (Barquín and Death, 2011; Brantschen et al., 2022; Von Fumetti et al., 2007). The first hypothesis is supported by the almost complete absence of the order Ephemeroptera in the eucrenal, as this group avoids the eucrenal or occurs there only at low density. A sequential separation of spring and stream sections along the first ordination axis is evident in the systems Posa, VF5, VF3 and VF2. In the hypocrenal and epirhithral sections, the occurrence described in the literature is confirmed (Barquín and Death, 2011; Cantonati et al., 2006). *Baetis alpinus* is the only species that was also detected in the eucrenal sections of the systems Spoel 2, VF3, Posa, VF5, VF2 and VA2. The eucrenal occurrence of *Baetis alpinus* can be explained by its developmental optimum at low temperatures, which prevail in the eucrenal of alpine springs (Gerecke et al., 2005; Küry et al., 2017). In contrast, species of the orders Plecoptera and Trichoptera show a stronger association with the crenal, which is evident across all systems particularly for *Leuctra alpina*, *Nemoura sinuata, Nemurella pictetii* and *Wormaldia occipitalis*. In the systems Posa, VF5 and VF3, most species of the family Nemouridae were detected in all three sections. This can be explained by the broader ecological valence of some species, the higher elevational gradient compared to other systems and potentially increased flow conditions (Gerecke et al., 2005; Von Fumetti et al., 2007; Wigger et al., 2015).

Across all investigated systems, a cumulative increase in the number of detected taxa with increasing distance from the eucrenal was observed, except in the two systems Spoel 1 and Spoel 2. In total, 265 species were detected in the eucrenal, increasing to 540 species in the hypocrenal and reaching a maximum of 677 species in the epirhithral. This cumulative increase in diversity can be explained by increasing habitat complexity, greater temperature amplitudes and the admixture of rhithrobiont species. (Barquín and Death, 2011; Küry et al., 2017; Staudacher and Füreder, 2007). The low detection numbers in Spoel 1 and Spoel 2 can only be partially explained. In both systems, very low discharge was observed in the eucrenal, which increased in the downstream sections but remained lower than in other systems. This may have led to dilution of eDNA concentrations in addition to potentially low population sizes, thereby increasing the probability of false negative detections (Curtis et al., 2021). Wigger et al. (2015) showed that in alpine springs, species richness decreases significantly with elevation, which is consistent with the trend observed in the highest system Bescha (2225 m a.s.l.).

Diptera, and especially the family Chironomidae with 96 identified species, represent the most species rich and widespread group. Their dominance can be attributed to their extreme ecological adaptability and use of small scale habitat structures (Cantonati et al., 2006a). The species *Parametriocnemus stylatus*, *Tvetenia bavarica* and *Corynoneura lobata* were consistently detected in all systems. In Bluogls, Posa, VF5 and Bescha, most dipteran species were found mainly in the hypocrenal and epirhithral, whereas in Spoel 1 and Spoel 2 they were detected mainly in the eucrenal.

A potential accumulation of eDNA in the epirhithral may cause downstream integration of genetic signals from upstream communities. In particular chironomid DNA, which is likely to be most abundant in the eucrenal of Spoel 1 and Spoel 2, may become incorporated into more complex downstream communities (Barquín and Death, 2011; Cantonati et al., 2006a; Mächler et al., 2021).

Diptera also represent important hosts for parasitic Hydrachnidia larvae (Cantonati et al., 2022, 2006a). Water mites showed the second highest diversity in the dataset, which may be related to the high availability of Diptera as transport vectors or food sources (Cantonati et al., 2022). Water mites are consistent indicators of hydrological stability because taxa such as *Partnunia* or *Sperchon* are considered crenobiont and do not tolerate desiccation (Cantonati et al., 2022; Di Sabatino et al., 2000).

Dominant alpine species such as *Sperchon mutilus*, *Sperchon squamosus*, *Sperchon thienemanni* and *Sperchon violaceus* were detected in all systems except Spoel 1 and Bescha. These taxa are described as cold stenothermic alpine species with a strong preference for spring habitats, which was confirmed in Spoel 2, VF3, VF5 and VA2 (Di Sabatino et al., 2000). This pattern supports the findings of Wigger et al. (2015) that higher elevation systems host temperature adapted specialists, while overall taxon richness declines with altitude. In systems such as Bescha diversity is therefore dominated by specialised taxa such as *Lebertia schechteli* (Wigger et al., 2015).

The hypothesis based on Illies (1961) that crenobionts occur primarily in the eucrenal and in much lower numbers in the hypocrenal could only be partially confirmed, illustrating the complexity of eDNA signals in running waters. Only in Spoel 2 was this pattern fully supported, as *Partnunia cf. steinmanni* B LB 2019 and *Sperchon mutilus* were detected exclusively in the eucrenal. *Partnunia cf. steinmanni* was detected in the eucrenal of VF3 and VF2 and *Partnunia cf. steinmanni* A LB 2019 in Posa.

The second hypothesis was formulated to test whether crenobionts can also be detected in the epirhithral using eDNA, and this was supported by the detection of *Protonemura lateralis*, *Dictyogenus fontium* and *Sperchon mutilus*. However, this result must be interpreted cautiously, because distances between sections differ among systems and the effects of eDNA retention and resuspension cannot be resolved (Shogren et al., 2017). Nevertheless, the high sensitivity of eDNA compared to conventional kick net sampling represents a major advantage for detecting rare and taxonomically challenging species (Brantschen et al., 2022; Leese et al., 2021)

5. Conclusion

This study demonstrates that eDNA metabarcoding is an effective tool for resolving the biological complexity of alpine spring ecosystems and, in some systems, their longitudinal zonation. The results indicate that biological gradients along alpine stream systems can be reliably captured using eDNA based approaches. In several systems (VA2, VF5 and VF3), a clear sequential differentiation between the eucrenal, hypocrenal and epirhithral was detected, closely corresponding to the classical concept of longitudinal zonation in running waters (Illies, 1961; Von Fumetti et al., 2007).

The restriction of crenobionts to the eucrenal was only partially supported. Although some crenobiontic taxa were confined to crenal sections, most crenobionts were also detected in the hypocrenal and epirhithral. This pattern can be attributed to multiple factors and resulted in partial confirmation of the second hypothesis. Future research should therefore focus on more comprehensive verification of crenobiontic taxa to improve ecological interpretation.

A key strength of eDNA metabarcoding lies in its high sensitivity for detecting small and taxonomically challenging organisms, as illustrated by the mites and chironomid dipterans identified in this study (Di Sabatino et al., 2000; Leese et al., 2021). Moreover, the non-invasive nature of the method provides a substantial advantage for long term monitoring in protected areas (Blattner et al., 2021; Cantonati et al., 2022)

Despite these advantages, several methodological limitations were identified. Future studies could address these by adjusting the sampling period to later in the season in order to reduce snowmelt induced dilution effects, combined with direct measurements of local discharge (Curtis et al., 2021; Mächler et al., 2021). Sampling sections should be defined in closer accordance with local environmental conditions and sampled at finer spatial resolution. Continuous monitoring of abiotic parameters, in addition to discharge, would further improve the delineation of zonal boundaries The application of more taxon specific primer sets, such as fwhF2/EPTDr2n (Leese et al., 2021), may further enhance the detection of zonation patterns in EPT taxa while reducing bioinformatic noise. Finally, the inclusion or exclusion of singleton sequences should be carefully tailored to the specific research question to ensure robust interpretation (Laroche et al., 2017).

In the long term, eDNA metabarcoding holds strong potential for establishing alpine spring ecosystems as sensitive indicators of climate driven change. Given their high sensitivity to thermal and hydrological shifts, standardized long term genetic monitoring could provide early warning signals of changes in spring specific biodiversity (Cantonati et al., 2022; Küry et al., 2017).

References

Andrews, S., 2010. A Quality Control Tool for High Throughput Data.

BAFU, 2017. BLN 1915 Schweizerischer Nationalpark und angrenzende Gebiete. BAFU.

Bahlburg, H., Breitkreuz, C., 2017. Grundlagen der Geologie. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-54931-5

Barquín, J., Death, R.G., 2011. Downstream changes in spring-fed stream invertebrate communities: the effect of increased temperature range? J Limnol 70, 134. https://doi.org/10.4081/jlimnol.2011.s1.134

Beng, K.C., Corlett, R.T., 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. Biodivers Conserv 29, 2089–2121. https://doi.org/10.1007/s10531-020-01980-0

Blattner, L., Ebner, J.N., Zopfi, J., Von Fumetti, S., 2021. Targeted non-invasive bioindicator species detection in eDNA water samples to assess and monitor the integrity of vulnerable alpine freshwater environments Ecological Indicators.

Blattner, L., Lapellegerie, P., Courtney-Mustaphi, C., Heiri, O., 2024. Sediment Core DNA-Metabarcoding and Chitinous Remain Identification: Integrating Complementary Methods to Characterise Chironomidae Biodiversity in Lake Sediment Archives, Molecular Ecology Resources. 1Department of Environmental Sciences, Geoecology, University of Basel, Basel.

Blattner, L., Lucek, K., Beck, N., Berner, D., Von Fumetti, S., 2022. Intra-Alpine Islands: Population genomic inference reveals high degree of isolation between freshwater spring habitats. Diversity and Distributions 28, 291–305. https://doi.org/10.1111/ddi.13461

Bossley, J.P., Smiley, P.C., 2019. Impact of student-induced disturbance on stream macroinvertebrates differs among habitat types. Sci Rep 9, 1447. https://doi.org/10.1038/s41598-018-38210-1

Brantschen, J., Pellissier, L., Walser, J., Altermatt, F., 2022. Evaluation of primer pairs for eDNA-based assessment of Ephemeroptera, Plecoptera, and Trichoptera across a biogeographically diverse region. Environmental DNA 4, 1356–1368. https://doi.org/10.1002/edn3.342

Cantonati, M., Bonettini, A.M., 1996. Macroinvertebrate assemblages of springs of the River Sarca catchment (Adamello-Brenta Natural Park, Trentino, Italy).

Cantonati, M., Gerecke, R., Bertuzzi, E., 2006a. Springs of the Alps – Sensitive Ecosystems to Environmental Change: From Biodiversity Assessments to Long-term Studies. Hydrobiologia 562, 59–96. https://doi.org/10.1007/s10750-005-1806-9

Cantonati, M., Gerecke, R., Bertuzzi, E., 2006b. Springs of the Alps – Sensitive Ecosystems to Environmental Change: From Biodiversity Assessments to Long-term Studies. Hydrobiologia 562, 59–96. https://doi.org/10.1007/s10750-005-1806-9

Cantonati, M., Lichtenwöhrer, K., Leonhardt, G., Seifert, L., Mustoni, A., Hotzy, R., Schubert, E., Blattner, L., Bilous, O., Lotz, A., Poschlod, B., Gerecke, R., 2022. Using Springs as Sentinels of Climate Change in Nature Parks North and South of the Alps: A Critical Evaluation of Methodological Aspects and Recommendations for Long-Term Monitoring. Water 14, 2843. https://doi.org/10.3390/w14182843

Cantonati, M., Segadelli, S., Spitale, D., Gabrieli, J., Gerecke, R., Angeli, N., De Nardo, M.T., Ogata, K., Wehr, J.D., 2020. Geological and hydrochemical prerequisites of unexpectedly high biodiversity in spring ecosystems at the landscape level. Science of The Total Environment 740, 140157. https://doi.org/10.1016/j.scitotenv.2020.140157

Curtis, A.N., Tiemann, J.S., Douglass, S.A., Davis, M.A., Larson, E.R., 2021. High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. Diversity and Distributions 27, 1918–1931. https://doi.org/10.1111/ddi.13196

Das UNESCO-Biosphärenreservat Engiadina Val Müstair [WWW Document], 2026. URL https://www.gr.ch/DE/institutionen/verwaltung/ekud/anu/themen/paerke-unesco/biosphaerenreservat/Seiten/info.aspx (accessed 2.9.26).

Di Sabatino, A., Gerecke, R., Martin, P., 2000. The biology and ecology of lotic water mites (Hydrachnidia). Freshwater Biology 44, 47–62. https://doi.org/10.1046/j.1365-2427.2000.00591.x

Elbrecht, V., Vamos, E.E., Meissner, K., Aroviita, J., Leese, F., 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods Ecol Evol 8. https://doi.org/10.1111/2041-210X.12789

Erman, A., Erman, D.C., 1995. Spring Permanence, Trichoptera Species Richness, and the Role of Drought. J. Kansas Entomol. Soc. 50–64.

Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32, 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Gerecke, R., Cantonati, M., Spitale, D., Stur, E., Wiedenbrug, S., 2011. The challenges of long-term ecological research in springs in the northern and southern Alps: indicator groups, habitat diversity, and medium-term change. J Limnol 70, 168. https://doi.org/10.4081/jlimnol.2011.s1.168

Gerecke, R., Di Sabatino, A., Gledhill, T., Smit, H., 2010. Süßwasserfauna von Mitteleuropa, Bd. 7/2-2 Chelicerata, Acari 2. Spektrum Akademischer Verlag Heidelberg, Heidelberg.

Gerecke, R., Martin, P., Gledhill, T., 2018. Water mites (Acari: Parasitengona: Hydrachnidia) as inhabitants of groundwater-influenced habitats - considerations following an update of Limnofauna Europaea. Limnologica 69, 81–93. https://doi.org/10.1016/j.limno.2017.11.008

Gerecke, R., Meisch, C., Stoch, F., Acri, F., Franz, H., 1997. Eucrenon-hypocrenon ecotone and spring typology in the Alps of Berchtesgaden (Upper Bavaria, Germany).

Gerecke, R., Meisch, C., Stoch, F., Schrankel, I., 2005. Die Fauna der Quellen und des hyporheischen Interstitials in Luxemburg (No. 41), Ferrantia. Travaux scientifiques du Musée national d'histoire naturelle Luxembourg, Luxemburg.

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E., Taberlet, P., 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol 7, 1299–1307. https://doi.org/10.1111/2041-210X.12595

Haller, H., Eisenhut, A., Haller, R. (eds), 2013. Atlas des Schweizerischen Nationalparks – die ersten 100 Jahre Zur ersten Orientierung. Topografie des Schweizerischen Nationalparks. Haupt Verlag, Bern.

Harvey, C.J., Fisher, J.L., Samhouri, J.F., Williams, G.D., Francis, T.B., Jacobson, K.C., deReynier, Y.L., Hunsicker, M.E., Garfield, N., 2020. The importance of long-term ecological time series for integrated ecosystem assessment and ecosystem-based management. Progress in Oceanography 188, 102418. https://doi.org/10.1016/j.pocean.2020.102418

Hering, D., Meier, C., Rawer-Jost, C., Feld, C.K., Biss, R., Zenker, A., Sundermann, A., Lohse, S., Böhmer, J., 2004. Assessing streams in Germany with benthic invertebrates: selection of candidate metrics. Limnologica 34, 398–415. https://doi.org/10.1016/S0075-9511(04)80009-4

Hernandez, C., Bougas, B., Perreault-Payette, A., Simard, A., Côté, G., Bernatchez, L., 2020. 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in Eastern Canada. Environmental DNA 2, 373–386. https://doi.org/10.1002/edn3.89

Illies, J., 1961. Versuch einer allgemeinen biozönotischen Gliederung der Fließgewässer. Intl Review of Hydrobiology 46, 205–213. https://doi.org/10.1002/iroh.19610460205

Illies, J., Botosaneanu, L., 1963. Problèmes et méthodes de la classification et de la zonation écologique des eaux courantes, considerées surtout du point de vue faunistique. Für Theoretische Und Angewandte Limnologie: Mitteilungen, 12(1), 1–57.

Info fauna Carto [WWW Document], 2026. . Info fauna Nationales Daten- und Informationszentrum der Schweizer Fauna. URL https://lepus.infofauna.ch/carto/ (accessed 1.29.26).

Kuefner, W., Ossyssek, S., Geist, J., Raeder, U., 2020. The silicification value: a novel diatom-based indicator to assess climate change in freshwater habitats. Diatom Research 35, 1–16. https://doi.org/10.1080/0269249X.2020.1722246

Küry, D., Lubini, V., Stucki, P., 2017. Temperature patterns and factors governing thermal response in high elevation springs of the Swiss Central Alps. Hydrobiologia 793, 185–197. https://doi.org/10.1007/s10750-016-2918-0

Laroche, O., Wood, S.A., Tremblay, L.A., Lear, G., Ellis, J.I., Pochon, X., 2017. Metabarcoding monitoring analysis: the pros and cons of using co-extracted environmental DNA and RNA data to assess offshore oil production impacts on benthic communities. PeerJ 5, e3347. https://doi.org/10.7717/peerj.3347

Leese, F., Sander, M., Buchner, D., Elbrecht, V., Haase, P., Zizka, V.M.A., 2021. Improved freshwater macroinvertebrate detection from environmental DNA through minimized nontarget amplification. Environmental DNA 3, 261–276. https://doi.org/10.1002/edn3.177

Leyer, I., Wesche, K., 2008. Multivariate Statistik in der Ökologie: eine Einführung, Korrigierter Nachdruck. ed, Springer-Lehrbuch. Springer, Berlin Heidelberg.

Liu, M., Clarke, L.J., Baker, S.C., Jordan, G.J., Burridge, C.P., 2020. A practical guide to DNA metabarcoding for entomological ecologists. Ecological Entomology 45, 373–385. https://doi.org/10.1111/een.12831

Lubini, V., Knispel, S., Cordillot, F., Nöthiger-Koch, U., Brachypteratrifasciata, S., 2010. Rote Listen Eintagsfliegen, Steinfliegen, Köcherfliegen.

Lubini, V., Stucki, P., Vicentini, H., Küry, D., 2016. Bewertung von Quell-Lebensräumen in der Schweiz. Im Auftrag des Bundesamtes für Umwelt BAFU.

Mächler, E., Salyani, A., Walser, J.-C., Larsen, A., Schaefli, B., Altermatt, F., Ceperley, N., 2021. Environmental DNA simultaneously informs hydrological and biodiversity characterization of an Alpine catchment. Hydrol. Earth Syst. Sci. 25, 735–753. https://doi.org/10.5194/hess-25-735-2021

Mahé, F., Czech, L., Stamatakis, A., Quince, C., De Vargas, C., Dunthorn, M., Rognes, T., 2021. Swarm v3: towards tera-scale amplicon clustering. Bioinformatics 38, 267–269. https://doi.org/10.1093/bioinformatics/btab493

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Technical Notes 1: 10-12.

Moog, O. (Ed.), Sporka, F., Hödl, W., Eder, E., Gaviria, S., Herzig, A., Forró, L., Bauernfeind, E., Weichselbaumer, P., 2002. FAUNA AQUATICA AUSTRIACA, Teil III, Katalog zur autökologischen Einstufung aquatischer Organismen Österreichs. Wasserwirtschaftskataster, Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Wien.

Morinière, J., Hendrich, L., Balke, M., Beermann, A.J., König, T., Hess, M., Koch, S., Müller, R., Leese, F., Hebert, P.D.N., Hausmann, A., Schubart, C.D., Haszprunar, G., 2017. A DNA barcode library for Germany′s mayflies, stoneflies and caddisflies (Ephemeroptera, Plecoptera and Trichoptera). Molecular Ecology Resources 17, 1293–1307. https://doi.org/10.1111/1755-0998.12683

Myers, M.J., Sperling, F.A.H., Resh, V.H., n.d. Dispersal of Two Species of Trichoptera from Desert Springs: Conservation Implications for Isolated vs Connected Populations.

Pawlowski, J., Apothéloz-Perret-Gentil, L., Mächler, E., Altermatt, F., 2020. Anwendung von eDNA-Methoden in biologischen Untersuchungen und bei der biologischen Bewertung von aquatischen Ökosystemen, Umwelt-Wissen UW. BAFU, Bern.

Qiagen Group, 2018. UCP Multiplex PCR Kit Handbook Ultra-Clean Production master mix for multiplex hot-start PCR and microbiome applications.

Reiss, M., Martin, P., Gerecke, R., Von Fumetti, S., 2016. Limno-ecological characteristics and distribution patterns of spring habitats and invertebrates from the Lowlands to the Alps. Environ Earth Sci 75, 1033. https://doi.org/10.1007/s12665-016-5818-8

Robinson, C.T., 2009. Long-term Biomonitoring of Alpine Waters in the Swiss National Park. ecomont 1, 23–34. https://doi.org/10.1553/eco.mont1s23

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584

Rozkosny, R., František, G., 2004. Süßwasserfauna von Mitteleuropa, Insecta: Diptera: Muscidae. Spektrum Akademischer Verlag, Heidelberg.

Schmedtje, U., Colling, M., 1996. Ökologische Typisierung der aquatischen Makrofauna, Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft.

Schuhmacher, H., Schmidt-Kloiber, A., Graf, W., 2001. freshwaterecology.info [WWW Document]. Macro-invertebrates. URL https://www.freshwaterecology.info/fwe_info.php?p=b2c9bXpiI3BhcmFtPTE%3D&utm

Schweizer Pärke [WWW Document], 2025. . Biosfera Val Müstair. URL https://www.parks.swiss/de/die-schweizer-paerke/uebersicht/biosfera-val-muestair (accessed 10.9.25).

Schwoerbel, J., Brendelberger, H., 2021. Einführung in die Limnologie, 11. Auflage. ed. Springer Spektrum.

Shogren, A.J., Tank, J.L., Andruszkiewicz, E., Olds, B., Mahon, A.R., Jerde, C.L., Bolster, D., 2017. Controls on eDNA movement in streams: Transport, Retention, and Resuspension. Sci Rep 7, 5065. https://doi.org/10.1038/s41598-017-05223-1

Spinnler, D., Feichtinger, L., Siegrist, D., Peter, F., Frohn, T., Oertli, A., Schwyzer, Y., Merz, K., 2020. Charta 2021 – 2030 Naturpark Biosfera Val Müstair Gesuch um Verleihung des Parklabels, Teil C. Managementplan für den Betrieb eines Regionalen Naturparks. Gemeindeversammlung Val Müstair, Tschierv.

Staudacher, K., Füreder, L., 2007. Habitat Complexity and Invertebrates in Selected Alpine Springs (Schütt, Carinthia, Austria). International Review of Hydrobiology 92, 465–479. https://doi.org/10.1002/iroh.200610988

Studemann, D., Landolt, P., Sartori, M., 1992. Ephemeroptera (deutsche Ausgabe). https://doi.org/10.5169/SEALS-1006760

Sweeney, B.W., Battle, J.M., Jackson, J.K., Dapkey, T., 2011. Can DNA barcodes of stream macroinvertebrates improve descriptions of community structure and water quality? Journal of the North American Benthological Society 30, 195–216. https://doi.org/10.1899/10-016.1

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., Kawabata, Z., 2012. Estimation of Fish Biomass Using Environmental DNA. PLoS ONE 7, e35868. https://doi.org/10.1371/journal.pone.0035868

Van der Kamp, G., 1995. The Hydrogeology of Springs in Relation to the Biodiversity of Spring Fauna: A Review.

Von Fumetti, S., Bieri-Wigger, F., Nagel, P., 2017. Temperature variability and its influence on macroinvertebrate assemblages of alpine springs. Ecohydrology 10, e1878. https://doi.org/10.1002/eco.1878

Von Fumetti, S., Blattner, L., 2016. Faunistic assemblages of natural springs in different areas in the Swiss National Park: a small-scale comparison, Hydrobiologia (2017). Springer International Publishing.

Von Fumetti, S., Nagel, P., Baltes, B., 2007. Where a springhead becomes a springbrook a regional zonation of springs. fal 169, 37–48. https://doi.org/10.1127/1863-9135/2007/0169-0037

Weigand, H., Beermann, A.J., Čiampor, F., Costa, F.O., Csabai, Z., Duarte, S., Geiger, M.F., Grabowski, M., Rimet, F., Rulik, B., Strand, M., Szucsich, N., Weigand, A.M., Willassen, E., Wyler, S.A., Bouchez, A., Borja, A., Čiamporová-Zaťovičová, Z., Ferreira, S., Dijkstra, K.-D.B., Eisendle, U., Freyhof, J., Gadawski, P., Graf, W., Haegerbaeumer, A., Van Der Hoorn, B.B., Japoshvili, B., Keresztes, L., Keskin, E., Leese, F., Macher, J.N., Mamos, T., Paz, G., Pešić, V., Pfannkuchen, D.M., Pfannkuchen, M.A., Price, B.W., Rinkevich, B., Teixeira, M.A.L., Várbíró, G., Ekrem, T., 2019. DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. Science of The Total Environment 678, 499–524. https://doi.org/10.1016/j.scitotenv.2019.04.247

Wigger, F.W., Schmidlin, L., Nagel, P., Von Fumetti, S., 2015. Macroinvertebrate assemblages of natural springs along an altitudinal gradient in the Bernese Alps, Switzerland. Ann. Limnol. - Int. J. Lim. 51, 237–247. https://doi.org/10.1051/limn/2015018

Zizka, V.M.A., Geiger, M.F., Leese, F., 2020. DNA metabarcoding of stream invertebrates reveals spatio-temporal variation but consistent status class assessments in a natural and urban river. Ecological Indicators 115, 106383. https://doi.org/10.1016/j.ecolind.2020.106383

Tools:

ChatGPT Version 5.2, OpenAI: openai.com

Translation and rephrasing of phrases, programming in RStudio

NotebookLM, Google: notebooklm.google

Translation and rephrasing of phrases

Appendix

Table 2. List of detected taxa

Tab. 2: List of detected taxa
Presence (+) or absence (–) of the identified taxa, including representatives of EPT-taxa (dark grey) and crenobiont species (light grey)

	Spoel_1_1_1	Spoel_1_1_2	Spoel_1_2_1	Spoel_1_2_2	Spoel_1_3_1	Spoel_1_3_2	Spoel_2_1_1	Spoel_2_1_2	Spoel_2_2_1	Spoel_2_2_2	Spoel_2_3_1	Spoel_2_3_2	Bluogls_1_1_1	Bluogls_1_1_2	Bluogls_1_2_1	Bluogls_1_2_2	Bluogls_1_3_1	Bluogls_1_3_2	VF3_1_1_1	VF3_1_1_2	VF3_1_2_1	VF3_1_2_2	VF3_1_3_1	VF3_1_3_2	Posa_1_1_1	Posa_1_1_2	Posa_1_2_1	Posa_1_2_2	Posa_1_3_1	Posa_1_3_2	VF5_1_1_1	VF5_1_1_2	VF5_1_2_1	VF5_1_2_2	VF5_1_3_1	VF5_1_3_2	VF2_1_1_1	VF2_1_1_2	VF2_1_2_1	VF2_1_2_2	VF2_1_3_1	VF2_1_3_2	VA2_1_1_1	VA2_1_1_2	VA2_1_2_1	VA2_1_2_2	VA2_1_3_1	VA2_1_3_2	Bescha_1_1_1	Bescha_1_1_2	Bescha_1_2_1	Bescha_1_2_2	Bescha_1_3_1	Bescha_1_3_2	Bescha_1_1_N	Bluogls_1_1_N	VF2_1_1_N
Arachnida																																																									
Trombidiformes																																																									
Anisitsiellidae																																																									
Bandakia cf. concreta A LB-2019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-
Aturidae																																																									
Ljania cf. bipapillata B LB-2019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Feltriidae																																																									
Feltria minuta	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Halacaridae																																																									
Soldanellonyx chappuisi	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrovolziidae																																																									
Hydrovolzia placophora	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Hydryphantidae																																															-	-					-	-	-	-	-
Panisus torrenticolus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parathyas palustris	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Partnunia angusta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Partnunia cf. steinmanni A LB-2019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Partnunia cf. steinmanni B LB-2019	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Partnunia cf. steinmanni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Protzia invalvaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wandesia cf. racovitzai A LB-2019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wandesia cf. racovitzai B LB-2019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hygrobatidae																																																									
Hygrobates cf. norvegicus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebertiidae																																																									
Lebertia cuneifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebertia glabra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebertia lativentris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebertia maculosa	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lebertia reticulata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Lebertia schechteli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Lebertia sefvei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sperchontidae																																																									
Sperchon mutilus	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Sperchon squamosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sperchon thienemanni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Sperchon violaceus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Branchiopoda																																																									
Diplostraca																																																									
Chydoridae																																																									
Alona guttata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Continuation: List of detected taxa
Presence (+) or absence (–) of the identified taxa, including representatives of EPT-taxa (dark grey) and crenobiont species (light grey)

	Spoel_1_1_1	Spoel_1_1_2	Spoel_1_2_1	Spoel_1_2_2	Spoel_1_3_1	Spoel_1_3_2	Spoel_2_1_1	Spoel_2_1_2	Spoel_2_2_1	Spoel_2_2_2	Spoel_2_3_1	Spoel_2_3_2	Bluogls_1_1_1	Bluogls_1_1_2	Bluogls_1_2_1	Bluogls_1_2_2	Bluogls_1_3_1	Bluogls_1_3_2	VF3_1_1_1	VF3_1_1_2	VF3_1_2_1	VF3_1_2_2	VF3_1_3_1	VF3_1_3_2	Posa_1_1_1	Posa_1_1_2	Posa_1_2_1	Posa_1_2_2	Posa_1_3_1	Posa_1_3_2	VF5_1_1_1	VF5_1_1_2	VF5_1_2_1	VF5_1_2_2	VF5_1_3_1	VF5_1_3_2	VF2_1_1_1	VF2_1_1_2	VF2_1_2_1	VF2_1_2_2	VF2_1_3_1	VF2_1_3_2	VA2_1_1_1	VA2_1_1_2	VA2_1_2_1	VA2_1_2_2	VA2_1_3_1	VA2_1_3_2	Bescha_1_1_1	Bescha_1_1_2	Bescha_1_2_1	Bescha_1_2_2	Bescha_1_3_1	Bescha_1_3_2	Bescha_1_1_N	Bluogls_1_1_N	VF2_1_1_N
Insecta																																																									
Coleoptera																																																									
Dytiscidae																																																									
Agabus guttatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroporus discretus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroporus marginatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroporus nevadensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Elmidae																																																									
Elmis latreillei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Helophoridae																																																							-	-	-
Helophorus glacialis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Hydraenidae																																																									
Hydraena lapidicola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limnebius truncatellus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrophilidae																																																									
Laccobius bipunctatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psephenidae																																																									
Eubria palustris	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Scirtidae																																																									
Elodes hausmanni	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Diptera																																																									
Blephariceridae																																																									
Liponeura cinerascens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ceratopogonidae																																																									
Atrichopogon paulus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceratopogon grandiforceps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicoides brunnicans	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Culicoides delta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicoides fascipennis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicoides furcillatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicoides jurensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicoides kibunensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicoides cf. grisescens	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Dasyhelea incisurata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dasyhelea lucida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dasyhelea modesta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Forcipomyia nigra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palpomyia flavipes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Palpomyia puberula	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palpomyia serripes	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Serromyia femorata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-

Continuation: List of detected taxa
Presence (+) or absence (–) of the identified taxa, including representatives of EPT-taxa (dark grey) and crenobiont species (light grey)

	Spoel_1_1_1	**Spoel_1_1_2**	**Spoel_1_2_1**	**Spoel_1_2_2**	**Spoel_1_3_1**	**Spoel_1_3_2**	**Spoel_2_1_1**	**Spoel_2_1_2**	**Spoel_2_2_1**	**Spoel_2_2_2**	**Spoel_2_3_1**	**Spoel_2_3_2**	**Bluogls_1_1_1**	**Bluogls_1_1_2**	**Bluogls_1_2_1**	**Bluogls_1_2_2**	**Bluogls_1_3_1**	**Bluogls_1_3_2**	**VF3_1_1_1**	**VF3_1_1_2**	**VF3_1_2_1**	**VF3_1_2_2**	**VF3_1_3_1**	**VF3_1_3_2**	**Posa_1_1_1**	**Posa_1_1_2**	**Posa_1_2_1**	**Posa_1_2_2**	**Posa_1_3_1**	**Posa_1_3_2**	**VF5_1_1_1**	**VF5_1_1_2**	**VF5_1_2_1**	**VF5_1_2_2**	**VF5_1_3_1**	**VF5_1_3_2**	**VF2_1_1_1**	**VF2_1_1_2**	**VF2_1_2_1**	**VF2_1_2_2**	**VF2_1_3_1**	**VF2_1_3_2**	**VA2_1_1_1**	**VA2_1_1_2**	**VA2_1_2_1**	**VA2_1_2_2**	**VA2_1_3_1**	**VA2_1_3_2**	**Bescha_1_1_1**	**Bescha_1_1_2**	**Bescha_1_2_1**	**Bescha_1_2_2**	**Bescha_1_3_1**	**Bescha_1_3_2**	**Bescha_1_1_N**	**Bluogls_1_1_N**	**VF2_1_1_N**
Insecta																																																									
Diptera																																																									
Chironomidae																																																									
Brillia bifida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-
Cardiocladius capucinus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chaetocladius acuminatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Chaetocladius laminatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chaetocladius longivirgatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Chaetocladius suecicus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corynoneura lobata	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
Diamesa bertrami	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Diamesa bohemani	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Diamesa cinerella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
Diamesa dampfi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
Diamesa hamaticornis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
Diamesa hyperborea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Diamesa incallida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Diamesa kasymovi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Diamesa latitarsis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Diamesa modesta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
Diamesa tonsa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-
Diamesa zernyi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Eukiefferiella brevicalcar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eukiefferiella claripennis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Eukiefferiella coerulescens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-
Eukiefferiella devonica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Eukiefferiella devonica gr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Eukiefferiella tirolensis	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Gymnometriocnemus brevitarsis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Gymnometriocnemus marionensis	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gymnometriocnemus subnudus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heterotrissocladius zierli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
Krenopelopia binotata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+
Krenosmittia boreoalpina	-	+	-	-	+	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	+	-	-	+	+	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
Limnophyes difficilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Limnophyes edwardsi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Limnophyes minimus	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Limnophyes natalensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Limnophyes pentaplastus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Continuation: List of detected taxa

Presence (+) or absence (–) of the identified taxa, including representatives of EPT-taxa (dark grey) and crenobiont species (light grey)

	Spoel_1_1_1	Spoel_1_1_2	Spoel_1_2_1	Spoel_1_2_2	Spoel_1_3_1	Spoel_1_3_2	Spoel_2_1_1	Spoel_2_1_2	Spoel_2_2_1	Spoel_2_2_2	Spoel_2_3_1	Spoel_2_3_2	Bluogls_1_1_1	Bluogls_1_1_2	Bluogls_1_2_1	Bluogls_1_2_2	Bluogls_1_3_1	Bluogls_1_3_2	VF3_1_1_1	VF3_1_1_2	VF3_1_2_1	VF3_1_2_2	VF3_1_3_1	VF3_1_3_2	Posa_1_1_1	Posa_1_1_2	Posa_1_2_1	Posa_1_2_2	Posa_1_3_1	Posa_1_3_2	VF5_1_1_1	VF5_1_1_2	VF5_1_2_1	VF5_1_2_2	VF5_1_3_1	VF5_1_3_2	VF2_1_1_1	VF2_1_1_2	VF2_1_2_1	VF2_1_2_2	VF2_1_3_1	VF2_1_3_2	VA2_1_1_1	VA2_1_1_2	VA2_1_2_1	VA2_1_2_2	VA2_1_3_1	VA2_1_3_2	Bescha_1_1_1	Bescha_1_1_2	Bescha_1_2_1	Bescha_1_2_2	Bescha_1_3_1	Bescha_1_3_2	Bescha_1_1_N	Bluogls_1_1_N	VF2_1_1_N
Insecta																																																									
Diptera																																																									
Thienemannia libanica	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Thienemanniella caspersi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Thienemanniella cf. clavicornis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-
Tokunagaia tonollii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-
Tvetenia bavarica	-	-	-	-	+	-	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-
Tvetenia calvescens	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culicidae																																																									
Anopheles funestus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ochlerotatus communis	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Limoniidae																																																									
Dicranomyia stigmatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eloeophila maculata	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Eloeophila mundata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eloeophila submarmorata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gonomyia simplex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molophilus flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Neolimnophila carteri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paradelphomyia fuscula	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pediciidae																																																									
Dicranota subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
Pedicia rivosa	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
Tricyphona alticola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tricyphona immaculata	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Tricyphona livida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Simuliidae																																																									
Prosimulium latimucro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Prosimulium rufipes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simulium argyreatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simulium aureum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Simulium beltukovae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
Simulium carthusiense	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Simulium cryophilum	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	+	-	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Simulium maximum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simulium monticola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simulium variegatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simulium vernum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Syrphidae																																																									
Eristalis cryptarum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eristalis tenax	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-

Continuation: List of detected taxa

Presence (+) or absence (–) of the identified taxa, including representatives of EPT-taxa (dark grey) and crenobiont species (light grey)

	Spoel_1_1_1	Spoel_1_1_2	Spoel_1_2_1	Spoel_1_2_2	Spoel_1_3_1	Spoel_1_3_2	Spoel_2_1_1	Spoel_2_1_2	Spoel_2_2_1	Spoel_2_2_2	Spoel_2_3_1	Spoel_2_3_2	Bluogls_1_1_1	Bluogls_1_1_2	Bluogls_1_2_1	Bluogls_1_2_2	Bluogls_1_3_1	Bluogls_1_3_2	VF3_1_1_1	VF3_1_1_2	VF3_1_2_1	VF3_1_2_2	VF3_1_3_1	VF3_1_3_2	Posa_1_1_1	Posa_1_1_2	Posa_1_2_1	Posa_1_2_2	Posa_1_3_1	Posa_1_3_2	VF5_1_1_1	VF5_1_1_2	VF5_1_2_1	VF5_1_2_2	VF5_1_3_1	VF5_1_3_2	VF2_1_1_1	VF2_1_1_2	VF2_1_2_1	VF2_1_2_2	VF2_1_3_1	VF2_1_3_2	VA2_1_1_1	VA2_1_1_2	VA2_1_2_1	VA2_1_2_2	VA2_1_3_1	VA2_1_3_2	Bescha_1_1_1	Bescha_1_1_2	Bescha_1_2_1	Bescha_1_2_2	Bescha_1_3_1	Bescha_1_3_2	Bescha_1_1_N	Bluogls_1_1_N	VF2_1_1_N
Insecta																																																									
Ephemeroptera																																																									
Baetidae																																																									
Baetis alpinus	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	-	+	-	-	+	+	-	-	-	-	-
Baetis rhodani	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Baetis cf. rhodani	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heptageniidae																																																									
Ecdyonurus helveticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Ecdyonurus insignis	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epeorus alpicola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemiptera																																																									
Corixidae																																																									
Sigara lateralis	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-
Plecoptera																																																									
Leuctridae																																																									
Leuctra alpina	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	+	+	-	-	-
Leuctra armata	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-
Leuctra fusca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leuctra geniculata	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leuctra inermis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leuctra cf. inermis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Leuctra rauscheri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Leuctra rosinae	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Leuctra teriolensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nemouridae																																																									
Amphinemura standfussi	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Nemoura mortoni	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-
Nemoura obtusa	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-
Nemoura sinuata	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-
Nemurella pictetii	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Protonemura brevistyla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-
Protonemura lateralis	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-
Protonemura nimborum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protonemura nitida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-
Perlodidae																																																									
Dictyogenus fontium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
Isoperla grammatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoperla rivulorum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Perlodes intricatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

Continuation: List of detected taxa
Presence (+) or absence (–) of the identified taxa, including representatives of EPT-taxa (dark grey) and crenobiont species (light grey)

	Spoel_1_1_1	Spoel_1_1_2	Spoel_1_2_1	Spoel_1_2_2	Spoel_1_3_1	Spoel_1_3_2	Spoel_2_1_1	Spoel_2_1_2	Spoel_2_2_1	Spoel_2_2_2	Spoel_2_3_1	Spoel_2_3_2	Bluogls_1_1_1	Bluogls_1_1_2	Bluogls_1_2_1	Bluogls_1_2_2	Bluogls_1_3_1	Bluogls_1_3_2	VF3_1_1_1	VF3_1_1_2	VF3_1_2_1	VF3_1_2_2	VF3_1_3_1	VF3_1_3_2	Posa_1_1_1	Posa_1_1_2	Posa_1_2_1	Posa_1_2_2	Posa_1_3_1	Posa_1_3_2	VF5_1_1_1	VF5_1_1_2	VF5_1_2_1	VF5_1_2_2	VF5_1_3_1	VF5_1_3_2	VF2_1_1_1	VF2_1_1_2	VF2_1_2_1	VF2_1_2_2	VF2_1_3_1	VF2_1_3_2	VA2_1_1_1	VA2_1_1_2	VA2_1_2_1	VA2_1_2_2	VA2_1_3_1	VA2_1_3_2	Bescha_1_1_1	Bescha_1_1_2	Bescha_1_2_1	Bescha_1_2_2	Bescha_1_3_1	Bescha_1_3_2	Bescha_1_1_N	Bluogls_1_1_N	VF2_1_1_N
Insecta																																																									
Plecoptera																																																									
Taeniopterygidae																																																									
Rhabdiopteryx neglecta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoptera																																																									
Apataniidae																																																									
Apatania helvetica	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beraeidae																																																									
Beraea pullata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brachycentridae																																																									
Micrasema morosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Goeridae																																																									
Lithax niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Hydropsychidae																																																									
Smicridea turrialbana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Limnephilidae																																																									
Allogamus uncatus	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Anisogamus difformis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
Drusus discolor	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	-	-
Drusus monticola	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-
Drusus muelleri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Halesus rubricollis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limnephilus extricatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melampophylax melampus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Metanoea rhaetica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Parachiona picicornis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Potamophylax cingulatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudopsilopteryx zimmeri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Philopotamidae																																																									
Wormaldia copiosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Wormaldia occipitalis	-	+	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polycentropodidae																																																									
Plectrocnemia brevis	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plectrocnemia conspersa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Plectrocnemia geniculata	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Rhyacophilidae																																																									
Rhyacophila bonaparti	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Rhyacophila intermedia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhyacophila stigmatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhyacophila tristis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhyacophila vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Malacostraca																																																									
Amphipoda																																																									
Gammaridae																																																									
Gammarus fossarum	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Gammarus cf. Fossarum	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Ostracoda																																																									
Podocopida																																																									
Candonidae																																																									
Candona candida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Bivalvia																																																									
Venerida																																																									
Sphaeriidae																																																									
Euglesa personata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Overview of sampling sites

Sample ID	River section	Study area	Coordinates CH1903/ LV03	Distance to spring (m)	Altitdue (m.a.s.l)	Image
Spoel_1_1_1	Section_1	Plan Periv	2‘810‘185.00, 1‘169‘246.00	0	1825.9	
						
Spoel_1_2_1	Section_2	Plan Periv	2‘810‘105.00, 1‘169‘155.00	220	1769.3	
						
Spoel_1_3_1	Section_3	Plan Periv	2‘809‘937.00, 1‘169‘064.00	440	1709.6	
						
Spoel_2_1_1	Section_1	Plan Periv	2‘810‘453.00, 1‘169‘475.00	0	1937.8	
						
Spoel_2_2_1	Section_2	Plan Periv	2‘810‘292.00, 1‘169‘427.00	210	1890.3	
						

Table 3. Continuation

Sample ID	River section	Study area	Coordinates CH1903/ LV03	Distance to spring (m)	Altitdue (m.a.s.l)	Image
Spoel_2_3_1	Section_3	Plan Periv	2‘810‘084.00, 1‘169‘287.00	440	1809.3	
						
Bluogls _1_1_1	Section_1	God da Bass	2‘816‘116.00, 1‘170‘558.00	0	1953	
						
Bluogls _1_2_1	Section_2	God da Bass	2‘815‘936.00, 1‘170‘597.00	240	1947.1	
						
Bluogls _1_3_1	Section_3	God da Bass	2‘815‘721.00, 1‘170‘612.00	490	1936.5	
						
VF3_1_1_1	Section_1	God sur il Fuorn	2‘810‘881.00, 1‘172‘515.00	0	1938.4	
						

Table 3. Continuation

Sample ID	River section	Study area	Coordinates CH1903/ LV03	Distance to spring (m)	Altitdue (m.a.s.l)	Image
VF3_1_2_1	Section_2	God sur il Fuorn	2'810'765.00, 1'172'475.00	220	1884.9	.
						
VF3_1_3_1	Section_3	God sur il Fuorn	2'810'731.00, 1‘172‘447.00	310	1870.2	
						
Posa_1_1_1	Section_1	Alp Cham-patsch	2'823‘303.00, 1‘169'785.00	0	2133.5	
						
Posa_1_2_1	Section_2	Alp Cham-patsch	2'823‘309.00, 1‘169'577.00	270	2056.1	
						
Posa_1_3_1	Section_3	Alp Cham-patsch	2'823‘244.00, 1‘169‘388.00	510	2003.3	
						

Table 3. Continuation

Sample ID	River section	Study area	Coordinates CH1903/ LV03	Distance to spring (m)	Altitdue (m.a.s.l)	Image
VF5_1_1_1	Section_1	God sur il Fuorn/ Ergèr	2‘811‘741.00, 1‘171‘941.00	0	1804.9	
						
VF5_1_2_1	Section_2	God sur il Fuorn/ Ergèr	2‘811‘710.00, 1‘171‘873.00	100	1782.3	
						
VF5_1_3_1	Section_3	God sur il Fuorn/ Ergèr	2‘811‘681.00, 1‘171‘833.00	160	1774.8	
						
VF2_1_1_1	Section_1	God sur il Fuorn	2‘810‘888.00, 1‘172‘427.00	0	1907.9	
						
VF2_1_2_1	Section_2	God sur il Fuorn	2‘810‘850.00, 1‘172‘390.00	70	1886.3	
						

Table 3. Continuation

Sample ID	River section	Study area	Coordinates CH1903/ LV03	Distance to spring (m)	Altitdue (m.a.s.l)	Image
VF2_1_3_1	Section_3	God sur il Fuorn	2‘810‘834.00, 1‘172‘368.00	120	1872.7	
						
VA2_1_1_1	Section_1	Plan da l‘Acqua Suot	2‘809‘585.00, 1‘169‘070.00	0	1713.5	
						
VA2_1_2_1	Section_2	Plan da l‘Acqua Suot	2‘809‘669.00, 1‘169‘069.00	100	1685	
						
VA2_1_3_1	Section_3	Plan da l‘Acqua Suot	2‘809‘720.00, 1‘169‘074.00	160	1658.4	
						
Bescha_1_1_1	Section_1	Val Müstair	2‘821‘575.00, 1‘165‘995.00	0	2225.8	
						

Table 3. Continuation

Sample ID	River section	Study area	Coordinates CH1903/ LV03	Distance to spring (m)	Altitdue (m.a.s.l)	Image
Bescha _1_2_1	Section_2	Val Müstair	2‘821‘649.00, 1‘166‘122.00	274	2173.4	
						
Bescha _1_3_1	Section_3	Val Müstair	2‘821‘787.00, 1‘166‘237.00	480	2136	
						

Universität Basel

Philosophisch-Naturwissenschaftliche Fakultät

Erklärung zur wissenschaftlichen Redlichkeit und Veröffentlichung der Arbeit (beinhaltet Erklärung zu Plagiat und Betrug)

Titel der Arbeit: DNA Metabarcoding of Marcoinvertebrates in Alpine fresh water Ecosystems: Implication for Stream Zonation

Name Beurteiler*in: Stefanie von Fumetti

Name Student*in: Lauro Müller

Matrikelnummer: 22-063-697

Ich bezeuge mit meiner Unterschrift, dass ich meine Arbeit selbständig ohne fremde Hilfe verfasst habe und meine Angaben über die bei der Abfassung meiner Arbeit benützten Quellen in jeder Hinsicht der Wahrheit entsprechen und vollständig sind. Alle Quellen, die wörtlich oder sinngemäss übernommen wurden, habe ich als solche gekennzeichnet.
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Eine Überprüfung der Arbeit auf Plagiate und KI-gestützte Programme – unter Einsatz entsprechender Software – darf vorgenommen werden. Ich habe zur Kenntnis genommen, dass unlauteres Verhalten zu einer Bewertung der betroffenen Arbeit mit einer Note 1 oder mit «nicht bestanden» bzw. «fail» oder zum Ausschluss vom Studium führen kann.

Ort, Datum: 04.02.2026 Student*in: Lauro Müller

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☐ Nein

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Ort, Datum: ________________ Beurteiler*in: ________________

Diese Erklärung ist in die Bachelor-, resp. Masterarbeit einzufügen.

September 2023