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# 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

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**Abstract:** Spring ecosystems are diverse transition zones between ground- and surface-water habitats. Due to their characteristics and vulnerable species assemblages, springs are considered indicator systems for monitoring environmental change. In particular, climate change is expected to alter spring-ecosystem features, such as water temperature and discharge, affecting otherwise typically stable biotic and abiotic conditions. However, reliable trend-development recognition and analysis require a uniform methodology and comparable data series over long periods of time. Spring research findings in the Berchtesgaden National Park and the Adamello-Brenta Nature Park have been consolidated to develop methodological recommendations to create lasting societal-added value. The successful transfer of the methodology to the Bavarian Forest National Park and the experienced contribution of the Bavarian Association for the Protection of Nature (Bavarian Climate Alliance) strongly improved method validations. Our resulting, newly developed recommendations for long-term spring monitoring have a focus on climate change impacts and aim at providing a decision-making basis for establishing programs in similar ecological and climatic zones. Uniform site-selection criteria and selected climate-sensitive parameters are indicated. This includes documenting the spring's environment and structure, measuring abiotic parameters, and determining selected floristic and faunistic groups. We recommend measurement and sampling-survey intervals ranging from 3(4) times yearly to every 5 years, depending on the parameter. We further suggest a database system that integrates all monitoring parameters to ensure consistent data management and storage. Analysing the data resulting from our new holistic spring monitoring methodology should provide critical knowledge about putatively changing ecosystems that can then be used as evidence of climate-change impact on spring ecosystems.

**Keywords:** springs; climate-change effects; monitoring; long-term ecological research; discharge; temperature; biodiversity; Berchtesgaden National Park; Adamello-Brenta Nature Park; Bavarian Forest National Park

## 1. Introduction

### 1.1. An Interdisciplinary View on the Spring System

A spring is defined as a "locally limited groundwater emergence site that, at least temporarily, leads to discharge" [1]. Permanent springs are the only locations in the hydrographic landscape where groundwater flows continuously without mixing with surface water. Precipitation-originated water may enter the spring area only during heavy rainfall, especially in exposed open terrain on waterlogged soil. Therefore, springs differ significantly from streams, rivers, ponds, and lakes, all characterized by input from surface water and precipitation. Additionally, they also show fundamental differences in contrast to groundwater-influenced areas that belong to larger water bodies: There, the properties of the groundwater are diluted by mixing directly at the contact zone with the water body. In the spring system, on the other hand, groundwater enters entirely different environmental conditions on the Earth's surface, experiencing a process of change in contact with the atmosphere and substrate [2].

Springs are characterized by two separate systems: the groundwater reservoir and the above-ground spring area. As part of the water cycle of a region, the groundwater reservoir is influenced by climatic conditions on the regional scale. Its characteristics determine the quantity and quality of the water flowing at the spring's mouth. The above-ground spring area stretches from the spring's mouth, i.e., the groundwater exit point, to the beginning of the spring stream. This area's properties are shaped not only by internal factors (e.g., water quality and substrate composition) but also by external factors such as the properties of the adjacent terrain, the surrounding vegetation and, above all, the climatic conditions at the point of emergence. The biodiversity at a spring is significantly influenced by discharge amount and substrata diversity [3–5]. The availability of many microhabitats in the spring area enables the establishment of a differentiated algae, moss and vascular-plant flora in the transition area between water and land, favouring diverse colonisation by invertebrates.

In addition to an overview of important physical and chemical parameters, understanding the entire spring system is necessary for climate-impact research. Information about the catchment area (elevation, natural conditions in the recharge area) and the nature of the aquifer, which transports the water from the catchment area to the outflow at the spring mouth, and the conditions near the spring is of fundamental importance; i.e., the spring habitat has to be considered with an ecohydrogeological (sensu [6]) approach.

A ten-year study in the Berchtesgaden National Park [4], which provided comprehensive biological data for the first time in the northern Alpine region, resulted in three critical insights into spring biocoenoses:

- A surprising taxonomic diversity, with the discovery of numerous animal species that are rare or new to science: Individual study sites can differ greatly regarding species diversity and fauna spectrum. Only after examining a large number of ecologically different springs can the contribution of these habitats to the biodiversity of a geographic region (landscape) be estimated (compare [7]).
- A high stability in the organismic colonisation: During annual repeat investigations in three selected spring complexes (1994 to 2005), no changes in the relative abundance patterns of the representatives of essential animal groups (snails and mussels, crustaceans, mites and caddisflies) could be determined.
- A deficit in terms of available data on settlement history: While changes in the distribution of flora or various animal groups (birds, beetles and butterflies) that have traditionally been observed intensively in large-scale landscape structures allow con-

clusions to be drawn about ecological developments over the past 200 years, there is a complete lack of corresponding information for spring biocoenoses.

Due to the interplay of hydrogeology, groundwater, soils, climatology, hydrology, water chemistry, biology and possible human impacts, spring ecosystems can be considered as complex systems. As a result of the spatial heterogeneity of the influencing factors and the different temporal scales involved, climate-change-related alterations will occur differently.

### *1.2. Spring Types*

Different spring types, e.g., [8], play an essential role in analysing the habitat's ecological and climatic changes. Depending on the nature of the substrate and the flow conditions, springs can be classified, e.g., in the following system established in limnology (Steinemann–Thienemann classification) [9,10]:

- Rheocrenes (Figure 1A): Water emerges at well-defined, often point-like spots and forms a clearly recognizable spring stream after a short distance. Compared to helocrenes (see below), they show a reduced species diversity due to a lower substratum differentiation but often show a pronounced biocenotic longitudinal gradient. In the immediate spring mouth area, populations of stygobiontic species can occur. In the following, often moss-rich actual source area, communities of crenobiontic and crenophilous species develop. Finally, in the subsequent transitional area spring/spring-fed rivulet also representatives of the typical brook fauna, e.g., filtering or larger predatory species, thrive. In springs of this type, it is expected that climate-related changes in living conditions are most likely to be reflected in modifications of the settlement structure. In addition to shifts in the boundaries in the longitudinal zonation and the immigration and emigration of stygobiontic/rhithrobiontic species and larger-scale zoogeographic migration movements could also be documented well here. A particular type of rheocrenes is characterised by the spring's mouth shifting in elevation, dependent on the level of the groundwater reservoir. Such springs are of interest from a hydrographic point of view, since tendencies in groundwater capacities can be observed here. However, the instability of the emergence point severely limits possibilities for biological observations.
- The structural diversity is particularly pronounced in helocrenes (Figure 1B). They occur in flat terrains or, with steeper gradients, as hillside spring mires and are characterised by diffuse, low discharge, with the formation of extensive shallow water areas. In springs of this type, biocoenoses of particular zoogeographical and biological interest have been discovered in recent decades, e.g., [11]. In addition to species that are strictly bound to springs (crenobionts), there are also several taxa with a preference for wet areas developing between water and land. Climate-related changes in the living conditions in such springs are difficult to document, because helocrenic springs can react to changes in temperature, discharge or chemistry by means of shifts within their complex structure.
- Limnocrenes (Figure 1C) are characterised by water damming up at the spring's mouth. The external factors affecting the groundwater discharge can be negligible if the dammed area is small. Still, they can become relevant with the increasing size of the pool (temperature stratification, formation of stagnant bank areas, etc.). Springs with relatively small pools at the spring mouth can be considered a special case of rheocrenes. As in rheocrenes, climate-related changes are likely to result in the emigration and immigration of species.


**Figure 1.** (**A**) Rheocrenic spring in the Adamello-Brenta Nature Park; (**B**) helocrenic spring in the Bavarian Forest National Park; (**C**) limnocrenic spring the Berchtesgaden National Park.

Due to its ease of application, the Steinemann–Thienemann classification is still widely used in the hydrobiological literature. Since the existing monitoring data in Berchtesgaden National Park and Adamello-Brenta Nature Park were recorded on the basis of this basic classification, we also stick to it in this context. Apart from this tripartite classification, transition types (e.g., rheohelocrenes) can be identified. A detailed classification system integrating experience from many studies in various geographical parts of Central Europe is provided by [3]. Stevens et al. [12] provide a comprehensive overview of different spring type classifications based on a historic literature review. Although the available data exclude globally representative coverage, they show examples from North America, South America, Africa, Japan, and Europe. They propose an updated conceptual classification key, which is tested for 244 springs in the climate zones of North America.

### 1.3. Weather and Climate Impacts on Spring Systems

The effects of climate change on ecosystems are manifold and have both global and regional character: The temperature on the Earth's surface increased globally by approx. +1.1 °C since 1850–1900 [13]. Twice as much warming (ca. +2 °C) is observed in the Alps [14]. This difference in temperature development can be partly explained by the fact that the land's surface is warming more (+1.6 °C) than the oceans. A further rise in air temperature comparable to the development of the past decades is to be expected. According to different scenarios of the IPCC-AR6 (SSP1-1.9 to SSP5-8.5), a global temperature increase of 1.4 °C to 4.4 °C on average is expected by 2100 compared to 1850–1900 [13].

The spatial and temporal distribution and the intensity of precipitation are also affected by climate change. Heavy rain events in Bavaria, the Alps, and large parts of Europe are projected to occur more intensively and frequently [15]. Furthermore, the flow regime of pre-Alpine rivers North of the Alps is projected to change drastically [16], mainly due to alterations in the snow's dynamics.

Compared to riverine systems, it has been assumed that springs are habitats with stable abiotic conditions and biotic colonisation that do not change much over time [17].

However, this stability is endangered by the influences of climate change: Springs are groundwater outlets and, thus, a part of a region's water cycle. The aquifer, typically largely unaffected by ephemeral, diurnal and seasonal weather events, reflects progressive environmental changes, e.g., climate change [18]. These environmental changes in the aquifer propagate to the spring habitat and cause climate-related changes in the spring system.

The underground water's residence time characterises the spring system's retention. If the water stays in the groundwater body for an extended period, a delayed impact of climatic changes on the spring habitat is expected. Correspondingly, groundwater bodies with a larger volume have higher resilience to increasing temperatures than smaller ones with the same flow velocity. Therefore, springs that drain small groundwater bodies are likely to be affected more quickly by climate change. The elevation of the recharge area is as well of great importance. With increasing elevation, snow depth and snow cover duration increase. Since the snow cover acts as a source of water for the spring catchment, the cool meltwater leads to increased discharge and cooler water temperatures. A study from Scandinavia study already shows an increase in mean spring temperatures since 2000 [19]. Based on projections of regional climate models, which predict a decrease in snow cover [20], the water temperature and the discharge regime of the springs are expected to change: springs with catchment areas characterised by snow dynamics will show earlier discharge peaks due to snowmelt, whereas the peak volume is expected to decrease.

Changes in the quantity and seasonality of precipitation also impact the spring system. In the course of the spring monitoring between 2017 and 2019 in the Bavarian Forest National Park, it was found that springs that had never previously gone dry dried in the autumn months after the low-precipitation years of 2018 and 2019. Faunistic analyses of these springs before the dry years showed the presence of communities typical of permanent springs [21].

The effects of small-scale weather extremes, e.g., [22], on springs have so far received little attention in the literature: It can be expected that the increasing frequency and intensity of extreme weather events will increase the temperature variability of helocrenes and small rheocrenes. In addition, we expect changes in the spring habitat due to surface runoff after heavy rainfall, which may carry larger amounts of fresh rainwater into the spring mouth. These influences of microclimatic conditions on the spring habitat must be understood and observed in the long term to recognize the effects of climate change and to be able to assess them differently depending on the spring's location.

In summary, it can be assumed that climate-change-related influences in the catchment area and the vicinity of the spring contribute to the following developments:

(1) Increases in water temperature and change in temperature amplitudes over the year;
(2) Changed discharge behaviour in terms of seasonality and discharge volume;
(3) Changed physical and chemical characteristics of the spring water;
(4) Shifts in the distribution limits of species with narrow temperature tolerances to higher altitudes;
(5) Spread of thermophilic species;
(6) Immigration of species that were previously unable to gain a foothold due to other ecological limitations (e.g., duration of the growing season and food preferences);
(7) Diffuse, randomly controlled immigration of species of different ecological valence due to the extended vegetation period.

The main goal of this contribution is to provide recommendations to facilitate using springs as sentinel systems of climate change, based on a report in German resulting from the project "Springs in the Bavarian National Parks as Indicators of Climate Change (SpringNPB)" [21], integrated by data, observations and expertise gained in the Adamello-Brenta Nature Park (southeastern Alps, Trentino, Italy), e.g., [11,23–25], since the 1990s. This spring monitoring method, which pays special attention to climate change effects, was developed to produce a decision-making basis for planning and establishing climate-related spring monitoring. It includes the following aspects: criteria for selecting suitable sites; structural description of the springs studied; selection of abiotic parameters, their assessment and measurement; selection of biotic parameters, their assessment and measurement; time intervals to be observed for repeated examinations; sustainable data storage in a database created for long-term source monitoring.

We suggest that spring monitoring must be designed on a national extended geographical basis: In Bavaria, spring research was first transferred from the Berchtesgaden National

Park (high-elevation mountains) to the Bavarian Forest National Park (middle-elevation mountain ranges). Additionally, the methodology is now being transferred to the Rhön Biosphere Reserve and other Bavarian middle-elevation mountain ranges (Fichtelgebirge, Spessart, and Steigerwald).

## 2. Recommendations for the Long-Term Monitoring of Spring Habitats Targeting Climate-Change Effects

Three aspects are essential for climate-related spring monitoring:

- Site selection;
- Mapping of the spring's structure and the surrounding environment;
- Recording of abiotic and biotic parameters.

These are explained in detail in the following, with an explanatory introductory text for each parameter.

Since the methods described below primarily require in situ measurements, care must be taken to comply with the locally applicable hygiene protocols (see [26] for an example).

### *2.1. Selection of the Springs to Be Studied*

The following criteria are to be used for the selection of reference sites:

- Hydrogeology: All critical hydrogeological units in the study area need to be considered. The porosity, location and dimension of the aquifer strongly influence the abiotic parameters of the spring water. Springs from deep aquifers show more stable thermal conditions than those from near-surface aquifers [27].
- Geography: The essential characteristic landscape units are to be covered. In addition to the (hydro-)geological structuring, the diversity of surface forms and the substrate and vegetation conditions characterize the spring types. Due to their structure, specific landscape units must be disregarded if only spring types unsuitable for standardized monitoring (large limno- or helocrenes and linear rheocrenes) are available.
- Elevation gradient: For a landscape unit, the selected locations must represent the entire vertical gradient over which springs are distributed in the area. The temperature gradient associated with the elevation gradient is crucial in climate observation.
- Structural types: Flowing springs are best suited for standardized monitoring: Here, a low influence of external factors enables the most vital imprint by the aquifer. In addition, the reference points should be as diverse as possible (coarse substrate-high discharge and fine substrate-low discharge). In geographic areas without rheocrenes, helocrenes and limnocrenes can also be included in the monitoring by considering the following criteria.
- Discharge: Springs with sporadically or seasonally interrupted discharge are unsuitable for long-term monitoring. Perennial discharge must be checked before selecting reference points (inspection after long dry phases or after long periods of frost when precipitation only occurs as ice or snow). Particularly in karst areas, the examination must be carried out several times, at least once several months after a drought, since discharge can be highly dynamic (see Figure 2) and can also be subject to latency with respect to meteorological conditions. As a rule, in low-altitude mountain ranges, early autumn and, in high-elevation mountains, winter are most suitable for verifying discharge stability. In questionable cases, a biological examination of a substrate sample is helpful: The presence of surface water microcrustaceans or species-rich colonization by mites or caddisflies indicate long-term bed stability; a poor fauna characterizes unstable sources, occasionally with the dominance of subterranean amphipods and turbellaria species, which retreat to groundwater in dry seasons. Observations show that, in contrast to running waters in Europe, no animal species are specially adapted to temporarily discharging springs. Resettlement occurs primarily through species that are not typical of the source (depending on the typology: river dwellers, species of temporary small bodies of water and groundwater species).

**Figure 2.** Discharge dynamics at the spring Schwarzbachloch in the Berchtesgaden National Park; the catchment area shows a high degree of karstification.

- Temperature: Springs with stable temperatures over the year are particularly suitable for monitoring long-term changes in the groundwater system. However, locations with substantial daily and seasonal temperature variability also provide useful data, such as changes in amplitudes or shifts in maxima and minima.
- Ecological integrity: Only near-natural (natural) springs are suitable for long-term, monitoring including biological aspects.
- Limestone-Precipitating Springs (LPS): Due to their peculiar chemistry, "petrifying springs" (flora-fauna-habitat 7220) have relatively poor fauna [28]. Only a few animal species colonize the extreme habitat with lime coatings (Ger. "*Sinter*"). However, they deserve attention because climate change can affect carbonate precipitation. If LPS are characteristic of a landscape, at least one habitat of this type should be included in the long-term monitoring.
- Logistics: For the practicability of a monitoring program, the accessibility of the locations is one of the selection criteria. To ensure regular data collection, the springs should be easily accessible, at least when there is no snow.
- Data situation: If the suitability is otherwise equal, preference should be given to locations for which comparable results from earlier investigations are available. Such data from before the start of the monitoring extend the time series and increase the value of the study. If several similar sources are available, the site with the highest number of reference species should be preferred, or the availability of data from the catchment area (e.g., groundwater measuring points) should be decisive.
- Comparability: Extreme habitats should not be selected as reference points, even if their characteristics possibly represent the particular trait of an area (e.g., rock-face springs, LPS, springs with a unique chemistry and thermal springs).

### 2.2. Overview on Monitoring Parameters

The following parameters and associated monitoring intervals (Table 1) form the core of the method for climate-related spring monitoring recommended here.

**Table 1.** Overview on monitoring parameters.

| Parameter | Measurement Method | Monitoring Interval |
|---|---|---|
| Environment and structure mapping | Mapping sheet | every 5 years |
| | Physical-chemical water properties | |
| Discharge | Measurement container | 4(3) times per year (May, July, October) |
| Temperature (water and air) | Data logger | Hourly to four-hourly (loggers) |
| pH value | Multisensor device | 4(3) times per year |
| Electrical conductivity | Multisensor device | 4(3) times per year |
| Oxygen content (concentration and saturation) | Multisensor device | 4(3) times per year |
| Electrolytes/Nutrients | Ion chromatography | Once per year |
| | Biological monitoring of aquatic organisms | |
| Microbiology | Agar nutrient medium | Once per year |
| Botany—diatoms | Morphotaxonomy | every 5 years |
| Zoology—zoobenthos | Morphotaxonomy | every 5 years |

### *2.3. Mapping of the Spring Structure and of the Surrounding Environment*

At the beginning of the monitoring, the structural characteristics of the spring area must be documented so that developments can be easily traced during long-term observations. In the interest of uniform recording, the standards of the Bavarian spring monitoring sheet [29], including sketch and photographic documentation, should be followed.

Each biological sampling shall also include repeat mapping at five-year intervals, comparing all details with the initial survey and noting observed changes (e.g., climate-induced changes, anthropogenic disturbance and changes in sediment structure or shading).

#### Mapping Method

According to the documentation sheet, after entering the header data for the topographical, local governmental and nature conservation characterisation of the site (coordinates, catchment area, ownership and protection regulations), the size and structure of the spring are characterised (situation, type of outlet and substrate characteristics). Furthermore, the spring's condition is documented based on a selection of morphological characteristics and considering possible impairments. The surrounding area (5–50 m radius) is also recorded regarding its structure, vegetation, shading and possible disturbances. Spring structure, drainage and surroundings are depicted in a sketch using uniform symbols (Figure 3 left).

The latter contains all important structures that perform the following:

(a) Serve the recognition of the site;

(b) May be subject to change during long-term observations.

To be able to determine potential structural changes with each mapping, marking the spring's mouth is recommended, as shown in Figure 3 (right).

### *2.4. Monitoring of Abiotic Parameters*

The recommended physical and chemical monitoring consist of several components that allow the effects of climate changes on abiotic parameters in the spring water to be observed from various perspectives. In addition to water temperature and discharge, three critical parameters for this issue, pH, electrical conductivity and oxygen content are to be recorded in situ. In order to obtain more background knowledge about the dynamics of hydrochemical processes and to record climate-related changes in detail, electrolytes and nutrients should also be measured at longer intervals, if possible annually.

Most parameters are measured directly at the spring's outlet; only when recording the discharge is an additional determination made at the confluence of all spring branches on whether a system comprising several spring outlets is involved. During the measurement, the sensors must be completely covered with water (Figure 4). We propose to use a batterypowered flow pump. The maintenance of the individual measuring devices should be timed with monitoring intervals.


**Figure 3.** Sketch of the spring area (**left**) and marking of the spring mouth in Berchtesgaden National Park (**right**).

**Figure 4.** Application of a flow pump (marked by the red arrow) during winter low-discharge conditions in the field in the Berchtesgaden National Park to keep measuring sensors constantly covered with water.

The main parameters are briefly discussed in the following. For their general features, meaning, and measurement methods in springs, we suggest, e.g., the following sources [25,30,31]. The suggested monitoring intervals are available in Table 1.

The water temperature is measured continuously with data loggers. In contrast, the in situ parameters such as conductivity, pH value and dissolved oxygen content are taken at the beginning of each month in January, May, July and October—i.e., once per season. If there is no access to the spring during the winter months, measurement dates must be limited to springtime, summer and autumn. It is recommended to measure chemical parameters, which are not detectable in situ, once a year in summer.

### 2.4.1. Discharge

The discharge is defined as the volume of groundwater leaving a spring per unit of time [32]. Depending on the characteristics of the aquifer, the residence time can range from a few weeks (in extreme cases of karst springs, even days) to many months or even years. The flow velocity, discharge volume and source substrate shape the sediment composition and is essentially responsible for the structure of the riverbed and its colonisation.

Groundwater recharge, which influences spring discharge, can be impacted by climate change directly (precipitation amount and duration of snow cover) or indirectly (vegetation and land cover [33]). A changing climate can alter the discharge's dynamics—longer dry periods can change the spring type or lead to ultimate desiccation.

### 2.4.2. Water Temperature

An essential property of springs is their considerable long-term water temperature stability compared to streams or rivers, ponds or lakes. During percolation, the water temperature gradually approaches the annual average temperature of the area concerned, which also prevails in the entire groundwater body. External disturbances can cause deviations from this norm: Increased temperatures in low-lying strata influenced by geothermal heat or by rising thermal water, as well as lowered temperatures due to winter surface contact or meltwater flowing in rapidly from higher elevations may occur [34]. In addition, direct climatic influences at the spring mouth are important factors, particularly in the case of springs with weaker discharges. The temperature response is also decisively influenced by the size and porosity of the groundwater body. The longer the residence time of the water in the groundwater body, the more strongly the maxima and minima of the temperature amplitude can be shifted against the course of the outside temperature, up to an exactly opposite direction, with maximum temperatures in the winter [35].

Temperature conditions play a formative role as a controlling factor for the composition of biotic communities in springs. Since temperatures used to be primarily observed in situ during the vegetation period, spring water is usually attributed such as "cold" or "cool", and not infrequently, even crenobiontic organisms bound to springs are automatically assigned the ecological term of cold stenothermy (cold binding). However, the decisive phenomenon in this context is more likely to be the relative warmth of the habitat during the cold season [2]. For an adequate analysis of this connection, evidence from laboratory experiments is lacking in most cases. For the spring-typical caddisfly *Crunoecia irrorata* Curtis, Ebner et al. [36] could not observe a specific temperature preference.

Apart from these ecological interpretations, the temperature factor is a decisive variable for long-term climate observations at springs. Global warming inevitably leads to temperature increases in the Earth's groundwater reservoirs [18]. On the one hand, such developments can be expected to be reflected in the annual average temperature of springs (albeit with a delay that can vary greatly depending on the catchment area and subsurface). On the other hand, changes in the frequency and amplitude of temperature fluctuations can also be expected (e.g., through direct influences in the spring area). In the context of a warmer climate, temperature and precipitation extremes are increasingly projected. Hence, changes in the spring water's daily and annual temperature amplitudes deserve special attention.

During the initial recording of the spring, measurements should be taken with a handheld thermometer along longitudinal and transverse transects through the entire spring area to determine the exact location of the groundwater discharge (with the lowest water temperature in summer and the highest in winter). Such additional hand measurements should be repeated during subsequent fieldwork as part of the monitoring as soon as structural changes are perceptible. Furthermore, the air temperature in the source area should be recorded.

By applying data loggers, the water's temperature is measured using continuous data recording. If possible, data loggers should be attached close to the spring outlet. Depending on the discharge, they can be fixed with a plastic or metal rod (Figure 5left). The devices should be protected against turbulence during heavier discharge by a housing and installed so deep that they do not dry out during low water levels (Figure 5right). Nevertheless, experiences from the Rhön Biosphere Reserve have shown that in operational monitoring, it can happen that the data loggers float up after heavy precipitation or snowmelt. In contrast, temperature loggers can dry out during shallow flow despite careful site selection.

**Figure 5.** Metal rod, chain and metal basket to fix and protect the data logger against bed load (**left**). Attachment of a data logger in a spring (**right**).

In addition to the data logger in the spring water, air temperature is to be documented using another data logger near the spring's area.

Although these are good methods to monitor temperature changes from the groundwater source, they may not provide enough information about temperature in the spring's habitat. Depending upon the discharge rate, the area of the spring habitat and the temperature difference between the spring water and the surrounding area, impacts on within-spring meso- and microhabitats may not be easily detectable by only measuring the water temperature right at the spring outlet. A suggestion that we still have to test ourselves might be using infrared photos taken periodically in a consistent manner and location to complement the outlet's temperature measurements.

### 2.4.3. pH

In areas with weakly buffered soils (a situation often aggravated in pure spruce stands), Brehm [37] observed the increasing acidification of spring waters with increasing humidity, partly explainable by increased humification processes at the expense of mineralisation processes. During the time of the study, this was intensified by acid inputs from precipitation. The concentration of hydrogen ions in the spring water decisively influences chemical processes, e.g., the adsorption and dissolution behaviour of elements. At pH values below 4, the solubility and leaching of heavy metals increase drastically [38].

Acidity plays a vital role as a controlling factor for the composition of the spring fauna: At low pH values, the conditions of existence of species dependent on lime become more complex—molluscs may be completely absent and crustaceans are only represented by specially adapted species. In water with a high pH value, sinter formation occurs at springs from highly calcareous catchment areas; this occurs occasionally already shortly below the spring mouth but often only after a longer flow distance. The lime encrustations on all sediment components limit colonisation possibilities for many spring organisms enormously and, in extreme cases, lead to severe species impoverishment.

Climate-related changes in the catchment area and spring environment (vegetation change and change in precipitation regime) can impact pH values. If trends are long-term, it can be expected that such developments will be reflected in a changed composition of the biotic communities.

#### 2.4.4. Conductivity

Conductivity is a sensitive indicator of the hydrogeological situation, seasonal changes and potential long-term trends. In seasons when the outdoor temperature is close to the annual average temperature, identifying the groundwater discharge in the source area can also be performed by determining the areas with the highest conductivity instead of measuring the temperature. In red sandstone and silicate, it can assume values close to 0 (e.g., Finland, on granite: 10–30 µS/cm, [35]); in sedimentary rocks, it is significantly higher (examples from the Swiss Plateau [39]: in the Jura, it is 510–530 µS/cm; in the Lias, it is also significantly higher; in Sandstone-Keuper, it is around 670 µS/cm; in Muschelkalk, it is 600–1300 µS/cm), and in the Gipskeuper, it can increase to values typical for marine brackish water (around 2200 µS/cm). In springs, the conductivity amplitude can indicate the influence of inflowing surface water [40].

Conductivity integrates the entire electrolyte content and is, thus, particularly important for a general assessment of the spring water. For example, the seasonal pattern of conductivity values can provide valuable information for estimating the origin of groundwater [41]; conductivity also provides valuable information for interpreting the mean residence times of water in the subsurface or changing flow paths due to climatic influences.

#### 2.4.5. Dissolved Oxygen

Depending on the nature of the infiltration zone, dissolved oxygen is more or less consumed by organic processes during groundwater recharge, so its concentration essentially develops in the opposite direction to $CO_2$, and groundwater tends to be low in oxygen. However, residual amounts are usually present: The metabolism of stygobiont animals is adapted to long-lasting $O_2$ deficiency situations but is very much dependent on $O_2$ respiration [42]. The nutritional basis of heterotrophic organisms in groundwater consists of locally enriched minute quantities of organic material, which can arise autochthonously (chemoautotrophy) or be introduced. Spring water is, therefore, typically low in oxygen at the mouth of the spring, but its $O_2$ content adjusts to the quantitative conditions in the air after a few meters of flow. The speed of this process depends on the turbulence of the outflow. Turbulence in flowing springs often leads to pressure equalisation with the atmosphere immediately at the outlet (e.g., [25]). However, measurements taken directly at the spring mouth often show an $O_2$ content close to saturation. This indicates characteristics of the groundwater's body: (1) a relatively small aquifer, with rapidly flowing groundwater in which no significant respiration of introduced organic matter takes place; (2) exchange with atmospheric air in fractured aquifers or at least in the area close to the spring; (3) infiltration of oxygen-rich surface water in the spring area. Although no reliable laboratory studies are available yet, it can be assumed that spring-bound plant and animal species are not polyoxybiont but at least have tolerance towards, perhaps even a preference for, water with low oxygen saturation. Such phenomena have been demonstrated in groundwater organisms in laboratory tests (e.g., [43]). On the other hand, their oxygen depletion could make springs unattractive as habitats for polyoxybiont stream dwellers.

Dissolved oxygen is in a characteristic equilibrium with other chemical factors in groundwater, which shifts as it exits the spring. Similarly to $CO_2$ contents and pH values, it is under the influence of external factors in the groundwater body's catchment area and spring mouth. Since its solubility in water depends, among other things, on the temperature and the flow rate, a changed $O_2$ regime in springs can also be expected under climatic change.

### 2.4.6. Electrolytes and Main Algal Nutrients (N, P, S, Si)

Measurements of other chemical parameters (electrolytes and nutrients) are essential for understanding the spring system. Due to the increased ion dissolving capacity of the, usually $CO_2$-rich, groundwater and the correspondingly shifted equilibria, electrolyte concentrations in springs are already higher than in the surrounding waters. This also applies to nitrate, where, in addition to the often low filtration capacity of the soil [44], nitrification may also cause higher concentrations. Indicators for an increase in nitrate levels can be eutraphentic or tolerant algae at the spring mouth [45]. Similarly, high sulphate or hydrogen sulphide concentrations can also occur in springs [46], especially from Cretaceous and Keuper aquifers. Such special habitats, even if their chemism is of natural origin, are not suitable as reference sites. The chloride content is particularly interesting for understanding external influences' effect on groundwater reservoirs. Since chloride does not undergo any biological reactions and is usually already completely dissolved out of aquifers, it can be assumed that the discharge in the spring water directly reflects input in the catchment area [41]. Accordingly, a gradient in increases from the Alpine zones (at 4 mg/L or below) to the Tertiary of the Alpine foothills (>14 mg/L) can be observed in Bavaria [34]. Strongly increased winter values may be caused by the spreading of de-icing salt on roads. Springs with elevated chloride levels compared to surrounding waters are not considered reference sites.

The relative concentration of different components of the electrolyte spectrum is one of the factors contributing to the high individuality of single springs. Even springs emerging not far from each other can differ significantly in this respect. Rarely explored, but probably considerable given the high biological diversity, is the significance of this "electrolytic fingerprint" of an individual source for its organismal colonisation.

To determine detailed changes in spring-water chemistry over more extended periods, the observation of essential ions must be part of the monitoring. In high alpine areas, increased heavy metal concentrations have been observed as a consequence of climate change: Rising temperatures can increase the melting of block glaciers and thus lead to the input of highly ionised water into water bodies [47]. As already mentioned, for the oxygen content and the pH value, climatic change can also be expected to lead to changes in the input and balance of electrolytes, which in some cases can have an essential influence on the trophic state of the spring water.

Due to financial reasons and staff efforts, we recommend the measurement of essential ions once a year. A certified external laboratory must be commissioned if an in-house laboratory is unavailable. Sampling is either to be carried out by external contractors themselves or by their processor according to precise instructions.

## *2.5. Biological Monitoring*

Research into springs' plant and animal colonization has only recently entered a path of continuous improvement. There are many scattered published individual records from the 19th and early 20th centuries. Still, after the early monograph on three springs in Graubünden by Nadig [48], larger-scale surveys only went around the turn of the last century. The monographs that are now available document the strong individuality of these habitats. The springhead-colonizing communities often show a high proportion of springs-dependent (crenobiontic) species in comparison with all subsequent stretches of the running water system, and in alpine terrain with steep slopes, there is also a significantly higher diversity [23]. In springs of low-elevation mountain ranges, on crystalline substratum or, above all, on Buntsandstein (Bunter sandstone, lower Triassic), the diversity can be lower than in the spring-fed stream.

In addition to the presence of specially-adapted species (crenobionts), springs are typically characterized by the absence of animal groups that represent the stream fauna:

fish can only be found exceptionally in large springs with extensive dammed areas, and filtering organisms are often entirely absent and are most likely to appear in seepages with higher organic input; mayflies are often missing or are only represented by small populations of single species.

Sampling of biological materials

The challenge of spring biology lies in the small spatial extent of the object under investigation. The use of limnological standard techniques, such as those that have proven themselves for the removal of phytobenthos and zoobenthos from lakes or rivers (microbiological sampling is a special case, see Section 2.5.1), would leave the habitat destroyed; accordingly, the removal of the substratum must be limited to relatively small volumes. In turn, limiting the sample size carries the risk that species with low individual densities, often found in springs, remain undetected. Large-scale biological monitoring should be two-pronged: (1) quantitative observation of species that dominate the community in larger populations; (2) qualitative observation of rarer species using less invasive methods (hand collection and genetic methods). Rapid and continuous development of molecular genetic methods accompanies the realization of these recommendations. We have therefore decided to devote separate chapters to classical morphological and molecular biological methods. The conventional methodology discussed first is also the one with which the basis for these guidelines was developed. We recommend a combined approach in which both method fields are used in parallel for new monitoring. The combination of yearly genetic assessments of selected species with periodic community-wide morphotaxonomy-based evaluations is the most promising solution with reference to the information it can provide.

Sampling periodicity

Microbiological investigations should be carried out annually at the same time as the chemical parameters are recorded. Sampling for botanical and zoological analyses (phytobenthos, meiobenthos and macrobenthos) should be performed at five-year intervals. The time interval should be reduced accordingly if interesting developments emerge during the evaluation. In the event of organizational or financial bottlenecks, the sampling interval should not be changed. Samples from an annual survey can be kept frozen and analysed later. In view of the rapidly advancing possibilities in the field of molecular biology, the best possible fixation and maintenance must be discussed with specialists. A storage system with protection against power failures is essential for such samples.

### 2.5.1. Microbiology

Microbiological investigations in groundwater and springs have thus far largely focused on the usability of the drinking-water resource (determination of the total germ count, germ counts of coliforms and enterococci; increased values indicate anthropogenic disturbances in the catchment area and are important as exclusion criteria when selecting reference sources). However, the actual microbiological diversity in groundwater and springs has not yet been adequately documented. Many protistic and mycological taxonomic units ("OTUs") can be found in eDNA investigations (see Section 2.5.4).

The microbiological diversity in springs and groundwater is a research field with great potential but is usually not yet considered within the framework of standardized monitoring. However, the observation of selected parameters established in drinking water analysis conveys important signals about the development of these communities as well.

It can be assumed that an increase in temperature in ground- and spring-water leads to an increase in microbial cell density, as it enhances growth conditions for certain colony-forming strains [49].

Measuring Method

There is a large variety of methods for microbiological water analyses. In recent spring research, membrane filtration in connection with an agar culture medium has proven itself. The recording of parameters relevant to drinking water (see above) should be carried out as a minimum program, both during the initial examination and as part of long-term monitoring. Alternatively, eDNA 16S amplicon sequencing can provide information about microorganism communities and is relatively easy to combine with CO1 metabarcoding approaches. If no laboratory with a processor is available, external contractors must be commissioned who will also take over the quality assurance for the sampling procedure or carry it out themselves. The crucial thing when taking samples is that you do not cause any turbulence in the substratum and thus purely determine the microbiological conditions in the water column. The microbiological examination should be carried out annually at the same time as the chemical parameters are recorded.

### 2.5.2. Botany

Spring colonization by macrophytes varies greatly: Locations that are less suitable for monitoring for other reasons (e.g., helocrenes) are characterized by a higher species richness (e.g., [50]). At the beginning of the monitoring, a vegetation survey should be carried out according to the specifications in [51], and changes are to be logged in repeat investigations. The study of the algal flora is of particular interest for understanding the structure and variability of the phototrophic sector of the community (Figure 6), which can be very diverse and species-rich in springs [51]. Indicator species for important factors such as water-flow stability, acidification, nutrient balance or light climate can be identified.

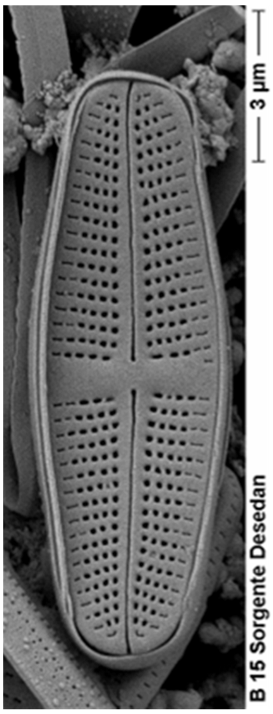


**Figure 6.** The diatom *Achnanthidium dolomiticum* Cantonati et Lange-Bertalot observed under a scanning electron microscope. This mainly epiphytic species (in springs typically found on bryophytes) is on the one hand characteristic of inland waters with above-average magnesium concentrations and on the other hand an excellent indicator of discharge fluctuations, an ecological factor particularly important for the monitoring of climate change.

If it is not possible to assess the entire algal flora, the diatoms (*Bacillariophyta*) are the predestined indicator group due to their wealth of species and the already thorough documentation of their habitat requirements. In addition to the permanently moist immediate vicinity of springs, they also colonize areas affected by fluctuations in the bed that are only periodically moistened, e.g., spray-water zones. Diatom communities can provide indications on various trends that are of particular importance against the background of climate change: They not only react sensitively to the continuity and variability of discharge conditions but also to changes in the chemical composition of the water, e.g., nitrate and organic matter concentrations or changes in acidity [52–56]. Spring specialists have not yet been discovered among diatoms, but species that can be used to distinguish between groundwater-influenced and stream-typical flowing water sections do exist [57]. Most species react very sensitively and precisely to the factors most important for the classification of spring types and possible climate-related changes (flow rate, mineral content, substratum composition and light conditions) [58].

Phytobenthos Sampling

The following description is based in many details on Cantonati et al. [51]. The entire eucrenal should be included in the sampling. A period with average, stable discharge conditions is particularly suitable for assessments, which should in no case be carried out shortly after heavy precipitation (often late summer in the Alps and low mountain ranges). Using an underwater-viewing device ("aquascope") is useful in larger, turbulent rheocrenes.

Field Work

Since several algae form macroscopically visible covers that differ in terms of size, growth form, coloration and consistency, a provisional assignment or even identification is already possible in the field. The sampling is therefore preceded by a recording in which all macroscopically differentiable algae layers are assessed and documented photographically and with the aid of hand-drawn sketches, accompanied by an estimate of the degree of cover and layer thickness. A key and ecological reference data for macroalgae in springs are given by Cantonati et al. [51]. In any case, it is necessary to take specimens of the species classified in this way and to verify them in the laboratory.

All macroalgae assessed and mixed microalgae samples are removed and stored separately by substratum type. Since the selection of the substrata sampled is based on their degree of coverage, there is automatically a clear differentiation determined by the spring type: While coarser mineral substrata dominate in rheocrenes, springs of other kinds show higher proportions of mineral and organic fine sediments—the latter often particularly richly populated.

Smaller stones and other hard substrata are collected as a whole. Growth forms that can only be found on large blocks or bedrock are removed, if necessary, with the help of a scraper or brush, and stored separately. In three places with fine surface sediment, the top 2 mm is sucked off. In addition to lithic surfaces, bryophytes as a growth substratum in springs often contribute to a higher diversity of diatoms and deserve special consideration. Apical parts are not only taken from them but also from submerged organs of macrophytes (withering leaves and roots) and the material is crushed so that firmly attached diatoms do not escape observation. If it is necessary to save processing time and costs, sampling can be limited to the substratum types with the highest degree of coverage.

Lab Work

All collected material are transported to the laboratory for further processing, as cool and dark as possible. If timely processing cannot be guaranteed, samples can be stored at refrigerator temperatures for a few days or frozen for a few weeks. Storage for even longer periods is possible after adding formaldehyde to a final concentration of 2–3% or ethanol to >40%. However, the former leads to morphological changes in some species: the latter to discoloration in the medium to long term. If the sample material has not come into contact with laboratory chemicals at all, parts of it can also be dried and herbarized, with the advantage that such materials can still be analyzed molecular-biologically decades later [51].

The relative frequencies are determined with different methods depending on whether all algal taxa or only the diatoms are processed (see [59] for further details). Relative macroalgae covers are estimated based on observations in the field and, if necessary, corrected based on random-sample observations in the laboratory. Two steps are required to determine the frequency of (mixed) microalgae (apart from diatoms): The relative proportions of the total coverage of the mixed microalgae populations macroscopically recognisable during the fieldwork are determined according to their complete coverage and then converted concerning the overgrown area. The relative frequency of the species that form assemblages is specified in the laboratory (relative surface area of the individual microalgae taxa in 5–10 preparations per type), and the final relative frequency of the individual species is then calculated according to the ratio of the respective vegetation type to the overall degree of coverage. To determine the abundance of diatoms, the material to be examined is first digested with hydrogen peroxide or various strong acids (see [51]) to remove organic components. Then, at least 400 valves are counted from each choriotope sub-sample at $\times$1000 magnification, and other rarer taxa that are only recorded qualitatively are identified in a further inspection of the remaining material.

The algal flora should be examined every five years, at the same time as the zoobenthos described below. This time interval is proposed on an empirical basis, and it is a compromise between indications emerging from our own data (e.g., [11]) and the financial support that can currently be hoped for this type of environmental monitoring.

### 2.5.3. Zoology

In contrast to floristic knowledge, orders and classes of small-dimensioned invertebrates (meiozoobenthos, e.g., Rotatoria, Oligochaeta, Nematoda; microzoobenthos, Protozoa) are widely underdocumented. These groups may also have a high potential as ecological indicators in springs (e.g., for Oligochaeta [60]), and the application of molecular techniques could contribute to an improved understanding on their diversity.

Currently, the faunistic documentation of Central European springs is widely satisfactory for many macrozoobenthos groups. To achieve a broad-based documentation of life conditions, a faunistic analysis of a spring should comprise, in addition to the essential insect orders of Odonata, Heteroptera, Ephemeroptera, Plecoptera, Coleoptera and Trichoptera, also the Acari (Figure 7), Crustacea and Mollusca.

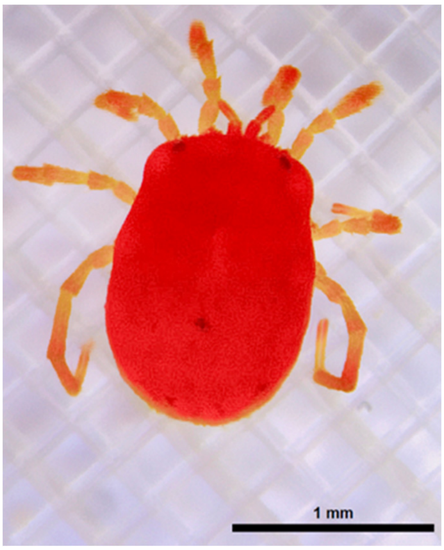


**Figure 7.** The invertebrate group of true freshwater mites (Acari and Hydrachnidia) is particularly relevant for spring monitoring due to their high share of specialized species exclusive to these habitats (crenobionts) and their dependence on continuous flow and stable conditions. The picture shows *Partnunia steinmanni* Walter: In a study on genetic exchange among populations of this species in National Parks of the Alps, Blattner et al. [61,62] showed that Alpine springs are relatively isolated habitats with island characteristics.

In springs, most insects belong to a segment of the fauna that, at the adult stage, is exposed to the conditions characterising the nearer or wider terrestrial surroundings of their habitat. Some beetle species make an exception with a submersed life at all free-living stages. Thus, insects are the group best indicating the interlocking between the proper spring habitat and its terrestrial surroundings. At the same time, they have no particular relationship to groundwater in Central Europe except for the stygophile beetle *Hydroporus ferrugineus* Stephens.

The flies and midges (Diptera) are of particular significance for understanding the aquatic-terrestrial ecotone, with many families being species-rich in spring habitats. For many of these species, it is still unclear if their larvae and pupae develop in submerged habitats or in the more or less wet terrestrial marginal areas. Such gaps in our knowledge are still hampering the use of the high indicator value of dipteran diversity in and around springs. An appropriate documentation of the dipteran species in springs is impossible without the exposition of emergence traps over an entire season (with some specialized species hatching only during snow cover in winter). For the solution of questions concerning dipteran diversity in springs, molecular techniques are a promising tool.

Similar to insects, also most water mites pass a part of their life cycle in the terrestrial environment. However, in contrast to insects, at the larval stage, the adults are strictly bound to the perennial presence of water. In the European fauna, no other group of animals has such a high share of species strictly bound to spring habitats (springs-dependent species) [63]. The reason for this phenomenon is still unclear, but it makes water mites a top indicator group for the ecological monitoring of springs. As in insects and also among mites, no species are known to have a strict binding to groundwater. Due to the parasitic relationship with insects at the larval stage, populations of true freshwater mites (Hydrachnidia) are indirectly influenced by the conditions in the surroundings of springs [64]. In addition, their life cycle includes two pupa-like resting stages, which are sensitive to oxygen depletion and colmatation in sediments.

Some microcrustaceans are, at least during certain life stages, resistant to drought and adapted to aerial dispersal with vertebrates. In contrast to insects and mites, many others, e.g., species of Amphipoda, Bathynellacea or Isopoda, are bound to water during their entire life cycle, and many of them have a particular close relationship to groundwater, as well as many Copepoda and Ostracoda. Microcrustacean species assemblages variously adapted to seeping or running springs are best suited for characterizing substratum and organic production conditions, respectively. The presence or absence of the drought-sensitive *Gammarus* species allows statements about flow stability but may be conditioned also by hydrological events that took place long ago (e.g., glaciations). In general, the presence of stygophilous or stygobiont crustaceans allows statements about the characteristics of the groundwater reservoir feeding the spring.

Representatives of Mollusca are generally poor in spring-dependent species numbers, and due to their need of calcium for building their shells, they are often completely absent from acidic waters. As active fine-particle filter-feeders, larger populations of mussels indicate drift of organic fine material. Among snails (Gastropoda), several genera include crenobiontic or crenophilic (sometimes stygophilic) species. They are all grazers of bacterial and algal periphyton, and many show restricted local distributions and are, in some cases, difficult to separate taxonomically. In view of their potential to be detected also in subfossil sediment layers, Mollusca should be integrative part of any biotic spring monitoring.

Reference Taxa

During the first stage of long-term faunistic monitoring, it makes sense to select, for the geographical area under observation, significative reference taxa and species expected to be particularly sensitive against changes in climatic conditions. The applicability of as many as possible of the following 12 criteria indicates the particular sensitivity of a selected taxon against climate change consequences (Table 2).

**Table 2.** Criteria for the selection of reference species.

| Ecology | Zoogeography |
|---|---|
| Restricted nutritional range | Restricted or scattered distribution |
| Parasitic/symbiotic relations to other organisms | Population at border of distribution area |
| Sensitivity against increased nutrient contents | Changes in distribution during the past decades |
| Particular water chemistry requirements | Binding to particular vegetation ranges |
| Temperature sensitivity | Binding to particular elevation belts |
| Preference for a particular spring typology | Intraspecific variability |

Based on these criteria, the following ten higher-level reference taxa results best suit faunistic long-term monitoring in Central Europe: Mollusca; Acari; Copepoda; Ostracoda; Amphipoda; Ephemeroptera; Plecoptera; Trichoptera; Coleoptera; Diptera. Out of these taxa, the best-suited reference species should be selected for each geographic subunit taken into consideration. Obviously, the appearance of representatives of other taxa generally uncommon in springs always merits particular attention.

It must be underlined, however, that information on habitat preference or zoogeography of species is incomplete for many representatives of the spring fauna. Sometimes, data were produced tautologically (e.g., stating for a species stenothermy just because it is found mostly in springs). More in detail, "cold stenothermy" is often attributed to spring dwelling taxa because fieldwork is performed in summer. The relatively warm water temperature in comparison with the surroundings during winter might be of much higher significance for the binding of species to spring habitats.

In addition to disappearing reference species, the new appearance of alien species and their interpretation under ecological and zoogeographical points of view is obviously an aspect of particular weight for the interpretation of climate change.

Once the reference species are defined, a representation of as many of these as possible is an important criterion when individual springs are selected for long-term monitoring, and changes in their population size merit particular attention.

In certain limits, the "climate change vulnerability score" (CCVS) proposed by Hershkovitz et al. [65] for stream-dwelling species of the insect orders Ephemeroptera, Plecoptera and Trichoptera is useful also for the interpretation of spring habitats. An extension of this tool to spring dwelling species and representatives of other invertebrate groups would be highly desirable.

The potential, however, to reduce efforts and costs of taxonomic work, and to accelerate it by reducing the long-term monitoring of a set of reference species, finds its limitations: recognizing and separating the species in question requires a broad knowledge of the entire diversity of a taxonomic group, and the diagnostic characters of reference species are often difficult to recognize. Furthermore, a continuously updated knowledge of general developments in ecological and zoogeographical research on spring-dwelling species is a prerequisite for an appropriate interpretation.

At least some of the restrictions might be overcome by applying molecular techniques (see Section 2.5.4.). However, once such methods are established, it would not make sense to delimit the investigation to a search for selected species in samples of sediment or assorted specimens. The production of entire species lists considering all important groups will not mean more effort than searching for selected species. Under such conditions, large parts of the fauna could be monitored, also allowing a continuous reconsideration and updating of the reference species lists.

Zoobenthos Sampling

Given the problem of producing representative, surface-correlated data from small habitats such as springs, Zollhöfer (s.a. [66,67]) proposed the extraction of mini-samples from all over the eucrenal, using size-reduced Surber samplers (s.a. [68]). Franz et al. [69]

apply for the collection of one hand net sample integrating over all characteristic substrata along a transect line through the eucrenal (Figure 8). The Surber technique combines the two advantages of having an area reference and the possibility of obtaining detailed information on species distribution within microhabitats. However, because of the generally high heterogeneity of substrata, elaborating a representative area reference is de facto widely impossible. Documenting microhabitat preferences of selected species is an interesting topic by itself but is of no relevance for the purpose of climate change studies. Given its distinctly lower input, both in the field and during sorting in the lab, we propose the integrative method of Franz et al. [70] as a standard. However, most of the work steps proposed in the following would correspond to each other with both methods.

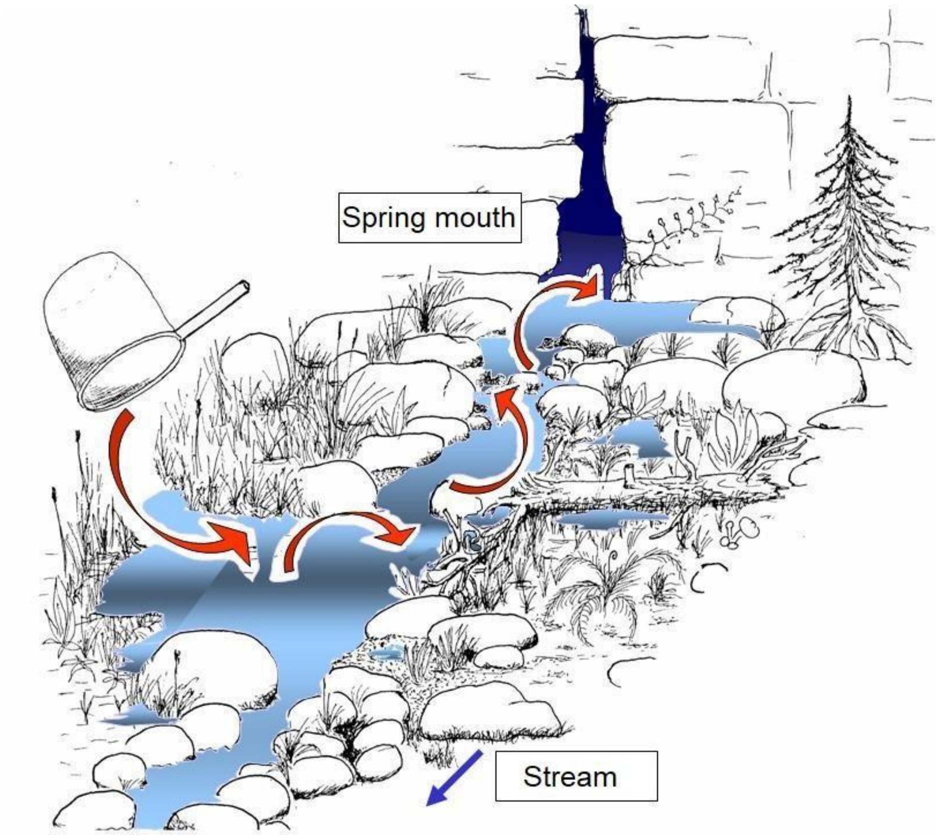


**Figure 8.** Integrative zoobenthos sampling in spring habitats.

Field Work

At the beginning of the successful application of any method stands a thorough mapping of the spring area, estimating the proportional coverage of all important choriotopes (mineral substrata sorted by granulometry, organic substrata such as wood, roots, detritus, algae, moss and higher plants). During an integrative net sampling, as well as when positioning Surber samplers, collecting intensities on various substrata should reflect their relative importance for the structuring of the habitat. Another important aspect is the representative consideration of sunny and shadowed parts (obviously taking into account potential changes in the course of the day).

For an integrative net sampling [69], the material of all characteristic substrata in the eucrenal is washed in a hand net (mesh size 250 µm). The removal of sediments takes place in a mosaic pattern along a transect all over the eucrenal, considering that, in summary, only a small part of the area is affected. In doing so, potential damages that could concern the spring and, consequently, the next following samplings in the monitoring, are kept as low as possible.

Carefully washing and homogenizing the net contents are performed in the subsequent spring brook or another uninvestigated waterbody nearby. Rough material such as wood or stones is replaced after being rubbed or brushed off. The final sample volume is reduced to about 1 L and then separated, with the help of a sieve (mesh width 1.000 μm), into a coarse and finer fraction. Animals in the coarse fraction should be sorted on the spot from the living material. In parallel, this fraction may be enriched by additional hand sampling of larger-sized invertebrates, such as adult amphipods and beetles or mature insect larvae or pupae. As integrative sampling is of a semiquantitative character, such additional sampling does not impose a noteworthy influence on the result, but it may contribute to completing the species list by adding larger sized or quickly escaping representatives of species that easily escape the standard integrative sampling.

From the fine fraction, sand should be released by repeated stirring and the organic material representing the fraction to be further investigated should be poured. At the end, for the sake of delimiting time-consuming laboratory work, the volume of material stored after intensive mixing should not exceed 100 $cm^3$. To maintain all specimens in conditions suitable for molecular analysis, storage must be performed in 100% undenatured ethanol.

Laboratory Work

Materials collected with the methodology described above allow further interpretations mainly under two aspects: (1) the identification of dominant taxa; (2) the elaboration of a species list that includes also rare species t are present in low density, but occasionally of high indicator potential.

Sorting from the fine fraction is performed at the level of classes, for insects of orders, and within the latter at least for Diptera at family level. Since taxa with low abundance may also be indicative in springs (e.g., stygobiont crustaceans or crenobiont mites), subsampling is not advisable. We suggest the following procedure: The fine fraction is carefully homogenised and then subdivided into three fractions differing from each other in a low, medium and high percentage of specimens with higher body mass. A total number of 100 specimens of all invertebrate groups is each sorted out from part 1 and part 3. In the case that part 1 plus 3 contain in total less than 200 specimens, the total material of part 2 is sorted as well. Thereafter, the entire remnant of the fine fraction is checked, but only representatives of previously undetected taxa (that will enrich the total taxonomic spectrum) are removed for further investigation. As it is desirable that, in the end, for each morphospecies, several specimens per site are available; this last step of sorting should be performed in an unsparing manner.

As soon as taxonomic dominance values are calculated from the "200-individuals sample", the specimens from the coarse samples separated in the field are sorted to the same taxonomic level. Thereafter, all specimens sorted out are kept separate, taxon by taxon, for further processing in the labs of specialists.

Similar results may be also obtained when a flat bowl, e.g., a petri dish, is subdivided into an equal number of sectors of which only one is considered for quantitative picking out, while qualitative sorting is conducted using the others. Both procedures allow sparing working efforts, combined with gaining a representative cross-section of the complete fauna. As, during mixing and bottling of samples, representatives of various groups of animals are unequally distributed in the sample according to their various body mass, considering parts of all weight fractions within the fine material sample is a prerequisite for not overlooking parts of the taxonomic diversity.

It is recommended to repeat the faunistic analysis in the same frequency as the botanical studies every five years.

#### 2.5.4. Environmental DNA as a Tool to Monitor Spring Biota

Molecular genetic tools, particularly those focusing on environmental DNA (eDNA), are increasingly crucial for ecosystem monitoring and nature conservation [70,71]. In particular, two distinct approaches, metabarcoding whole species assemblages and targeted species detection, have gained particular importance and are regularly applied to various freshwater habitats. Environmental DNA metabarcoding is a powerful tool for identifying entire sets of species in parallel that are present in a particular habitat (see, e.g., [72,73]). It allows massive parallel sample throughputs and can be considered a cost- and time-effective supplement compared to traditional approaches [74]. In contrast to metabarcoding, the targeted detection of single species using methods such as qPCR offers sensitive and highly species-specific detection procedures [75]. Thus, these targeted approaches are particularly interesting when aiming at invasive or rare species detection [76]. Although distinct and focusing on different scales of species detection, both methods can be applied to eDNA samples, which has the major benefit of being entirely non-invasive in contrast to traditional approaches [77].

Although the above outlined molecular genetic methods are well established and tested for different freshwater habitats, data on their suitability for spring ecosystem assessment are still scarce. First, data and preliminary spring eDNA metabarcoding investigations based on CO1 amplicon sequencing of sediment samples from springs in the Berchtesgaden National Park (unpublished data) have shown that enriching reference sequence databases constitutes a significant challenge. Many spring-dependent species expected to be present according to previous data were not reliably recorded with eDNA metabarcoding due to lacking sequence database entries. Improving spring species sequence reference databases and a more systematic survey and comparison between traditional and metabarcoding methodologies would first be necessary to generate conclusive evidence on the suitability of eDNA metabarcoding in springs. In contrast, single species detection in filtered spring water eDNA samples with targeted qPCR assays has proven to be a good biomonitoring approach [60].

### 2.6. Long-Term Data Storage and Evaluation

In long-term monitoring, sustainable data management is of great importance. To prevent possible losses or double storage, it must be created in a centralized manner. For the storage of the monitoring data, we propose a database system for which backups are made regularly. Cloud storage solutions may increase accessibility and reliability for environmental monitoring [78]. In the case of the Berchtesgaden National Park, a customised MS Access is developed, which enables user-friendly data import and export using MS Excel data sheets. Before the current monitoring data are fed into the database, an automated quality control is carried out [79].

In addition to the database system, the location information of the monitored springs is stored in a central GIS.

Access to the data for research purposes can be granted upon request to the respective protected area. The publication of the data is planned for the future.

### 2.7. Framework Conditions for Monitoring at Springs

Although longer time intervals are recommended for biotic aspects, a continuous control of data collection and management is necessary from the very beginning of monitoring only for the abiotic measurements series. Data collection in the form of consecutive short projects completed in steps cannot guarantee the continuity required for this research topic, although there might be value in looking at short-term projects periodically for additional context to the long-term data. Since this is a climate-related monitoring that takes place over a very long period according to fixed standards, this long-term nature must be guaranteed as best as possible even before monitoring is established. The implementation of the guidelines can only be carried out successfully by trained specialists.

The following prerequisites are necessary for the measurement and determination of the described parameters:

Acquisitions:

- Data logger for continuous measurement of water temperature.

- Measuring devices according to the defined standards for the in situ measurement of physical and chemical parameters.
- Sample containers for physical, chemical and biological investigations.
- Standard collection equipment for biological sampling.
- Equipment for collection and preservation of eDNA samples (filter capsules, disposable syringes and isolation boxes with dry ice).

Laboratory equipment: Complete equipment for all chemical and microbiological analyses and the standard maintenance of all measuring devices. If there is no adequate laboratory equipment with qualified personnel, a contractual integration of external laboratories was applied.

Expert network: Regardless of whether the biological aspects of long-term monitoring are performed using traditional methods or genetic barcoding, the availability of experienced biological experts is essential. If specialists responsible for certain animal and plant groups are not available in the Institutions involved, external specialists should assume long-term responsibilities for their specialty area.

Data storage should be at the top of the project's hierarchy and geared towards long-term operation. This is where time-series management takes place, decisions are made about the type of storage media, the comparability of the data structures is ensured, and the availability of the data is coordinated.

## 3. Findings from Previous Spring (Long-Term) Research in the Alpine Region: Relevance of Standardized Long-Term Spring Monitoring in the Context of Climate Change

The collection of data (in the Berchtesgaden National Park and Bavarian Forest National Park) strictly adhering to the methodology we described in the present contribution started in 2018. However, particularly in the Berchtesgaden National Park and in the Adamello-Brenta Nature Park, some long-term data have been collected with very similar methods and included in publications over the years [4,11,80]. In particular, Cantonati et al. [58] could show a clear segregation of the older spring-data in non-parametric diatom-based ordinations, suggesting a strong potential for the use of spring diatoms in studies aiming at tracking the effects of climate change. Moreover, there is another paper in progress (recently submitted), in which one of us (GL) is involved as an author, about the abiotic data series and monitoring intervals (temperature and electrical conductivity) of the Berchtesgaden springs (data analysis on different monitoring intervals of the parameters). We have chosen to use these processed and published data series as a preliminary validation of the spring monitoring strategy presented here.

To be able to understand and predict climate change effects on springs, comprehensive long-term observations of all necessary physical and chemical parameters [81] and of the development of flora and fauna are indispensable prerequisites (e.g., [17]). In the Berchtesgaden National Park, there is a >30 years tradition of hydrogeological research involving springs. Numerous hydrogeologic tracer experiments and isotope research were carried out to unveil subsurface pathways [82–84]. Later, a detailed physically based water-balance modeling was performed focusing on climate change, snowmelt, and groundwater [85,86].

In addition, physical and chemical data (since 2000) and faunistic recordings (since 1994) on springs were collected for monitoring purposes within the area using different methods resulting in time series and practical knowledge in spring observation covering almost 30 years. Until now, no significant trends could be detected, probably due to the limited availability of temporally high-resolution data [87].

However, the long-term observation of springs and water cycle in the Berchtesgaden National Park and exchange with other protected areas made clear that until now there is no comparable and standardized method or guideline for observing springs.

In northern Italy, the Adamello-Brenta Nature Park supported pioneering efforts in spring ecology at the beginning of the 1990s [23,52,88] and then fostered research on spring research, including repeated but uncontinuous support for long-term observations [52]. First ideas and concepts on long-term ecological research at springs were published as early as 1998 [24]. Several springs (ranging from three to nine) were sampled yearly with standardised methods since 1998 (however, the first data for these sites are from 1993 or 1996; Gerecke et al. [11] and references therein).

In the Black Forest National Park, the processes controlling spatial and temporal dynamics of spring water chemistry were studied as a baseline for future studies of hydrochemistry in this protected area [89].

Various faunistic and floristic projects have also started in other areas of the Alps over the past two decades to document springs as habitats. In most cases, the focus was on the documentation of a hitherto little-known biodiversity (Austria, Upper Austria, Kalkalpen National Park [90], Styria, Gesäuse National Park [91,92], Ausseerland [93], Switzerland, NP Graubünden [94] and entire Swiss Alps [27]). During these investigations, new insights into the floristics, faunistics and biogeography of Alpine springs could be obtained, accompanied by the discovery of many species new to science. For Swiss springs, Lubini et al. [95] developed an assessment procedure that allows statements about anthropogenic disturbances and provides the basis for renaturation measures. Using the example of selected groups of insects from the Swiss high Alps, Küry et al. [27] presented an analysis of spring inhabitants' sensitivity to climate change for the first time. The springs were examined at elevations between 1700 and over 2500 m a.s.l. Based on this research, the Swiss Federal Office for the Environment (FOEn) published a working basis for the recording, protection and renaturation of the country's springs in 2021.

An overview of corresponding research in North America, where springs have as well become an important object of environmental sciences research, was provided by Stevens et al. [96]. These research efforts also include the development of spring ecosystem assessment protocols: a recent example, supplied by Kurzweil et al. [97], is a simple adaptation of well-established methods to evaluate ecological indicators of springs and identify areas of concern. In addition to the detection of changes, the analysis of the monitoring data enables an improved understanding of the system of the investigated springs [81].

## 4. Conclusions

A near-natural spring represents a small-scale transition area between the subterranean groundwater system and the surface-water network. The emerging groundwater forms a sensitive ecotone with distinct and diverse habitats.

Climate change, both on a global and a regional scale, will affect the temperature and water cycle of spring catchments and, consequently, impact the physical, chemical and ecological properties of the system. The monitoring networks in the Berchtesgaden National Park, the Adamello-Brenta Nature Park and the newly implemented monitoring in the Bavarian Forest National Park have the potential to contribute to the investigation of climate change impacts on spring ecosystems. Here, we used our data series and experience (published over the years and currently being published) for a critical evaluation of methodological aspects and to develop suggestions for spring-monitoring programs in the Alpine area. If a spring is repeatedly examined over a long period of time, considering the parameters and applying the monitoring intervals recommended in this guide, the resulting data series serve to identify significant trends in habitat conditions and communities that can allow statements to be made about the influence of climate change on the spring habitat.

We refined methodological approaches to observe springs and monitor climate change impact on aquatic ecosystems. In an interdisciplinary project, together with experts from hydrogeology, hydrology, zoology, botany and geography, we have conceived and tested a procedure for long-term monitoring of springs, which makes possible the use of these sensitive habitats as indicators of climate change.

The present recommendations have been tested in practice for applicability, and we hope the recommendations can facilitate future spring-monitoring programs in the Alps.

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