

# Technical Guidelines for assessing and monitoring the condition of Annex I habitat types of the Directive 92/43/EEC

Caves not open to the public (8310)



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Technical Guidelines for assessing and monitoring  
the condition of Annex I habitat types of the  
Directive 92/43/EEC

**Caves not open to the public  
(8310)**

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## Glossary and definitions

### Habitats

**Natural habitats:** are terrestrial or aquatic areas distinguished by geographic, abiotic and biotic features, whether entirely natural or semi-natural (Habitats Directive).

**Habitat condition:** is the quality of a natural or semi natural habitat in terms of its abiotic and biotic characteristics. Condition is assessed with respect to the habitat composition, structure and function. In the framework of conservation status assessment, condition corresponds to the parameter “structure and function”. The condition of a habitat asset is interpreted as the ensemble of multiple relevant characteristics, which are measured by sets of variables and indicators that in turn are used to compile the assessments.

**Habitat characteristics:** are the attributes of the habitat and its major abiotic and biotic components, including structure, processes, and functionality. They can be classified as abiotic (physical, chemical), biotic (compositional structural, functional) and landscape characteristics (based on the Ecosystems Condition Typology defined in the SEEA-EA; United Nations et al., 2021).

### Species

**Characteristic species:** are species that characterise the habitat type, are used to define the habitat, and can include dominant and accompanying species.

**Typical species:** are species that indicate good condition of the habitat type concerned. Their conservation status is evaluated under the structure and function parameter. Usually, typical species are selected as indicators of good condition and provide complementary information to that provided by other variables that are used to measure compositional, structural and functional characteristics.

### Variables

**Condition variables:** are quantitative metrics describing individual characteristics of a habitat asset. They are related to key characteristics of the habitat that can be measured, must have clear and unambiguous definition, measurement instructions and well-defined measurement units that indicate the quantity or quality they measure. In these guidelines, the following types of condition variables are included:

- **Essential variables:** describe essential characteristics of the habitat that reflect the habitat quality or condition. These variables are selected on the basis of their relevance, validity and reliability and should be assessed in all Member States following equivalent measurement procedures.
- **Recommended variables:** are optional, additional condition variables that may be measured when relevant and possible to gain further insight into the habitat condition, e.g. according to contextual factors; these are complementary to the essential variables, can improve the assessment and help understand or interpret the overall results.
- **Specific variables:** are condition variables that should be measured in some specific habitat types or habitat sub-groups; can thus be considered essential for those habitats, which need to be specified (e.g. salinity for saline grasslands, groundwater level for bog woodlands, etc.).

**Descriptive or contextual variables:** define environmental characteristics (e.g. climate, topography, lithology) that relate to the ecological requirements of the habitat, are useful to characterise the habitat in a specific location, for defining the relevant thresholds for the condition variables and for interpreting the results of the assessment. These variables, however, are not included in the aggregation of the measured variables to determine the condition of the habitat.

**Reference levels and thresholds:** are defined for the values of the variables (or ranges) that determine whether the habitat is in good condition or not. They are set considering the distance from the reference condition (good). The value of the reference level is used to re-scale a variable to derive an individual condition indicator.

**Condition indicators:** are rescaled versions of condition variables. Usually, they are rescaled between a lower level that corresponds to high habitat degradation and an upper level that corresponds to the state of a reference habitat in good condition.

**Aggregation:** is defined in this document as a rule to integrate and summarise the information obtained from the measured variables at different spatial scales, primarily at the local scale (sampling plot, monitoring station or site).

## Abbreviations

EU: European Union

IAS – Invasive Alien Species

MS: Member State

EU Member States acronyms:

Austria	(AT)	Estonia	(EE)	Italy	(IT)	Portugal	(PT)
Belgium	(BE)	Finland	(FI)	Latvia	(LV)	Romania	(RO)
Bulgaria	(BG)	France	(FR)	Lithuania	(LT)	Slovakia	(SK)
Croatia	(HR)	Germany	(DE)	Luxembourg	(LU)	Slovenia	(SI)
Cyprus	(CY)	Greece	(EL)	Malta	(MT)	Spain	(ES)
Czechia	(CZ)	Hungary	(HU)	Netherlands	(NL)	Sweden	(SE)
Denmark	(DK)	Ireland	(IE)	Poland	(PL)		



## Executive summary

These guidelines cover cave natural habitats protected under the Habitats Directive, which are not open to the public. Terrestrial underground caves, cave systems and passages including water bodies represent unique and diverse habitats that are characterized by the partial or complete absence of light and often extreme environmental conditions, which can occur in a wide variety of rock types and caused by widely differing geological processes.

The Habitats Directive includes cave habitats under the habitat type 8310 Caves not open to the public, including their water bodies and streams, hosting specialised or high endemic species. Their ecological characterization is given by climatic conditions such as temperature, humidity, airflow and other atmospheric conditions typical of the interior of a cave, as well as the lithology and substrate characteristics. These caves are usually devoid of vegetation of higher plants but can host mosses (e.g., *Schistostega pennata*) and algal carpets, rarely ferns at their entry. They also host very specialised and highly endemic cavernicolous fauna, including underground relic forms of a fauna which has been diversified outside. This fauna is mainly composed of invertebrates which exclusively live in caves and underground waters.

The analysis of existing methodologies across EU Member States reveals significant variability in assessing and monitoring these habitats. The most common characteristics being monitored across the different consulted methodologies are morphological characteristics of the cave (e.g. morphology and volume, speleothems) and presence of fauna (e.g. invertebrates and bats). Thresholds for interpreting these metrics and procedures are often absent or based on expert judgement, which often is poorly justified. Aggregation procedures are not included in the majority of methodologies analysed; those that have presented an aggregation system exhibit notable differences. Monitoring procedures are largely based on periodic field observations.

A set of essential, recommended and specific variables for monitoring caves are proposed. They are categorized into abiotic (e.g., air temperature, relative humidity, CO<sub>2</sub> concentration, soil pH, etc.), biotic (e.g., presence of moss, algae, invertebrates, bats, etc.), structural (e.g., vegetation cover, etc.), functional (e.g., primary production, population dynamics, etc.), and landscape (e.g., patch size, fragmentation). The main criteria and guide for reference values and critical thresholds determining favourable condition are provided, but specific values should consider their specifics, regarding the considered variable, the particular habitat, the contextual biogeographical gradients, and the historical, cultural and socio-economic background.

The guidelines outline several priority areas for future efforts, including maintaining the exchange of information between Member States, moving to the definition of common methods, for instance, by training and evaluation programs, exploring new data sources (e.g., climatic ones) and incorporating remote sensing methodologies, integrating climate change impacts into monitoring. Strengthening knowledge exchange, technology sharing, and coordinated efforts is critical to fostering an inclusive, collective approach to conservation. Aligning methodologies with EU biodiversity policies, particularly the Nature Restoration Regulation, will be vital in achieving restoration and conservation goals.

## 1. Definition and ecological characterisation

### 1.1 Definition and interpretation of habitats covered

According to the Interpretation Manual, caves not open to the public (habitat 8310) are defined as “underground cavities not open to the public for tourism or beneficial uses, comprising the entrance and exit zones, as well as internal galleries and halls, and including both natural and artificial cavities” (European Commission, 2013). In comparison, Weaver and Johnson (1980) define caves as natural underground openings extending beyond the zone of light and large enough to permit the entry of humans; however, this excludes small, inaccessible cavities that may harbour a large number of small animal species (Elliott & Ashley, 2005). Another definition considers a cave or cavern as any natural cavity in the subsoil, whether accessible or not, and regardless of its dimensions, layout, origin or lithology (Renault 1987, in Robledo et al., 2019a). Such cavities can range from a few to thousands of meters in length and are generally characterized by the absence of sunlight and a limited supply of nutrients (De Waele & Gutiérrez, 2022). Importantly, the exclusion of sunlight is not a requirement for Annex I habitat caves; in fact, the official definition includes entrance zones that may still have some light, and many species typical of cave ecosystems are found primarily or exclusively in these transition areas, while others inhabit only the completely dark zone.

Caves occur in a wide variety of rock types and form through diverse geological processes, resulting in sizes that range from small chambers to extensive networks of interconnecting passages stretching for many miles (Figure 1). The geomorphological characteristics of caves are determined by the structural and geological context in which they develop. Although most caves are found in carbonate rocks such as limestone, dolomite, and marble, they can also form in quartzite, sandstone, and volcanic rocks. Carbonate rocks are particularly prone to cave formation because they dissolve in mildly acidic rainwater, which absorbs carbon dioxide from the atmosphere and soil. As this acidic water infiltrates the carbonate mass, it enlarges existing pores, faults, fractures, and fissures by dissolution.

Caves commonly exhibit distinctive features and processes, including speleothems, unique mineral assemblages, fossils, multiple levels, and the presence of both permanent and intermittent water flows, which can be either turbulent or diffuse (Pérez-Alberti et al., 2009). Subterranean habitats can be broadly categorised into three main types: interstitial spaces (small cavities within the substrate), large cavities such as caves, and shallow subterranean habitats consisting of voids near the surface. Despite their structural differences, these environments share two fundamental characteristics: the absence of natural light and reliance on nutrients supplied from the surface (Culver & Pipan, 2019).

Subterranean habitats across Europe support exceptionally distinct biological communities, reflecting the unique conditions found in each environment. Hypotelminorheic seeps, which are shallow groundwater features common in limestone regions, are often inhabited by diverse amphipods, stygobiotic isopods, and molluscs (Culver & Pipan, 2019). Epikarst systems—consisting of the fragmented, weathered upper layer of karst—are especially rich in aquatic copepods, but also house amphipods and isopods. These habitats display high diversity, with numerous species uniquely adapted to fissures above classical cave zones.

Caves and groundwater habitats, especially in karst regions, are home to a remarkable diversity of species that are highly specialized for life underground. These ecosystems are considered biodiversity hotspots, yet most research has focused on regions in Europe and the

USA, with much less data available on a global scale (Pipan et al., 2020; Zagmajster et al., 2021).

Subterranean ecosystems display minimal or negligible primary productivity, largely due to the absence of sunlight. They serve as valuable systems for studying ecosystem functioning, particularly with regard to the interplay between natural selection and genetic drift in evolutionary processes. Although these environments are extreme, they are typically characterised by long-term environmental stability. This combination offers a unique perspective on adaptation and evolution within isolated and resource-limited habitats (Culver & Pipan, 2019).

These habitats can provide important refuges for many species that need protection from drought, cold and, in the case of animals, predators. The most complete faunal records are from caves and, worldwide, more than 10,000 species are restricted to this habitat. Hundreds of species, especially bats, depend on caves for some part of their life cycle. Furthermore, the vegetation of karst regions in general and caves in particular is crucial for maintaining fragile local ecosystems (Biondi et al., 2011).

These caves are usually devoid of vegetation of higher plants but can host mosses (e.g. *Schistostega pennata*) and algal carpets, rarely ferns at their entry. They also host highly endemic cavernicolous fauna, including underground relic forms of a fauna which has been diversified outside. This fauna is mainly composed of invertebrates which exclusively live in caves and underground waters.

Taken together, these environments each support communities governed by their specific hydrological and geological attributes, underscoring the complexity and ecological importance of subterranean habitats in Europe and the need for their conservation.

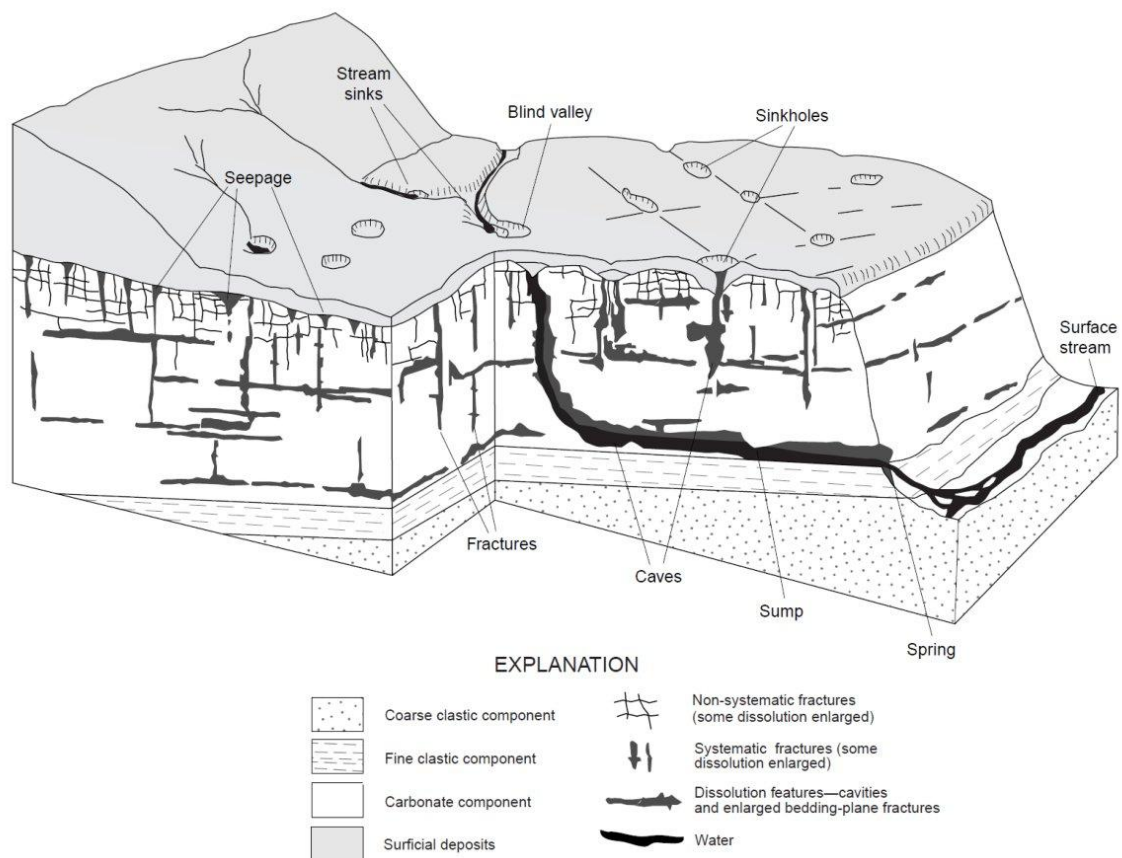
A classification of caves based on their characteristics developed by Robledo et al., (2019a) is presented in Table 1.

**Table 1. Classification of caves based on their characteristics**

Adapted from Robledo et al. (2019a)

Feature	Categories
<b>According to their lithology</b>	Karstic: carbonate, evaporite or quartzite other lithologies (igneous rocks, etc.)
<b>According to the type of development</b>	Horizontal Vertical
<b>According to their location</b>	Inland caves Coastal caves (karstic or marine)
<b>According to their altitude</b>	Alpine caves Mesoalpine caves Low-altitude caves
<b>According to the hydrogeological relationship of the aquifer waters</b>	Caves in vadose zone Caves in epi-phreatic zone Caves in phreatic zone

**Figure 1. Karst drainage (black) and related landforms**



Source: Lively (2020)

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Caves not open to the public are those that, at present, are not exploited by tourism and do not present human activity. They are protected under the Habitats Directive as habitats of Community interest and are defined in the Interpretation Manual of EU habitats (European Commission, 2013) as: Caves not open to the public, including their water bodies and streams, hosting high endemic species, or that are of paramount importance for the conservation of Annex II species (e.g., bats, amphibians).

The habitat type is present in 23 EU Member States: Austria (AT), Belgium (BE), Bulgaria (BG), Cyprus (CY), Czechia (CZ), Germany (DE), Estonia (EE), Spain (ES), France (FR), Greece (GR), Croatia (HR), Hungary (HU), Ireland (IE), Italy (IT), Lithuania (LT), Luxembourg (LU), Latvia (LV), Malta (MT), Poland (PL), Romania (RO), Sweden (SE), Slovenia (SI), and Slovakia (SK).

## 1.2 Environmental and ecological characterization and selection of variables to measure habitat condition

The condition of the habitat represented by caves not open to the public (8310) can be analysed considering their main abiotic and biotic characteristics, which can be measured by relevant associated variables, as described in the sections below.



The description of key characteristics of and the corresponding variables to measure habitat condition in these guidelines follows the ecosystem condition typology (ECT) defined in the System of Environmental Economic Accounting – Ecosystem Accounting (SEEA-EA) (United Nations, 2021), which is also proposed in the EU wide methodology to map and assess ecosystem condition (Vallecillo et al., 2022). According to this framework, ecosystem condition is the quality of an ecosystem measured in terms of its abiotic and biotic characteristics. The SEEA ecosystem condition typology establishes a common language to support increased comparability among different ecosystem condition studies. The ECT has six classes of characteristics: abiotic physical, abiotic chemical, biotic compositional, biotic structural, biotic functional and landscape.

The condition of caves is primarily determined by the stability of their microclimate. Variations in parameters such as temperature, humidity, ventilation, CO<sub>2</sub> levels, radon, and the physicochemical properties of water (if present) affect caves. These factors also influence the presence of cave fauna and the growth of organisms that can modify cave features, like green disease or moonmilk. In certain endokarst systems, the cave-aquifer relationship is critical, as intensive exploitation, contamination, or hydrodynamic changes directly impact the cave. Key drivers of environmental changes include contamination of infiltration waters (e.g., nitrates, pesticides), which disrupt chemical balances controlling dissolution and precipitation and may cause fauna loss. Temperature fluctuations affect both geochemical processes and fauna survival. Changes in CO<sub>2</sub> concentrations influence water chemistry and associated cave processes. Human interventions altering cave ventilation, such as opening new entrances, can disrupt cave conditions and fauna. Furthermore, modifications to water flow and infiltration—due to vegetation loss, urban development, or other actions in recharge zones—alter water volume entering the cave, impacting the cave ecosystem.

### 1.2.1 Ecological characterization of forests

The abiotic characteristics of subterranean habitats encompass geological, geomorphological, climatic, hydrogeological, and edaphic components. In cave environments, geological, geomorphological, and hydrogeological features are particularly influential. The habitat extends beyond the physical cave itself, being shaped by water dynamics—whether turbulent or diffuse—as well as by flooded zones within the cave, which can constitute distinct habitat types (Robledo et al., 2019a).

### Geology and geomorphology

The features that define caves are closely linked to the lithology of the host rock and its degree of fracturing. Caves predominantly form in carbonate rocks such as limestone and dolomite, as well as in evaporites like gypsum, anhydrite, and various salts. In tropical climates, caves can also develop in quartzites; if these are present in Europe, their existence suggests past climatic conditions markedly different from those of the present. Additionally, in Europe, caves occur in siliceous mountain ranges, where they are often smaller and structurally distinct from their carbonate counterparts, sometimes forming along fractures or geological boundaries rather than exclusively due to historical tropical climates.

The principal process responsible for cave formation is karstification—traditionally seen as the decarbonation of carbonate rocks. However, broader definitions include the dissolution of salts, hydrolysis, or even silica dissolution under specific conditions. Caves and cavities can develop in virtually any compact substrate, but their frequency and extent are far greater within karst massifs developed on soluble rocks such as gypsum, limestone, and dolomite. Nonetheless,

non-karstic processes can also generate caves—for example, in volcanic areas, where caves are mainly formed by the differential solidification of magmatic flows (Pérez-Alberti et al., 2009). Furthermore, significant cave systems have been documented in siliceous-clayey flysch rocks (such as in the Outer Carpathians), often resulting from gravitational deformation of slopes., as well as caves formed by the accumulation of granite blocks in the northwest of the Iberian Peninsula (Vidal-Romaní et al., 2010).

It is important to recognise that caves rarely exist as isolated geological forms. Most are components of complex systems where processes, forms, and products are interconnected, with water acting as the primary driving force of these networks. In this context, permeability plays a critical role, controlling the rate and pathways of fluid circulation through the rock. Permeability, defined as a rock's ability to transmit fluids (water, oil, gas) under certain pressure and temperature conditions, is thus fundamental to both the formation and the ecological function of caves. As such, cavities can be characterised and quantified by their pore volume and permeability (Robledo et al., 2019a).

### Climate characteristics

Cave climate encompasses key climatic factors within caves. These depend on latitude and elevation, which govern overall temperature, as well as cave geometry—including the size and number of entrances—which influences temperature variation, airflow, and humidity. Occasionally, the types and volumes of gases present can also affect the cave's climate.

The climate regime determines subsurface processes such as infiltration, dissolution, and precipitation. In arid and semiarid regions, limited precipitation slows cementation, causing minimal changes in porosity. In contrast, temperate and tropical regions with higher rainfall experience accelerated geochemical processes and greater porosity changes (Choquette & James, 1987).

Temperature in caves generally establishes with depth. Caves with a single small entrance are most stable, while those with multiple entrances show increased airflow and temperature fluctuations. Despite the general increase in underground temperature with depth, caves are usually cold because percolating meteoric waters in the vadose zone maintain internal temperatures close to the outside environment (Colucci et al., 2016). Karst massifs tend to be cold and unaffected by geothermal heat, as cave systems increase permeability and lower rock temperatures.

Internal morphology can also create thermal stratification by trapping air in ascending or descending branches, producing thermal hotspots near cave entrances (Colucci et al., 2016). For example, Cucchiara Cave (Sicily) displayed ceiling temperatures of 36.6°C and ground temperatures of 26.3°C at 2 meters depth, indicating significant thermal layering.

**Temperature** is a crucial environmental parameter for bats. Breeding colonies can tolerate up to 38–40°C, but hibernation shelters require more specific ranges—typically 2–8°C in caves and around 0°C in hollows. Tolerances differ by species: *Barbastella barbastellus* withstands -2°C to 4°C, *Myotis* species prefer 2–6°C, *Miniopterus schreibersii* 5–9°C, and *Rhinolophus* species 7–10°C (Valenciuc & Valenciuc, 1973; Ransome, 1990).

**Relative humidity** in caves is also extremely important for bats, with optimal levels near saturation (100%) to prevent dehydration through water loss over the patagium (Borda & Borda, 2009).

**Carbon dioxide** (CO<sub>2</sub>) significantly affects cave microclimates and ecosystem health. Elevated CO<sub>2</sub> may result from organism respiration or organic matter decay. At illuminated cave entrances, CO<sub>2</sub> levels can indicate the photosynthetic activity of autotrophs like algae and mosses, as lower CO<sub>2</sub> suggests higher rates of photosynthesis. Abnormal concentrations may reveal ecological disturbances, such as changes in species composition or increased organic decay.

CO<sub>2</sub> also plays a vital role in nutrient cycling and chemical weathering by driving mineral dissolution, crucial for speleothem formation and other geological processes (Martin-Pozas et al., 2022; Xu et al., 2024). It supports diverse microbial communities operating various CO<sub>2</sub>-fixation pathways, such as the Calvin-Benson-Bassham cycle, which supports chemoautotrophic processes (Ortiz et al., 2013; Tebo et al., 2015). Methanotrophic and other CO<sub>2</sub>-utilising microbes contribute further to carbon sequestration and nutrient cycling (Martin-Pozas et al., 2022; Xu et al., 2024).

Moreover, CO<sub>2</sub> influences microbial community composition and processes like methane oxidation and moonmilk formation, a calcite deposit of microbial origin (Martin-Pozas et al., 2022, Carmichael et al., 2015). Environmental factors including pH and ions like Ca<sup>2+</sup> and NO<sub>3</sub><sup>-</sup> also affect microbial diversity and carbon cycling in caves (Xu et al., 2024).

### Hydrogeological characteristics

A very important component in caves is water circulation. In the hydrological processes of caves in karst areas there is significant variability in water circulation, which is the result of both the characteristics of the karst itself and the temporal variability of the climate. This, together with precipitation on the surface and the discharge within the cavity, means that the dynamics of karst systems are not linear (Baker & Brunsdon, 2003) and can only be modelled using non-linear models (Labat et al., 2000a; 2002b; 2000c), due to both the physical karst heterogeneity (in space) and the dynamic variability (in time) together with non-Gaussian inputs (rain) and outputs (flow).

### Edapho-sedimentary characteristics

Sediment accumulations commonly occur inside many caves, resulting from both internal processes such as dissolution and chemical precipitation, and external processes related to water circulation. These sediments serve as important environmental and sedimentary archives and provide substrates for colonising species. Research on rock shelters and caves has investigated the climatic variability recorded in these sedimentary layers. Two primary factors determine a cave's usefulness as an environmental archive: the temporal resolution of the sediment deposition and the sensitivity of the environment to change (Woodward & Goldberg, 2001).

Clastic sediments within caves are composed of rock fragments that have been transported and redeposited, either by water flow—moving gravels and mud—or by gravity-driven flows such as slides and detrital movements. Sedimentology and stratigraphy enable the identification of sediment origins and transport mechanisms by jointly analysing stratification, grain size, sorting, mineralogy, geochemistry, and sedimentary structures. Specific sedimentary facies are then identified based on the combination of these diagnostic features, reflecting the governing depositional processes or forces (Springer, 2019).

**Table 2. Overview of abiotic characteristics relevant to habitat type 8310, including links to habitat condition and relevant measurement variables**

Abiotic characteristics	Link to habitat condition	Relevant variables to measure
<b>1. Physical state characteristics</b>		
<b>Geological and geomorphological</b>		
<b>Lithology</b>	Influences cave formation and biodiversity.	Rock type (e.g., limestone, gypsum), degree of fracturing.
<b>Speleogenesis</b>	Determines cave morphology and species niches.	Rate of karstification, presence of karst features.
<b>Cave morphology</b>	Affects airflow, temperature gradients, and moisture retention.	Internal cave structure (chambers, passages), ceiling height.
<b>Hydrogeological characteristics</b>		
<b>Water flow dynamics</b>	Affects nutrient transport and sediment distribution.	Water flow rates, types of water flow (turbulent vs. diffuse).
<b>Hydrology</b>	Influences ecological dynamics and nutrient cycling.	Water levels and discharge rates, seasonal variations.
<b>Climatic characteristics</b>		
<b>Temperature</b>	Stability affects species distribution and behaviour, especially bats.	Temperature inside the cave, at various depths, seasonal variations.
<b>Humidity</b>	High levels are crucial for preventing dehydration in cave fauna.	Relative humidity levels, changes with airflow.
<b>Airflow</b>	Influences temperature and humidity, impacting the cave microclimate.	Airflow velocity and direction, number and size of entrances.
<b>CO<sub>2</sub></b>	Critical component of nutrient cycles plays a significant role in both the chemical weathering of rocks and the availability of nutrients for microbial communities.	CO <sub>2</sub> concentration measured with gas analysers.
<b>Edapho-sedimentary characteristics</b>		
<b>Stratigraphy</b>	Reflects past environmental conditions and influences habitat suitability.	Sediment layer thickness and composition, temporal resolution.
<b>Sediment composition</b>	Serves as ecological archives and substrates for species.	Types of sediments (clastic, chemical), origin and transport.
<b>2. Chemical state characteristics</b>		
<b>Chemical properties of soil and sediments</b>	Influence nutrient availability and ecosystem health.	pH and soil nutrients, including nitrogen, phosphorus and organic matter content.
<b>Water chemical characteristics</b>	Influence aquatic life and overall ecosystem health.	Presence of chemical elements in (e.g., calcium, magnesium, iron). Concentration of nutrients: nitrates and phosphates.





Agttelek karst cave in Hungary.  
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### 1.2.2 Main ecological characteristics and identification of variables to measure habitat condition

Caves are environments devoid of natural daylight and are thus inhabited by species specially adapted to life in darkness. Microclimatic conditions vary considerably both within and among caves, influencing the composition and distribution of fauna and flora. Many cave species rely on detritus originating from the surface for sustenance, while others are. The ecological importance of cave habitats lies in their support of highly specialized and often endemic organisms, some of which are protected under Annex II of the Habitats Directive. This is particularly true for species such as bats, which use caves as breeding and shelter sites, and certain amphibians.

Cave-dwelling species are typically categorized into three ecological groups (Howarth & Moldovan, 2018):

- **Troglobionts:** Obligate cave-dwellers that exhibit specialized morphological traits, such as loss of pigmentation and regressed eyes.
- **Troglophiles:** Facultative cave species that can establish permanent populations in caves but are also found in surface habitats.
- **Trogloxenes:** Species that utilize caves for only part of their life cycle, often for shelter or reproduction.

Due to the absence of light, photosynthetic organisms are generally confined to the entrance zones of caves. In these transitional areas, vascular plants typical of rocky habitats and

requiring high humidity—such as certain ferns (*Phyllitis*, *Polypodium*)—may be found, alongside mosses like *Eucladium verticillatum* and algal carpets. Vascular plants are usually restricted to well-lit areas near cave entrances, while mosses and algal mats can extend further into the cave. Deeper sections, where light is absent, support primarily non-photosynthetic organisms. The cave flora thus consists mainly of mosses and algae. Additionally, microbial communities such as bacteria and archaea contribute to the cave's ecological balance (Barton & Northup, 2007).

However, in show caves or artificially lit environments, lampenflora, a term for plant and microbial growth stimulated by artificial lighting, can alter natural cave dynamics (Figure 2). While the entrance zone undergoes natural ecological succession, the artificially lit dark zones experience lampenflora-induced biodeterioration, which may threaten cave walls, speleothems, and endemic biota (Kwaśnicka et al., 2022).

Cyanobacteria serve as effective indicators of nutrient levels and water quality in cave habitats due to their sensitivity to environmental changes and their ability to produce specific pigments and toxins. They can provide insights into the ecological conditions of these environments.

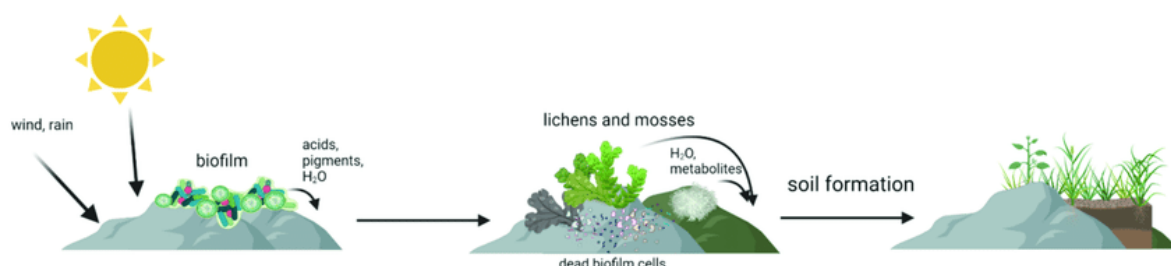
Changes in cyanobacterial community structure, such as species richness and diversity indices, can indicate variations in nutrient levels. For instance, a decrease in species richness and diversity is often associated with increased nutrient loads, as seen in running waters (Douterelo et al., 2004). In caves, cyanobacteria diversity is influenced by seasonal changes and environmental parameters like humidity and water content in biofilms (Popović et al., 2020). The prevalence of certain cyanobacterial orders, such as Oscillatoriales in nutrient-rich environments and Nostocales in nutrient-poor areas, can signal nutrient levels (Douterelo et al., 2004). This differentiation can be used to assess the nutrient status of cave waters.

Cyanobacteria produce pigments like chlorophyll f and d, which allow them to thrive in low-light conditions typical of caves. These pigments can indicate the presence of specific light conditions and nutrient availability (Behrendt et al., 2020). Although not all cyanobacteria produce toxins, the presence of genes related to cyanotoxin production, such as cylindrospermopsin, can indicate potential water quality issues. However, the absence of certain toxin genes, like microcystins, in cave samples suggests a lower risk of toxin-related water quality problems in these environments (Jablonska et al., 2024).

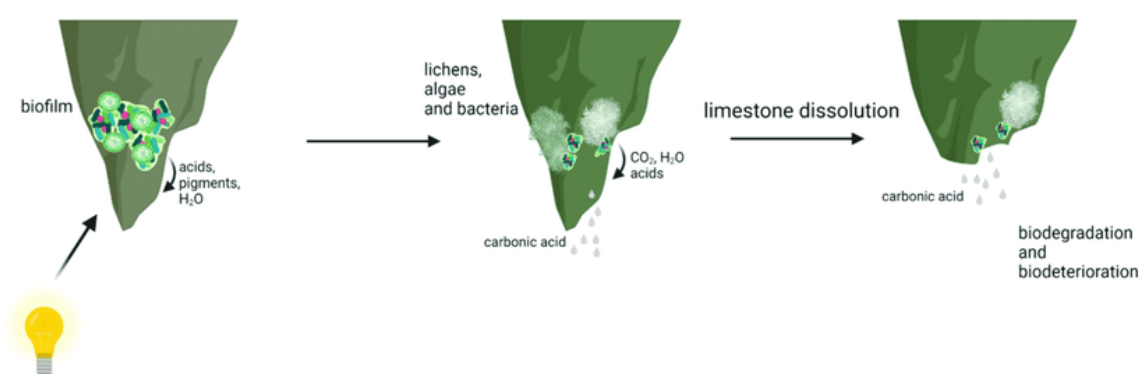
One of the most fascinating aspects of the cave environment is the presence of highly endemic fauna. Cave-dwelling fauna mainly comprises terrestrial or aquatic invertebrate species, many of which are restricted to subterranean habitats. These organisms inhabit either the terrestrial sections of caves or water bodies within the endokarst system. Invertebrate groups include beetles, crustaceans, arachnids, and molluscs, with many species exhibiting extremely limited or endemic distributions due to the isolated and restrictive nature of their habitat. Among terrestrial Coleoptera, most species belong to the Bathysciinae and Trechinae subfamilies; these are carnivorous beetles with very narrow geographical ranges. Aquatic invertebrates also exhibit a high degree of endemism and are dominated by crustaceans such as isopods, amphipods, syncarids, and copepods. Some are considered living fossils. Aquatic molluscs of the Hydrobiidae family are also commonly found.

**Figure 2. Differences between natural succession in cave entrance zone and lampenflora-induced biodeterioration in cave dark zone**

**A** Ecological succession at cave entrance zone



**B** Lampenflora-induced biodeterioration



The figure illustrates the contrasting ecological dynamics between the cave entrance zone (A), where natural succession promotes the growth of autotrophic algae and higher plant life, and the dark zone (B), where lampenflora-induced biodeterioration leads to the degradation of rock surfaces by phototrophic microorganisms. The presence of autotrophic algae in the entrance zone supports the development of a diverse biofilm ecosystem, facilitating nutrient cycling and providing essential resources to heterotrophic organisms, while the dark zone's microbial activity disrupts the substrate, paving the way for higher forms of life like lichens and mosses to establish themselves on the altered limestone.

Source: Kwaśnicka et al., (2022).

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Regarding vertebrates, caves serve as hibernation sites for many bat species, several of which are endangered. These species, many of which are listed in Annex II of the Habitats Directive, are highly vulnerable, thereby increasing the conservation value of caves as habitats of Community interest (European Commission, 1992). Other iconic hibernators that depend on caves to optimize their energy balance during winter are brown bears (*Ursus arctos*). These large carnivores require specific den characteristics for successful overwintering in the Central Alps; namely, a small entrance, suitable length, location in wooded areas, and, higher internal temperatures during winter (Chirichella et al., 2018). Caves are also inhabited by rare amphibians, such as the blind cave salamander *Proteus anguinus*, which is among the most iconic and endangered cave vertebrates in Europe (Kostanjšek et al., 2023).

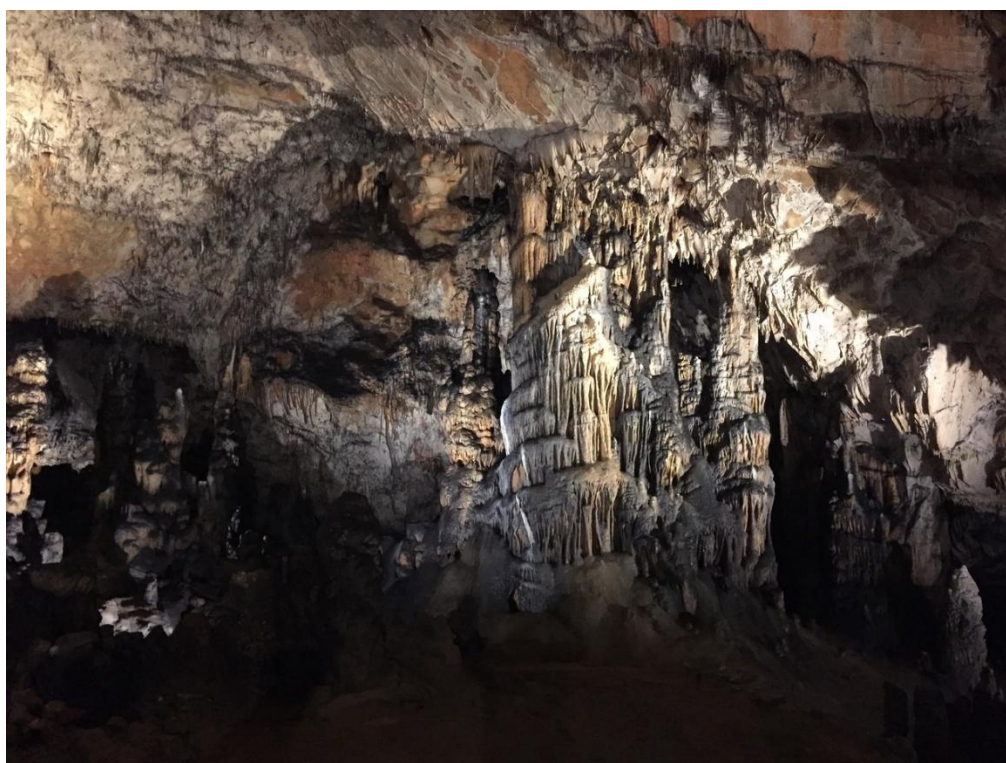
Species adapted to subterranean life often exhibit similar morphological traits, known collectively as the troglomorphic phenotype, which evolved independently from surface-dwelling (epigean) ancestors (Ribera et al., 2018). These traits typically include loss of pigmentation, eye regression, and elongation of appendages. Troglomorphic species also tend to have very limited distributions, largely due to their low dispersal capacity. Until recently, the prevailing theory suggested that such traits evolved independently in geographically distant



species, implying a form of convergent or orthogenetic evolution. However, recent advances in ecological and phylogenetic studies have challenged this view. Evidence now suggests that epigean and subterranean environments form a continuum, with troglomorphic species capable of occupying different zones along this gradient, depending on ecological conditions. Moreover, molecular phylogenetic analyses have revealed monophyletic lineages composed exclusively of troglomorphic species. This supports the hypothesis of a single colonisation event followed by subterranean adaptation, dispersal, and diversification.

The cave flora and fauna of northern Europe are relatively impoverished when compared with those of southern Europe. This is largely due to the glaciation events of the Pleistocene, which rendered large parts of northern Europe uninhabitable for cave fauna, resulting in the dominance of more recent colonisers. In contrast, much of southern Europe escaped glaciation, apart from specific mountainous areas, and consequently hosts a richer community of relict troglobites. Although caves not open to the public are distributed throughout Europe, they are especially common in the limestone regions of southern Europe. These southern European caves often support a diverse array of troglobitic species, many of which are endemic.

On the sensibility of caves to invasive alien species impact, a systematic literature survey conducted by Nicolosi et al. (2023) on invasive alien species on subterranean habitats, indicated that despite the extreme environmental filters of subterranean ecosystems (i.e. perpetual darkness and scarce food resources), which drive specialized adaptations, reports of alien species in these habitats have increased in recent decades. The study reveals that the impact of alien species in caves is still unknown in most cases with only a 22.7% of the cases reviewed reporting negative biological consequences. Invertebrates, mainly insects and arachnids, were found to be the dominant alien species (Nicolosi et al., 2023). The biotic characteristics discussed in the preceding section are summarized in Table 3.



Agttelek karst cave in Hungary.  
© Jozef Sibik, 2017.



**Table 3. Overview of the biotic characteristics relevant to habitat type 8310**

Biotic characteristics	Link to habitat condition	Relevant variables and measurement methods
<b>1. Compositional state characteristics</b>		
<b>Presence of ferns, mosses, lichens, vascular plants</b>	Indicates habitat diversity and ecological stability.	Species surveys, quadrat sampling
<b>Presence of key vertebrate species</b>	Indicates biodiversity and conservation value of the habitat.	Fauna surveys, trapping methods, visual counts, acoustic monitoring for bats.
<b>Diversity indices of invertebrates</b>	Higher diversity suggests a more stable and resilient ecosystem.	Collection and identification, diversity metrics
<b>Invasive species presence</b>	Degradation of cave ecosystem	Inventory of species present and assessment of invasiveness. Impact on native species and ecosystem functioning
<b>2. Structural state characteristics</b>		
<b>Cover and distribution of mosses, algae and vascular plants</b>	influences biodiversity and ecological interactions	Percentage cover of mosses and algae on rock surfaces and in damp areas within the cave
<b>3. Functional state characteristics</b>		
<b>Breakdown of organic matter by decomposers</b>	Indicates decomposition activity in caves	Rates of decomposition (e.g., litter decomposition rates) Abundance and diversity of decomposer organisms (e.g., fungi, bacteria)
<b>Microbial community diversity</b>	Essential for nutrient cycling and overall cave ecosystem functioning.	Diversity of microbial taxa (e.g., bacteria, archaea). Functional traits of microbial communities (e.g., nitrogen-fixing capabilities). Molecular techniques
<b>Trophic relationships and interactions</b>	Essential for understanding food web dynamics and ecosystem interactions.	Feeding relationships and dietary composition of key species (e.g., bats, invertebrates). Field observations, stable isotope analysis, eDNA

Landscape characteristics, including adjacent habitats and the vegetation cover of surrounding areas, significantly influence cave systems. These aspects are often disrupted by human activities such as mining, quarrying, agriculture, and urbanisation, leading to impacts on the movement of cave species between the cave and external environments, changes in water infiltration, and increased groundwater contamination. An overview of the landscape characteristics and other anthropic disturbances are presented in Table 4.

**Table 4: Landscape characteristics and disturbance alterations relevant to habitat type 8310**

Characteristics	Link to habitat condition	Relevant variables and measurement methods
<b>Landscape characteristics</b>		
<b>Matrix quality / edge effects</b>	influences habitat quality, species movement and survival	Land use types and human impact in the surrounding area. Habitat quality assessments of areas adjacent to the caves.
<b>Other characteristics: Disturbances</b>		
<b>Contamination of infiltration waters</b>	Impact water quality and ecosystem health.	Presence of pollutants in water. Concentrations of nitrates, pesticides, and other contaminants. Water quality assessments (e.g., chemical analysis)
<b>Modifications in water flow</b>	Changes in the natural hydrology that can affect water supply and ecological processes within the cave	Water flow rates and patterns. Impact assessment on cave biota due to altered flow conditions.
<b>Alterations in infiltration zones</b>	Influencing the volume and quality of water supply.	Changes in the volume of water that infiltrates into the cave. Human activities that impact areas where water enters the cave.
<b>Access and visitation impacts</b>	Degradation of cave ecosystem and disturbance to species inhabiting the cave	Signs and effects of visitors (trampling, fire, etc.). Assessment of damage to cave formations and habitats

### 1.3 Selection of typical species for condition assessment

The Habitats Directive uses the term ‘typical species’, but it does not give a definition for use in reporting. For a habitat type to be considered in favourable conservation status, the Habitats Directive requires that both its structure functions and its ‘typical species’ be in favourable status (Article 1(e)).

The assessment of typical species is part of the assessment of the structure and function parameter, however little guidance has been provided on how to use the typical species in this assessment.

The formulation of Art. 1(e) would suggest that the assessment of typical species could be carried out separately and complement the assessment of structure and function. In this regard, the selection of typical species should be as robust and appropriate as possible.

The Guidelines for Article 17 reporting<sup>1</sup> (European Commission, 2023) provide some definitions and interpretations regarding typical species, such as the following:

- The assessment of typical species is part of the assessment of the structure and function parameter; however, a full assessment of the conservation status (as for species listed in Annexes II, IV and V) of each typical species is not required.
- The selection of 'typical species' should reflect favourable structure and functions of the habitat type.
- Typical species should include species which are good indicators of favourable habitat quality, they should include species sensitive to changes in the condition of the habitat ('early warning indicator species').
- Typical species may be drawn from any species group and, although often most species reported were vascular plants, consideration should be given to also selecting lichens, mosses, fungi, and animals, including birds.
- The sum of sites and occurrences of each habitat type should support viable populations within the region being assessed of the typical species on a long-term basis for Structure and functions to be favourable.
- Given the ecological and geographical variability of the Annex I habitat across their range, even within a single biogeographical region, it is very unlikely that all typical species will be present in all examples of a given habitat type, particularly in large Member States. Indeed, even within one Member State different species may be present in different parts of the range of a habitat type or in different subtypes.
- Some species may be typical for several habitats (including non-Annex I habitats) and not dependent on a single Annex I habitat type.

**All MSs have communicated a list of typical species for each habitat type<sup>2</sup>**, although usually they have not provided any justification or rationale for their selection. The variability of the selection of typical species by MSs seems to indicate that different interpretations are done on the concept of typical species. Mostly, plants are proposed as typical species (> 90% of the selected species). However, species from other taxonomic groups are also considered (e.g., lichens, insects, birds, mammals...)

It is advisable to select species from other taxonomic groups than plants, such as fungi, lichens, animals, micro-organisms. This is a challenge as plant data is more abundant and accessible.

It can be useful to consider key functional groups for the selection of typical species, considering the habitat's ecology, the role of typical species as bioindicators (e.g. decomposers, trophic and symbiotic relationships, etc.) and their sensitivity to changes. Table 5 provides an illustrative list of species' groups that can be used as indicators to assess caves habitat condition.

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<sup>1</sup> Guidelines for Article 17 reporting: [https://cdr.eionet.europa.eu/help/habitats\\_art17/Reporting2025/FinalGuidelines\\_Art.17\\_2019-2024.pdf/](https://cdr.eionet.europa.eu/help/habitats_art17/Reporting2025/FinalGuidelines_Art.17_2019-2024.pdf/)

<sup>2</sup> The list (for all habitat types and MSs) is available at: <https://cdr.eionet.europa.eu/help/habitatsart17>

Troglobites (obligate cave-dwellers with morphological adaptations) are the most specialised species and could therefore be considered as species typical of the habitat, but collecting them requires specialists which also explain the lack of surveys and knowledge about these species. Troglobites include species of plathelminthes (*Dendrocoelum collini*), oligochaetes (Enchytraeidae, Lumbriculidae, Tubificidae), molluscs (*Avenionia roberti*), cladoceran crustaceans (e.g. *Alona phreatica*), copepod crustaceans (notably of the genera *Acanthocyclops*, *Diacyclops*, *Eucyclops*), amphipod crustaceans (Crangonyctidae, Gammaridae, Niphargidae), isopod crustaceans of the Asellidae (notably *Proasellus cavaticus*, *P. hermallensis*, *P. meridianus*), ostracode crustaceans (Candonidae, Cyprididae), springtails (*Schaefferia willemi*, *Tomocerus unidentatus*), beetles (*Tychobythinus belgicus*) (Hendrickx et al, 2021).

Troglophilous species (which can carry out their entire development cycle in caves without being totally dependent on them) include flatworms (plathelminthes) such as the *Fonticola notadena*, spiders such as the very common *Meta menardi*, myriapods, and gastropods (*Oxychilus draparnaudi*) (Hendrickx et al, 2021).

**Table 5. Selecting typical species for monitoring habitat 8310**

Species Group	Sub-type	Ecological role: bioindicator	Sensitive to changes in quality
<b>Mosses</b>	<i>Fontinalis</i> spp.	Indicate moisture levels and heavy metal contamination in aquatic ecosystems	Sensitive to changes in humidity and nutrient levels (Say & Whitton, 2004).
<b>Algae</b>	Cyanobacteria	Indicate nutrient levels and water quality	Sensitive to changes in light conditions and pollution
<b>Bacteria</b>	<i>Pseudomonas</i> spp.	Indicate organic matter decomposition and nutrient cycling	Sensitive to changes in organic pollution and nutrient loads
<b>Invertebrates</b>	Collembola (Springtails)	Biodiversity	Highlighting the rich biodiversity and the importance of these organisms in cave ecosystems (Baquero et al., 2023).
	Cave Spiders ( <i>Centromerus</i> spp.)	Biodiversity	Critically endangered cave spiders have been recorded in the Madeira caves, emphasizing the need for monitoring to ensure their conservation
	Cave-dwelling amphipods	Indicate habitat health and water quality	Sensitive to changes in water chemistry and habitat structure
<b>Amphibians</b>	<i>Proteus anginus</i>	Indicate ecosystem health and moisture availability	Sensitive to changes in humidity, temperature, and pollutants
<b>Bats</b>	Cave-roosting bats	Indicators of landscape integrity	Their diversity and community structure can reflect the health of both underground and aboveground ecosystems



## 2. Analysis of existing methodologies for the assessment and monitoring of habitat condition

The analysis presented in this section has been done based on the methodologies available from Austria, Belgium (Wallonia), Bulgaria, Czechia, Germany, Italy, Lithuania, Poland, Romania, and Spain. Greece and Slovenia also include habitat 8310 in their methodologies, although the variables proposed are not specific for caves but apply also to other rocky habitats or to all open habitats. Therefore, they have not been considered in this analysis.

A table with a detailed description of the main variable used by the Member States, including metrics, measurement methods and thresholds, is presented in Annex 1.

### 2.1 Variables used, metrics and measurement methods, existing data sources

The majority of variables included in existing methodologies for monitoring habitat 8310 primarily focus on the measurement of physical characteristics. Chemical variables are largely absent, with only one national methodology, from Spain (Robledo et al., 2019b), including such parameters. In terms of biotic variables, those that are described focus mainly on the monitoring of invertebrates, bats, bryophytes, and mosses. Only one methodology proposes a variable related to microbial biodiversity, indicating a significant gap in current monitoring approaches.

Only one variable identified in this review relates to landscape characteristics, which involves recording the transitional nature of adjacent habitats. Additionally, several methodologies include variables aimed at detecting anthropogenic disturbances, further highlighting the growing awareness of human impacts on subterranean systems across EU Member States.

All the methodologies analysed include at least some **physical variables** for the monitoring of caves not open to the public (see Table 6). Cave climate conditions, including those of microcavities, are measured in four national methodologies: Spain (Robledo et al., 2019b), Romania (Vlaicu et al., 2013), Poland (Urban & Piksa, 2015), and Belgium – Wallonia (Hendrickx et al., 2021). Air temperature and microcavity temperature are measured using thermometers placed in different cave sections and at multiple times throughout the year, enabling the documentation of both seasonal and annual variations. Humidity is also measured at various points within the cave, corresponding to its distinct microclimatic zones. Modern thermohygrometers are commonly used, with measuring points determined by the cave's morphology, number of entrances, and ventilation type. As a general recommendation, measurement stations should be positioned near the proximal zone of the cave.

Ventilation is explicitly monitored in Romania (Vlaicu et al., 2013), due to its critical influence on both temperature and humidity. In caves with a single entrance, ventilation is bidirectional and may be permanent or seasonal, requiring measurements at both floor level and vault level to capture the vertical stratification of airflows. For instance, during summer, warm air enters the cave through the vault while cooler internal air exits at floor level. This pattern reverses in winter. In ascending or descending caves, ventilation remains seasonal and unidirectional, limited to a single season—summer for ascending caves and winter for descending ones. In both cases, airflow measurements are required at both vertical levels.

Water accumulation and source identification are assessed in two MSs through expert field assessment. Romania's methodology (Vlaicu et al., 2013) distinguishes between precipitation-based microdroplets and waterfall-fed sources.

Several methodologies also include the analysis of typical cave structures and speleothems, conducted through expert field evaluations. This is the case for methodologies from Spain (Robledo et al., 2019b), Poland (Urban & Piksa, 2015), Germany (BfN, 2017), Belgium – Wallonia (Hendrickx et al., 2021), and the Czech Republic (Lustyk, 2023). Cave morphology and volume are likewise recorded during fieldwork, with changes assessed by comparison against historical data (e.g., Romania: Vlaicu et al., 2013; Poland: Urban & Piksa, 2015; Bulgaria: MOEW, 2013).

Lithuania's methodology (Rašomavičius, 2015) is the only one to include the percentage of cave shading as a variable. However, no information is provided regarding the method of measurement.

With regard to **chemical variables**, only the Spanish methodology (Robledo et al., 2019b) includes detailed protocols for measuring chemical characteristics within its cave monitoring programme. Specifically, seasonal and annual CO<sub>2</sub> concentrations are recorded with four measurements per year, although the measurement method is not specified. In caves containing endokarst lakes, physicochemical parameters of the water—such as pH, conductivity, and temperature—are also monitored on a quarterly basis. Additionally, the ionic and cationic composition of water, as well as the presence of heavy metals, is measured twice per year at different locations within the cave system.

**Compositional variables** in the reviewed methodologies focus primarily on invertebrate species and bats. Monitoring targets include troglobitic species such as crustaceans, carabid beetles, and colevids. Angelini et al. (2016) describe several capture techniques for these taxa: visual observation; the use of sleeved nets, aspirators, and tweezers; various types of trapping (e.g., submerged pots for aquatic crustaceans and baited traps for beetles); and continuous water filtering to collect fauna from dripping water or resurgences. For caves with endokarst lakes, Robledo et al. (2019b) recommend the monitoring of coliform bacteria and nematodes, though no specific methodologies are provided. Similarly, while the monitoring of wintering bat populations is indicated in the Polish methodology (Urban & Piksa, 2015), no details regarding the methods of data collection are included.

The vegetation composition outside the cave and in its immediate surroundings is addressed in Angelini et al. (2016), which includes general assessment but no detailed sampling procedures. The presence of alien species is noted exclusively in the Lithuanian methodology (Rašomavičius, 2015), though again, neither the specific methods nor a list of targeted species is provided.

In the same Lithuanian methodology, a number of **structural variables** are listed for habitat assessment, yet no measurement techniques or threshold values are defined. These variables include the coverage of mosses, lichens, and exposed rock, as well as the presence of expansive species, shrubs, trees, and the percentage of bare rock.

Regarding the only **landscape** variable found in the methodologies considered in this analysis, the presence of natural or semi-natural plant communities and the use of adjacent areas is described (width of the transition zone, type of adjacent communities, use of adjacent territories, etc.) and the transition zone, including its width, is supposed to be measured, but no methods not thresholds are provided (Rašomavičius, 2015).

Variables related to anthropogenic **disturbances** have been incorporated into several national monitoring methodologies. These variables primarily address factors such as cave accessibility, the presence of pollution, and various other forms of human disturbance, including recreational use. Their inclusion reflects a growing recognition of the pressures exerted by human activity on subterranean environments.

A summary of the main characteristics and variables used in the national methodologies reviewed in this analysis is presented in Table 6 provides further details on the corresponding measurement methods and, where available, threshold values.

**Table 6. Main characteristics and variables used in national methodologies for the assessment and monitoring of habitat 8310**

Ecological characteristics	Variables	AT	BE	BG	CZ	DE	ES	IT	LT	PL	RO
<b>1. Abiotic characteristics</b>											
<b>1.1 Physical state characteristics</b>											
<b>Climatic</b>	Air temperature										
	Humidity										
	Ventilation										
	CO2										
<b>Hydrology</b>	Hydrological regime, piezometry (amount of groundwater)										
<b>Cave morphology and shape</b>	Speleothems, morphology, volume, diversity of biotopes										
<b>1.2 Chemical state characteristics</b>											
<b>Water chemical status</b>	pH, conductivity, temperature, ions and cations, heavy metals										
<b>2. Biotic characteristics</b>											
<b>2.1 Compositional state characteristics</b>											
<b>Microbial components</b>	Fecal coliforms, nematodes, etc.										
<b>Vegetation</b>	Vegetation at the cave entrance										
<b>Fauna</b>	Presence and number of bats and invertebrates										
<b>Invasive alien species</b>	Presence of IAS										

Ecological characteristics	Variables	AT	BE	BG	CZ	DE	ES	IT	LT	PL	RO
<b>2.2 Structural state characteristics</b>											
<b>Vegetation cover</b>	Cover of mosses										
<b>3. Landscape characteristics</b>											
<b>Surrounding conditions</b>	Natural communities and use in adjacent areas										
<b>4. Other</b>											
<b>Anthropic pressures, pollution</b>	Human access Signs of pollution Fire										

## 2.2 Definition of ranges and thresholds to obtain condition indicators

Some national methodologies include thresholds for variable assessment, typically expressed using qualitative categories. These are often aligned with established conservation status frameworks. For example, thresholds may be categorised as excellent (A), good (B), or medium-poor (C) (BfN, 2017; Ellmauer, 2005), or may correspond to the three categories used in the overall assessment of conservation status parameters under the Habitats Directive: favourable (FV), unfavourable–inadequate (U1), and unfavourable–bad (U2) (Urban & Piksa, 2015). However, in most cases, the methodologies do not explain how these thresholds are determined, limiting their transparency and reproducibility.

In some instances, thresholds appear to be based on observed changes in selected variables over time. For example, in the Polish methodology (Urban & Piksa, 2015), the absence of significant changes in cave temperature and humidity is assessed as favourable (FV). In contrast, major alterations that impact the thermal regime, microclimate, or air circulation are classified as unfavourable–bad (U2), while moderate changes fall under unfavourable–inadequate (U1).

Additional examples of thresholds applied to specific variables in the national methodologies reviewed are provided in Annex. In some cases, these thresholds are expressed as clear boundaries, such as the absence, moderate presence, or minimal presence of negative indicators. Similarly, changes in certain parameters may be categorised as none, moderate, or significant. In other cases, particularly those involving the presence or abundance of species, assessments are conducted through visual inspection, with thresholds applied using expert judgement rather than quantitative metrics.

## 2.3 Aggregation methods at the local scale

The aggregation of the results obtained from the measurement of the variables proposed for the assessment and monitoring of caves is not always described in the methodologies analysed.

The aggregation of results from measured variables used to assess and monitor cave habitats is not consistently described across the methodologies analysed. In some cases, the overall



assessment framework remains vague, with no clear indication of how individual variable outcomes are synthesised to determine habitat conservation status.

In the Polish methodology (Mróz, 2017), the overall conservation assessment is primarily based on the lowest value among the variables measured. For instance, three variables rated as favourable (FV)—or possibly two FV ratings and one not assessed (XX)—will result in an overall assessment of FV. If one or more variables are rated as unfavourable—inadequate (U1), but none as unfavourable—bad (U2), the overall assessment becomes U1. If any variable receives a U2 rating, the final assessment is U2. However, this general rule is only partially applicable, as the methodology distinguishes two cardinal parameters that hold primary importance in determining the outcome: (1) changes in cave microclimate and (2) anthropopression and cave accessibility. If either of these parameters is rated poorly, it automatically lowers the rating of the “structure and function” parameter, overriding more favourable scores in other areas.

In contrast, the methodologies from Austria and Germany employ an aggregation method based on categorical majority rules (Kroiher et al., 2017). These approaches define pre-established combinations of ordinal condition categories—such as excellent, good, or bad condition—to evaluate habitat status. Variables are grouped into thematic sets to generate partial scores, typically based on: (1) the completeness of typical habitat structures, (2) the presence of habitat-typical species, and (3) the degree of impairments. The final assessment is then derived from a combination of these partial scores and generally reflects the majority condition across the grouped variables.

#### **Box 1. Example of categorical majority rules (combination of qualitative assessment of variables) in Germany**

First, criteria (variables) are included in the following three groups: 1) completeness of the typical habitat structures, 2) completeness of the habitat typical species inventory, 3) impairments.

Then, a categorical status is given to each of these variable groups: **Excellent, Good and Medium-poor.**

Subsequently, the following rules are applied considering the status of the three criteria:

- All three criteria share the same status -> common status condition
- Two criteria share the same status -> in general, more common status condition, e.g. 2 Excellent and 1 Good -> Excellent
- But, if there is a C rating, an overall rating of A is not possible, so 2 Excellent and 1 Medium-Poor -> Good status
- 1 Excellent, one 1 Good and 1 Medium-poor -> Good status condition

## **2.4 Aggregation at biogeographical scale**

Although some older methodologies could have considered different aggregation methods at the biogeographical scale, in the last reporting period (2013-2018) all MSs should have followed the Article 17 reporting guidelines, which established that “if 90% of habitat area is considered as in ‘good’ condition, then the status of ‘structure and functions’ parameter is

'favourable'. If more than 25% of the habitat area is reported as 'not in good condition', then the 'structure and functions' parameter is 'unfavourable-bad'".

## 2.5 Selection of localities

Overall, the methodologies considered in this analysis do not clearly indicate the number of localities nor the specific criteria and methods followed to select them. In some case, these seems to have been chosen based on their representativity and conservation importance.

In Germany, caves from the Alpine region were selected based on available information and caves from the Continental region were selected by random sampling (BfN, 2017).

The methodology available from Austria, indicates that caves that host or endemic species or are of high importance for the conservation of Annex II species (e.g., amphibians, bats) must be monitored (Ellmauer, 2005).

## 2.6 General monitoring and sampling methods

Regarding monitoring protocols, sample sizes and frequency of sampling, most of the consulted methodologies do not provide complete information.

In Italy (Angelini et al., 2016), surveys on the aquatic component must be carried out twice a year, in clean waters (basic waters) or in rainy periods (vadose waters), those on terrestrial fauna are carried out in winter and spring visits. The trapping times vary from 24 hours (pots in water) to at least one month (bait). Monitoring requires adequate knowledge of cavity progression techniques and the assistance of local speleological groups. Given the constancy of the cave environment over time, a single survey over the six years of monitoring is generally sufficient.

The monitoring conducted in Poland (Urban & Piksa, 2015) is carried out covering the entire cave, with detailed inspections focused on selected observation points and sections. General monitoring is conducted in a five-year cycle, twice a year, in summer and winter, preferably in February and August. Detailed monitoring consists in observing changes in parameters in relation to the basic state at the appropriate stages: winter and summer. In the case of morphological features (the shape and microrelief of the walls and ceiling of cave voids, condition of dripstones on the ceiling and walls and the bottom of the cave), the basic, reference state is the state from the first stage (one-year cycle) of monitoring, while in the case of hydrological and climatic parameters and features, the basic state is the average or dominant values (states of features) from the first two or three stages of monitoring. The total length of sections and observation points (which can be assigned a size of up to 5 m) of detailed monitoring should not be less than 10% for caves with corridors up to 100 m long, 7% of the corridor length – for caves up to 500 m long, and 3% – for longer caves. The selection of sites and sections monitored in detail should take into account: – the diversity of cave voids (e.g. both narrow corridors and larger rooms should be taken into account), differentiation of other morphological, hydrological and microclimatic features of the cave (e.g. both "wet" and "dry" sections of the cave, climatically static and dynamic parts of the cave should be taken into account), diversity of anthropogenic pressure (e.g. both easily accessible fragments near the entrance and difficult to access deeper parts of the cave should be taken into account), occurrence of fauna (sections where fauna is concentrated should be taken into account, e.g. troglodite communities in parts near the entrance of caves). As a rule, the observation (measurement) point is at least one of the cave openings.

The monitoring of caves in Romania (Vlaicu et al., 2013) is strongly oriented to monitor bat populations. Monitoring stations are strategically placed in caves that serve as important habitats for bats, including both hibernation and breeding sites. The monitoring process involves regular visits to these caves, typically twice a year – once during the hibernation period (winter) and once during the active season (summer). Inside the caves, the stations are organized into three main categories to measure abiotic conditions:

- **Outer station:** This station is located outside the cave, typically near the entrance. It measures external environmental conditions such as temperature, humidity, and potentially other meteorological parameters.
- **Entry station:** This station is positioned at the cave entrance. It serves as a transitional point between the external environment and the cave interior, measuring conditions in this intermediate zone.
- **Underground station:** These stations are located within the cave itself. They are typically placed in areas frequented by bats, such as roosting sites, hibernation spots, or maternity colonies. Multiple underground stations may be set up in different parts of the cave to capture the variability of conditions throughout the cave system.

In Lithuania (Rašomavičius, 2015) one single location is monitored with square sample plots. Observations of the structure and functions of the cave are carried out within the natural limits of the habitat. The preferred sample period is June and the second half of July.

**Table 7. Examples of the general monitoring and sampling methods based on the available data for Habitat 8310**

Country	Monitoring method	Frequency	Details
<b>Italy</b>	Surveys on aquatic and terrestrial components	General: Six-year cycle. Aquatic: Twice a year; Terrestrial: Winter and Spring	Aquatic surveys in clean or rainy periods; terrestrial fauna monitored in winter and spring. Single survey over six years generally sufficient.
<b>Poland</b>	General and detailed monitoring of caves	General: Five-year cycle, twice a year (February and August)	Detailed monitoring focused on selected observation points. Morphological features compared to baseline state. Monitoring sites should represent cave diversity and anthropogenic pressure.
<b>Romania</b>	Monitoring bat populations with strategic stations	Twice a year (Winter and Summer)	Stations include outer (outside cave), entry (at cave entrance), and underground (inside cave) to measure abiotic conditions related to bat habitats.
<b>Lithuania</b>	Monitoring at a single location using square sample plots	Preferred: June and second half of July	Observations of cave structure and functions carried out within natural limits of the habitat.

## 2.7 Other relevant methodologies

The 6<sup>th</sup> EuroSpeleo Protection Symposium, dedicated to cave monitoring practices in Europe, was part of an ongoing series of events organized by the European Cave Protection Commission (ECPC). These symposia aim to address diverse aspects of cave conservation and protection. The primary theme of this event was “Assessing, monitoring, and protecting cave biotopes and geotopes through Sysmo action”.

During the symposium, participants evaluated the current state of cave monitoring across Europe, revealing significant differences in methods and approaches among countries and regions. In addition to assessing the conservation status of caves, the symposium served as a platform to explore innovative strategies for enhancing future monitoring efforts.

The outcomes of this event are particularly valuable, as they shed light on key challenges in cave protection while offering actionable guidance for standardizing and improving monitoring practices across Europe. The recommendations emphasize the need for a holistic, technology-driven, and community-engaged approach to monitoring and conservation.

One of the developments discussed was the use of smart digital tools to streamline and improve ecological assessments. For instance, the German CaveLife app (Vogel et al., 2017) enables direct digital data entry in the field, significantly enhancing data accuracy and efficiency.

The CaveLife App is a smartphone app for cave data collection on the field that aims to contribute to the assessment of the condition of habitat 8310 (Zaenker et al., 2022), developed in Germany by the Verband der deutschen Höhlen- und Karstforscher e.V. (VdHK, German Speleological Federation) and the Hesse Federation for Cave and Karst Research.

The app allows amateurs speleologist to upload the collected data to a centralised database, making the data available for authorities across Europe. The user can collect data on the description of the surroundings (e.g. forest border), width and high of the cave entrance, type of rock and degree of humidity. Then, there are three major fields to register data on taxa, habitat structures and threats. For the first one, a list of species and animal groups is available to allow its selection after visual observation. The habitat structures field include a variety of structures such as standing and flowing waters, cave walls, cervices or sediment deposits. Users are then able to assess the condition of the structure following Natura 2000 standard, rating the individual habitat structures into A (excellent developed), B (developed good to medium) and C (largely missing). Then, the app assigns an overall score. Threats include visits, waste deposits, intervention in the groundwater regime or quarrying and its assessment is similar to that of habitat structures. However, the overall assessment of threats must be justified by an expert opinion.

## 2.8 Conclusions

This chapter presents a comprehensive analysis of the methodologies employed across various European countries — namely Austria, Belgium (Wallonie), Bulgaria, Czechia, Germany, Italy, Lithuania, Poland, Romania, and Spain — for assessing and monitoring the condition of habitat 8310, specifically focusing on caves not open to the public. The review highlights significant variability in the methodologies, particularly in the range of variables measured, the metrics used, and the methods of data collection.

Most methodologies prioritise physical characteristics of the cave habitats, with limited inclusion of chemical and biotic variables. Notably, while some methodologies incorporate monitoring of invertebrates and bats, as well as bryophytes, there remains a lack of comprehensive biotic assessments, particularly concerning microbial biodiversity. Moreover, only a few methodologies address landscape characteristics or anthropogenic disturbances, indicating a gap in holistic habitat evaluation.

The analysis reveals that climate conditions, such as air temperature and humidity, are consistently monitored across several methodologies, using thermohygrometers positioned



strategically within cave environments. Furthermore, methodologies from Poland and Romania stand out for their detailed monitoring protocols, which include specific guidelines for seasonal assessments and the establishment of monitoring stations to capture variability in abiotic conditions.

The chapter also identifies the need for defined thresholds for interpreting monitoring results, with some methodologies offering qualitative categories to assess habitat conditions. However, there is a noted absence of clear methods for establishing these thresholds, which could enhance the reliability of assessments.

Aggregation methods for results at both local and biogeographic scales vary among methodologies, with Poland's approach of determining overall assessments based on the lowest value of measured variables being particularly noteworthy. The selection criteria for monitoring localities also appear inconsistent, with some methodologies emphasizing representativity and conservation importance while others rely on random sampling.

Finally, the insights from the 6<sup>th</sup> EuroSpeleo Protection Symposium underscore the necessity for a more integrated approach to cave monitoring. The recommendations emphasize the importance of involving local communities, utilizing innovative digital tools, and expanding the focus of cave biomonitoring to encompass broader biological diversity. These findings provide a crucial foundation for future improvements in methodologies and practices aimed at preserving the ecological integrity of cave habitats across Europe.

### 3. Guidance for the harmonisation of methodologies for assessment and monitoring of habitat condition

#### 3.1 Selection of condition variables, metrics and measurement methods

This section aims to provide a selection of a minimum common set of variables for the assessment of habitat condition, including abiotic (physical and chemical), biotic (composition, structure, and function) and landscape variables, with a rationale for their selection.

Regarding key abiotic characteristics, the assessment and monitoring of caves requires the consideration of several important factors.

A proposed list of **descriptive, essential, recommended and specific variables** for caves not open to the public is presented in Table 8, including metrics and general measurement methods. The list is based on the main characteristics of caves (as described in section 1.2) and the analysis of methodologies available from EU MSs for the assessment of habitat 8310 condition. The proposed variables are consistent with those already used by the various MSs, as described in section 2.1.

**Essential variables** (E) correspond to key characteristics that need to be measured to properly assess the habitat condition. **Recommended variables** (R) correspond to common variables which are relevant but are optional and can be neglected in some contexts. **Specific variables** (S) should be measured in some specific habitats due to their particular characteristics. **Descriptive variables** (D) are measured in order to obtain contextual information needed to understand the environmental gradients that must be considered in the assessment. Such variables do not directly determine the habitat condition but are useful for the definition of thresholds and for the interpretation of results.

The proposed **descriptive variables** provide foundational information about the cave's physical structure and geological context. They can be measured once at the beginning of the monitoring cycle since these measurements help establish a baseline and should be repeated only when substantial changes are observed. Substrate type, which includes rock, sediment, clay, and guano, is assessed through geological mapping or ground-penetrating radar (GPR). The presence of speleothems, such as stalactites, stalagmites, and flowstones, is recorded using photomonitoring, ideally at annual intervals, or through 3D scanning techniques like LIDAR (as noted by Gillieson et al., 2022). Cave volume and shape can be measured using LIDAR or Terrestrial laser scanning (TLS) technologies, allowing the possibility of generation 3D models of the cave (Gallay et al., 2015)

As regards **abiotic variables**, temperature, including both ambient air and water (when relevant), and relative humidity are similarly recorded through electronic sensors. Soil pH, representing the acidity or alkalinity of soils and water, that can influence microbial communities, is measured using pH meters. CO<sub>2</sub> concentration, often measured using data loggers, can indicate changes in air exchange or anthropogenic disturbance. Specific variables are proposed for caves with an important presence and influence of water, such as springs or standing water bodies. Measurement of water turbidity, water flow, dissolved oxygen content and chemical content is proposed.

In terms of **biotic compositional variables**, several essential variables are used, including the presence and identification of species, especially those endemics to cave ecosystems, such as stygobionts, troglobionts, troglaphiles, and key invertebrate taxa, including Coleoptera, Lepidoptera, Mollusca, and Araneae. Recording of presence of invasive species is also

indicated as a variable to monitor cave condition, with an increase in the number of invasive species present signalling bad condition. Due to their role as bioindicators and their presence in cave environments, the presence of bat is proposed as an essential variable.

Regarding **structural variables**, the number of bat colonies and the type of use they do of the cave needs to be recorded (wintering and breeding populations). Monitoring the presence of mosses, ferns, lichens, and vascular plants is also proposed as an essential variable, both to measure compositional and structural biotic characteristics. However, because these groups are generally less prevalent and ecologically significant in cave environments compared to other organism groups, direct assessment of their coverage is recommended rather than required. This measurement should be conducted when the expert team determines it to be relevant to the specific cave context.

Two **functional variables** are proposed. Trophic group assignment, which categorizes species based on their ecological roles, such as detritivores and predators, provides information on the ecological functioning and relations between cave faunal communities. Analysis of microbial community and fungi is proposed as a functional variable due to the role of these communities in the cave trophic complex. The inclusion of these unrepresented taxa groups in monitoring programmes is one of the recommendations made by the 6<sup>th</sup> EuroSpeleo Protection Symposium to improve habitat condition assessment of habitat 8310 (Weigand et al., 2022). This variable is proposed to be measured using eDNA.

Regarding the measurement of **landscape characteristics** of caves, land use of the surrounding areas is proposed to be measured. Changes in land use or fragmentation of the habitats surrounding the cave, can affect cave fauna dispersion and connectivity, affecting the biological community of the cave (Bento et al., 2024). Thus, mapping of the land use cover around the cave area is proposed as an essential variable.

Human-related pressures are covered through the evaluation of tourism intensity signs, constructed trails, or infrastructure, habitat alterations, such as broken speleothems or substrate degradation, and pollution indicators, such as oil films or solid waste.

Karstic caves require emphasis on hydrological variables due to their sensitivity to water quality changes. Therefore, **specific variables** measuring water flow characteristics, turbidity, water pH and dissolved oxygen are proposed for this type of cave following Gillieson et al. (2022).

**Table 8. Proposals for condition variables for assessing and monitoring habitat type 8310 (caves not open to the public)**

The variables are included in the types recognized in the SEEA EA methodology. Metrics may show several options

Characteristics	Variables	Metrics	App	Standardised measurement procedures	Considerations relating to methodologies
<b>1. Abiotic characteristics</b>					
<b>1.1 Physical state characteristics</b>					
<b>Climate</b>	<b>Temperature (ambient air and water)</b>	°C	Essential	Digital thermometers or data loggers.	
	<b>Relative humidity</b>	%	Essential	Hygrometers or data loggers.	
<b>Water flow dynamics</b>	<b>Water flow</b>	m³/s	Specific	Water level loggers, flow meters.	For caves with important presence of water (standing water, dripping, spring)
	<b>Water turbidity</b>	NTU = Nephelometric Turbidity Unit (standard light angle) FNU = Formazin Nephelometric Unit (ISO method) or STT= Total Suspended Solids (mg/L)	Specific	Turbidity can be measured with sensor or turbidimeters.	For caves with important presence of water (standing water, dripping, spring)
<b>Lithology</b>	<b>Substrate type</b>	Rock type, sediment, clay, guano, etc	Descriptive	Geological mapping or GPR.	Measurement should be conducted at the beginning of the first monitoring period.
<b>Cave morphology</b>	<b>Presence of speleothems (stalactites, stalagmites, flowstones, etc.)</b>	Presence and types	Descriptive	Photomonitoring. LIDAR.	A monitoring interval of one year may be appropriate for many show caves.
	<b>Cave volume and shape</b>	m³	Descriptive	Terrestrial laser scanning (TLS) technologies	Measurement should be conducted at the beginning of the first monitoring period, and repeated if any substantial or evident changes occur.



Technical Guidelines for assessing and monitoring the condition of  
Caves not open to the public (8310)

Characteristics	Variables	Metrics	App	Standardised measurement procedures	Considerations relating to methodologies
<b>1.2 Chemical state characteristics</b>					
Soil/water characteristics	pH (acidity or alkalinity of soils/sediment and water)	pH	Essential	pH meter.	
Atmospheric conditions	CO <sub>2</sub> concentration	mg/L	Essential	Data loggers.	
Water characteristics	Dissolved oxygen	mg/L	Specific	Data loggers.	For caves with important presence of water (standing water, dripping, spring)
	Presence of chemical elements in water	mg/L	Specific	Water sampling and laboratory analysis	Detection of nutrients, heavy metals and other pollutants
<b>2. Abiotic characteristics</b>					
<b>2.1 Compositional state characteristics</b>					
Presence and abundance of biological communities and species	Presence of invertebrate species (Coleoptera, Lepidoptera, Mollusca, Arachnidae)	Number of species and % of each species	Essential	Traps and visual surveys.	Surveys based on local inventories and reference lists.
	Presence of bat species	Number of species and % of each species	Essential	Acoustic monitoring and mist netting for bat species. Faunal activity evidence (e.g. bat guano)	Surveys based on local inventories and reference lists.
	Presence of invasive alien species	Number of species and % of each species	Essential	Traps and visual surveys.	Surveys based on local inventories and reference lists.
	Presence of fern, moss, lichens or vascular plant species	Number of species and % of each species	Essential	Visual surveys.	Surveys based on local inventories and reference lists.
<b>2.2 Structural state characteristics</b>					
Pattern of occupancy of	Number of bat colonies	Number of wintering/breeding colonies per cave	Essential	Visual assessment. Colony counts.	Wintering, breeding...

Technical Guidelines for assessing and monitoring the condition of  
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Characteristics	Variables	Metrics	App	Standardised measurement procedures	Considerations relating to methodologies
<b>communities and species</b>	<b>Coverage of fern, moss, lichens or vascular plant species</b>	% of coverage	Recommended	Quadrants if possible.	Section of the cave where present to be recorded (on the walls, at the entrance...).
<b>2.3 Functional state characteristics</b>					
<b>Dynamics and natural processes</b>	<b>Trophic interactions</b>	% of trophic groups	Recommended	Food web analysis and/or stable isotope tracking	To be conducted after assessment of compositional variables.
	<b>Composition of microbial and fungal communities</b>	Presence and % of different species	Essential	eDNA.	Regular monitoring of biofilm composition, extent, and microbial succession in different cave zones.
<b>3. Landscape characteristics</b>					
<b>Landscape</b>	<b>Land use in the surrounding area</b>	Presence and classification	Essential	Satellite imagery, GIS mapping.	To record land uses and habitat types in the surrounding area of the cave. Information can be retrieved from land use maps.
<b>4. Other variables</b>					
<b>Disturbances</b>	<b>Presence of human disturbances</b>	Presence	Essential	Visual assessment inside the caves	Signals and extent of disturbance to be record down. Disturbances: littering, trampling...
	<b>Habitat alterations</b>	Extent of the alteration.	Essential	Visual assessment.	Signals and extent of disturbance to be record down. To record the presence of broken speleothems, degraded substrates and the impact of such disturbance on other elements.
	<b>Pollution indicators</b>	Presence	Essential	Visual assessment.	Signals and extent of disturbance to be record down: oil films, waste...

App: Application.

### 3.2 Guidelines for the establishment of reference and threshold values, and obtaining condition indicators for the variables measured

The measured variables need to be compared with reference values and critical thresholds to assess the condition. A reference level is the value of a variable under reference conditions, against which it is meaningful to compare past, present or future measurements. The difference between a variable's measured value and its reference level represents its distance from the reference condition.

Reference levels should be defined consistently across different variables within a given ecosystem type, and for the same variable across different ecosystem types. This ensures that derived indicators are compatible and comparable, and that their aggregation is ecologically meaningful (United Nations, 2021).

Reference levels are typically defined with upper and lower values reflecting the endpoints of a condition variable's range, which can then be used in re-scaling. For instance, the highest value may represent a natural state, while the lowest value may represent a degraded state where ecosystem processes fall below the threshold required to maintain function (Keith et al., 2013, in United Nations, 2021). For example, pH values in freshwater ecosystems clearly indicate whether biological life can be sustained, while soil nutrient enrichment beyond a certain threshold can lead to the loss of sensitive species.

Establishing reference values and thresholds is essential for determining whether habitats are in good condition or have become degraded. Reference values represent the desired state of an ecosystem, typically reflecting intact or minimally disturbed conditions. These values serve as benchmarks for assessing habitat condition.

These guidelines do not aim to prescribe specific threshold values. Rather, they outline the main criteria and provide guidance for establishing reference values that support the determination of good or not-good condition, while accounting for the ecological variability of habitats across their range.

With regard to the variables, the harmonisation of reference values and thresholds should consider a set of **common requirements**:

- For a given habitat, the final assessment of its condition and trend over time – based on the reference values and thresholds of the variables characterising the habitat – should be equivalent across Member States, after accounting for the contextual factors specific to each MS (e.g., climate).
- Thresholds, limits, and reference values should be tested using sufficiently robust datasets that represent the full range of habitat conditions, from degraded to high-quality sites.
- Thresholds must account for the natural variability of habitats across their range. Consequently, different threshold or reference values for the same habitat type may be appropriate in different MSs or in different regions within a single MS.
- Establishing reference values requires information external to the evaluated site, which can provide insight into the condition of the habitat and be translated into variable values that characterise that condition.
- Reference values should meet the criteria of validity (ecological relevance), robustness (reliability), transparency, and applicability (Czúcz et al., 2021; Jakobsson et al., 2020).

- 
- Each MS should provide a clear, justified, and comprehensible description of the methodology used to establish threshold and reference values for each variable.
  - The methodologies should be designed for regular evaluation and improvement, based on the best available scientific knowledge. Any modifications made – and their implications for past monitoring data – must be communicated transparently.
  - A reference library and indicator thresholds should be developed for different habitat types across regions, taking into account their ecological characteristics and natural variability.
  - Joint training or guidance on setting threshold and reference values should be offered to experts from the different MSs in order to achieve ensure harmonised approaches.

Several approaches have been recognised for estimating reference values to assess habitat condition (Stoddard et al., 2006; Jakobsson et al., 2020; Keith et al., 2020). These can be broadly synthesised into six categories: (1) absolute biophysical boundaries, (2) comparison to reference empirical cases – i.e., areas or communities considered to be in good condition, (3) comparison to undisturbed cases, (4) modelling and extrapolation of variable-condition relationships, (5) statistical assessments, and (6) expert judgement.

All approaches should be grounded in scientific literature. Methods that use values from a single baseline year as a reference for good condition are not recommended, as the selected year may not reflect favourable conditions, and historical data may be unreliable or incomplete (Jakobsson et al., 2020). The use of historical period (e.g., pre-industrial) as a reference state, as proposed by Stoddard et al. (2006) and Keith (2020) aligns with the baseline approach but also overlaps with comparisons to undisturbed cases (see below). If conditions during a specific baseline year are well documented as favourable, they may be useful for trend analyses. Likewise, where historical pristine conditions are clearly documented, they may serve as valid reference states under the undisturbed comparison approach.

### **Absolute biophysical boundaries**

These refer to situations in which observed values of variables exceed the physical and chemical limits (e.g., pH, bare soil cover, critical loads for eutrophication or acidification) or biotic limits (e.g., presence of alien species) that define the habitat. When such limits are exceeded, the habitat cannot be in good condition (Jakobsson et al., 2020). These thresholds therefore indicate negative impacts on the favourable condition of the habitat.

- Advantages: This approach provides robust and transparent criteria that are clearly linked to the ecological integrity of the habitat.
- Disadvantages: It is applicable to a limited number of variables, typically those with direct negative impacts on habitat condition.

### **Comparison to empirical cases considered to be in good condition**

This approach is based on identifying areas or communities considered to be in good condition (Stoddard et al. 2006; Jakobsson et al. 2020; Keith et al. 2020). These serve as reference cases from which the reference values can be derived. Therefore, their careful selection – and the availability of a sufficient number of such cases – is essential for ensuring the reliability of the reference value estimates (Soranno et al., 2011). While this method may appear straightforward, it is often limited by the scarcity of suitable sites, especially in landscapes that have been historically modified.

- Advantages: Providing that sufficient data from high-quality cases are available, this approach offers empirical validity and reliability by directly linking variable values to habitat condition.
- Disadvantages: Methodological challenges arise due to the difficulty of identifying a sufficient number of suitable reference sites in historically altered environments.

### **Comparison to cases with a natural disturbance regime**

This approach is closely related to the previous one, based on the assumption that most human-induced disturbances reduce habitat quality. This assumption is generally valid in human-modified landscapes and can be linked to historical reference conditions when human pressures were less pronounced (Stoddard, 2006). However, disturbances that are part of a natural disturbance regime may actually indicate naturalness and thus good habitat condition. In fact, a certain level of disturbance can be beneficial, supporting microhabitat formation, enhancing biodiversity, and promoting regeneration of habitat-characteristic species (Keith et al., 2020).

Historical reference criteria may include the absence of human intervention or management, as found in “primary” forests (*sensu* Sabatini et al., 2017), and are often directly connected to climax communities such as old-growth or primeval forests (Wirth et al., 2009; Burrascano et al. 2013; Buchwald, 2005), which are typically assumed to be in good condition. However, in regions with long-standing anthropogenic pressure, it may be difficult to identify unaltered or naturally disturbed habitats for certain types (Keith et al., 2020). Additionally, defining the undisturbed state based on a relatively short time period may overlook disturbance legacies that persist over longer timescales (Alfaro-Sánchez et al., 2019).

- Advantages: This approach provides transparent and empirically grounded criteria for defining reference conditions and can benefit from large-scale information on disturbance and land-use history.
- Disadvantages: The assumption that any disturbance reduces habitat quality may not always be valid. Moreover, identifying sufficient undisturbed or naturally disturbed reference areas can be challenging for some habitat types.

### **Modelling the relationships between variables and condition**

This approach assumes a relationship between variable values and habitat condition. When determining threshold and reference values, models that describe these relationships share a conceptual basis with methodologies based on dose-response curves. Such models assume that certain cases of good condition correlate with specific levels of a condition variable.

The advantage of modelling is that it allows reference values to be inferred where empirical examples of good condition or undisturbed condition are lacking. In these situations, information from known empirical examples can be extrapolated to other contexts, such as locations along a climatic gradient.

Various modelling procedures are available. Functional relationships – linear, saturated, or humped – can be applied (Stoddard et al., 2006; Jakobsson et al., 2020). For instance, deadwood volume in pristine forests can be modelled along productivity gradients to establish reference values in climatic conditions where unaltered forests no longer exist (Jakobsson et al., 2020). Correlative climate niche models can also be used to estimate the suitability of species sets (i.e., variables that characterise the habitat) at different points along the climatic gradient (Jakobsson et al., 2020).



Although these approaches offer a functional basis for establishing reference values, they involve several assumptions that often require expert judgement. It is also possible to create models in which condition is inferred from variables other than the condition variable itself – for example, biodiversity-related condition variables may be inferred from pollution levels. However, this approach should be used with caution to avoid tautological inferences involving variables that reflect pressures.

- **Advantages:** Modelling approaches are flexible, transparent, and encompass a variety of procedures based on functional relationships between variables and condition, drawing on scientific knowledge from multiple disciplines. They can also be applied to obtain reference values when empirical examples of good or undisturbed condition are lacking.
- **Disadvantages:** The information available to build models is often insufficient or unreliable for many variables. Outputs are highly sensitive to the chosen modelling procedure and underlying assumptions, and expert judgement is ultimately required at multiple stages of the modelling process.

### Statistical assessments

This approach is based on quantitative data from databases, such as habitat inventories, which report the distribution of variables within a given habitat. It assumes that higher values of certain variables correspond to good condition when a positive relationship exists, and vice versa. For such variables, high percentile values or confidence intervals (e.g., 95%, Jakobsson et al., 2020), or differences from the maximum observed values (Storch et al., 2018), may be used.

For variables with a negative impact on habitat condition, low (e.g., 5%) or minimum values are applied, while for variables that show a hump-shaped (non-linear) relationship with condition – peaking at intermediate values (e.g., gap occurrence, browsing) – a combination of high and low percentiles may be used.

This approach is particularly suited to variables obtainable from forest inventories (Storch, 2018; Pescador et al., 2022), and is useful when empirical examples of good condition are lacking. However, it may provide limited insight into the state of habitats that are in poor condition throughout the entire assessed territory. In other words, this approach is not directly based on reference situations of good condition, but on statistical inferences subject to the constraints of the sampling used to build the reference database.

- **Advantages:** This approach can be applied with reasonable ease by users with statistical training. It is transparent, replicable, and minimally subjective.
- **Disadvantages:** The existence of appropriate, quantitative datasets representing the reference state is essential for this method. Its reliability depends on the distribution of condition classes (from bad to good) in the dataset and on how well this distribution corresponds to empirical situations of good condition. As a result, it may lead to under- or overestimation of good condition and may be less reliable for habitats that are poorly represented in the dataset.

### Expert judgement

Setting of reference values and thresholds based on expert judgement is common practice, particularly where other sources of information are lacking – for instance, in certain non-abundant habitats where experts have developed empirical knowledge of habitat condition. However, this approach is often criticised for its limited transparency, and the level of expertise

may be insufficient in some cases. For this reason, it is sometimes considered a last-resort option for many variables.

Nonetheless, for certain variables – such as assemblages of characteristic species, successional stages, the presence of microhabitats, or regeneration characteristics – expert judgement may be appropriate for establishing thresholds and reference values. In other cases, it can also serve as a complement to other approaches.

In all situations, it is advisable to apply expert judgement through protocols based on consensus and consultation with multiple experts of comparable experience. This should include clear procedures (e.g., standardised questionnaires) and transparent documentation of how conclusions were reached (Stoddard et al., 2006). A further limitation is the lack of available experts for certain habitats, which can hamper the correct application of this approach.

- Advantages: This approach is easy to apply and is commonly used.
- Disadvantages: It entails a high degree of subjectivity and low transparency, which limits replicability and reliability. Its use may also be constrained by the scarcity of suitable experts for particular habitats and Member States.

These approaches are drawn from methodologies applied by Member States and documented in the literature. Given the uncertainties involved in setting reference levels, a combination of approaches is generally recommended to improve reliability. The approaches described are not mutually exclusive, and are often applied in combination. For example, expert judgement is typically required when defining reference cases for good condition or when making modelling decisions about the relationship between variables and condition. Similarly, modelling-based approaches can complement those based on empirical cases of good or undisturbed condition and may also be integrated with statistical methods.

Habitat condition assessments are based on determining whether the variables used indicate good or not good condition. However, it is common practice to define more than two categories for each variable – e.g., good, medium, and bad – as observed in the analysis of methodologies used by MSs. The criteria for assigning these condition categories vary depending on the characteristics of each variable. For example, categorical variables may involve thresholds such as “no alien species allowed”, while quantitative variables may follow linear or non-linear relationships with condition (Jakobsson et al., 2020).

This classification of variable values – whether quantitative or categorical – into condition categories (e.g., good, not good; or good, medium, bad) corresponds to the scaling process needed for joint evaluation through aggregation procedures, as described in the following section. Condition categories can be translated into numerical values (e.g., good = 2, medium = 1, bad = 0). Alternatively, where quantitative values for the variables are available, these can be directly standardised for use in aggregation.

Owing to the different metrics and magnitudes used for the variables that characterise habitats, the values obtained from their measurement require some form of standardisation – e.g., through re-scaling – in order to build indicators that combine multiple variables. In habitat condition assessments, each characteristic and its associated variable is likely to be measured in a different unit. These values are normalised using reference levels and reference conditions, allowing comparison across variables. Measurement values are scaled in relation to their reference levels, thereby normalised to a common scale and aligned direction of change. They can then be combined to form a composite index or used to obtain an overall

condition result through appropriate aggregation approaches (see further details in Section 3.3. on Aggregation).

Thresholds, limits and reference values must be tested against sufficiently broad data sets, covering the full range of habitat conditions – from degraded to high-quality examples. A reference library should be established, and indicator thresholds identified across mire types and regions.

### 3.3 Guidelines for the aggregation of variables at the local level

Ecological assessments require the integration of physical, chemical, and biological quality elements. The choice of aggregation method for combining these partial assessments into an overall evaluation has been widely discussed within the scientific community, as it can significantly influence the final outcome. Various approaches can be used to integrate the values of measured variables into an overall index reflecting the condition of habitat types at the local scale (e.g., monitoring plot, station, or site).

Applying appropriate aggregation approaches is essential for categorising condition at the local scale as good or not good, since the proportions of habitat type area in good/not good condition is the key information needed for evaluating the conservation status of structure and functions at the biogeographical level.

#### 3.3.1 Overview of aggregation methods

Based on the literature (e.g., Langhans et al., 2014; Borja et al., 2014), two main aggregation approaches can be distinguished: the one-out, all-out rule (minimum aggregation) and additive aggregation (e.g., addition, arithmetic mean, geometric mean).

Further information on aggregation approaches and methods is provided below.

##### **Minimum aggregation, or the One-out, all-out rule**

For the minimum aggregation, the aggregated value is calculated as the minimum of the values of the measured variables.

The one-out, all-out (OOAO) rule has been recommended for assessing ecological status under the Water Framework Directive (CIS, 2003). The principle behind this minimum aggregation method is that a water body cannot be classified as having good ecological status if any of the measured quality elements fail to meet the required threshold. This is considered a precautionary and rigorous approach, but it has also been criticised for potentially underestimating the true overall status.

A precautionary OOAO approach is also used in the aggregation of parameters when assessing conservation status under the Habitats Directives, the IUCN Red List of Species and the IUCN Red List of Ecosystems.

##### **Conditional rules**

Conditional rules require that a certain proportion of variables meet their respective thresholds in order for the overall assessment to achieve a good condition rating. For example, the overall status may be considered as not good when a specific number of variables fail to meet their thresholds.

## Simple additive methods and averaging approaches

Simple additive methods calculate an aggregated value as the sum of the  $n$  values ( $v_i$ ) of the variables.

Averaging approaches are among the most commonly used methods for aggregating indicators. These include straightforward calculations such as the arithmetic mean, weighted average, median, or combinations thereof, to produce an overall assessment value.

## Weighting

Differential weighting of indicators may be applied when calculating sums, means, or medians. The choice of weighting system should reflect the relative importance of each indicator in determining the overall condition of the ecosystem. Ideally, the approach should be supported by a clear scientific rationale and informed by input from ecologists with expertise in the relevant ecosystem types.

However, a robust basis for assigning weights is not always available. In such cases, weighting often relies on expert judgment, which can be subjective, as expert opinions may differ considerably.

## Normalization of variables values (rescaling)

In the assessment of habitat condition, each characteristic and associated variable is likely to involve the use of different measurement units. To ensure comparability, the measured values of variables are often normalised to a common scale (e.g., 0 to 1 or 0 to 100). This involves rescaling the raw data based on reference values or thresholds that define the boundary between good and not good condition for each variable. By rescaling the condition variables, indicators are standardised to the same scale, making it possible to aggregate them into condition indices that reflect the overall condition at a given plot or location.

**Figure 3. Example of deriving condition indicators by rescaling the values obtained for variables, based on upper and lower reference levels**



$$\text{Condition indicator} = \frac{(V - VL)}{(VH - VL)} \quad [\text{Equation 1}]$$

Where:

- $V$  is the measured/observed value of the variable,
- $VH$  is the high condition value for the variable (upper reference level),
- $VL$  is the low condition value (lower reference level).

Source: Vallecillo et al. (2022)

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### 3.3.2 Proposal for the aggregation of measured variables

A quantitative aggregation method should be applied to integrate all essential and specific variables measured to assess the habitat condition. The method should be applied consistently across the habitat range in order to obtain comparable results. The main steps for aggregation are described below.

#### Step 1 – Normalisation of the variables

The quantitative values obtained for each variable should be normalised by rescaling based on reference values (as described above). The value of each variable will be thus in the range from 0 to 1.

#### Step 2 – Aggregation of normalised variables

The aggregated value is then calculated by the aggregation of the normalised values of the variables. For the sake of simplicity, and owing to the difficulty to suggest a more complex method or index, we describe here a preliminary proposal for aggregation based on the arithmetic mean with normalisation of the values obtained for each of the measured variables, which could be used to determine the habitat condition at the local scale, as summarised in the following equation:

$$Local\ condition = \sum_{i=1}^n v_i / n$$

Where  $n$  is the number of variables,  $v_i$  the rescaled value of the corresponding variable (between 0 and 1). As a consequence, the aggregated value should range between 0 and 1.

Another possible method would be to use the weighted average, in which the weight of each variable should be decided, justified and agreed upon for each habitat type by all the MSs that would apply the method. This method can be formulated with the following equation:

$$Local\ condition = \sum_{i=1}^n v_i * w_i / n$$

Where  $n$  is the number of variables,  $v_i$  the rescaled value of the corresponding variable (between 0 and 1) and  $w_i$  the corresponding weight, with  $\sum w_i = 1$ . As consequence, the aggregated value should range between 0 and 1.

This second method, however, poses serious difficulties in assigning weights to the variables, which must be based on a proper evaluation of their importance and influence on the habitat condition, based on a robust scientific knowledge. It also requires reaching a consensus on the weights assigned to the variables measured for each type of habitat, among all the countries that must assess its condition. This is a crucial aspect to obtain comparable results in the assessments carried out by all the Member States.

#### Step 3 – Identify the threshold to determine good/not good condition at the local scale

Finally, a threshold must be applied to the aggregated value to distinguish between good and not good overall condition. This is a crucial step and, wherever possible, this threshold should be established based on empirical data from reference localities in good condition and from localities showing a degraded state. Where such reference localities are not fully available, modelling to obtain such thresholds could be applied.





### 3.4 Guidelines for aggregation at the biogeographical region scale

This chapter outlines the procedures for aggregating condition indices obtained at the local scale to the biogeographical region scale for habitat type 8310- caves not open to the public.

The aggregation of condition indices is essential for understanding the overall health of habitat type 8310 at a broader scale. According to Article 17 reporting guidelines (European Commission, 2023), the following general rules have been established for habitat assessment:

- If 90% of the habitat area is considered to be in 'good' condition, then the status of the 'structure and functions' parameter is deemed 'favourable'.
- If more than 25% of the habitat area is reported as 'not in good condition', then the 'structure and functions' parameter is classified as 'unfavourable-bad'.

These guidelines serve as a minimum requirement for the aggregation process, allowing for the assessment of habitat quality across various biogeographical regions.

### 3.5 Guidelines on general sampling methods and protocols

These guidelines are intended to provide a comprehensive, standardised framework for sampling design and monitoring that can be implemented consistently across EU member states. These approaches have been chosen because they are grounded in methodologies that have demonstrated wide effectiveness and success across diverse EU contexts.

After identifying the principal habitat locations within each biogeographical region, a stratified sampling design should be established to ensure that all major ecological zones within the region are represented. This approach allows for comprehensive coverage of the habitat type under assessment, capturing variation both within and outside Natura 2000 sites. All sampling locations must be precisely georeferenced, with photographs taken of both the plots themselves and their immediate surroundings to facilitate future monitoring and verification.

Effective cave monitoring should follow a structured, multi-tiered approach tailored to the cave's size, morphology, climate, and ecological importance.

Monitoring should be carried out twice annually, once in winter and once in summer, preferably in February and August (Urban & Piksa, 2015). This timing allows for the observation of seasonal variations in both environmental conditions and biological activity. In some cases, monitoring may be focused during the summer, specifically in June and the second half of July, to coincide with peak biological processes (Rašomavičius, 2015).

The entire cave should be included in the general monitoring, with more focused, detailed inspections conducted at selected observation points and sections. These points should be chosen to reflect the diversity of the cave system. This includes a variety of morphological features, such as narrow corridors and large chambers, as well as hydrological and microclimatic differences, including both wet and dry zones and areas with stable versus fluctuating airflows. Sections affected by human activity, such as those near entrances or well-travelled paths, as well as remote interior areas should also be included. In addition, areas of ecological significance, especially those that serve as habitats for cave-dwelling fauna like bats, should be prioritized.

The total length of sections and number of observation points should be defined based on the size of the cave, ensuring the proper representation of its diversity. From this observation points, visual assessment of the biotic compositional variables will be conducted. The size and

number of sample plots used to for the structural variables, will also be defined by the characteristics of the cave and the distribution of the relevant taxa.

In terms of monitoring methodology, general observations help track broad trends across the cave, while detailed monitoring focuses on comparing current conditions to a baseline state.

For morphological features like wall shape, microrelief, and dripstone formations, the initial one-year monitoring cycle serves as the reference point (Urban & Piksa, 2015). For hydrological and climatic parameters, the baseline is defined by average or dominant values recorded over the first two to three monitoring cycles (Urban & Piksa, 2015).

An effective monitoring setup should include a system of stations positioned to capture environmental variability. Stations should be located outside the cave to measure external weather conditions and at the cave entrance to monitor the transition zone (Vlaicu et al., 2013). Additional stations should be established inside the cave, especially in areas frequented by bats or the rest of the fauna being monitored. These stations help document internal temperature, humidity, and other key abiotic factors.

The proposed monitoring cycle is of six years, in order for it to be aligned with the Article 17 reporting period. This period length is aligned with five-year and six-year conducted by Angelini et al., (2016) and Urban and Piksa (2015).

### 3.6 Selecting monitoring localities and sampling design

The selection of sampling localities – along with the sample size (number of plots) and power – is essential to ensure that the results of assessment and monitoring are representative for each habitat type at the biogeographical scale.

**Identifying and selecting localities for sampling** requires a systematic approach to ensure that the chosen sites provide comprehensive and representative data on habitat condition within the biogeographical region. Sampling localities should reflect the full range of habitat diversity, as well as environmental gradients, including variations in elevation, soil types, and climate. Moreover, sites should be selected both inside and outside protected areas. This requires a sound understanding of the distribution and variability of each habitat across its range, including the identification of ecotypes or subtypes, where relevant. The main criteria for selecting monitoring localities are summarised below:

- **Ecological variability:** Localities must represent the full range of ecological diversity and variability within the habitat type. Selection should include different ecotypes or subtypes, successional stages, and reflect key environmental gradients such as altitude, soil type, moisture levels, geomorphological features, and topography.
- **Spatial Coverage:** Adequate spatial coverage is essential to capture habitat heterogeneity. Localities should be selected across the full geographical range of the habitat type within the region, ensuring they are well distributed and represent a significant proportion of the habitat's total occupied area.
- **Degree of conservation and exposure to pressures and threats:** The selection of monitoring localities should include areas with varying degrees of conservation and degradation, in order to capture the full range of habitat condition across its distribution. This includes both well-conserved areas with minimal human impact, and areas affected by degradation and subject to different pressures. To reflect the diversity of pressures acting on the habitat, localities should span a range of intensity levels – from

low to high – and account for different sources of disturbance, such as urbanisation, agriculture, and climate change.

- **Presence inside and outside Natura 2000 sites:** The assessment and monitoring of habitat conservation status must be carried out both inside and outside Natura 2000 sites. This requires selecting localities – and an appropriate number of plots – that reflect the proportion to the habitat's distribution within and outside the Natura 2000 network.
- **Habitat fragmentation at landscape scale:** Localities should be selected based on landscape metrics such as patch size and connectivity. Including both isolated and well-connected sites allow for the assessment of fragmentation effects on habitat condition. Understanding these patterns is essential for developing strategies to mitigate the negative impacts of habitat fragmentation.
- **Lack of Information:** Including areas where data are lacking contributes to building a more comprehensive dataset. Selecting localities in historically under-sampled regions ensures a more balanced and complete understanding of habitat condition across its range. This helps to address data gaps and supports more informed conservation planning.
- **Accessibility and practicality:** Monitoring localities should be accessible for regular field visits, taking into account logistical factors such as distance from roads and ease of access. Practical considerations also include the safety of field personnel and the feasibility of transporting equipment to and from the site.
- **Historical data and existing monitoring sites:** Making use of existing monitoring sites with historical data can strengthen the understanding of long-term trends and changes in habitat condition. Such sites provide valuable baselines for comparison and support more robust trend analyses over time.

Once sampling localities have been identified for each habitat type, the minimum number of plots per locality – and across the biogeographical region – must be calculated to balance sampling effort with the need for representative data.

The **size of the sample** influences two statistical properties: 1) the precision of the estimates and 2) the power of the assessment to draw meaningful conclusions. The number of plots must be **statistically sufficient** to detect changes and trends with the desired level of confidence. Appropriate statistical methods should be applied to determine an adequate sample size.

Considering the heterogeneity of habitat types, it is highly recommended to consult a sampling statistician when determining sample size – that is, the minimum number of plots required to ensure representativity and statistical significance.

Some key elements for ensuring proper representation of habitat condition in the sample are summarised below:

#### **Sample size and distribution:**

- The number of localities/transects etc. should be sufficient to provide a statistically robust sample size. This ensures that the data collected can be generalized to the entire habitat type within the region.
- Statistical methods such as stratified random sampling are often used to ensure that all habitat subtypes and environmental gradients are adequately represented.

#### Sampling design:

- Within each sampling area or locality, multiple plots are established to collect detailed data on benthos, infauna, mobile species and other ecological indicators. The distribution and number of sampling stations depend on the variability and size of the habitat patch. Sampling areas (plots, transects) are laid out considering the existing main ecological gradients, e.g., exposure to waves/currents/tides, depth, sediment characteristics.

#### Replication and randomization:

- Replication of sampling units within each locality and randomization of sampling plots location help to reduce bias and increase the reliability of the data.
- Randomized plot locations ensure that the sampling captures the natural variability within the habitat.

### 3.7 Use of available data sources, open data bases, new technologies

A number of important cave biodiversity databases were also highlighted as valuable resources for conservation planning and research:

- **CroSpeleo Database**<sup>3</sup> – Developed by the Institute for Environment and Nature of the Croatian Ministry of Economy and Sustainable Development (2022).
- **Database of Romanian Cave Invertebrates**<sup>4</sup> – Includes a Red List of cave species and a list of hotspot and coldspot caves (Moldovan et al., 2020).
- **Cave Biodiversity of Georgia (CBG)**<sup>5</sup> – A comprehensive resource on Georgian cave fauna (Barjadze et al., 2019).
- **SubBIOCODE Database**<sup>6</sup> – Focused on subterranean biodiversity in Bosnia and Herzegovina (SubBioLab, 2022).
- **Cave Fauna of Greece Database** – A key reference on Greek subterranean fauna (Paragamian et al., 2018).

A notable recent initiative is the Stygofauna Mundi project, which is currently being developed by the Water Research Institute (CNR, Italy) (Martínez et al., 2024). It is an ongoing project building a worldwide database gathering distribution and evolutionary data on subterranean aquatic animal diversity easily accessible to everyone. The aim is to provide a framework for international collaboration and facilitate monitoring objectives.

Collectively, these databases and digital tools underscore the growing momentum toward data-driven and collaborative cave conservation across Europe.

New technologies and modelling approaches or mapping, monitoring, and assessing habitat type 8310 are evolving with advancements in digital tools and data analysis techniques.

These new technologies and approaches include **Digital Tools and Open Data** that integrate new digital tools and open FAIR (Findable, Accessible, Interoperable, and Reusable) data infrastructures that can be recommended for cave monitoring. This includes the use of **DNA-**

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<sup>3</sup> <https://crospeleo.mingor.hr/>

<sup>4</sup> <https://bdi.pensoft.net/article/53571>

<sup>5</sup> <https://cbg.iliauni.edu.ge/en/>

<sup>6</sup> <https://subbiocode.net/>

**based tools** for biomonitoring, which can enhance the understanding of subterranean biodiversity (Weigand et al., 2022).

An example of the new tools currently used in cave monitoring is the aforementioned app CaveLife (Vogel et al., 2017).

The Object-Based Image Analysis (OBIA) approach, which uses multi-scale image segmentation and rule-based hierarchical modelling of habitats, is recognized for its potential to map the complex ecosystems within caves (Strasser & Lang, 2015). This method may be adapted for advanced ecological mapping techniques in subterranean environments.

Autonomous aerial systems are being used for cave mapping, enabling exploration and documentation in environments that are inaccessible or hazardous for humans. Aerial robots, such as autonomous aerial vehicles equipped with depth cameras, LiDAR sensors, and lighting, are capable of mapping intricate cave networks in total darkness. These robots can generate high-resolution 3D maps even during communication dropouts, as demonstrated in Laurel Caverns and in a wild cave in West Virginia (Tabib et al., 2021).

Regarding advanced photogrammetric and laser scanning techniques that can contribute to cave mapping, Cross-Polarized Structure-from-Motion (SfM) Photogrammetry is effective for capturing difficult surfaces, such as ice within caves, by using polarizing filters to eliminate surface reflections, resulting in increased point cloud density and accuracy (Bartoš et al., 2023). Terrestrial Laser Scanning (TLS) is another standard method for producing detailed 3D cave models, valued for its high point density and precision, and is often combined with other modalities for robust mapping outcomes (Bayarri et al., 2023; Oludare & Pradhan, 2016). Ground-based stereo photogrammetry combined with UAV aerial photography is also used to map remote or hard-to-reach cave passages, providing detailed geodetic georeferencing and producing robust cartographic resources (Shcherbakov et al., 2024).

Mobile mapping systems, such as portable 3D laser scanners, allow users to walk through accessible areas of caves and efficiently generate high-resolution maps. This approach has been effective in Australian cave systems, streamlining the documentation process (Zlot & Bosse, 2014).

Finally, integrated geomatics techniques, which combine methods like 3D laser scanning, UAVs, and ground-penetrating radar, provide comprehensive records of cave morphology and fluid dynamics, which are important for conservation, as demonstrated in the Altamira Cave project (Bayarri et al., 2023). UAV-based 3D mapping uses drone systems equipped with sensors, laser range finders, and cameras to create real-time 3D cave maps. These systems address limitations of traditional mapping methods (Laczkó et al., 2021).



## 4. Guidelines to assess fragmentation at appropriate scales

The spatial distribution and connectivity of cave habitats has an effect on species movement, genetic diversity, and local microclimates. Landscape fragmentation caused by the presence of anthropic land uses in the surrounding areas, such as urbanization, agriculture, industrial and extractive activities, can have an impact on the caves. These activities can lead to changes in the vegetation composition of the adjacent areas, and alteration of groundwater quality, which can affect cave communities.

Assessing habitat condition should therefore include landscape characteristics that reflect fragmentation. Key factors include proximity to other caves, land use in surrounding areas, and the degree of connectivity to natural habitats.

When cave systems are isolated, species like bats may struggle to move between them, reducing genetic exchange and increasing vulnerability to environmental change. A five-year study (2018–2023) on bat diversity in 91 caves across Minas Gerais, Brazil, found that surrounding land-use types significantly shape bat communities. Caves in natural landscapes supported higher species richness and diversity. In contrast, caves near urban areas or eucalyptus plantations hosted bat communities with different compositions, even when species counts were similar. This suggests functional fragmentation, where sensitive species are replaced by disturbance-tolerant ones (Bento, 2024). Another study on the same mining area of Brazil (Gómes et al., 2019), investigated the impact of surrounding habitat fragmentation and land use on cave environmental stability. The findings highlighted the influence of the adjacent vegetal cover and land use in supporting cave communities.

These findings underscore the ecological consequences of landscape characteristics. Even when cave habitats remain physically intact, changes in the surrounding landscape can alter species composition and ecosystem function.

To effectively integrate landscape characteristics and fragmentation into habitat condition assessments, standardized methodologies should be applied. Geographic information systems (GIS) can be used to map cave locations and calculate relationships with land use cover data of the surrounding areas, while field surveys provide critical ecological data. These indicators should be incorporated into existing habitat monitoring frameworks through composite indices that combine traditional measures, such as species diversity, with spatial and landscape-level fragmentation data. This approach enables a more comprehensive evaluation of habitat quality and ecological resilience.

## 5. Next steps to address future needs

These guidelines recommend standard methods for assessing and monitoring the condition of caves not open to the public with the aim of promoting harmonised procedures across the EU Member States. To ensure that habitat condition assessments are comparable across countries, it is essential to define a common set of variables/indicators with well-defined metrics and standard measurement procedures. These should include physical, chemical, compositional, and functional variables to comprehensively evaluate the health of cave habitats.

To implement these guidelines, the following next steps are suggested:

- **Test the proposed set of variables** with agreed measurement procedures and monitoring methods. Use common protocols for sampling, while considering the particularities of cave habitats and the existing contextual factors at local and country level; this testing would be useful to identify gaps of knowledge, flaws of applicability and robustness and reliability of results. The evaluation should provide recommendations to be further integrated in the harmonised procedure, as needed.
- Develop further, test and standardise the methods for the establishment of **reference values and thresholds to determine good condition**. Defining ecological thresholds based on proper habitat characterisation is essential. These thresholds will indicate the health and quality of these rocky habitats, aiding in the monitoring of changes over time. They will also facilitate the assessment of impacts of climate change, human activities, and invasive species, providing critical insight for conservation efforts.
- Develop further, test and standardise the methods for the **aggregation of results obtained** from all the variables measured at the local scale and for each biogeographical region.
- Develop further and test the criteria for the **selection of monitoring localities** and sampling design to ensure a sufficiently representative sample that allows for proper implementation of the aggregation of results at the biogeographical region level.
- Promote harmonised methods for the use of **typical species**: Typical species provide a practical way to evaluate habitat status, reflecting specific ecological conditions. Clear criteria should be defined for selecting these species, along with the methodologies to assess their status and integrate the results into overall condition assessment for each habitat.

The current proposal should be viewed as a starting point and may be adapted where more suitable alternatives are identified based on national experience or ecological requirements.

Caves that are not open to the public, particularly those containing unique water bodies and streams that host specialized or highly endemic species, play a critical role in the conservation of European biodiversity, including Annex II species as bats and amphibians. Addressing future needs for these sensitive ecosystems is essential for ensuring their protection, understanding their ecological dynamics, and facilitating informed management practices. This chapter outlines key areas of focus and potential measures to address these needs.

Future cave monitoring efforts in Europe are guided by the need to address significant inconsistencies and data gaps across national approaches. At the 6<sup>th</sup> EuroSpeleo Protection Symposium, participants outlined a clear path forward that recognises both the ecological

complexity of caves and the challenges of standardised monitoring within the framework of the EU Habitats Directive and the Emerald Network (Weigand et al., 2022).

Monitoring should encompass both biological and geological diversity. Bio- and geodiversity are deeply interconnected in subterranean ecosystems, and their integration is critical for effective conservation. This requires interdisciplinary collaboration among biologists, geologists, speleologists, and other experts (Wynne et al., 2021).

Local communities should be actively involved in monitoring and protection strategies. Community engagement, through public education, co-management agreements, and local stewardship, has proven effective in cases such as Vjetrenica Cave in Bosnia and Herzegovina, where locals, scientists, and municipal authorities jointly manage and protect one of Europe's most biodiverse caves (Ozimec & Lucic, 2009; Culver et al., 2021).

Moreover, caves should be understood as accessible entry points to broader subterranean ecosystems, rather than isolated features. Monitoring must consider the surrounding aquifers and geological features that support unique cave-adapted biota (Mammola, 2018).

A major recommendation is the adoption of new digital technologies and open data infrastructures following the FAIR principles (Findable, Accessible, Interoperable, Reusable). Tools such as the CaveLife smartphone app enable standardised, in-field data collection by trained volunteers and facilitate broader data sharing across borders (Vogel et al., 2017; Wilkinson et al., 2016). Furthermore, biodiversity monitoring should expand beyond a narrow focus on Annex II species, primarily bats, to include the broader cave fauna, particularly invertebrates, fungi, and microbes, which often play critical roles in ecosystem function but are underrepresented in conservation policy (Zaenker et al., 2020; Saccò et al., 2019).

The use of DNA-based techniques also offers exciting potential. DNA barcoding and environmental DNA (eDNA) methods allow for the identification of cryptic or juvenile life stages and the detection of rare species in hard-to-reach habitats. These tools have already been applied to species such as *Proteus anguinus*, the Alabama cave crayfish, and several stygobiont amphipods (Gorički et al., 2017; Niemiller et al., 2017; Trontelj et al., 2009). Metabarcoding further enhances our ability to monitor whole subterranean communities, including bacteria, protists, and fungi, which are essential for cave ecosystem stability (Alaoui-Sosse et al., 2021; Saccò et al., 2022).

In conclusion, the future of cave monitoring depends on embracing a holistic, multidisciplinary, and inclusive approach. While natural variation between cave systems requires flexibility, greater consistency in data collection methods, species coverage, and legal definitions is essential. Reviving and implementing the long-neglected Recommendation No. 36 of the Bern Convention could offer a common legal foundation for harmonised action across Europe (Bern Convention, 1992; Haslett, 2007).

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## 7 Annex 1. Summary overview of consulted methodologies

Examples of variables, measurement methods and thresholds used by the Member States for assessment and monitoring of habitat type 8310  
Caves not open to the public

Variable names	Metrics	Measurement methods	Exmaples of thresholds	MS and references
<b>1. Abiotic characteristics</b>				
<b>1.1 Physical state characteristics</b>				
Temperature	°C	Determine the annual and seasonal temperature (four measurements per year). Microcavity temperature: measured by thermometer outside, at the cave entrance and underground in the different zones (vestibular, disturbed or stability zone)	No changes = FV; Significant changes, affecting thermal regime, microclimate zones or air circulation = U2; Smaller changes = U1 (Urban & Piksa, 2015)	ES: Robledo et al., 2019b RO: Vlaicu et al., 2013 PL: Urban & Piksa, 2015. BE-W: Hendrickx, 2021.
Humidity	%	Measured by psychrometer or electronic thermos-hygrometers at the cave entrance and underground in the different zones (vestibular, disturbed or stability zone)	No changes = FV; Significant changes, affecting thermal regime, microclimate zones or air circulation = U2; Smaller changes = U1 (Urban & Piksa, 2015)	ES: Robledo et al., 2019b RO: Vlaicu et al., 2013 PL: Urban & Piksa, 2015
CO2 (analysis of the microclimatic environment of the cavity)	Not provided	Determine the annual and seasonal CO2 average (four measurements per year).	Not provided	ES: Robledo et al., 2019b
Piezometry (water status analysis in caves with endokarst lakes)	Not provided	Determine variations in piezometric levels (four measurements per year).	Not provided	ES: Robledo et al., 2019b;
Speleothems and forms of dissolution (distribution and typology). Shape and microrelief of selected parts of the cave	Not provided	Analysis of the forms of dissolution and speleothems inside the cavity and catalogue of the same, according to the generic type.	Not provided	ES: Robledo et al., 2019b; PL: Urban & Piksa, 2015
Cave morphology and volume	Not provided	Field survey and expert assessment, comparison with historical data	Cave volume not decreased = FV. Cave volume slightly decreased = U1. Cave volume significantly decreased = U2 (Urban & Piksa, 2015)	RO: Vlaicu et al., 2013 PL: Urban & Piksa, 2015. BG: MOEW, 2013
Ventilation	Not provided	The direction of the air current will be observed directly and established in relation to the reference input: E-I (exterior–interior); I-E (interior–exterior).	Not provided	RO: Vlaicu et al., 2013

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Variable names	Metrics	Measurement methods	Exmaples of thresholds	MS and references
<b>1. Abiotic characteristics</b>				
<b>1.1 Physical state characteristics</b>				
Typical habitat structure of cave	Not provided	Expert judgement with reasoning. Diversity of microbiotopes present (basins, organic deposits, cracks, etc.)	DE: A = excellent development of structures; B = good to moderate development of structures; C = structures mostly missing	DE: BfN, 2017 BE-W: Hendrickx, 2021 CZ: Lustyk 2023
Hydrological regime: water accumulation water intrusion and hydrological changes	Not provided	Field survey and expert assessment. Presence of underground lake, pool of water formed in a calcific crust or excavation of the bedrock, Puddling of water on bedrock deposits. Flow of water into the cavity, dripping and/or seeping of water into the cavity.	PL: No changes = FV; New or disappearing watercourse = U1; New or disappearing watercourse – permanent change (at least by 3 consecutive years); water level changes > 0.5m, visible or sniffable pollution of waters = U2	RO: Vlaicu et al., 2013 PL: Urban & Piksa, 2015
Source of microdroplets	Not provided	Filed survey. Microdroplets from precipitation, from waterfalls, from showers	Not provided	RO: Vlaicu et al., 2013
Cave shade	%	Percentage of shade of the cave is evaluated	Not provided	LT: Rašomavičius, 2015
<b>1.2 Chemical state characteristics</b>				
Physicochemical (water status analysis in caves with endokarst lakes)	Not provided	Determine the physicochemical basis of the water, pH, conductivity and temperature (four measurements per year).	Not provided	ES: Robledo et al., 2019b
Ionic and cationic (Water status analysis in caves with endokarst lakes)	Not provided	Determine the ionic and cationic chemistry of the water using important components such as Ca, Mg, Cl, NO <sub>3</sub> , etc. (two measurements per year, if the endokarst water surface is important, they will be taken in two different parts of the cave).	Not provided	ES: Robledo et al., 2019b
Heavy metals (Water status analysis in caves with endokarst lakes)	Not provided	Determine the chemistry of heavy metals such as As, Hg, Ar, Pb, Cd, etc. (two measurements per year, if the endokarst water surface is important they will be taken in two different parts of the cave).	Not provided	ES: Robledo et al., 2019b

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Variable names	Metrics	Measurement methods	Exmaples of thresholds	MS and references
<b>2. Biotic characteristics</b>				
<b>2.1 Compositional state characteristics</b>				
Microbiological (water status analysis in caves with endokarst lakes)	Not provided	Microbiological analyses (fecal coliforms, etc.).	Not provided	ES: Robledo et al., 2019b.
Analysis of the vegetation at the entrance and in the surrounding area	percentage coverage	For the vegetation of the entrances, the vegetation strips present must be graphically represented (in longitudinal and/or vertical section); the surveys focus on the expeditious characterization of the main floristic groupings identified.	Not provided	IT: Angelini et al., 2016
Species inventory in caves: bats, plants and other species (invertebrates)	Presence	DE: Expert judgement with reasoning according to list of species. Verification of the presence and consistency of troglobiontic populations, with crustaceans (aquatic fauna) and carabidae and colebids (terrestrial fauna) as target groups. IT: Faunal sampling can be carried out: (1) on sight, using a sleeved net, aspirator and tweezers; (2) with trapping (pots for aquatic crustaceans or baits for beetles); (3) by continuous filtering (for dripping or resurgence waters). In any case, these are qualitative or semi-quantitative techniques	A = bat species presence in cave above average and permanent bat habitat; B = average or lesser importance; C = cave is not bat habitat or below average presence of bats compared to potential of cave (BfN, 2017)	DE: BfN 2017 PL: Urban & Piksa, 2015 IT: Angelini et al., 2016 CZ: Lutsyk 2023
Bats monitoring: wintering bats	%	Expert assessment, bats monitoring	Number of species and population stable or increasing = FV. Population < 50% of population recorded by last control = U2 (if data available). Average population in last 10 years < 40% of the maximal recorded population = U1	PL: Urban & Piksa, 2015
Number of invertebrate species	Number of species of invertebrates	Detected and assigned to categories a to c based on literature. Categories: a. troglloxenes; b. trogllophiles; c. trogllobionts.	Not provided	BE: Hendrickx et al., 2021. BG: MOEW, 2013
Number of species and number of bats using the caves (cave-loving species)	Not provided	Not provided	Not provided	BG: MOEW, 2013
Typical species	%	The coverage of each species in the field is evaluated (%) based on a reference list of species.	Not provided	LT: Rašomavičius, 2015

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Variable names	Metrics	Measurement methods	Exmaples of thresholds	MS and references
<b>2. Biotic characteristics</b>				
<b>2.2 Structural state characteristics</b>				
Coverage of mosses	%	The coverage of mosses is evaluated by visual inspection.	Not provided	LT: Rašomavičius, 2015
<b>3. Landscape characteristics</b>				
Ecotones and adjacent areas	Presence	The nature of the transition of the habitat to other natural or semi-natural plant communities is described (width of the transition zone, type of adjacent communities, use of adjacent territories, etc.)	Not provided	LT: Rašomavičius, 2015
<b>4. Other characteristics</b>				
Human access	Presence	Not provided	Access excluded or very rare = FV. Access possible but difficult, low pressure = U1. Access easy, significant pressure = U2	PL: Urban & Piksa, 2015
Anthropogenic pressure and pollution of caves	Presence	Not provided	No pollution or visual indicators of pressure = FV; Only remnants of open fire = U1; Significant pollution = U2	PL: Urban & Piksa, 2015 RO: Vlaicu et al., 2013
Disturbance and degradation indicators	Presence	Impairments can be determined by field inspections on site or by researching official authorisations (e.g. extractive activities).	A: Low: no impairments (e.g. recreational use, construction, etc.) visible. B: Medium: minor impairments (e.g. occasional driving) visible. C: High: significant impairments (e.g. regular driving; fire)	AT: Ellmauer, T (ed), 2005. DE: BfN, 2017 CZ: Lustyk, 2023. RO: Vlaicu et al., 2013 PL: Urban & Piksa, 2015



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