



**IMPLEMENTATION OF THE STRATEGIC ACTION PROGRAMME (SAP) OF THE  
DINARIC KARST AQUIFER SYSTEM: IMPROVING GROUNDWATER  
GOVERNANCE AND SUSTAINABILITY OF RELATED ECOSYSTEMS**

**OUTPUT 3.1.1**

**Methodology for biodiversity monitoring in subterranean  
environment**

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## Introduction

In identifying groundwater-dependent ecosystems in Croatia, special attention is given to the role of groundwater in the environment and how it can transmit the effects of anthropogenic pressures (pollution of groundwater or reduction in groundwater flow or level) within groundwater bodies (GWBs). Groundwater is practically all water located below the earth's surface. However, for the purpose of assessing the condition of groundwater within which ecosystems connected or dependent on groundwater exist, it is necessary to primarily consider groundwater in the saturated zone of the aquifer. Only such groundwater and the intensive anthropogenic impacts on it whether pollution or groundwater abstraction—within the total observed groundwater can negatively affect groundwater-dependent ecosystems.

In Bosnia and Herzegovina, the Water Framework Directive (WFD) recognizes two types of ecosystems associated with groundwater. The first type refers to groundwater-associated aquatic ecosystems (where groundwater interacts with surface water). The second type includes terrestrial ecosystems that depend on groundwater for their survival and functioning.

Bosnia and Herzegovina has a rich subterranean biodiversity, reflecting the ecological uniqueness of the Dinaric karstic region, especially in karstic cave systems and underground rivers. These habitats are home of a wide variety of specialized and very often, endemic species.

Bosnia and Herzegovina's underground ecosystems are considered among the most biologically diverse in Europe, with species such as cave-dwelling amphipods, isopods, and the olm highlighting the ecological significance of these hidden environments (Delić et al., 2023, Falniowski et al., 2021).

Limited research has been conducted, primarily focusing on specific groups such as subterranean gastropods, but these represent only a small fraction of the actual biodiversity present. Much of the subterranean environment's diversity in Bosnia and Herzegovina is still undiscovered and poorly understood. There are achieved studies (Delić et al, 2023, Falniowski et al., 2021, Stanić Koštroman et al, 2021) and projects focused on subterranean or groundwater-related biodiversity and ecosystems in Bosnia and Herzegovina.

Some of projects are:

**Cepf, 2016-Protection of the Underground Biodiversity in the Neretva River Catchment Area;**

**Rufor 2016-2017-Assessment of Distribution and Conservation Status of Some Endemic Freshwater snail in Bosnia and Herzegovina,**

**Ruford 2012-Assessment and Promotion of Biodiversity Values of Three Kars Poljes in Bosnia and Herzegovina;**

**Dimfe 2023-2024-Enhancing the Knowledge Base on Dabarsko and Fatničko Karstic Fields and Underground Water Systems and Reducing the Threats from Unsustainable Water Use in Bosnia and Herzegovina Rufor project,**

**CroRis (2023-2027)-Fungi of hydrologically active Dinaric cave ecosystems and their bioactive potential etc.**

In Albania Subterranean habitats within the dinaric karst area are almost not studied; limited studies have been carried out during 2006-2016 focused only to subterrenian gastropods (Grego J. et al,

2017), which represents a very small fraction of the actual species richness. Albanian subterranean environment diversity is still not discovered.

The Water Framework Directive (WFD) recognizes two types of ecosystems that may be associated with groundwater. One is groundwater-associated aquatic ecosystem, which refers exclusively to the connection of groundwater with surface waters and the ecosystems within them. The other type includes terrestrial ecosystems dependent on groundwater.

Karst terrains are characterized by high permeability of fractured carbonate rocks, allowing infiltration of significantly larger amounts of precipitation than other less permeable terrains. As a consequence, surface watercourses are very rare in karst areas, and the majority of discharge occurs underground. One karst-specific feature is the presence of extensive closed depressions, or karst fields, often with sinking rivers, which are also a peculiarity of such areas.

Considering that groundwater plays a key role in the sustainability of many terrestrial ecosystems, it is critically important not only for human life and socioeconomic development but also for numerous flora and fauna species (Gibert et al., 2008). Although considered a renewable natural resource, groundwater has been depleted in many parts of the world or its ecological status permanently degraded over the last 50 years. As a refuge for a large number of diverse organisms, complex relationships between groundwater and subterranean biota generate dynamics in many ecological systems (Danielopol et al., 2003). Over the past 20 years, analyses related to groundwater ecology have significantly increased, integrating both hydrological and ecological research frameworks. Most surface waters originate from groundwater, and the majority of the Earth's freshwater is stored in aquifers (Gibert et al., 2008).

In the Dinaric region of Croatia, about twenty larger karst fields are located at average altitudes between 250 and 700 m. Morphologically, they are usually elongated parallel to the Dinaric geological structures (NW-SE) and situated between mountain or highland massifs also elongated in the same direction.

**In the Dinaric region of Bosnia and Herzegovina, numerous large karst fields (polja) are found at altitudes typically ranging from 300 to 800 meters above sea level.** These fields are morphologically elongated along the northwest-southeast axis, following the dominant orientation of the Dinaric geological structures. They are situated between mountain ranges or highland massifs that also extend in the same structural direction. Notable karst poljes in Bosnia and Herzegovina include **Livanjsko, Duvanjsko, Glamočko, Nevesinjsko, Gatačko,** and **Popovo polje**, many of which are seasonally or permanently flooded and serve as important hydrological and ecological systems within the Dinaric karst region.

It is estimated that around two-thirds of Bosnia and Herzegovina's groundwater resources are stored in karst aquifers, which supply a substantial portion of the country's water for public consumption, agriculture, and industry. It is estimated that several dozen springs have average discharges exceeding 100 liters per second, with a smaller number exceeding 1,000 liters per second. Also is important to note that nationwide statistics vary.

In the Albanian karst there are roughly 110 springs with an average discharge exceeding 100 l/s. Of these, 17 have discharges exceeding 1,000 l/s. Most of them are in the Dinaric part of the country

(north from Vjosa). It is estimated that 2/3 of groundwater resources in the entire country are in karstic aquifers which provide more than 60% of the water consumed in Albania (DIKTAS TDA, 2013).

In Albania, out of 60 identified GWBs, 18 are classified as 'carbonate'. It is estimated that highly karstified rocks and productive karst aquifers crop out over an area of about 6,500 km<sup>2</sup> or ¼ of Albania's total surface area. Large karstic areas in the Ionian and Kruja zones and in the Adriatic basin are covered by flysch and molasses deposits (DIKTAS TDA, 2013).

Karst fields are characterized by the occurrence of springs on one side and the development of sinking streams on the opposite side of the field. Springs may be permanent or intermittent, fed by upstream mountainous areas which usually have higher precipitation. The waters of these springs form streams flowing toward the opposite, regularly lower-lying side of the field where they sink underground. Quaternary and sometimes Neogene or older deposits in the substratum of specific fields contribute to the formation and sustainability of these streams. The largest sinking rivers in Croatian karst are the Lika, Gacka, and Dobra. During high water periods, outflows can greatly exceed the capacity of the sinks, causing occasional flooding of the lowest parts or entire karst fields. The sinking waters of karst fields drain toward the nearest local erosion base, i.e., a lower river valley, or toward the absolute erosion base, i.e., springs and brackish water outlets by the coast.

In Bosnia and Herzegovina, the Dinaric karst covers a significant portion of the national territory, particularly in **Herzegovina, western Bosnia, and parts of central and southern Bosnia**, extending from intermontane basins to the high Dinaric mountains (DIKTAS TDA, 2013; Džajić & Milanović, 2012). The most important karstic springs in Bosnia and Herzegovina include **Buna and Bunica**, as well as **Trebišnjica and Neretva, Lištica and Trebižat** springs.

In Montenegro, the Dinaric karst covers nearly 60% of the national territory, extending from the Adriatic coast to the mountainous hinterland (Petrović et al., 2019). The karst landscape includes extensive plateaus such as Durmitor, Orjen, and Lovćen, and large closed depressions such as the Nikšićko, Grahovsko, and Cetinjsko poljes. Notable caves include Lipska Cave near Cetinje and Đalovića Cave near Bijelo Polje, one of the largest known cave systems in the Western Balkans. Karst aquifers play a key role in Montenegro's hydrology and water supply, providing over 70% of the country's drinking water resources (DIKTAS TDA, 2013; Radulović et al., 2021). The most important karst springs are Bolje sestre, the main source of the regional water supply for the Montenegrin coast (average discharge >3 m<sup>3</sup>/s), as well as Biogradsko, Rijeka Crnojevića, and Morača springs. The hydrogeological system is dominated by conduit-type aquifers with strong seasonal fluctuations caused by the Mediterranean–continental climatic gradient.

The project karstic watershed in Albania is represented by the transboundary area of Cemi/Cijevna watershed shared between Albania and Montenegro is of special ecological importance due to its high groundwater connectivity.

In Montenegro, the importance of preserving the hydrological, geological, and biodiversity values of the Cijevna River has been formally recognized, and since 2017 the river has been designated as a protected natural asset. This recognition is further supported by hydrological and bioecological studies conducted under the DIKTAS and GEF projects emphasize the need for continued investigation and protection of these shared karst systems (Radulović et al., 2021).

A common phenomenon in Dinaric karst is stepped drainage, where sinking waters from one field feed springs located in a lower-neighbouring karst field. In this way, waters infiltrated in the upper parts of the catchment change their character several times on their path to the final recipient, switching from

groundwater to surface water and vice versa. Flow conditions in karst are very complex, and part of the groundwater may not emerge at a lower water step but drain directly through deeper aquifer zones to the main regional erosion base.

In Croatian karst areas, groundwater and surface waters are multiply connected. The focus is primarily on karst aquifers, i.e., caves exclusively dependent on groundwater, as well as other ecosystems connected to groundwater in karst, such as karst springs, rivers, and lakes. Surface waters in fractured terrain sink underground, flow through it, and upon reaching less permeable layers (barriers), re-emerge forming surface streams. A typical example is groundwater discharge at contacts with less permeable rocks, the formation of surface streams at karst fields, and sinking streams where they meet fractured carbonate rocks. The dominant groundwater-dependent ecosystem in this part of Croatia consists of freshwater karst cave habitats (according to the national habitat classification, NKS), i.e., caves and pits closed to the public (according to NATURA 2000), many of which are connected to natural groundwater discharge locations (springs). As mentioned above, only speleological objects that reach the groundwater level in the saturated part of the karst aquifer are considered. All sites listed in the Natura 2000 habitat list of Croatia were selected, as well as a small number not listed but containing findings of targeted bioindicator species, covering various types of subterranean aquatic habitats in the karst areas of the Pannonian and Dinaric parts of Croatia within the aquifer zone or anhydrophylline caves with groundwater inflow.

In Bosnia and Herzegovina focus is primarily on **karst aquifers**, i.e., caves and subterranean habitats fully dependent on groundwater, as well as other ecosystems linked to groundwater in karst, such as **karst springs, sinking rivers, and polje systems**. Surface waters in fractured and karstified terrain sink underground, flow through conduits and fractures, and upon encountering less permeable layers or bedrock barriers, reemerge as springs or temporary surface streams. Some examples include **groundwater discharge at contact zones with less permeable rocks**, formation of **surface streams in karst poljes**, and sinking streams where rivers or streams encounter highly fractured carbonate rocks. The dominant groundwater-dependent ecosystems in Bosnia and Herzegovina include **freshwater karst cave habitats**, according to the national habitat classification, i.e., caves, pits, and siphons often closed to the public. Many of these are directly connected to **natural groundwater discharge points**, particularly karst springs such as **Buna, Bunica, and Trebižat**, which act as hotspots of subterranean biodiversity.

The dominant groundwater-dependent ecosystems in Bosnia and Herzegovina consist of **freshwater karst cave habitats**, according to the national habitat classification, i.e., caves, pits, and siphons often closed to the public.

Only speleological objects that reach the **groundwater level in the saturated zone of the karst aquifer** are considered. Sites included cover locations listed in **Natura 2000** as well as additional caves, springs, and sinking streams not officially listed but containing findings of **targeted bioindicator species**. This selection represents a variety of **subterranean aquatic habitats** in the Dinaric karst regions of Herzegovina, western Bosnia, and parts of central and southern Bosnia, encompassing cave systems, siphons, ponors, and groundwater-fed aquatic habitats.

Speleological objects such as caves, pits, and karst springs are protected under **national legislation**, including entity-level environmental and nature protection laws, which recognize these sites as **natural and cultural goods of general interest** and regulate their management and conservation. Ongoing projects at the national and regional levels focus on **mapping karst springs, caves, sinking rivers, and groundwater-dependent habitats**, particularly within areas identified for potential

inclusion in the **Natura 2000 network**. Protection and management measures are implemented in accordance with the **Water Framework Directive (2000/60/EC)**, the **Habitats Directive (92/43/EEC)**, and relevant national and entity-level environmental regulations.

Efforts include monitoring hydrological regimes, controlling land use in karst catchments, and conserving biodiversity hotspots linked to subterranean waters.

Karst is intensively developed in wide horizontal or gently sloping carbonates mainly of massive and thick-bedded Triassic and Cretaceous formations. The karst morphology is very rich with karren fields, sinkholes, uvalas, poljes, blind valleys, karst plateaus, tower karst, and vertical shafts and caves. During the period 1991–2014, nearly 100 International speleological expeditions are organized in Albania (Zhallov, 2015). In the karst areas, a strong contrast between the remarkable frequency and variety of karst landforms at the ground surface, and the limited depth and length of most of the explored caves is observed; the caves are with a mostly vertical development (Eftimi, 2020).

In Montenegro, speleological objects such as caves, pits, and speleothems are protected by national legislation under the Law on Nature Protection (Official Gazette of Montenegro, No. 27/16), which recognizes them as goods of general interest and regulates their management and conservation. Ongoing national projects focus on mapping karst springs, caves, and groundwater habitats within Montenegro's Natura 2000 candidate network, along with establishing baseline data for groundwater-dependent ecosystems (GDEs). Protection measures are implemented in accordance with the Water Framework Directive (2000/60/EC) and the Habitats Directive (92/43/EEC).

## 2. Ecological Aspects of Groundwater-Dependent Ecosystems

Many ecosystems are directly or indirectly dependent on groundwater, so it is necessary to first define the main ecological characteristics and interactions of groundwater-dependent ecosystems.

Groundwater-dependent ecosystems include:

- Terrestrial ecosystems seasonally or periodically dependent on groundwater;
- Riverine habitats including aquatic, hyporheic, and riparian zones;
- Karst aquifers and cave ecosystems;
- Wetlands constantly dependent on groundwater;
- Springs;
- Estuaries and marine ecosystems dependent on groundwater.

In the karst region of Croatia, groundwater and surface waters are multiply interconnected. The focus here is on karst aquifers and caves exclusively dependent on groundwater, as well as other ecosystems connected to groundwater in karst such as springs, rivers, and lakes. Surface waters sink underground in fractured areas, flow underground, and discharge at less permeable layers, forming surface streams. The dominant groundwater-dependent ecosystem here is freshwater cave habitats (NK classification) and caves and pits close to the public (NATURA 2000), many linked to natural groundwater outlets.

Only those speleological objects reaching the groundwater level in the saturated part of the karst aquifer are considered. All sites listed in Croatia's Natura 2000 habitats and additional sites with records of specific bioindicator species are included, covering types of subterranean aquatic habitats in Karst areas of Dinaric Croatia or anhydrophaline caves receiving groundwater.

Springs belong exclusively to groundwater-dependent ecosystems. Biological classification of springs is based mainly on prevailing abiotic factors. There are few attempts to classify springs based on fauna composition (Gerecke et al., 1998; von Fumetti and Nagel, 2011), but classifications exist based on habitat type (Glazier, 2009) and glaciation exposure (Martin and Brunke, 2012). A more complex classification considering morphology, hydrogeology, and hydrobiology was conducted in Poland (Galas, 2005).

Currently, the most common biological classification (Hynes, 1970; Reiss and Chiffard, 2015), included in Croatia's national habitat classification (NKS), groups springs morphologically into three types

1. **Rheocrenic springs:** typical of mountainous regions where pressurized water emerges at one point, creating a turbulent spring stream. These springs are well-aerated with rocky substrates, dominated by moss vegetation.
2. **Limnocrenic springs:** resemble lakes as water emerges in a deep depression, forming a pond that overflows forming a stream. Characterized by low flow, sedimented bottoms with fine calcareous particles and micro- or macrophyte vegetation.
3. **Helocrenic springs:** diffuse water emergence through mud, organic detritus or permeable rocks, usually forming swampy areas with dense hydrophilous vegetation. This type corresponds to Natura 2000 habitat 7220 (tufa-depositing springs with Cratoneurion), characterized by moss-dominated formations.

Like neighboring countries, Bosnia and Herzegovina's karst aquifers form critical connections between surface and groundwater systems. Sinking rivers and streams recharge underground flows, which later emerge at springs such as Buna, Bunica, Trebižat, and other regional sources, feeding rivers, wetlands, and seasonal polje systems. These ecosystems support groundwater-dependent fauna, including troglobiont and stygobiont species, and represent key biodiversity hotspots within the Dinaric karst region.

Cave and subterranean aquatic habitats in Bosnia and Herzegovina occur primarily within karst aquifers and host specialized stygobiont fauna, including endemic amphipods, isopods, and oligochaetes. Many caves and pits are documented in national speleological records, and a subset is recognized and protected under Natura 2000 or national conservation regulations. Biological monitoring often targets endemic and bioindicator species, reflecting the health of groundwater-dependent ecosystems (GDEs). Karst springs in Bosnia and Herzegovina are abundant and morphologically diverse. Their classification follows Croatia's. Some springs exhibit oligotrophic conditions with very low nutrient loads, supporting sensitive mosses, invertebrates, and stygobiotic communities. wetlands, particularly those associated with large karst poljes (e.g., Livanjsko, Duvanjsko, Popovo) or glacial/intermontane basins, depend almost entirely on groundwater inputs. These habitats sustain diverse assemblages of hydrophilous plants, amphibians, and freshwater algae and invertebrates (Stanić Koštroman et al., 2021). Numerous hydrological, biological, and geological studies confirm the ecological importance and heterogeneity of springs (Džajić & Milanović, 2012;

Gottstein et al., 2009, Stanić Koštroman et al., 2022). Consequently, each spring is considered unique, and existing classification schemes serve as approximate models to study their natural diversity

In Montenegro, groundwater-dependent ecosystems are equally diverse and closely tied to the country's complex karst and mountainous landscapes. Karst formations cover large areas of the Dinaric region, creating a network of subterranean rivers, caves, and springs that sustain unique habitats. Similar to Croatia, Montenegro's karst aquifers form critical connections between surface and groundwater systems, where sinking streams recharge underground flows that later emerge as springs, feeding rivers, lakes, and wetlands.

Cave and subterranean aquatic habitats occur primarily in karst aquifers and are home to specialized stygobiont fauna. Many caves are documented in national speleological records, and some are protected under Natura 2000 and national conservation regulations. Biological monitoring often focuses on endemic and indicator species, such as groundwater amphipods and oligochaetes. Springs in Montenegro are abundant and morphologically diverse. Their classification largely mirrors Croatian systems (rheocrenic, limnocrenic, helocrenic), although regional studies have emphasized differences in hydrochemistry and flow regimes due to variable precipitation and karst permeability. Some high-altitude rheocrenic springs are characterized by exceptionally low nutrient loads and oligotrophic conditions, supporting sensitive moss and invertebrate communities. Certain wetlands, especially those associated with large karst poljes or glacial valleys, depend entirely on groundwater inputs. These habitats support rich assemblages of hydrophilous plants, amphibians, and freshwater invertebrates. Riverine ecosystems in Montenegro often exhibit hyporheic zones strongly influenced by groundwater, with seasonal flow fluctuations creating dynamic habitats. Along the Adriatic coast, submarine groundwater discharge contributes to localized estuarine ecosystems, affecting salinity gradients and nutrient dynamics in small coastal lagoons and bays.

Springs serve as ecological transition zones, integrating features of adjacent ecosystems and acting as biodiversity hotspots. In Montenegro, as in Croatia, the classification of springs often relies on abiotic parameters such as flow, morphology, and substrate composition. However, ongoing studies also emphasize biotic indicators, including endemic stygofauna and moss communities, as key factors in assessing ecological integrity. Each spring, as a transitional interface, carries features of adjacent ecosystems unique to the region. Numerous biological, hydrological, and geological studies confirm this (Gottstein et al., 2009). Hence, each spring is unique, and existing classification criteria are approximate models to study their natural diversity. Understanding these ecosystems is crucial for water management and conservation planning, especially given the increasing pressures from tourism, urban development, and climate variability. Protecting groundwater-dependent habitats requires integrated approaches that consider the connectivity of subterranean and surface waters, the unique biodiversity they sustain, and the ecological services they provide.

### 3. Key Species within Groundwater-Dependent Ecosystems

River streams with intergranular aquifers, hyporheic zones, and riparian vegetation host a very large diversity of fauna and flora dependent on good qualitative and quantitative groundwater status. Due to the lack of recent faunistic research on porous aquifers in Croatia, available results from benthic macroinvertebrate studies in river ecosystems and targeted research of the hyporheic zone are included. Key indicators of good river system status are numerous aquatic macroinvertebrates, particularly stoneflies (Plecoptera) and decapod crustaceans of Austropotamobius genus (*A. pallipes* and *A. torrentium*). Perlodidae family (Plecoptera) is especially indicative of good hyporheic zone status.

**Karst and intergranular aquifers in Croatia support diverse obligate groundwater fauna, most dominantly crustaceans from Amphipoda, Copepoda, Decapoda, Isopoda, Ostracoda, Syncarida, and Thermosbaenacea groups. Other important and highly adapted groundwater invertebrates valuable for ecosystem monitoring include protozoa, micrometazoa (Gastrotricha, Nematoda, Nemertina, Rotifera, Tardigrada), sponges (Porifera), flatworms (Turbellaria), snails (Gastropoda), oligochaetes, polychaetes, leeches (Hirudinea), aquatic insects (Insecta), water mites (Hydrachnellae), and vertebrates such as amphibians and fish (Gottstein 2010; Gottstein et al. 2001).**

Many are narrowly distributed endemic species with limited ranges, complicating the choice of universal indicator species for monitoring multiple groundwater-dependent ecosystems. However, findings of many subterranean fauna species in adjacent groundwater-dependent ecosystems are excellent indicators of these systems' reliance on groundwater and their ecological stability and conservation status, supporting their use in monitoring.

Groundwater crustaceans, as a dominant group, are relatively under-researched in Croatia. New species and subspecies are expected to be discovered, especially in subterranean habitats. The genus *Niphargus* is very species-rich, including surface-dwelling representatives dependent on high-quality groundwater (included here). One endemic genus of isopods included in the analysis is the troglobiont *Monolistra*, which inhabits karst springs and caves with flowing groundwater. It reaches about 10 mm length with distinct habitus and is suitable for biomonitoring. It coexists with the largest subterranean isopod in Croatia, *Sphaeromides*, another suitable species for ecological monitoring of groundwater and groundwater-dependent ecosystems. The genus *Troglocaris* is also widespread across the Dinarides of Croatia.

The genus *Congeria* is another subterranean bioindicator organism for groundwater-dependent systems. Croatia has 16 known cave mussel sites, but living populations are confirmed at only six. *Congeria kusceri* inhabits the Neretva River basin in southern Dalmatia and Herzegovina (three known sites), while *Congeria jalzici* inhabits the Lika River basin (Markov ponor, Dankov ponor, Lukina jama–Trojama system).

The olm (*Proteus anguinus*) is especially noteworthy, inhabiting caves and sinkholes of karst fields from Istria to Dubrovnik. It is considered endangered and listed in Croatia's Red Book of cave fauna as a vulnerable species (VU B1+2bc; C2a).

*Proteus anguinus* is strictly protected by nature protection laws, included in the EU Natura 2000 Annex II and IV. Main threats include uncontrolled urbanization, changes in groundwater regimes due to hydrotechnical interventions (dam construction, water abstraction), and groundwater pollution. It is highly suitable as a bioindicator species for groundwater-dependent ecosystems.

Table 1.1: Lists of bioindicator organisms (many taxa not listed to species level here) of groundwater ecosystems with their corresponding habitats according to NKS included in the analysis and assessment of groundwater karst ecosystems.

Natura 2000 code	8310			8330
NKS code	H. 1.3.	H 1.3.1.	H. 1.3.2.1.	H 1.4.
Habitat type (NKS)	Freshwater karst cave habitats	Underground streams	Underground lakes	Anchialine karst caves
<i>Congerina</i>	x		x	
<i>Enapus</i>	x	x	x	
<i>Lanzaia</i>	x			
<i>Marifuga</i>	x	x	x	
<i>Monolista</i>	x	x	x	
<i>Niphargus</i>	x	x	x	x
<i>Proteus</i>	x	x	x	x
<i>Saxurinator</i>	x			
<i>Stigodiptomus</i>	x		x	
<i>Spheromides</i>	x		x	x
<i>Troglocaris</i>	x		x	x

Karst aquifers in Bosnia and Herzegovina support a highly diverse obligate groundwater fauna, with dominant groups including crustaceans such as Amphipoda, Copepoda, Decapoda, Isopoda, Ostracoda, and Syncarida. Other important and highly adapted groundwater invertebrates, valuable for ecosystem monitoring, include protozoa, micrometazoa (*Gastrotricha*, *Nematoda*, *Nemertina*, *Rotifera*, *Tardigrada*), sponges (Porifera), flatworms (Turbellaria), snails (Gastropoda), oligochaetes, polychaetes, leeches (Hirudinea), aquatic insects (Insecta), and water mites (Hydrachnellae).

Some vertebrate species, particularly endemic and relict amphibians (e.g., *Proteus anguinus* in certain Dinaric caves) and freshwater fish, are also closely linked to karst aquifers and associated springs. These groundwater-dependent organisms serve as bioindicators of ecological integrity and hydrological connectivity, reflecting both the quality and quantity of subterranean water resources across the Dinaric karst region of Bosnia and Herzegovina (Džajić & Milanović, 2012; Gottstein et al., 2001, Delić et al., 2023). The occurrence of stygobiont fauna, such as endemic amphipods (*Niphargus* spp.), crayfish (*Austropotamobius pallipes* and *A. torrentium*), and other cave-adapted invertebrates across adjacent karst springs, caves, and hyporheic zones, provides excellent indicators of groundwater dependence. These species reflect the ecological integrity, hydrological connectivity, and conservation status of these subterranean ecosystems, supporting their use in monitoring and management programs throughout the Dinaric karst of Bosnia and Herzegovina.

Groundwater crustaceans, as a dominant group in subterranean aquatic ecosystems of Bosnia and Herzegovina, remain relatively under-researched, and new species and subspecies are expected to be discovered, particularly in karst caves, springs, and intergranular aquifers. The genus *Niphargus* is highly species-rich and includes both surface-dwelling and strictly groundwater-dependent representatives, serving as excellent bioindicators of aquifer quality.

Among endemic isopods, the troglobiont *Monolistra* inhabits karst springs and caves with flowing groundwater (Delić et al., 2023). *Monolistra* often coexists with *Sphaeromides*, one of the largest subterranean isopods in the region, also used as an indicator species for ecological monitoring of groundwater and groundwater-dependent ecosystems. The genus *Troglocaris* is widespread across the Dinaric karst of Bosnia and Herzegovina, occupying numerous caves and spring systems. Another important groundwater bioindicator is the genus *Congeria*, a subterranean bivalve. In Bosnia and Herzegovina, *Congeria kusceri* has been reported from the Neretva River basin and associated karst poljes and caves, forming small but ecologically significant populations (Bilandžija et al., 2013). These species are sensitive to hydrological and water quality changes, making them valuable indicators for the status and conservation of groundwater-dependent ecosystems in the country.

The olm (*Proteus anguinus*) is a flagship species of subterranean aquatic ecosystems in Bosnia and Herzegovina, inhabiting caves, sinkholes, and subterranean rivers within the Dinaric karst, particularly in Herzegovina and parts of western and central Bosnia. It is considered endangered and is strictly protected under national nature protection laws. *Proteus anguinus* is also listed in the EU Natura 2000 Annex II and IV, highlighting its conservation importance at the European level.

Subterranean fauna in Albanian Karst/dinaric karst is almost not studied and limited to subterranean gastropods (Grego J. et al, 2017).

Subterranean biodiversity in Montenegro is extremely rich yet insufficiently explored. More than 300 species of subterranean fauna have been recorded, many of which are endemic to the Dinaric karst (Gottstein Matočec et al., 2001; Culver & Pipan, 2009). Typical groundwater-dependent species include *Niphargus*, *Monolistra*, *Troglocaris*, *Congeria kusceri*. Also, according Gorički et al. (2017), environmental DNA (eDNA) analysis has confirmed the presence of the *Proteus anguinus* in Montenegro, revealing previously unrecorded locations in subterranean waters. Malacological research by Glöer and Pešić (2008, 2012, 2015) has revealed numerous new species of freshwater gastropods (*Hydrobiidae*, *Moitessieriidae*) from Montenegrin karst springs and caves, particularly in the Skadar Lake basin, Zeta valley, and Boka Kotorska region. These taxa are mostly stygobiont or crenobiont organisms with very restricted distribution ranges, underlining the exceptional endemism of Montenegrin groundwater ecosystems. Their findings provide essential data for future groundwater-dependent ecosystem monitoring and conservation planning.

Aquatic bryophytes, as part of the macrophyte quality element under the EU Water Framework Directive, serve as important bioindicators of ecological status in karst rivers. Among the macrophytes species indicating good ecological status are bryophytes such as *Fontinalis antipyretica*, *Cinclidotus aquaticus* and *Rhynchostegium riparioides*. Observations of bryophyte communities at the entrances of underground karst features (caves) in river canyons are particularly valuable, as they can provide early indications of water quality before it enters subterranean systems.

## 4. Materials and Methods

Speleological research must be conducted using standard speleological equipment. Single Rope Technique (SRT) is used for vertical cave exploration. In investigated sites, microclimatic parameters must be measured: substrate, air, and water temperature (if water is present) and relative air humidity. Photo documentation of cave entrances selected underground spaces, bio-speleological surveys, fauna, and bryophytes is required. Underground spaces, fauna, bryophytes, and cave entrances are documented by digital camera. GPS coordinates of cave entrances are recorded.

In all localities to be sampled, as well as the frequency of sampling, will be standardized and recorded to ensure reproducibility of results.

Cave diving research must be done using standard cave diving techniques. Equipment includes diving regulators, air cylinders, underwater lamps for lighting. Divers should use dry trilaminar suits. Underwater photography of subterranean spaces and fauna is recorded with a camera.

Biological material in subterranean habitats is collected manually using exhaustors and tweezers, with detailed examination of cave habitats. Collected fauna is preserved in 70%, 75%, or 96% ethanol depending on conservation needs. Aquatic bryophytes will be manually collected and air-dried without pressing for further analysis. Each sample is properly and uniformly labelled with exact data on locality name, date of collection, collector, and medium. After preservation and labelling, the material is delivered to taxonomists for processing.

Additional abiotic parameters may also be recorded where applicable to better understand environmental conditions affecting biodiversity.

The basic physico-chemical parameters that are crucial to monitor in groundwater include the following:

- Water temperature, as it affects chemical processes in the aquifer and biological activities.
- pH value, which determines the acidity or alkalinity of water and effects on the solubility of substances and the availability of elements.
- Dissolved oxygen, essential for oxidation processes and for the life of microorganisms (bacteria) in water.
- Electrical conductivity, which indicates the concentration of dissolved ions in water and provides insight into mineralization.
- Total dissolved solids (TDS), which reflect the level of various ionic compounds.
- Concentration of basic cations (e.g.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ) and anions (e.g.  $\text{HCO}_3^{-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^{-}$ ).
- Parameters such as turbidity, organic and inorganic carbon (TOC, DOC)

Aquatic fauna in cascades and shallow lakes is collected with tweezers and aquarium nets; during diving, fauna is collected using a Sket bottle, while aquatic bryophytes are manually collected and air-dried without pressing for further analysis.

All collection and sampling procedures will follow ethical guidelines to minimize disturbance to cave ecosystems and species.

Collected biological data will be analyzed using statistical methods, including descriptive statistics, biodiversity indices, and comparison between localities, to assess species composition and ecological patterns.

## 5. Conclusion

A precise interpretation requires a good geological and hydrogeological knowledge of the site, including the interaction of water with the environment, which allows for an understanding of geochemical processes and anthropogenic impacts on groundwater. Equally important is the assessment and monitoring of biodiversity. Understanding the presence and dynamics of aquatic fauna, macrophytes (bryophytes) provides essential information on the ecological status and resilience of groundwater-dependent ecosystems, complementing geochemical and hydrological data.

Continuous monitoring is important for reliable analyses and drawing conclusions about the quality and condition of groundwater.

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