

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

Submerged or partially submerged sea caves (8330)



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

**Submerged or partially submerged sea caves
(8330)**

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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 MSs 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

HD: Habitats Directive

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)		

MSFD: Marine Strategy Framework Directive

SEEA EA: System of Environmental Economic Accounting- Ecosystem Accounting

WFD: Water Framework Directive

Executive summary

Submerged or partially submerged sea caves (habitat 8330) are present in all five EU Marine Regions. They may be formed in a variety of ways including by erosion of bedrock, the flooding of volcanic lava tubes which extend to sea level or when terrestrial caves have become totally or partially submerged due to changes in sea level. A general description of the habitat is provided including references to the definition in the Habitats Directive Interpretation Manual and EUNIS habitat types (level 4) and Annex I of the Nature Restoration Regulation.

A structured framework for the ecological characterization of submerged and semi-submerged cave habitats and the selection of appropriate variables for assessing their condition is set out in Section 1. Thirteen **key characteristics and corresponding variables essential for evaluating condition of the habitat** have been identified according to the framework proposed by the the System of Environmental Economic Accounting - Ecosystem Accounting (SEEA EA), an international standard for ecosystem accounts adopted by the United Nations Statistical Commission (Section 1.2 and Table 1).

All thirteen Member States that have reported habitat 8330 as present in their jurisdictions provide some information about the location and broad characteristics of the marine caves that have been given Natura 2000 status. Some of this stems from work that has been carried out specifically to fulfil monitoring and assessments for the Habitats Directive and in particular reporting under Article 17 but there are also relevant reports of marine cave surveys and assessments that can or have been used to inform such assessments although not directly stated as a reason for the work.

Only five **Member States** (Croatia, Ireland, Italy, Romania and Spain) have published specific **methodologies for assessment of structure and function of habitat 8330** and these provide varying degrees of detail. Out of the thirteen key characteristics of marine cave habitats described in Section 1, only three are specifically identified for recording in the methodologies of all these five Member States (Table 4). They are;

- topography/physical structure
- epifaunal and infaunal assemblages and characteristic species
- abundance and diversity of characteristic species

Many of the other key characteristics and associated variables may be recorded or described in general terms by these and other Member States even if not detailed in a specific methodology for marine cave assessments. Examples are geology, exposure to currents and wave action, water quality and associated fish and seals.

Reference values and thresholds are generally not defined in the methodologies available for review of condition monitoring of marine caves. Instead, the approach taken by Member States is to note trends in variables and rely on expert judgement to assess condition. Absence of pressures has been taken as an indication of favourable conservation status in some cases, where there are insufficient baseline data on which to fully describe the habitat or its typical species.

There is a lack of information on **sampling frequency** for monitoring marine caves. It is assumed that monitoring frequency is linked to the regular Article 17 reporting requirements, but this does not mean that all identified marine cave habitats are surveyed and monitored for each reporting period, nor that this is a necessity for all key characteristics.

No information is available on aggregation methods at a local scale or biogeographic scale specifically for marine cave habitats.

There is much commonality in approach across Member States in the study of marine caves but few studies on the issue of connectivity/fragmentation in marine caves. Advances in technology and in our understanding marine ecosystems leading to **new methodologies** to study and monitor marine caves.

The final part of document is focused on **guidance for harmonising methodologies** to ensure consistent data collection and assessment criteria across EU Member States. A proposed list of **essential, recommended and specific condition variables** is presented covering abiotic, biotic, and landscape/seascape characteristics. Potential approaches for **making assessments of condition** include comparison to undisturbed areas, hindcasting, modelling and expert judgement. Cross reference is also made to EU reference values in the Water Framework Directive and Marine Strategy Framework Directive that may be relevant.

1 Definition and ecological characterisation

1.1 Definition and interpretation of habitats covered

The Interpretation Manual of this habitat (European Commission, 2013) gives the following very brief description of 'Submerged or partially submerged sea caves' (code 8330):

"Caves situated under the sea or opened to it, at least at high tide, including partially submerged sea caves. Their bottom and sides harbour communities of marine invertebrates and algae."

The habitat is listed in the Interpretation Manual under Rocky Habitats and Caves and in the subcategory 'Other Rocky Habitats'. Also, in this subcategory are 'Caves not open to the public' (code 8310). Whilst the two cave habitats do share some characteristics, there are some very significant differences in their physical environments. There are also very specific monitoring techniques for investigation of underwater caves and significant differences in the associated communities of the two types of caves. For these reasons they have not been clustered for the purposes of this study.

Instead, we propose that, for this review of monitoring and assessment 'Submerged or partially submerged sea caves' are clustered with habitats listed under 'Open Sea and Tidal Areas'. The latter are a group of habitats that are in the Interpretation Manual under the category COASTAL AND HALOPHYTIC HABITATS. Our reasoning for proposing this clustering is that the category of 'Open Sea and Tidal Areas' includes habitats that have some very similar biotic and abiotic characteristics as well as similar issues with data collection and monitoring to habitat type 8330. In particular;

- Habitat types where major influences on the physical characteristics and associated biological communities are tidal conditions, degree of submergence, salinity and exposure to wave action and currents.
- Habitat types where the practicalities of monitoring, imposed by the need to work in a tidal and/or fully submerged environment, impose certain limitations on data collection even when compared to the monitoring of terrestrial caves.

According to the 2022 EUNIS marine habitat classification the following EUNIS habitat types (level 4) may be present as components of habitat type 8330: MA127, MA149, MA155, MB127, MB14E, MC126, MC146, MC152, ME152. One of these habitat types (MC126) is also listed in Annex II of the Nature Restoration Regulation.

'Submerged or partially submerged sea caves' are present in the following Member States: Bulgaria (BG), Cyprus (CY), Denmark (DK), Spain (ES), France (FR), Greece (GR), Croatia (HR), Ireland (IE), Italy (IT), Malta (MT), Portugal (PT), Romania (RO) and Sweden (SE).

The diversity of caves is strongly influenced by the underlying geology and oceanographic conditions, including tidal range.

In the **Mediterranean** most of the marine caves investigated to date are located in the northern Mediterranean basin particularly along eastern Adriatic, Aegean, Tyrrhenian, Provencal and Ionian coasts. Very dense concentrations are known from islands and rocky peninsulas such as around the Croatian and Balearic Islands, Corsica and Sardinia where they have developed in areas of limestone (Gerovasileiou & Bianchi, 2021).

In the **Macaronesian** region submerged and semi-submerged caves are common around the Canary Islands and the Azores. They have formed along coastlines that are predominantly rocky, and subject to strong erosion but, as in the case of the Canary Islands, some caves have also formed following saltwater incursion of volcanic tubes (lava tube caves).

In the **Atlantic** region, there are numerous sea caves around the coasts of Ireland formed in areas of geogenic hard rock habitats. The habitat is also present in France along the coast of Brittany and the Atlantic coasts of Spain and Portugal.

Both submerged and semi-submerged caves have been recorded from the northern and western parts of the **Black Sea** where they have developed in karstic and volcanic rocks (Ereskovsky et al., 2018).

In the **Baltic Sea**, there are sea caves in the cliff formations on the west coast of the Bornholm peninsula. These are basaltic dykes which have been eroded by waves leading to long and narrow, fissure-like sea caves such as the Sorte Gryde (Black Pot) cave which has a passage more than 40m in length¹.

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

Habitat 8830 is typically formed by erosion in areas of bedrock. Coastal and marine caves are also created by the flooding of volcanic lava tubes which extend to sea level as has occurred around some the Macaronesian islands, or when terrestrial caves have become totally or partially submerged due to changes in sea level.

Two subtypes, although not distinguished in the Habitat Interpretation Manual, are:

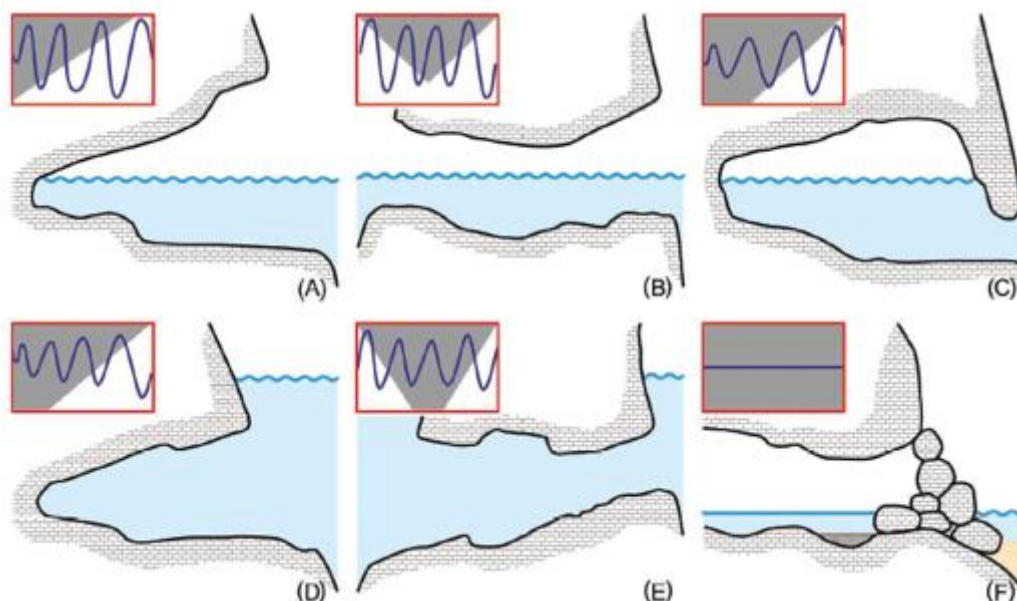
- **Anchialine caves** – caves which are constantly or intermittently under the influence of freshwater but with connections to the sea.
- **Euryhaline caves** – caves which are constantly under the influence of marine waters.

Caves can also be characterised based on their morphology e.g. semi-submerged blind-ended cave, submerged tunnel or cave with air dome (see Figure 1).

Light levels are highly influential on the associated biota. In semi-dark caves, where light levels are reduced compared to the open sea but not totally absent, algae may be present. At the other extreme in fully dark caves, especially where water circulation is reduced, there can be extreme oligotrophy and scanty sessile biota on nearly bare rock (Peres & Picard, 1964; Zabala et al., 1989).

¹ <https://www.showcaves.com/english/dk/caves/SorteGryde.html> [Accessed 30.01.2025]

Figure 1. Basic morphological types of marine caves



(A) semi-submerged blind-ended (cul-de-sac) cave; (B) semi-submerged tunnel; (C) cave with air dome; (D) submerged blind-ended cave; (E) submerged tunnel; and (F) marginal cave.

Source: Gerovasileiou & Bianchi (2021).

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The classification of the ecological characteristic and associated variables follows the UN-SEEA ecosystem condition typology (ECT), which has six classes: abiotic physical, abiotic chemical, biotic compositional, biotic structural, biotic functional and landscape/seascape characteristics (United Nations et al., 2021).

The key ecological characteristics which determine the structure and function of this habitat are mainly abiotic or biotic but there are also relevant features at a landscape scale.

1.2.1 Abiotic characteristics

Abiotic characteristics describe both the physical and chemical state of the habitat. The physical state characteristics of caves typically provide context and locational information. They are a useful starting point for both identifying where the habitat is likely to occur and describing its broad scale characteristics (e.g. Cinelli et al., 1977; Gerovasileiou & Bianchi, 2021; Gerovasileiou & Voultsiadou 2016; Gubbay et al., 2016, Parravicini et al., 2010). Geology and the method of formation, for example, determine many aspects of the physical characteristics of submerged and semi-submerged caves. In areas of limestone, caves may be formed by the dissolution of the bedrock for example, whereas structural weaknesses for example along fault lines, rock falls, tectonic movements and changes in sea level are other potential processes which lead to their formation and physical characteristics (e.g. Suric et al., 2010).

A second group of relevant physical state characteristics relate to the hydrographic conditions. They include exposure to wave action, current and surge and residence time of water in caves. These may be described in general terms, for example using existing data sources, such as bathymetric charts but in some cases these descriptions are supplemented with targeted monitoring both inside and outside caves. The same applies to chemical state characteristics. Where there is notable freshwater inflow, salinity measurements are particularly significant. Nutrient

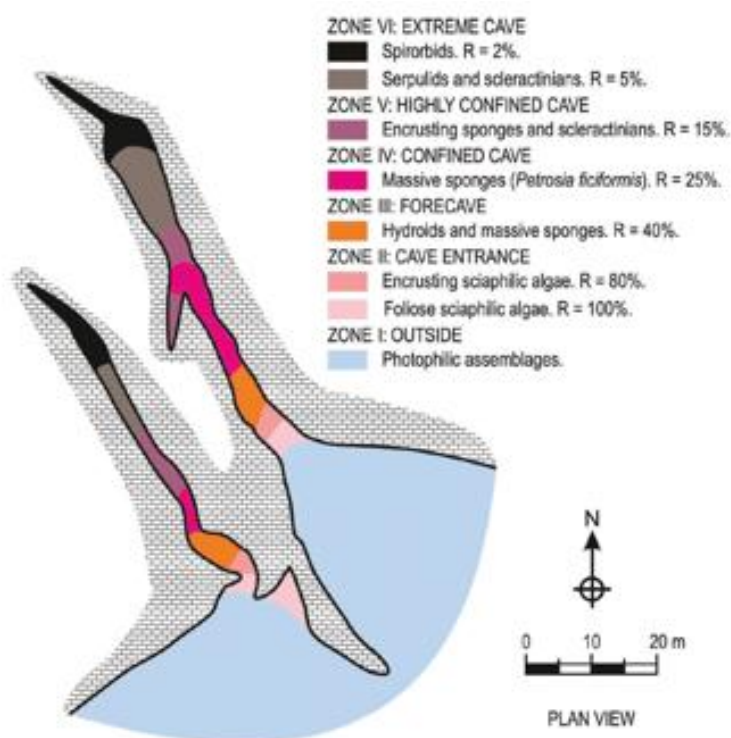
status is useful to measure but may only be done if there is some indication of the likelihood of poor water quality, for example due to the proximity of discharges such as storm overflows or of nutrient/sediment run off from surrounding agricultural land.

The degree of submergence, depth and exposure to currents, wave action, scour and surge as well as residence time of the water has a major influence on the species (epifaunal and infaunal assemblages) that colonise marine caves. In deep, sheltered caves where there is sufficient water movement, gorgonians and seafans may be present for example. In very exposed semi-submerged caves where there is considerable wave action and swell, the cave walls are more likely to be colonised by encrusting fauna such as barnacles, sponges, and hydroids. Light levels and turbidity are further influences, particularly on colonisation by algae which are typically found around cave entrances where light levels are sufficient but also influenced by exposure to wave action will determine the species that are present.

1.2.2 Biotic characteristics

The marine communities and typical species in this habitat differ depending on the type of cave (e.g. dark or semi-dark, fully submerged/partially submerged) the rock type, topography and location within the cave (e.g. light levels, position on ceiling, walls or floor) as strong environmental gradients result in marked biological zonation. Riedl (1966) identified six biotic zones in sublittoral cases and Peres & Picard (1964) used a model of two basic cave biocenoses (semi-dark and dark). Bianchi & Morri (1994) distinguished zones based on growth forms, trophic guilds, three-dimensional structure and biotic cover (Figure 2).

Figure 2. Zonation of the biotic assemblages of the twin caves ‘Grotte del Bue Marine in the Gorgona Island (Tyrrhenian Sea, Italy) according to the confinement gradient



R = percent cover. Cave depth is 6.5 m at the entrance and reaches 0 m in the terminal tract

Source: Gerovasileiou & Bianchi, 2021

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The species present include cave-specific species, species which are also found in suitable habitats outside caves, species which periodically move outside caves and those that may be carried into caves by chance. In fully submerged caves macroalgae, mostly rhodophytes will be limited to the area around the entrance where light levels are sufficient. Within the cave in the semi-dark and dark zones, where strong water movement is often a factor, filter feeding animals such as sponges, soft corals, hydroids, bryozoans, tunicates and polychaetes colonise the rock faces. Different species are generally present on the cave floor depending on the substrate (mud, sand, pebbles, boulders etc), level of turbulence and abrasion. They include polychaetes, molluscs, tube dwelling and burrowing anthozoans as well as both sessile (e.g. barnacles) and free-swimming crustaceans e.g. (mysids, amphipods, cumaceans thermosbaenacean). The only two species of Remipedia (an anchialine stygobitic) from Europe have been found in a submarine lava tube cave on Lanzarote (Koenemann et al., 2009).

The fish fauna can be a mix of speleophilic species², nocturnal species, species typical of cryptic habitats and fish found on rocky reefs that may enter or inhabit the entrance areas of the caves. In Mediterranean marine caves, 132 species have been recorded to date representing about 17% of the total Mediterranean fish species richness (Kovacic et al., 2024). They commonly included gobies, cardinal fish, conger eel, sea bream, and scorpion fish (Bussotti et al., 2015).

Some deep-water species secondarily colonise caves and, in extensive cave systems such as the flooded lava tubes in the Canary Islands, the extent of saltwater intrusion, its stratification and the residence time of seawater has resulted in a specialised fauna with pronounced morphological, physiological, biochemical and behavioural adaptations, such as the blind crab *Munidopsis polymorpha* (Wilkens et al., 1986).

In partially submerged caves, typical species from the surrounding rock may be present including macroalgae which can sometimes extend into cave entrances and some distance within. Where the caves are of sufficient size and have a suitable area which remains above the high tide at all times, the drying areas may be used by seals as haul out or pupping sites. This is the case for the Mediterranean Monk Seal which within EU Member States, uses caves in various locations along the coast of Cyprus (Beton et al., 2021) and around the Desertas Islands of Madeira (Pires et al., 2008).

Alien species have also been recorded within marine caves. In the Mediterranean, for example, 56 alien species (molluscs, cnidarians, bryozoans, polychaetes, crustaceans, macroalgae, tunicates and fishes) have been recorded in marine caves. Shipping and Lessepsian migration were considered the most common pathways of introduction followed by aquaculture and aquariums (Gerovasileiou et al., 2016).

Examples of variables that can be used to measure these characteristics when reporting on the condition of habitat type 8330 are summarised in Table 1.

² species preferentially or exclusively inhabiting caves

Table 1. Ecological characterisation and selection of variables used to measure habitat condition of habitat 8330 (submerged and semi-submerged caves)

Ecological characteristics	Types	Description	Examples of associated variables
Abiotic characteristics	Physical state characteristics	Degree of submergence/depth, tidal regime	Depth in relation to chart datum, tidal range
		Geology	Mineral/rock type
		Topography/physical structure	Physical dimensions, extent, longitude and latitudinal gradients, form.
		Hydrodynamics - Exposure to currents, wave action, scour and surge, circulation patterns	Current speeds, residence time
		Light levels	Irradiance
		Turbidity	Particle concentrations
	Chemical state characteristics	Salinity/freshwater influence/stratification	Salinity
		Water quality	Various parameters including nitrates & phosphates
Biotic characteristics	Compositional state characteristics	Invertebrates - Epifaunal & infaunal assemblages and characteristic species	Number of species/ biocenosis, presence (e.g., SACFOR ³ scale).
		Vertebrates - Associated fish and marine mammals (seals)	Fish species, seals using caves for breeding/haul out sites
	Structural state characteristics	Characteristic species	Percentage cover, biomass, Synthetic indicators – diversity index (M-AMBI, BENTIX etc ⁴)
	Functional state characteristics	Population dynamics	Population density and distribution of key species. Reproductive success and life cycle stages of resident species.
Landscape/ seascape characteristics		Connectivity/fragmentation	Addition/removal of barriers to water movement/species dispersal
Other		Disturbance	% free from damage due to human impact

1.2.3 Ecological processes that are relevant regarding proper functioning.

Hydrographic conditions are key to both the biological and physical characteristics and condition of coastal and marine caves. The marine communities and species which colonise or use caves are affected by water temperature, salinity, water circulation and residence time of water within the cave. In the Marseille area (France), for example, the disappearance of swarms of the stygophilic mysid *Hemimysis speluncola* was caused by two strong thermal

³ SACFOR is an abundance scale which has been used for both littoral and sublittoral taxa. (S=super-abundant, A=abundant, C=Common, F=frequent, O=occasional, R=rare) giving an indication of the % cover of individual species/colonies as well as growth forms such as crusts or turfs. <https://mhc.jncc.gov.uk/media/1009/sacfor.pdf>.

⁴ Marine Biotic Indices - multimetric indices of benthic habitat condition based on the macrozoobenthic community e.g. Paul et al., 2023

anomalies in 1997 and 1999 (Chevaldonné & Lejeusne, 2003). Water quality and the circulation of seawater in and around caves also affects larval dispersal and the input of suspended particulate organic matter which is an important source of food for filter feeding organisms.

In the case of the physical characteristics of caves, exposure to wave action, scour, surge and the tidal regime are major influences. Changes in these conditions and their effects do however need to be viewed in the context of the natural cycle of erosion and collapse of caves, with the rate of change depending on the geology and local conditions. This is particularly the case in friable rock but can also be caused by natural events such as storm damage leading to a cave collapse (JNCC, 2004). In the Marseille area (France) the disappearance of swarms of the stygophilic mysid *Hemimysis speluncola* was caused by two strong thermal anomalies in 1997 and 1999 (Chevaldonné & Lejeusne, 2003).



The blind crab *Munidopsis polymorpha* in La Corona volcanic tube (Lanzarote, Canary Islands)

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1.3 Selection of typical species for condition assessment

Typical species of the habitat are used to assess whether a habitat is at Favourable Conservation Status. The assessment of typical species is included as part of the assessment of the structure and function parameter, although a full assessment of the conservation status of each typical species is not required.

According to the guidelines for reporting under Article 17 (European Commission, 2023), the selection of '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'). Moreover, assuming that the habitat's structure and function are already being monitored, it is important that they provide any useful additional information.

Given the ecological and geographical variability of Annex I habitat types, different species can be selected as typical species for a habitat type in different marine regions. Indeed, even within one Member State different typical species may be present in different parts of the range of the habitat type or in different subtypes. On the other hand, many species may be typical for several habitats and not dependent on a single Annex I habitat type (European Commission, 2023).

Typical species may be drawn from any species group. A valuable reference list is the World Register of marine Cave Species (WoRCS) which also categorises species recording according to Phyla⁵.

The species used to monitor the condition of this habitat type differ depending on the geographical location and characteristics. Table 2 indicates frequently present groups from which species for monitoring may be selected, and the types of changes in quality they could be used indicate. They may be drawn from any species group and, in addition to plants, which are most often selected, consideration should be given to also selecting animals, including birds. For example, amphipod species (Crustaceans) are reliable bioindicators used in lab ecotoxicology tests as well as in field biomonitoring studies (Navarro-Barranco et al., 2020).

Anthozoa species such as cup corals and large sponges (Porifera) can be used as indicators of cave systems that are undisturbed, as they are long-lived as well as being relatively easy to recognise and relocate for monitoring if the caves systems are adequately mapped. Unscoured cave passages and tunnels can be particularly rich in sponges and dense growths of hydroids and ascidians. They probably vary in density and abundance seasonal but are likely to regrow in the same area of a cave each year. Salinity changes such as freshwater seepage into intertidal caves can be indicated by the development of green algae films and opportunistic low salinity -tolerate macroalgae and terrestrial mosses in place of fully marine communities where sufficient light reaches into the cave (JNCC, 2004).

⁵ <https://www.marinespecies.org/worcs/>

Table 2. Potential species groups from which to select typical species for monitoring habitat 8330 (submerged and semi-submerged caves)

Species Group	Sub-type	Ecological notes	Sensitive to changes in condition
Algae	(S-S) & (S)	Macroalgae may be present around cave entrances where light levels are sufficient. They are primary producers, absorbing nutrients, providing food, shelter and a surface for attachment by other species. The growth and viability of algae is affected by water movement, light levels and water quality.	Macroalgae may be present around cave entrances where light levels are sufficient. They are primary producers, absorbing nutrients, providing food, shelter and a surface for attachment by other species. The growth and viability of algae is affected by water movement, light levels and water quality.
Porifera	(S-S) & (S)	On cave walls in areas of good water flow. Sponges are sessile filter feeders playing a critical role in linking the pelagic environment to the benthos through nutrient cycling (Maldonado et al., 2012) They are able to accumulate hydrocarbons, PCBs and heavy metals (Perez, 2000). They can also provide a habitat for other species (e.g. crustaceans and polychaetes).	Turbidity, organic matter, water circulation, abrasion
Cnidaria - Anthozoans	(S-S) & (S)	On cave walls in areas of good water flow. They may be slow-growing and fragile therefore vulnerable to mechanical damage, for example by divers. Sediment re-suspension can also have a detrimental effect on sessile filter feeders.	Turbidity, organic matter, water circulation, abrasion.
Arthropoda - Crustaceans	(S-S) & (S)	Sessile and mobile. Ecological role as food source, detritivores, recycling nutrients.	Water quality, food webs
Mollusca	(S-S) & (S)	Sessile and mobile. Ecological role as food source, detritivores, recycling nutrients.	Water quality
Fish	(S-S) & (S)	Mobile. In all zones, Fish are useful indicators of environmental water quality because of their differential sensitivity to pollution.	Water quality, food webs, human disturbance
Seals	(S-S) & (S)	Monk seals shelter in coastal caves that have internal beaches, giving birth and raise their pups almost exclusively in this habitat.	Disturbance from human activities in and around caves used by this species is known to be a threat.

(S-S) Semi-submerged; (S): Submerged

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

All Member States that have reported habitat 8330 as present within their jurisdiction provide some information about the location and broad characteristics of the marine caves that have been given Natura 2000 status⁶. Some of this work has been carried out specifically to fulfil monitoring and assessments for the Habitats Directive with published guidance on the methodologies to be used (Italy, Croatia, Romania, Spain and Ireland) (La Mesa et al., 2019; Bakran-Petricioli, 2007; Zaharia et al., 2013; López-Bedoya & Pérez-Alberti, 2009; Scally et al., 2020).

There are also relevant reports of marine cave surveys and assessments carried out in other EU Member States that can or have been used to inform such assessments although not directly stated as a reason for the work (e.g. marine cave surveys carried out in Cyprus, France, Greece, Malta, and Portugal) (Jimenez et al., 2019; Rastorgueff, 2015; Digenis et al., 2022; Bussotti et al., 2015; Furlani et al., 2023; Knittweis et al., 2015; Monteiro et al., 2013).

All these methodologies have been considered in the following review albeit distinguishing between what is being done by Member States for reporting on habitat condition under Article 17 and what has been done as part of other initiatives.

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

A summary of the ecological characteristics and main variables used to measure habitat condition of submerged and semi-submerged caves is presented in Table 1 (Section 1).

Examples of the characteristics and variables used by Member States as part of the assessment of conservation status are presented in Table 3 and a summary analysis is given in Table 4, which indicates that only five Member States: Croatia (HR), Ireland (IE), Italy (IT), Romania (RO), Spain (ES) have published methodologies specifically relating to habitat type 8330.

⁶ as evidenced by the information on Standard Data Forms for designated sites where 8330 is a feature and Article 17 reporting for this habitat type.

Table 3. Examples of variables used by Member States to assess condition of habitat 8330 (submerged and semi-submerged caves)

Description	Examples of variables used by Member States	Notes
1. Abiotic characteristics		
1.1 Physical state characteristics		
Degree of submergence/depth, tidal regime	HR - Position, depth IT - Morpho-bathymetry RO – Location ES – Changes in sea level, tidal regime	Recorded by 4 Member States (MSs) that have published methodologies for assessing habitat 8330. In other MSs, whilst not highlighted in a specific assessment methodology for this habitat, the location of marine caves in relation to depth and tidal regime are typically included in the descriptions of the relevant Natura 2000 sites such as those provided in the SDFs as they have a major influence on form and associated species.
Geology	ES – Type of rock (karstic, metamorphic, igneous, volcanic, sedimentary). Weathering, karstification	Whilst only highlighted in one specific methodology for assessing habitat 8330, the geological characteristics of caves are typically included in the descriptions of them in relevant Natura 2000 sites (e.g. in SDFs) as a major influence on cave formation and form.
Topography/physical structure	HR - Shape and size, continuous or fragmented. IE – Cave length & width, surface area IT- Morpho-bathymetry RO - Number and internal dimensions ES –Orientation, type and dynamics of cliffs, internal dynamics, cave length	Recorded by all 5 MSs that have published methodologies for assessing habitat 8330. In other MSs whilst not highlighted in a specific assessment methodology the topography of caves and cave systems (caverns, tunnels, openings etc.) and in particular their dimensions are recorded in many cave surveys, although not necessarily comprehensively mapped or measured in great detail (depending on issues such as access, safety and available methodologies).
Hydrodynamics - Exposure to current, wave action, scour and surge, circulation patterns	IT- Morpho-bathymetry ES - Wave action ES – Modification of surface water and aquifers	Although only specified in two MSs assessment methodologies, these characteristics are typically included in the descriptions of the relevant Natura 2000 sites such as those provided in the SDFs although the information may be very general. Water circulation may also be noted in general site descriptions if limited water circulation is considered to be an issue affecting cave biocenosis.
Light levels	ES – Light intensity	Light levels will affect the presence, distribution and abundance of algae in and around cave systems. Only one MS has specifically indicated recording light levels (measurements could be made with a luxometer); however, this may also be determined indirectly when recording the distribution and abundance of shade and/or light tolerant species.
Turbidity	Not specified	Turbidity will affect the presence, distribution and abundance of epifauna within marine caves. No MSs have specifically indicated recording turbidity; however, this may be determined indirectly through species recording or more directly in water sampling

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Description	Examples of variables used by Member States	Notes
Salinity/freshwater influence/stratification	ES - Salinity	Only one MS has specifically indicated recording salinity, but this characteristic will typically be recorded if water quality sampling is undertaken (see below). Measurements could be made with CTD (Conductivity-Temperature-Depth) instruments that measure water characteristics such as salinity, temperature, pressure, depth and density.
Water quality	IT - Chemical, microbiological and physical parameters in water column ES – Pollution by fossil fuels and other chemical substances	Only specified by two MSs however this characteristic will typically be recorded if water quality sampling is undertaken (see below)
1.2 Chemical state characteristics		
Salinity/freshwater influence/stratification	ES - Salinity	Only one MS has specifically indicated recording salinity, but this characteristic will typically be recorded if water quality sampling is undertaken (see below). Measurements could be made with CTD (Conductivity-Temperature-Depth) instruments that measure water characteristics such as salinity, temperature, pressure, depth and density.
Water quality	IT - Chemical, microbiological and physical parameters in water column ES – Pollution by fossil fuels and other chemical substances	Only specified by two MSs however this characteristic will typically be recorded if water quality sampling is undertaken (see below)
2. Biotic characteristics		
2.1 Compositional state characteristics		
Invertebrates - Epifaunal & infaunal assemblages and characteristic species	HR - Species present IE – Characteristic flora and fauna, biotopes IT - Macrobenthic characterisation, identification of vagile fauna. RO - Characteristic species, number of biocenoses or presence of certain biocenoses ES – Number of characteristic species present in the cave. Presence of species that indicate environmental quality.	Recorded by all 5 MSs that have published methodologies for assessing habitat 8330. In other MSs, whilst not highlighted in a specific assessment methodology for this habitat, these characteristics are typically included in the descriptions of the relevant Natura 2000 sites such as those provided in the SDFs although the information may be very general.
Vertebrates - Associated fish and marine mammals (seals)	Not specified.	No MS have specifically indicated recording these characteristics, but they may be noted in general site descriptions.
Exotic and invasive species	ES – Presence and abundance of non-native species in the cave	Only one MS has specifically indicated recording non-native species in the cave, in order to measure the degree of naturalness and considering the possible negative effects that these species may have on the cave habitat.

Technical Guidelines for assessing and monitoring the condition of
Submerged or partially submerged sea caves (habitat type 8330)

Description	Examples of variables used by Member States	Notes
2.2 Structural state characteristics		
Characteristic species	HR - % cover IE – Species abundance (SACFOR scale) IT - Abundance and percentage cover of biota RO - Abundance of species ES - % of characteristic communities present in the cave	Recorded by all 5 MSs that have published methodologies for assessing habitat 8330. In other MSs, whilst not highlighted in a specific assessment methodology for this habitat, these characteristics are typically included in the descriptions of the relevant Natura 2000 sites such as those provided in the SDFs although the information may be very general.
2.3 Functional state characteristics		
Population dynamics	HR - Distribution/depth IE - Species surface area coverage IT - Abundance and percentage cover of biota RO - Spatial distribution of biocenoses inside cave ES - % of characteristic communities present in the cave	Relevant elements recorded by all 5 MSs that have published methodologies for assessing habitat 8330. In other MSs, whilst not highlighted in a specific assessment methodology for this habitat, some information on these characteristics may be included in the descriptions of the relevant Natura 2000 sites such as those provided in the SDFs although the information may be very general.
3. Landscape/seascape characteristics		
Connectivity/Fragmentation	Not specified.	No MS have specifically indicated recording these characteristics, but they may be noted in general site descriptions.
4. Other		
Disturbance	HR - Human influences e.g. waste disposal ES - Over-frequented of caves	Only specified by two MSs, however this characteristic is likely to be reported if disturbance issues are noted e.g. presence of litter, disturbance of wildlife, damage to epifauna.

Table 4. Main ecological characteristics and associated variables monitored in the assessment of structure and function of habitat 8330 (submerged and semi-submerged caves) by EU Member States

Ecological characteristics	Variables	Metrics	ES	HR	IE	IT	RO
1. Abiotic characteristics							
1.1 Physical state characteristics							
Degree of submergence / depth, tidal regime	Depth in relation to chart datum, tidal range	Metres (m), maximum & minimum with seasonal patterns					
Geology	Mineral/rock type	Mineral/rock type					
Topography / physical structure	Physical dimensions and form (e.g. presence of tunnels, air domes, drying areas)	Metres (m), degrees of slope (°), physical features from a reference list					
Hydrodynamics - Exposure to currents, wave action, scour and surge	Current speed, wave height, direction & frequency, circulation patterns	Knots (kn), Metres (m)					
Light levels	Irradiance	Watt/m ²					
Turbidity	Suspended particles, Light transmission through water sample	Nephelometric turbidity units (NTU), formazin turbidity units (FTU)					
1.2 Chemical state characteristics							
Salinity / freshwater influence / stratification	Salinity and conductivity	Parts per thousand Sodium and Chloride (0/00), depth/ boundaries (m) of different water bodies					
Water quality	Various substances including chemicals listed in the WFD and EQSD, nitrates & phosphates, oxygen, chlorophyll, dissolved solids.	pH, Chromophoric dissolved organic matter (CDOM), fluorescent dissolved organic matter (FDOM), total dissolved solids (TDS), dissolved oxygen (mg/l), oxygen saturation (%)					
2. Biotic characteristics							
2.1 Compositional state characteristics							
Invertebrates - Epifaunal & infaunal assemblages and characteristic species	Abundance and diversity of characteristic species	Number of biocenosis/taxa, presence & abundance of species (SACFOR scale), diversity index, (Shannon-Wiener diversity index, AMBI index) biomass, estimated % cover					
Vertebrates - Associated fish and marine mammals (seals)	Abundance, and diversity of characteristic species	Numbers, distribution, population structure, trophic composition (e.g. % omnivores/piscivores), seals using caves					

Ecological characteristics	Variables	Metrics	ES	HR	IE	IT	RO
2.2 Structural state characteristics							
Characteristic species	Condition	Percentage cover, biomass, density. Synthetic indicators (M-AMBI, BENTIX etc)					
2.3 Functional state characteristics							
Population dynamics	Population density and distribution of key species. Reproductive success and life cycle stages of resident species.	Percentage cover, biomass, present of different life stages					
3. Landscape/seascape characteristics							
Connectivity / Fragmentation	Addition/removal of barriers to water movement/species dispersal	Presence/absence of barriers.					
4. Other							
Disturbance	Footprint of activity, number and intensity of negative pressures	Presence/absence, % area directly affected by human activity pressures from a standardised list (graded High, Medium, Low, unknown)					

Methodologies used for assessment and monitoring of habitat condition and existing data sources

Monitoring the composition, structural and functional characteristics of marine caves is typically focused on recording the species that are present as well as their abundance and diversity. This may be on species groups (e.g. sponges) or on individual species (e.g. black corals, or the Mediterranean monk seal). Monitoring the biotic composition of caves has many similarities with monitoring reefs with transect sampling techniques suitable for monitoring the distribution of biocenoses in a cave (Zaharia, 2013). Metrics used include the SACFOR scale⁷, or more detailed assessment of area covered by encrusting species and estimates of biomass. An Ecosystem-Based Quality Index (EBQI), described below, is used in France to report on the condition of marine caves (Bissery et al., 2023). Accessibility, size and depth have a major influence on data gathering, including the level of detail in which the variables are recorded. Periodical monitoring should be considered to better address ecological studies and knowledge expansion on these key habitats (e.g. taxonomic studies on invertebrate communities, algal zonation, nutrient gradients).

Photographic techniques are commonly used to record the epifauna in caves. Still photography at pre-defined locations with a quadrat defining the sampling area has been extensively used. A further development of this technique has been video transects. In a study of caves in the Tremiti Islands MPA (Italy), for example, there was no requirement to use

⁷ Super-abundant, Abundant, Common, Frequent, Occasional, Rare, Present. <https://mhc.jncc.gov.uk/media/1009/sacfor.pdf>

plastic or steel frames to define quadrats as this task was guided using lasers. Acquisition of still images from video transects allowed diving time to be reduced and provided a continuous record of the benthic communities rather than spot information through direct photo-quadrats. The taxonomic macrogroups were identified; chlorophytes, rhodophytes, phaeophytes, sponges and cnidarians with percentage cover assessed for each still image (Spaccavento et al., 2022). Alternatively, samples may be collected for species identification as well as estimating biomass.

Table 5. lists some of the typical methodologies used to gather information on the key characteristics of marine caves.

Table 5. Survey methods used to investigate key characteristics of habitat 8330 (submerged and semi-submerged caves)

Key Characteristics	Methodologies
Degree of submergence/depth	Diving, shore & boat-based surveys
Geology	Existing high resolution geological maps, Aerial survey - Satellite/Drone imagery, shore & boat-based surveys,
Topography / Physical structure	Existing high resolution physical geography maps, Aerial survey - Satellite/Drone imagery, shore & boat-based surveys
Salinity / freshwater influence	Water chemistry data loggers
Light levels	Irradiance measurements
Hydrodynamics - Exposure to current, wave action, scour and surge	Existing bathymetric charts. Aerial survey - Satellite/Drone imagery, Current meters (ADCP)
Water circulation	Current meters
Turbidity / Sedimentation	Water sampling, Sediment sampling. Sediment traps
Epifaunal & infaunal assemblages	Photographic quadrats, video transects, visual census, direct sampling
Abundance of characteristic species	Photographic quadrats, video transects, visual census, direct sampling

Typical existing data sources on the physical characteristics of caves are geological maps, bathymetric charts, coastal survey data and photographic records. In terms of new data gathering, satellite imagery, aerial surveys using drones as well as shore and boat-based coastline surveys have all been used to locate both general and specific locations where this habitat may be present. An example of the outputs from Gozo, Malta are shown in Figure 3.

This is the case in Croatia, for example, where the following generic criteria used are; reduction in areas, number of localities where the habitat is present, changes in the qualitative and/or quantitative composition of taxa, and total known area in the country such that there is a real danger of complete disappearance of the habitat (Bakran-Petricioli, 2007).

The Article 17 reporting guidelines, indicate that for cave habitats their number can be used as a proxy to inform the conclusions on 'structure and functions' (European Commission, 2023).

2.3 Aggregation methods at the local scale

In most EU Member States, a common rather than habitat specific methodology is used to aggregate data on indicators at the local scale to provide a condition assessment at the level of the plot or monitoring locality. For example, a general approach taken in France is illustrated in Box 1.

However, no examples have been found of aggregation methods at a local scale to assess structure and function of marine cave habitats for Article 17 reporting.

Box 1. Aggregation of indicators at a local scale - France

In France, for evaluation at the scale of Natura 2000 sites three parameters (surface, structure and functions and alterations) are assessed against several criteria, themselves represented by one or more indicators filled in or calculated from metrics collected in the field. In this "PatriNat" method, each indicator assessed is compared with a threshold value.

Then each indicator is given a score (negative or zero) which is subtracted from the starting score of 100. A good indicator score will result in few points being subtracted, and a bad indicator score will result in more points being subtracted. Final scores indicate the overall status along a gradient (Table 6 & Figure 4 below).

Table 6. Example of scoring for three indicators A, B, C presenting different response modalities.

PARAMETERS	CRITERIA	INDICATORS	MODALITIES (threshold values)	GRADE
Parameter 1	Criterion X	A	0-3	0
			3-6	-5
			6-9	-10
Parameter 2	Criterion Y	B	80%-100%	0
			20%-80%	-10
			0%-20%	-15
		C	>1	0
			<0	-20
Final score (example)				100-0-15-20 = 65

Source: Delavenne & de Bettignies (2023) and Lepareur et al. (2018)

Figure 4. Determination of conservation status based on its overall score



Source: Delavenne & de Bettignies (2023) and Lepareur et al. (2018)

2.4 Aggregation at biogeographical scale

To assess the conservation status at a biogeographical scale, the area, quality and trends in the habitat need to be assessed. There is a lack of information on how Member States have undertaken aggregation at the biogeographical scale specifically for this habitat type but it is expected that the relevant guidance is followed. The most recent recommendation (for the reporting period 2019-2024) is 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 'unfavourable', then the 'structure and functions' parameter is 'unfavourable-bad' (European Commission, 2023).

2.5 Selection of localities

It is unclear how the number and distribution of localities to carry out the assessment and monitoring of marine caves is determined by Member States. Practical considerations such as the feasibility of accessing the habitat generally play a large part as will the need to cover the different cave types with selection based on their characteristics (caves in different geological strata, physiographic types etc.). In Italy, for example, monitoring sites are chosen to be representative of the characteristics of the individual regions, selected from the '*Catasto Speleologico*' (La Mesa et al., 2019). In Spain the sampling network must consider different environments or geographic areas within sea caves. Monitoring locations should include examples of caves of different origin, lithologies and forms that are subject to different climates and oceanographic parameters with a proposal for a preliminary list (by provinces, covering the Spain coasts), subject to review by experts (Lopez-Bedoya & Pérez-Alberti, 2009).

2.6 General monitoring and sampling methods

A six yearly cycle of reporting, as specified under Article 17, is required under the Habitats Directive. This includes reporting on the conservation status of habitats listed in Annex 1 of the Directive. It applies throughout the territory of the Member State concerned, not only where the habitat occurs within Natura 2000 sites. To inform this reporting, six-yearly monitoring of the relevant habitats would be the minimum required.

Submerged and semi-submerged caves are challenging habitats to survey and monitor. Most of the information is from shallow and/or semi-submerged caves which are generally easier to investigate but even in these cases there is a lack of baseline as well as time series data to identify any trends. Information about deeper caves is even more limited. One of the initiatives to try and improve this is the Dark Habitats Action Plan for the Mediterranean which includes

underwater caves as one of the habitat types for which more knowledge as well as conservation action is needed (UNEP/MAP – SPA/RAC, 2021).

There is much commonality in approach across Member States in the study of marine caves, from initial review of likely locations based on existing geological information and maps, followed by aerial surveys and further reconnaissance from boat and shore to gather more specific locational information. Variations in the methodologies used to assess and monitoring marine caves are mostly related to accessibility, size and depth at which the caves are located.

The feasibility and level of detail possible when recording the physical dimensions of caves, will depend on their complexity (galleries, tunnels, pits etc) and accessibility. Biological information is not always available. For example, in a review of data available on 77 marine caves known from along the coast of Liguria (Italy), geological, morphological and topographical information was found in virtually all cases, but biological information was only available for 13 of these caves (Canessa et al., 2014). When biological information is collected, it most commonly involves non-destructive techniques (diver/surveyor recording and photography) although there may be differences in sampling frequency, and the focus of analysis (e.g. species identification/ biomass estimates/ percentage cover).

There is a lack of information on sampling frequency for monitoring marine caves. It is assumed that monitoring frequency is linked to the regular Article 17 reporting requirements however this does not mean that all identified marine cave habitats are surveyed and monitored for each variable, for each reporting period.

In Ireland, for example, there are a very large number of sea caves around the coast that are completely submerged and unknown. Without considerable exploration it is unlikely that the full extent of the national resources will ever be known. Of the known sea caves, which are generally those that are partially submerged, there are insufficient baseline data on which to fully describe the habitat or its typical species and communities. Only a small sub-sample have been surveyed in detail, and this is not considered large enough to accurately assess their structure and function or extrapolate the likely total area of the habitat (Scaly et al., 2020).

In Italy, annual surveys are recommended where both water quality and biota are recorded (La Mesa et al., 2019). Observations on the species (biodiversity, abundance, percentage cover) are to be made through photographic surveys, aided by targeted samples for the identification of organisms photographed. Romania also recommends annual biological sampling and photographic transects from three sampling stations of this habitat type (Zaharia, 2013). The complexity of monitoring will depend on the physical dimensions of the cave and its location, as well as the number and variety of caverns in the system.

Long term time-series data are mostly lacking but there are examples albeit not linked to monitoring for the Habitats Directive. One of these is a study that examined long-term (26 years) changes in sessile benthic communities in the Grotta Azzurra cave of Capo Palinuro (Italy) along three main environmental gradients (confinement, depth and light). This revealed significant changes in the spatial organisation of benthic assemblages possibly linked to global warming and repeated summer heat waves (Montefalcone et al., 2023).

Another long-term study of changes in the Bergeggi marine cave (Italy) over 30 years revealed a decline in the cover of sessile organisms, matched by an increase of turf and sediment (Montefalcone et al., 2018). Comparing historical photographs from the 1980s of epibiotic communities in Kakoskali Cave, Cyprus is also being considered to provide a rough estimate of the rate of morphological changes in the biostolactites present in this cave (Jimenez et al., 2019).

2.7 Other relevant methodologies

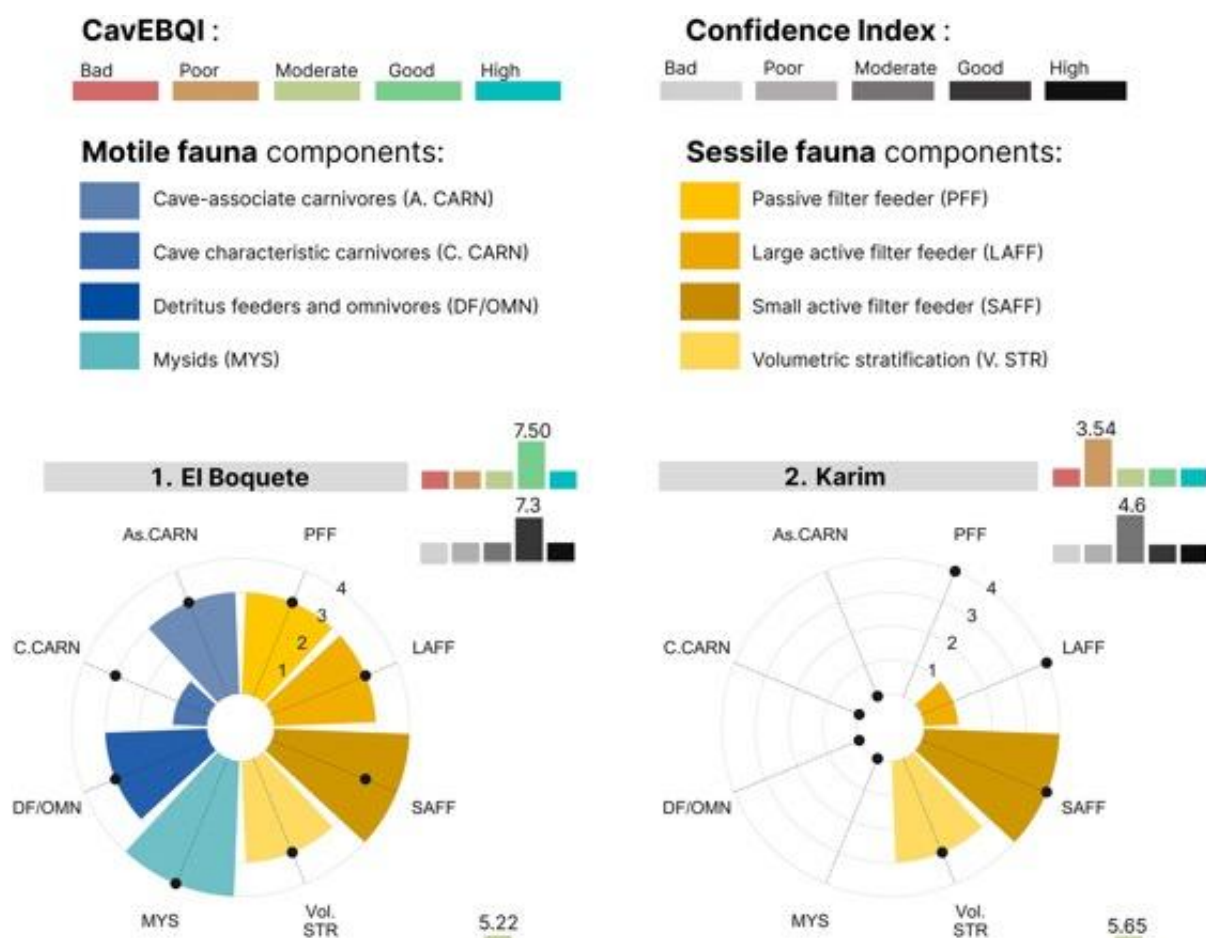
Advances in technology and in our understanding marine ecosystems are leading to new methods and approaches to study and monitor marine caves.

For example, a theoretical model of the structure and function of undersea caves has been developed and used to evaluate the ecological quality of several undersea caves in the Mediterranean (Rastorgueff et al., 2015). Where possible, a set of relevant parameters were evaluated on a semi-quantitative scale for each of these components. The model, adapted from a method developed for *Posidonia oceanica* (Personnic et al., 2014), was used to rank (from zero to 10) the status of cave ecosystems with the results presented as a Cave Ecosystem-Based Quality Index (CavEBQI).

An illustration of an adapted CavEBQI is shown in Figure 5, which applied this index in 21 caves of the Alboran Sea (Lanza-Arroyo et al., 2024).

This approach has subsequently been used to assess marine cave communities in Greece with further trials and cross-calibration exercises recommended (Digenis et al., 2022). It is also advocated in the Integrated Monitoring and Assessment Programme (IMAP) of the Dark Habitats Action Plan which considers that features indicative of high-quality status of underwater caves are “high spatial coverage of suspension feeders with a three-dimensional form and large filter feeders along with the presence of mysid swarms and several species of omnivorous and carnivorous fish and decapods” (Gerovasileiou et al., 2017). The Action Plan recommends that future monitoring schemes for marine caves should mainly consider common indicators related to biodiversity. Specifically, “habitat distributional range” and “condition of the habitat’s typical species and communities”.

Figure 5. Ecosystem-based ecological quality evaluation of undersea caves of the North Mediterranean Sea



Each chart corresponds to a different cave with the ecological status (bars) and the Confidence Index (points) for the components: PFF: Passive Filter Feeders; LAFF: Large Active Filter Feeders; SAFF: Small Active Filter Feeders; V.STR: Volumetric Stratification; MYS: Mysids; DF/OMN: Detritus Feeders /Omnivores; C.CARN: Characteristic Carnivores; A.CARN: Associated Carnivores. The Cave Ecosystem-Based Quality Index (EBQI) and its corresponding Confidence Index (CI) are shown at the top right of each chart.

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Source: Lanza-Arroyo et al. (2024)

Advances in technology and in data processing capabilities can modify and add to existing methodologies. One such example is the use of Structure from Motion (SfM) photogrammetry to characterize the sessile benthic communities in sublittoral caves. This technique was trialled in the Grotta Azzurra (Ancona, Italy) in 2021, and the results compared to a more traditional approach of photo quadrats (Pulido-Mantas et al., 2023). Both techniques gave similar results on species richness, percentage cover of identified taxa and most seascape metrics. The main difference was in estimates of taxa density with the photo-quadrats giving significant overestimates of the values. SfM does however involve much greater sampling, processing, and annotation time and, for large areas, cloud-based processing solutions may be needed to process the imagery data set. The study authors conclude that there is scope for it be considered an additional and complementary tool for marine cave monitoring.

Remotely Operated Vehicles (ROVs) have helped to locate caves in deeper waters, such as those discovered in the Linosa Trough (Mediterranean) and west of Gozo at depths of 270-795m but detailed study using these tools remains logistically challenging (Freiwald et al., 2009; Evans et al., 2016; Borg et al., 2017. In Gerovasileiou & Bianchi, 2021).

2.8 Conclusions

All Member States that have reported habitat 8330 as present in their jurisdictions provide some information about the location and broad characteristics of the marine caves that have been given Natura 2000 status. Some of this stems from work that has been carried out specifically to fulfil monitoring and assessments for the Habitats Directive and in particular reporting under Article 17 but there are also relevant reports of marine cave surveys and assessments that can or have been used to inform such assessments although not directly stated as a reason for the work.

Only five Member States (Croatia, Ireland, Italy, Romania and Spain) have published methodologies, in varying degrees of detail, specifically relating to habitat 8330. It is also the cases that out of the thirteen key characteristics of marine cave habitat described in Section 1.2 and Table 4, only three are specifically noted for recording in the methodologies of all these five Member States;

- topography/physical structure
- epifaunal and infaunal assemblages and characteristic species
- abundance and diversity of characteristic species

It should however be noted that many of the other key characteristics and associated variables may well be recorded or described in general terms by these and other Member States even if not detailed in a specific methodology for marine cave assessments. Examples are geology, exposure to currents and wave action, water quality and associated fish and seals.

Reference values and thresholds are generally not defined in the methodologies gathered for condition monitoring of marine caves. Only one MS has defined such thresholds for the proposed variables. Instead, the approach taken by most Member States is to note trends in variables and rely on expert judgement. Absence of pressures has also been taken as an indication of favourable conservation status in some cases, where there are insufficient baseline data on which to fully describe the habitat or its typical species.

There is a lack of information on sampling frequency for monitoring marine caves. It is assumed that monitoring frequency is linked to the regular Article 17 reporting requirements, but this does not mean that all identified marine cave habitats are surveyed and monitored for each reporting period, nor that this is a necessity for all key characteristics.

No information is available on aggregation methods at a local scale or biogeographic scale specifically for marine cave habitats.

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

3.1 Selection of condition variables, metrics and measurement methods

Variables identified for monitoring programmes need to be robustly associated to the key characteristics and processes (functions) that determine habitat condition and must be sensitive to natural threats or human pressures that decrease favourable condition (Maes et al., 2023). A set of variables associated with all types of characteristics (abiotic physical and chemical, biotic compositional, structural and functional, landscape variables) should be measured. The description of the condition variables, metrics and measurement methods need to be informed and clear so that they can be applicable in all Member States. The ecological characteristics, methodologies, variables and metrics used to investigate and assess the condition of habitat type 8330 are presented earlier in this report. A proposed list of essential, recommended and specific condition variables is presented in Table 7.

- **Essential** condition variables describe essential characteristics of the habitat, reflecting its conservation quality. Are selected on the basis of intrinsic and instrumental relevance, validity, reliability, availability, simplicity and compatibility, and should be assessed in each MS, following equivalent procedures.
- In addition, a set of **Recommended** condition variables are proposed as optional, additional or complementary variables that may need to be applied in some cases, according to contextual factors operating on habitats in the different MSs.
- There are also **Specific** condition variables which should be measured in some particular circumstances (e.g. salinity where there is notable freshwater inflow to caves).

On the other hand, some **descriptive or contextual variables** are included in this section. These variables define environmental characteristics (e.g. climate, topography, lithology) that can influence the habitat condition, are useful to define thresholds for the condition variables and interpret the results of the assessment but are not used in the aggregation of variables to determine the condition of the habitat.

Table 7 is based on the main characteristics of the cave habitats (described in section 1.2.1), together with the information provided by Member States about the assessment of the condition of the habitat, and habitat specific literature. The proposed metrics are intended to be easily but reliably obtained. The main **abiotic characteristics** are physical (describing the topography/physical structure, influencing factors such as tidal range, depth, exposure to currents light levels, temperature and turbidity) and chemical (related to water quality and whether there is any freshwater influence). The main **biotic characteristics** are compositional (associated species), structural (presence and condition of species) and functional (water circulation). The main **landscape/seascape characteristic** is connectivity, and a single variable, 'disturbance', has been proposed for monitoring in the category **other**.

Table 7. Proposals for condition variables for assessing and monitoring habitat 8330 (submerged and semi-submerged caves).

The variables are included in the types recognized in the SEEA EA methodology. Metrics may show several options. Abbreviations: ADCP - Acoustic Doppler Current Profiler, CTD – Conductivity, Temperature and Depth; DDV – Drop-drown video, ROV – Remotely Operated Vehicle

Characteristics	Variables	Metrics	Application	Standardised measurement procedures	Considerations relating to methodologies
1. Abiotic characteristics					
1.1 Physical state characteristics					
Degree of submergence / depth, tidal regime	- Depth in relation to chart datum - Tidal range	Metres (m), maximum & minimum with seasonal patterns	Essential	Hydrographic charts, tide gauges, ADCP. Modelling. Aerial survey (Satellite/Drone imagery)	Depth, together with topographical characteristics/geology and tidal regime influence the form and extent of this habitat types as well as on the development and stability of some features within them (e.g. passages and platforms). Boat and shore-based coastline surveys can be used to locate caves that have entrances at sea level. Depending on their depth and complexity, fully submerged caves may be located and surveyed by SCUBA, or ROVs.
Geology	- Mineral/rock type	Mineral/rock type	Descriptive	Existing high-resolution geological maps. Aerial survey (Satellite/Drone imagery). Shore & boat-based surveys. Depending on their depth and complexity, submerged caves may be surveyed by SCUBA or ROV.	Rock type together with topographical and hydrographical characteristics have a significant influence on the form and extent of cave habitats as well as on some species (e.g. bivalves that can bore into soft rocks).
Topography / physical structure	- Physical dimensions - Form (e.g. presence of tunnels, air domes, drying areas)	Metres (m), degrees of slope (°), physical features from a reference list	Essential, however practicalities will determine the extent and level of detail possible for monitoring the dimensions of cave habitats.	Existing high resolution physical geography/geological maps Aerial survey (Satellite/Drone imagery) Shore & boat-based surveys Depending on their depth and complexity of form, submerged caves may be surveyed by SCUBA or ROV.	The feasibility and level of detail possible when recording the physical dimensions of caves, will depend on their complexity (galleries, tunnels, pits etc) and accessibility. In some cases, detailed maps might be prepared whilst in more complex/less accessible submerged caves this may be limited to recording the dimensions around the entrance and any large chambers.

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Characteristics	Variables	Metrics	Application	Standardised measurement procedures	Considerations relating to methodologies
Hydrodynamics - Exposure to currents, wave action, scour and surge	<ul style="list-style-type: none"> - Current speed - Wave height - Direction, frequency - Circulation patterns/ residence time 	m/s, metres (m), time periods	Essential, however practicalities will determine the extent and level of detail possible for monitoring these variables in cave environments	Hydrographic charts, ADCP. Modelling. Aerial survey (Satellite/Drone imagery)	Current speed, wave height, direction & frequency, if measured directly, are most likely to be recorded in the vicinity/outside cave habitats. Getting an accurate measure of water circulation (direction, velocity, residence time etc.) in marine caves can be difficult because of the physical characteristics which can include blind tunnels, enclosed and semi-enclosed areas, and roof openings to the surface. Within caves, the colonising flora and fauna are indirect indicators of the strength of such variables therefore complimentary biological surveys are relevant to monitoring and reporting on these characteristics (see below). Seasonal changes and storm events will be apparent when recording these variables. Species composition is an indirect indicator of these variables.
Temperature	-Water temperature	-Temperature (°C)	Essential	CTD	Temperature is usually recorded as part of water quality sampling programmes, at different points of the cave.
Turbidity	<ul style="list-style-type: none"> - Suspended particles - Light transmission through water samples 	Nephelometric turbidity units (NTU), formazin turbidity units (FTU)	Essential	Turbidity sensor, Secchi disc, water chemistry data loggers. Aerial survey (Satellite data). Direct sampling (sediment sampling, sediment traps).	Different turbidity units would be used depending on tools used, therefore the same instrument should be used for comparability of data over time. Colonising species can be an indirect indicator of turbidity. Complementary biological surveys may therefore be considered for monitoring and reporting on this characteristic (see below).
Light levels	- Irradiance	Watt/m ²	Recommended, however practicality will determine the extent and level of detail possible for monitoring irradiance in cave environments	Spectrometers	Changing light levels within caves create a strong environmental gradient. This is particularly relevant to colonising algae which are indirect indicators of the irradiance. Complementary biological surveys may therefore be considered for monitoring and reporting on this characteristic (see below). Light levels if recorded, could usefully be determined from different locations within cave habitats depending on the physiographic features/cave type.

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Characteristics	Variables	Metrics	Application	Standardised measurement procedures	Considerations relating to methodologies
1.2 Chemical state characteristics					
Salinity / freshwater influence / stratification	- Salinity - Conductivity	Parts per thousand Sodium and Chloride (0/00), depth/ boundaries (m) of different water bodies	Specific	CTD, water chemistry data loggers	Where there is notable freshwater inflow to caves, salinity measurements will be particularly significant.
Water quality	- Various substances (including chemicals listed in the WFD and EQSD, nitrates & phosphates, oxygen, chlorophyll, dissolved solids)	Temperature (°C), pH, Chromophoric dissolved organic matter (CDOM), fluorescent dissolved organic matter (FDOM), total dissolved solids (TDS), dissolved oxygen (mg/l), oxygen saturation (%)	Essential	CTD with sensors to measure oxygen (saturated %) and dissolved (mg/l), pH, nitrate, chlorophyll, turbidity currents	Numerous parameters may be recorded under the variable "water quality". The standards set under the WFD for transitional waters will be particularly relevant for coastal marine caves. These include general parameters such as oxygenation, nutrients, nitrogen, phosphorus, as well as chemical and physico-chemical quality elements.
2. Biotic characteristics					
2.1 Compositional state characteristics					
Invertebrates - Epifaunal & infaunal assemblages and characteristic species	- Abundance of characteristic species - Diversity of characteristic species	Number of biocenosis/taxa, presence & abundance of species (SACFOR scale), diversity index, (Shannon-Wiener diversity index, AMBI index), biomass, estimated % cover. Density (ind/10 cm ²) and Shannon-Wiener for meiofauna	Essential, although recommended for meiofauna	Visual observation (Photographic quadrats, video transects, visual census, ROV imagery). Direct sampling. Meiofauna - diver-operated corer.	Monitoring the biotic composition of caves has many similarities with monitoring reefs. Non-destructive methods are likely to be favoured, and the methodology will depend on the species as well as factors such as the extent, location, and any seasonality. It may be possible to distinguish different biotic zones characterised by variables such as light levels and water circulation.
Vertebrates - Associated fish and marine mammals (seals)	- Abundance of characteristic species - Diversity of characteristic species	Numbers, distribution, population structure, trophic composition (e.g. % omnivores/piscivores), seals using caves	Essential	Aerial/boat-based surveys Photographic/satellite imagery. <i>in situ</i> observations, nets & traps (fish)	Methodology will depend on the species and will need to take account any seasonality in the presence of the species.

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Characteristics	Variables	Metrics	Application	Standardised measurement procedures	Considerations relating to methodologies
2.2 Structural state characteristics					
Characteristic species	- Condition	Percentage cover, biomass, density. Synthetic indicators (M-AMBI, BENTIX, etc.)	Essential	Visual observation (Photographic quadrats, video transects, visual census, ROV imagery)	Methodology will depend on the species and needs to take account any seasonality in the presence of the species.
2.3 Functional state characteristics					
Population dynamics	- Population size - Community structure	Percentage cover, biomass, density. Synthetic indicators (M-AMBI, BENTIX, etc.)	Specific	Visual observation (Photographic quadrats, video transects, visual census, ROV imagery)	Methodology will depend on the species and needs to take account any seasonality in the presence of the species
3. Landscape/Seascape characteristics					
Connectivity / Fragmentation	Presence / absence of barriers	Addition/removal of barriers to water movement/species dispersal	Recommended, however practicality will determine the extent and level of detail possible	Visual observation (Photographic quadrats, video transects, visual census, ROV imagery)	Methodology will depend on accessibility to cave system.
4. Other					
Disturbance	- Footprint of activity - Number and intensity of negative pressures	Presence/absence, % area directly affected by human activity pressures from a standardised list (graded High, Medium, Low, unknown)	Essential	Visual observation (video transects, visual census, ROV imagery)	Many different "types" of disturbance may be associated with marine caves, and they can be categorised in a variety of ways e.g. physical/chemical/biological; presence/absence. Some examples are litter, wastewater/industrial pollution runoff, abrasion/smothering of fauna on cave surfaces, and disturbance of seal haul outs. The significance of any of these on the structure and function of the habitat will depend on factors such as frequency, permanence, level and type of impact.

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

The observed measurements of the condition variables need to be compared to reference values and critical thresholds, in order to assess the condition for each variable. A reference level is the value of a variable at the reference condition, against which it is meaningful to compare past, present or future measured values of the variable. The difference between the value of a variable and its reference level represents the distance to the reference condition.

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

Reference levels are usually set with high and low levels reflecting the limits or endpoints of the range of a condition variable that can be used in re-scaling. For example, the high level may refer to a natural state and the low level may refer to a degraded state where ecosystem processes are below a threshold for maintaining function (Keith et al., 2013, in United Nations, 2021).

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

These guidelines do not intend to provide specific rules or values for these thresholds, but to define the main criteria and guide on the establishment of reference values that would help determining good or not good condition, considering the ecological variability of the habitats across their range.

In a review of approaches for setting reference conditions for assessing marine ecosystem quality, Borja et al. (2012) recommend that they should be defined/described with reference to:

- (1) Multiple sites with similar physical characteristics within an ecoregion or habitat type.
- (2) Ideally represent minimally impaired or undisturbed conditions (i.e. absence or minimal human pressure).
- (3) Provide an estimate of the variability in biological communities and habitat quality due to natural physical and climatic factors.

They identified four main approaches: crossing referencing pristine areas, hindcasting, modelling and best professional judgement.

Pristine areas: Reference values set against “pristine” areas could be developed with knowledge of either undisturbed habitats or habitats that are considered to be in good condition. However, for this to be credible, it would require comprehensive knowledge of the pressures and impacts on the different habitats, and the implications for their condition. Finding such locations is also likely to be problematic, especially as many examples of the habitats which are being assessed are adjacent to the coast or within territorial waters and therefore likely to have been subject to many pressures/impacts sometimes over significant periods of time.

Hindcasting: Using hindcasting to set reference levels requires cross reference to some historical reference condition. This may be a condition which is considered unimpacted (see above) or a set date (as with the Habitats Directive where 1994 is used as a baseline). Issues will arise around deciding when to set any baseline, the reliability and availability of historic data, and how to account for any natural oscillations in condition. For example, a habitat may not be in favorable condition in the selected baseline year, there may be a lack of sufficient data to inform decisions on the most appropriate baseline year, and global changes in recent decades could alter the former reference conditions making any comparisons with datasets from 50, or 100 years ago impossible.

Modelling: Modelling by extrapolating biological attributes can be used to summarise/simplify, visualize and explain actual or predicted situations e.g. the Driver-Pressure-State-Impact-Response (DPSIR) framework (OECD, 1993). There are, however, many considerations with developing and applying such models (Patricio et al., 2016). They include data availability, the level of confidence in the outcomes, how to scale up interpretations, for example from a site to a region, and how to assess cumulative impacts. There is an additional consideration that modelling approaches can be complex with less transparency and comprehensibility for stakeholders and policymakers.

Expert judgement: This is widely used when there is limited data but should ideally be underpinned by some clearly stated criteria and it has less transparency and comprehensibility for stakeholders.

The analysis carried out by Borja et al. (2012) (summarized in Table 8) considered that whilst using pristine or minimally impacted conditions was the best single method, others were also adequate when combined with expert judgement. Setting targets was seen as an alternative approach where none of the traditional reference conditions approaches were applicable, which implicitly indicates conditions where the indicator in question is not adversely affected or only slightly affected. Their conclusions, looking specifically at assessing benthic ecological status, were that a combination of methods in setting reference conditions is more adequate in obtaining final quality assessments related to the pressures on a habitat than one method alone.

Also relevant is the consideration that, regardless of the approach, there may be existing relevant thresholds and reference values set within legal obligations. For marine habitats this is the case at a European level, under the Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD).

Finally, the lack of experts in certain habitats can pose an additional difficulty for the correct use of this approach. The analysis carried out by Borja et al. (2012) (Table 8) was that whilst using pristine or minimally impacted conditions was the best single method, others were also adequate when combined with expert judgement.

Table 8. Evaluation of target and reference condition setting methods, regarding different issues

Methods / issues	Reference conditions			Expert judgement	Targets		
Main issues	Pristine areas	Historical data	Modelling	Best professional judgement	Baseline set in the past	Current baseline	Directional/ trends target
Legislation using/ proposing it	WFD, CWA	WFD, CWA, OSPAR	WFD, CWA	WFD, CWA	OSPAR	HD	OSPAR
Data needs	Moderate (2)	High (1)	High (1)	Low (3)	Moderate (2)	Moderate (2)	Moderate (2)
Scientific robustness	High (3)	Moderate (2)	Moderate/ high (2.5)	High (3)	High (3)	High (3)	High (3)
Confidence of the method	High (3)	Moderate (2)	Moderate/ high (2.5)	High (3)	Moderate (2)	High (3)	Moderate (2)
Applicability	High (3)	Low (1)	High (3)	High (3)	Moderate (2)	Moderate (2)	Moderate (2)
Practicality of the method within available time scales	High (3)	Moderate (2)	High (3)	High (3)	Moderate (2)	Moderate (2)	Moderate (2)
Transparency and comprehensibility	High (3)	High (3)	Low (1)	Low (1)	High (3)	High (3)	High (3)
Total scores	17	11	13	16	14	15	14

Source: Borja et al., 2012.

Note that scores are high: 3, moderate: 2, and low: 1, except in the case of data needs, which are opposite (the lowest data need the highest score). WFD: Water Framework Directive; HD: Habitats Directive; CWA: Clean Water Act; OSPAR: Oslo-Paris Convention.

General guidance on setting environmental thresholds is included in The Marine Strategy Framework Directive (MSFD) which requires that Good Environmental Status (GES) should be achieved in EU marine waters as described by eleven environmental Descriptors. At the core of the GES assessment lies the need for threshold values which enable a quantitative assessment of environmental status for the indicators and elements used for each GES Criterion.

Principles and guidelines on how these thresholds should be set are specified in Article 4(1) of Commission Decision (EU) 2017/848 (EU, 2017) (Box 2)¹⁰.

¹⁰ Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU) Article 13.

Box 2. Article 4 of Commission Decision (EU) 2017/848 (EU, 2017)

Article 4 - Setting of threshold values through Union, regional or subregional cooperation

1. Where Member States are required under this Decision to establish threshold values through Union, regional or subregional cooperation, those values shall:

- (a) be part of the set of characteristics used by Member States in their determination of good environmental status;
- (b) be consistent with Union legislation;
- (c) where appropriate, distinguish the quality level that reflects the significance of an adverse effect for a criterion and be set in relation to a reference condition;
- (d) be set at appropriate geographic scales of assessment to reflect the different biotic and abiotic characteristics of the regions, subregions and subdivisions;
- (e) be set on the basis of the precautionary principle, reflecting the potential risks to the marine environment;
- (f) be consistent across different criteria when they relate to the same ecosystem element;
- (g) make use of best available science;
- (h) be based on long time-series data, where available, to help determine the most appropriate value;
- (i) reflect natural ecosystem dynamics, including predator-prey relationships and hydrological and climatic variation, also acknowledging that the ecosystem or parts thereof may recover, if deteriorated, to a state that reflects prevailing physiographic, geographic, climatic and biological conditions, rather than return to a specific state of the past;
- (j) be consistent, where practical and appropriate, with relevant values set under regional institutional cooperation structures, including those agreed in the Regional Sea Conventions.

A review of the state of play with thresholds for MSFD criteria used by Member States, published in 2022, shows the progress made (e.g. Table 9 for Descriptor 5, Eutrophication) but it also indicates there is still some way to go before this is achieved for all eleven descriptors (Vasilakopoulos et al., 2022). No thresholds have been agreed as yet for D6 (sea floor pressures and impacts), for example.

Table 9. Agreed thresholds setting methods and values for Descriptor 5 (Eutrophication) criteria.

D5 Criterion	Compartment	Agreed threshold methods	Threshold Values available	Comments	Related regulations
D5C1	Coastal waters	Nutrient concentration in surface water or in the water column	From 10 to 13 MSs reported TVs for the nutrient categories	Strong input of WFD in coastal waters, some MSs TVs still missing, especially in the open sea.	WFD
	Open sea		From 7 to 14 MSs reported TVs for the different nutrient categories		
D5C2	Coastal waters	Chlorophyll-a in the water column	15 MSs reported TVs	Strong input of WFD in coastal waters.	WFD
	Open sea		17 MSs reported TVs		
D5C3	Coastal waters	Harmful algal blooms in the water column	Only Baltic MSs reporting a cyanobacteria bloom index	No index (e.g. red tides) in other marine regions	
	Open sea				
D5C4	Coastal waters	Photic limit (transparency) of the water column	11 MSs reported TVs		WFD
	Open sea		11 MSs reported TVs		
D5C5	Coastal waters	Dissolved oxygen at the bottom of the water column	12 MSs reported TVs	For some regions, TVs from project results and WFD are combined with expert evaluation. D5C5 may be substituted by D5C8.	WFD
	Open sea		14 MSs reported TVs		
D5C6	Coastal waters	Opportunistic macroalgae of benthic habitats	3 MSs reported TVs		WFD
	Open sea		None		
D5C7	Coastal waters	Macrophyte communities of benthic habitats	5 MSs reported TVs	Availability of TVs across regions is challenging	WFD
	Open sea		None		
D5C8	Coastal waters	Macrofaunal communities of benthic habitats	9 MSs reported TVs A	Availability of TVs across regions is challenging	WFD
	Open sea		None		

The colour in fourth column indicates the degree of achievement in setting threshold; green: high, yellow: moderate, red: low.

Source: Vasilakopoulos et al., 2022.

The Water Framework Directive (WFD) requires Member States to protect and where necessary restore water bodies in order to reach good status (chemical and ecological) and to prevent deterioration. Standards for priority substances and certain other pollutants are set out in the Environmental Quality Standards Directive (2008/105/EC)¹¹.

Apart from geology, all the variables identified for assessing the structure and function of habitat 8330, are covered in some way by the MSFD GES descriptors. Some WFD Environmental Quality Standards are also directly applicable.

A consistent approach, cross-referencing agreed thresholds for MSFD descriptors and WFD thresholds, with those that are also relevant to assessing the condition of the structure and function of marine and coastal habitats covered by the Habitats Directive is clearly desirable.

The harmonization of reference values and thresholds regarding the variables used for the assessment of habitat condition should consider the following **common requirements**:

- Thresholds need to consider the natural variability of the habitats across their range, and different threshold or reference values for the same habitat in different Member States or regions within a MS can be appropriated.
- Thresholds, limits and reference values need to be tested with sufficient data sets, which include full range of habitat conditions – from degraded habitats to best quality ones.
- The reference values should fulfil the criteria of validity (connection to relevant ecological integrity), robustness (reliability), transparency, and applicability (Czúcz et al., 2021; Jakobsson et al., 2020).
- A description of the methodology for establishing the threshold and reference values applied by each MS for each variable must be provided, justified and perfectly understandable.
- The methodologies should be suitable to be regularly evaluated and improved according to the best available scientific knowledge and any modifications made, and the impact these may have on previous monitoring work, must be communicated.
- Common training or guidance on setting threshold and reference values should be programmed for experts from the different MSs in order to achieve full harmonisation.

Table 10 makes some initial recommendations for setting reference/threshold values for the proposed variables for assessing and monitoring the condition of habitat type 8330 (marine caves).

¹¹ Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council

Table 10. Considerations for setting reference/threshold values for habitat 8330 (submerged and semi-submerged caves).

WFD Quality Elements: QE1 – Biological Quality Elements, QE1-2-1 - Macroalgae, QE1-2-2 - Angiosperms, QE1-2-3 – Macrophytes, QE1-2-4 – Phytobenthos, QE1-3 - Benthic invertebrates, QE1-4 - Fish, QE2 – Hydromorphological quality elements, QE2-1 – Hydrological or Tidal regime – QE2-3 – Morphological conditions, QE3 - Chemical and physico-chemical quality elements, QE3-1 – General parameters (Transparency, thermal, oxygenation, salinity, acidification, nutrient, Nitrogen, Phosphorus conditions), QE3-1-1 – Transparency, QE3-1-4 - Salinity conditions

MSFD Descriptors: D1 – Marine biodiversity, D3 – Commercial fish and shellfish, D4 – Food webs, D5 – Human-induced eutrophication, D6 – Seabed integrity, D7 – Hydrographical conditions, D8 – Contaminants, D10 – Marine litter, D11 – Energy, including underwater noise

Characteristics	Variables (application)	Reference/ Threshold type	Considerations relating to Reference Values	Relevant WFD EQS for Reference Values / Thresholds	Relevant MSFD Descriptors
1. Abiotic characteristics					
1.1 Physical state characteristics					
Degree of submergence / depth / tidal regime	- Depth in relation to chart datum - Tidal range (Essential)	Quantitative, Trend	Caves are formed by the erosion of bedrock through natural processes. Consequently, they are in a continuous state of development albeit at different rates depending on their geology and erosive forces. Depth is not static but subject to both diurnal and seasonal variation, as well as across the habitat being monitored. The existing status is therefore in equilibrium with the prevailing conditions. If this were to be disrupted to a significant degree, there can be major changes in both the physical and biological characteristics of the habitat.	QE2 (QE2-1, QE2-3)	D7
Geology	- Mineral/rock type (Descriptive)	Qualitative	Caves are formed by the erosion of bedrock through natural processes. Consequently, they are in a continuous state of development albeit at different rates depending on their geology and erosive forces. This variable will be descriptive with no changes foreseen in the underlying geology, therefore no proposals for reference values are recommended	-	-
Topography / physical structure	- Physical dimensions - Form (e.g. presence of tunnels, air domes, drying areas) (Essential; extent and level of detail to be determined)	Quantitative, Qualitative	Comparisons of imagery data over time can reveal gross changes in topography of the habitat; however, for the purposes of setting thresholds and reference values, any changes will need to be viewed in the context of "natural" changes as these habitats are subject to naturally occurring erosion and deposition as well as patterns of erosion and deposition which are the consequence of human activity. Threshold values will need to be set in the context of and with regard to knowledge of such changes	QE2 (QE2-1, QE2-3)	D7

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Characteristics	Variables (application)	Reference/ Threshold type	Considerations relating to Reference Values	Relevant WFD EQS for Reference Values / Thresholds	Relevant MSFD Descriptors
Hydrodynamics - Exposure to currents, wave action, scour and surge	<ul style="list-style-type: none"> - Current speed - Wave height - Direction & frequency - Circulation patterns, residence time (Essential; extent and level of detail to be determined)	Quantitative, Qualitative, Trend	Aside from seasonal changes, natural variation in these variables is likely to be low. However, if major changes do occur, they are likely to lead to significant changes in the species colonising cave habitats. Blocking/altering water circulation through and around caves has the potential to alter their chemical and biological characteristics as well as patterns of erosion and therefore their physical form. Reference values and thresholds will therefore need to distinguish between any natural change (e.g. from gradual erosion of passageways and ceilings) and significant change resulting from human activity (e.g. construction of structures such as sea wall which impede water flow). Whilst it would be possible to set quantitative reference values (e.g. a certain % change), trends are likely to be more informative as this is less likely to be skewed by single significant events.	QE2 (QE2-1)	D7
Temperature	-Water temperature (Essential)	Quantitative	Reference values for temperature might be carefully defined according to each subtype of cave and the seasonal cycle. Furthermore, need to take into account that thermal stress varies within species, assemblages and other physical (e.g. depth, distance from the entrance) or geographical parameters (e.g. latitude/longitude, currents).	QE3 (QE3-1-2)	D1, D5, D7
Turbidity	<ul style="list-style-type: none"> - Suspended particles - Light transmission through water samples (Essential)	Quantitative	Increasing turbidity can have an effect on the species that are present (e.g. reducing the number/extent of filter feeders) Reference levels relating to characteristic species and water quality would be complementary to this variable.	QE3 (QE3-1-1)	D5
Light levels	- Irradiance (Recommended; extent and level of detail to be determined)	Quantitative	Changes in light levels within cave environments are likely to be the result of changes in the physical characteristics of the cave (e.g. ceiling collapses, entrance blocking) and in the turbidity of the water. Reference levels relating to characteristic species and water quality could act as a proxy for this variable.	QE3 (QE3-1-1)	D7

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Characteristics	Variables (application)	Reference/ Threshold type	Considerations relating to Reference Values	Relevant WFD EQS for Reference Values / Thresholds	Relevant MSFD Descriptors
1.2 Chemical state characteristics					
Salinity / freshwater influence / stratification	- Salinity - Conductivity (Specific)	Quantitative	Changes in salinity attributed to human activity are the most relevant when setting thresholds and reference levels. This may be the result of ongoing activities and/or one-off events e.g. industrial discharge. Targets could be selected to represent the limits of the range of the characteristic species/biotope in key locations.	QE3 (QE3-1-4)	D7
Water quality	- Various substances (including chemicals listed in the WFD and EQSD, nitrates & phosphates, oxygen, chlorophyll, dissolved solids) (Essential)	Quantitative	The parameters and Environmental Quality Standards that apply under the WFD for transitional waters (e.g. in relation to chemical and physico-chemical quality elements) will be particularly relevant to determining water quality of caves in coastal environments and are already being used as reference values/thresholds by Member States.	QE3 (QE3-1)	D5, D8
2. Biotic characteristics					
2.1 Compositional state characteristics					
Invertebrates - Epifaunal & infaunal assemblages and characteristic species	- Abundance of characteristic species - Diversity of characteristic species (Essential, however recommended for meiofauna)	Quantitative, indices/ additional, scoring, linked to WFD	Reference values and thresholds for good condition will not only vary according to biogeographical region but also from location to location because of the factors such as depth, location, species etc. (see variables). Two types of thresholds should be considered. A threshold relating to extent, and a threshold relating to condition. <u>Extent</u> - change in distribution AND in the density/diversity of the relevant species. <u>Condition</u> - change in key features (e.g. length, biomass) and other evidence of declining condition such increasing prevalence of disease.	QE1 (QE1-2-4, QE1-3)	D1, D4, D6
Vertebrates - Associated fish and marine mammals (seals)	- Abundance of characteristic species - Diversity of characteristic species (Essential)	Quantitative, indices/ additional, scoring.	Reference values will need to take into account natural cycles of change, and to distinguish these from changes which are the result of human activity. .	QE1 (QE1-4; Fish)	D1, D3, D11

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Characteristics	Variables (application)	Reference/ Threshold type	Considerations relating to Reference Values	Relevant WFD EQS for Reference Values / Thresholds	Relevant MSFD Descriptors
2.2 Structural state characteristics					
Characteristic species	- Condition (Essential)	Quantitative, indices/ additional, scoring, linked to WFD	Reference values and thresholds for good condition will not only vary according to biogeographical region but also from location to location because of the factors such as depth, location, species etc. (see variables). Two types of thresholds should be considered. A threshold relating to extent, and a threshold relating to condition. <u>Extent</u> - change in distribution AND in the density/diversity of the relevant species. <u>Condition</u> - change in key features (e.g. length, biomass) and other evidence of declining condition such increasing prevalence of disease.	QE1 (QE1-2-4, QE1-3)	D1, D4, D6
2.3 Functional state characteristics					
Population dynamics	- Population size - Population structure (Specific)	Quantitative	Reference values will need to take into account natural cycles of change, and to distinguish these from changes which are the result of human activity.	QE1 (QE1-2-4, QE1-3)	D1, D4, D6
3. Landscape/Seascape characteristics					
Connectivity / Fragmentation	-Addition/removal of barriers to water movement/species dispersal (Recommended; extent and level of detail to be determined)	Quantitative, qualitative, expert judgement	Practicality will determine the extent and level of detail possible.	QE2 (QE2-1, QE2-3)	D7
4. Other					
Disturbance	- Footprint of activity - Number and intensity of negative pressures (Essential)	Quantitative, qualitative, expert judgement	For sites which are considered to be in favourable condition, the current state could be used as the reference value. Threshold values could be declines in condition or declines/changes which have an impact on the structure and function. Each location should characterise the major types of disturbance first and then for each of these consider what is considered significant.		D6; D8, D10

Table 11 indicates possible approaches for establishing thresholds and reference values applicable to the proposed variables, based on the procedures followed by MSs and the existing literature. In fact, a combination of approaches is suggested to better inform the setting of reference levels or thresholds, given the degree of uncertainty when setting reference levels. The different approaches described are not exclusive, they are often combined. For example, expert judgement is necessary when establishing reference cases for good condition or for certain decisions on modelling the relationship between variables and condition. In fact, modelling-based approaches complement those based on good condition or undisturbed cases and can also be combined with statistical approaches.

The evaluation of the condition of the habitats is based on determining whether the variables used in the assessment indicate good or not good condition. Different criteria are applied to attribute these condition categories according to the characteristics of each variable, for example, whether they are categorical (e.g. no alien species allowed), or quantitative variables which may obey to linear or non-linear relationships with the condition (Jakobsson et al., 2020). for the variables are available, they can be directly standardized to apply aggregation procedures.

Owing to the different metrics, measurement units and magnitudes applied to the variables that characterise the habitats, the values obtained from their measurement require some form of standardisation, e.g., by re-scaling, to build indicators combining different variables. The values obtained from the measurement of the variables can be scaled according to their reference levels, thus normalised to a common scale and direction of change, and can then combined to form a composite index or to obtain an overall result of the assessment using appropriate aggregation approaches (see further details below in Section 3.3 on Aggregation).

Table 11. Some initial recommendations for setting thresholds for the proposed variables

Description	Appli- cation	Compa- rison to undis- turbed areas	Compari- son to good condition areas	Hindcas- ting	Mode- lling	Expert judge- ment	EU Relevant existing reference values
1. Abiotic characteristics							
1.1 Physical state characteristics							
Degree of submergence/ depth / tidal regime	Essential						WFD, MSFD
Geology	Descriptive						
Topography/physical structure	Essential*						WFD, MSFD
Hydrodynamics - Exposure to currents, wave action, scour and surge	Essential*						WFD, MSFD
Temperature	Essential						MSFD, WFD
Turbidity	Essential						WFD, MSFD
Light levels	Reco- mmended*						WFD, MSFD

Description	Appli- cation	Compa- rison to undis- turbed areas	Compara- son to good condition areas	Hindcas- ting	Mode- lling	Expert judge- ment	EU Relevant existing reference values
1.2 Chemical state characteristics							
Salinity/freshwater influence/stratification	Specific						WFD, MSFD
Water quality	Essential						WFD, MSFD
2. Biotic characteristics							
2.1 Compositional state characteristics							
Invertebrates - Epifaunal & infaunal assemblages and characteristic species	Specific*						WFD, MSFD
Vertebrates - Associated fish and marine mammals (seals)	Essential						WFD, MSFD
2.2 Structural state characteristics							
Characteristic species	Essential						WFD, MSFD
2.3 Functional state characteristics							
Population dynamics	Specific						WFD, MSFD
3. Landscape/Seascape characteristics							
Connectivity /Fragmentation	Reco- mmended						WFD, MSFD
4. Other							
Disturbance	Essential						MSFD

*: check Table 7 for further information

3.3 Guidelines for the aggregation of variables at the local level

Ecological assessments require the integration of physical, chemical, and biological quality parameters. The choice of the aggregation method of such partial assessments into an overall assessment has been widely discussed within the scientific community, since the methodology can have a considerable influence on the outcome of the assessment. Different approaches can be used to integrate the values of the measured variables to give an overall value that indicates the overall condition of habitat types at the local scale, i.e. the monitoring plot, station or site.

An appropriate aggregation method is crucial to categorising local-scale condition into good/not good. This is because the proportion of the habitat type in **good/not good condition** is the main information required for assessment of the structure and function of the habitat type at the biogeographical level.

3.3.1 Overview of aggregation methods

In a review of methods for aggregating and integrating information when assessing the status of marine ecosystems under the MSFD, focusing mostly on the descriptors related to biodiversity, Borja et al. (2014) discussed the advantages and disadvantages of several different approaches used to combine a number of variables into an overall assessment. A review and discussion of advantages and disadvantages of several different aggregation methods for marine biodiversity status assessments has also been carried out by Barnard & Strong (2014).

The main approaches are summarised below.

One-out, all out (OOAO)

The OOAO rule has been recommended for assessment of Ecological Status under the Water Framework Directive (CIS, 2003). The logic behind this is that a water body could not achieve good ecological status if any of the quality elements measured fail. This means that an OOAO approach using the “worst case” scenario for its assessment. This can be viewed as a rigorous precautionary approach. One criticism, however, is that it could lead to an underestimation of the true overall status.

A precautionary one-out, all-out approach is also used in the aggregation of the parameters used in the assessment of conservation status under the Habitats Directives and the IUCN Red List of Species and the IUCN Red List of Ecosystems.

The OOAO rule is a rigorous and conservative approach which follows the precautionary principle, and works well where all the necessary information is available. In the marine environment, where there may be significant data gaps it is important to clarify the extent to which such an approach may be “preliminary”, “partial” or “incomplete” due to lack of data.

Averaging approach

The averaging approach is the most commonly used method to aggregate indicators (Shin et al., 2012) and consists of simple calculations, using methods such as arithmetic average, hierarchical average, weighted average, median, sum, product or combinations of those rules, to come up with an overall assessment. Differential weighting applied to the various indicators can be used when calculating means or medians. An adequate basis for assigning weights is not always available and assigning weights often involves expert judgment: However, expert opinions applied in such a way can show important differences.

This approach needs a normalisation of the obtained data to be used.

Conditional rules

Conditional rules are an approach where indicators can be combined in different ways to generate an overall assessment, depending on specified criteria. For instance, it can be formulated in a way that requires that specific proportion of the variables to achieve good status or if a certain number of variables do not meet the threshold, the overall status fails.

Scoring or rating

In this method different scores are assigned to a particular status for a number of different elements, e.g. ranging from 1 to 5 for poor to good). The scores may then be summed to

derive a total score which is then rated according to the number of elements taken into account. Different weights can be assigned to the various elements.

Multimetric indices to combine indicators

Within the WFD there are many examples of multimetric indices developed for different biological elements. Within the MSFD, the use of multimetric indices or multivariate techniques for integrating indicators of seafloor integrity have been recommended (Rice et al., 2010). Multimetric methods that are used to combine multiple parameters in one assessment may result in robust indicators, but ideally the various parameters should not be inter-correlated.

Multidimensional approaches

Multivariate methods, such as Discriminant Analysis or Factor Analysis combine parameters in a multi-dimensional space. Multivariate methods have the advantage of being more robust and less sensitive to correlation between indicators. However, interpretation is less intuitive than other methods, as information on individual indicators in each ecosystem is lost and links to management options are less obvious.

Decision tree

Decision trees provide the opportunity to apply different, specific, rules to combine individual assessments into an overall assessment. A decision tree allows implementing individual rules at each of its nodes and thus incorporates decisions at each step within the decision tree. The decision rules can be quantitative or qualitative as well as based on expert judgment. This gives room for a high degree of flexibility in reaching the final assessment.

Probabilistic approach

In some cases the results for each indicator may be uncertain due to several factors e.g., natural variation in the sampling sites, random variation in the samples, insufficient scientific understanding about what should be the reference value for good status, etc. If these uncertainties can be approximated, this gives rise to the possibility of taking this information into account when integrating the indicators. The more uncertain indicators will get less weight in the integrated assessment, while the more certain ones will be more reliable and hence get more weight.

High level integration

This approach, which includes the selection of an agreed reduced set of indicators and agreed weighting rules, could be considered a pragmatic compromise, reducing the risks associated with OOA while still giving an overall assessment. An example of a high-level integration, where assessments for several ecosystem components are merged into a final assessment, is the HELCOM-HOLAS project (HELCOM, 2010).

As seen in Section 2.3, across EU Member States, the aggregation at local-scale assessments relies on integrating information from multiple variables, though specific approaches varying by country and habitat. Several MSs however apply a conditional rule, whereby a number of relevant variables measured must reach or pass the defined thresholds for good condition, or even the one-out, all-out rule, which requires that all the variables reach the threshold, for the overall habitat condition at the local scale to be considered good.

3.3.2 Recommendations for the aggregation of the measured variables to determine the habitat type condition at the local scale

A common aggregation method to integrate all essential and specific variables measured to assess the habitat should be applied consistently across the habitat range in the EU in order to obtain comparable results.

Considering the various approaches described above and with the aim of harmonising the assessment of marine habitat types at the local scale, we suggest a two-step approach, in which a first aggregation is carried out separately for each group of variables associated to abiotic, biotic and landscape characteristics, and then, the results of such partial assessments are then integrated into the overall local assessment of the habitat condition following a one-out, all-out rule, as described below but being clear about where data is limited or insufficient to make such an assessment (Figure 1).

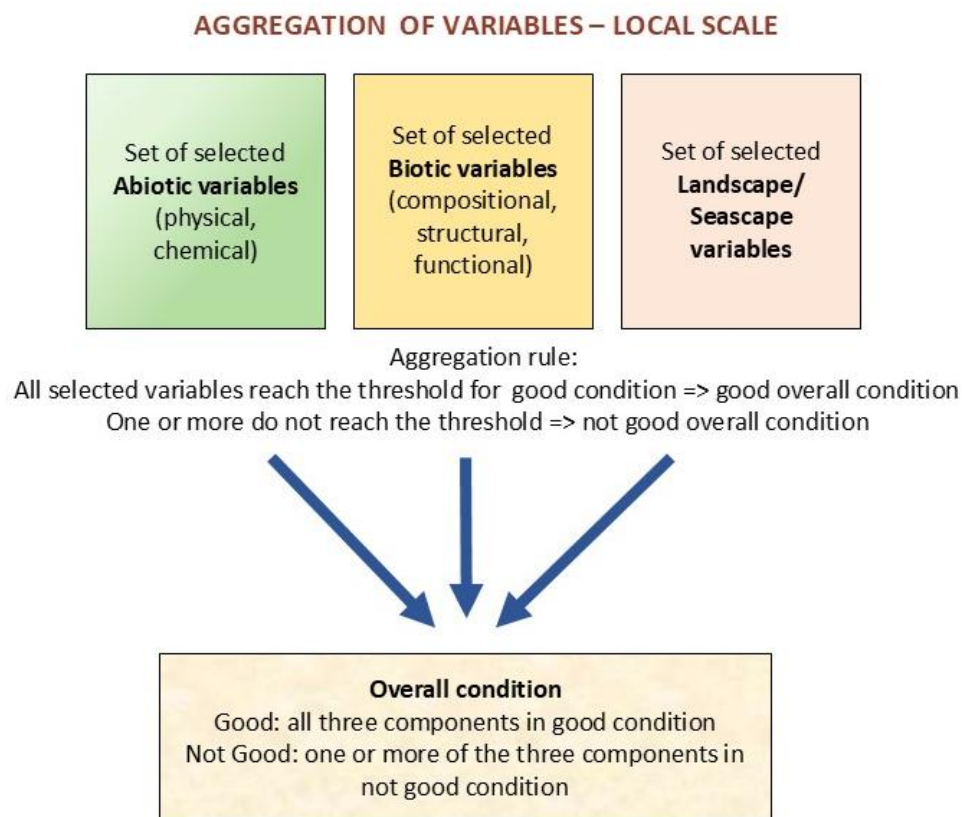
Step 1 – Aggregation of the variables measured in each group of characteristics

A first step would involve using a conditional rule. This would aggregate the variables for each group of characteristics whilst noting that a minimum set of essential variables in each group must reach/exceed the defined threshold for good condition. This would be done for each habitat component (abiotic, biotic, landscape). The selection of the set of variables that must reach the threshold is made considering their indicator value, i.e. their relative importance or relevance to determine whether the habitat is in good condition or not. These should be variables for which a clear threshold can be defined to distinguish good and not good condition. If any of those selected variables do not reach/exceed the minimum thresholds, the condition cannot be considered good for the corresponding component of the habitat (abiotic, biotic, landscape). If there are insufficient data on any particular variables to make such an assessment this should be noted.

Step 2 – Aggregation of the three groups of variables or habitat components

In a second step, the results achieved in each the three components or groups of characteristics (abiotic, biotic, landscape) would be aggregated following the “one-out, all-out” rule, which requires that all the three components have been assessed in good status for the overall condition of the habitat at the local scale to be considered good. If any of these components do not reach an overall good status, the condition of the habitat at the local scale cannot be considered good. Where there are data gaps on any of the habitat components (biotic, abiotic or landscape), a clarification should be provided to show that the assessment is incomplete or preliminary.

Figure 1. Scheme of the proposed aggregation of variables at the local level



3.4 Guidelines for aggregation at the biogeographical region scale

As a minimum requirement MSs must follow the recommendations from the Art. 17 reporting guidelines for the period 2013-2018, which establish 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'".

This rule highlights the importance of a sampling design that ensures sufficient representativeness of the total habitat area and diversity (see section 3.6 below).

3.5 Guidelines on general sampling methods and protocols

Harmonized monitoring protocols are crucial for assessing habitat conditions across Europe. These protocols should offer standardized methods for data collection, analysis and interpretation to ensure consistency and comparability over time and across regions. This section includes recommendations on sampling designs and monitoring protocols.

Large-scale survey techniques (e.g. charts, remote sensing) can be used to provide data for the whole feature across its range and assist with developing a stratified sampling programme to select a few locations to be investigated in detail. If so, the ability to relocate these sampling stations is essential (JNCC, 2004).

As the features across this habitat will not be uniform, for example, varying with position within the cave habitat, a single sample for a physical-chemical characterization is unlikely to provide a reliable description of the habitat as a whole. Multiple sampling and analysis may therefore be required, for example along transects and using quadrats to adequately record such heterogeneity.

Article 17 of the Habitat Directive requests a maximum period of 6 years. However, this period can be completed through different approaches depending on the resources of Member States. Thus, not all plots and not all variables need to be measured each 6 years. Regarding plots, Member States may establish a large number of monitoring sites, selecting a small number of them to be surveyed every season in order to gather a suitable number of plots with a complete monitoring at least every six years.

Within the six-year period, seasonality needs to be considered to avoid comparison of different time frameworks as the biotic and any associated macroalgae can change with seasons. Regarding variables, most variables are recommended to be seasonally surveyed (or at least annually), except tidal ranges with respect to LAT, that can be surveyed every 6 years. Adaptive monitoring is always recommended, allowing flexibility in frequency based on initial findings.

3.6 Selecting monitoring localities and sampling design

The **identification and selection of localities** for the assessment and monitoring of caves requires a systematic approach to ensure that the selected sites provide comprehensive and representative data. The selection of localities for sampling along with the sample size (number of plots) and power (statistical significance) are crucial to ensure the representativity of the results obtained in the assessment and monitoring of each habitat at the biogeographical scale.

Different approaches are recommended:

- **Geospatial Analysis:** remote sensing techniques (e.g. Acoustic surveys - side scan sonar, AGDS, MBES) as well as geographic information systems are essential tools to identify, analyse and integrate spatial data (e.g. extent, topography, and changes over time) and to identify areas of interest based on various criteria such as biodiversity, threats, and ecosystem services.
- **Field Surveys:** initial visits to potential sites are advisory to gather on-the-ground information about zonation patterns, accessibility, and logistics.
- **Review of existing data/knowledge:** reviews can help prioritise areas based on scientific knowledge.

Selecting a minimum number of localities for monitoring involves balancing several criteria to ensure comprehensive and effective coverage:

1. **Biogeographical or marine heterogeneity:** caves are present in all marine biogeographic regions but with different characteristics especially because of differences in tidal range and salinity profiles. Monitoring sites should be distributed across the entire area to represent the full range of ecological diversity and capture regional variations as well as habitat heterogeneity.

2. **Spatial Distribution:** monitoring sites must be distributed across the full geographical range of the habitat (subject to accessibility constraints) to avoid geographical bias and to capture regional variations and ensuring they represent a significant proportion of the habitat's area.
3. **Statistical Criterion:** It would be advisable to ensure that the number of sites is statistically sufficient to detect changes and trends with desired confidence levels (e.g., 95%). Multiple sites within similar ecological contexts should be included for data reliability and robustness.
4. **Existing data and monitoring sites:** Due to potential limitations in surface area and/or budget, previous research can help determine a more realistic number of monitoring locations. Making use of existing monitoring sites with historical data can also strengthen the understanding of long-term trends and changes in habitat condition. Such sites provide valuable baselines for comparison and support robust trend analyses over time.
5. **Degree of conservation and exposure to threat Levels:** Monitoring locations should include both protected and high-risk areas experiencing significant threats. The selection should include areas that show different degrees of conservation or degradation, in order to capture the existing variation in the habitat condition across its range. This requires including localities representing well-conserved habitat areas, with minimal human impact, as well as areas subjected to degradation and different pressures and threats. To capture the range of pressures affecting the habitat, localities should be selected across a spectrum of threat levels, from low to high and considering different sources of threats, such as water quality, disturbance/accessibility and resource extraction.
6. **Presence inside and outside Natura 2000 sites:** The assessment and monitoring of habitats conservation status must be done both inside and outside Natura 2000 sites, which requires selecting localities – and an appropriate number of sampling stations/transects – that reflect the proportion to the habitat's distribution within and outside the Natura 2000 network .
7. **Accessibility and practicality:** Monitoring localities should be accessible for regular visits, taking into account logistical factors and ease of access. Practical considerations also include the safety of field personnel and the feasibility of transporting equipment to and from the site.

Once the sampling localities have been identified for each habitat type, the minimum number of sampling stations in each locality and across the biogeographical region must be calculated in order to balance the sampling effort with representative data. The **size of the sample** influences two statistical properties: 1) the precision of our estimates and 2) the power of the assessment to draw conclusions. The number of sampling stations must be **statistically sufficient** to be able to detect changes and trends with desired confidence levels. Appropriate statistical methods should be used for determining an adequate sample size.

Considering the heterogeneity of habitat types, it is highly recommended to consult with a sampling statistician regarding the sample size, i.e. the minimum number of sampling stations/transects etc. required to ensure representativity and statistical significance. Some key elements to ensure a proper representation of the habitat condition in the sample are summarised below.

Key elements for statistical representation

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 and modelling

Regarding databases, marine caves have been recently subject to more attention with certain projects being the driving force behind current progress. Two examples are:

- Using the **World Register of marine Cave Species** (WoRCS¹²), a comprehensive taxonomic and ecological database of species known from marine and anchialine cave environments worldwide.
- An ongoing project, **Stygofauna Mundi** by the Water Research Institute (CNR, Italy) (Martinez et al., 2024) 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.

Regarding new technologies, various innovative tools are currently in use and under continuous development for monitoring and biotic/abiotic sampling in marine caves. Recent technological advancements that have significantly improved underwater cave monitoring include:

- **Illumination technologies** now include specialized LED systems with various colour options, particularly blue and red light, to minimise impact on marine life. However, researchers must carefully consider the effects of artificial light on fish behaviour.
- **Navigation technologies** which have progressed to overcome GPS limitations underwater, with innovations such as acoustic compasses or inertial navigation

¹² <https://www.marinespecies.org/worcs/> [Accessed 30th January, 2025]

systems. These developments enable more precise and less intrusive monitoring of cave ecosystems, enhancing our understanding of these unique environments while striving to minimize ecological impact.

- Development of **dry suits** and a better understanding of **gas blends** which are key elements for marine cave monitoring.

In-situ sampling in marine caves requires a high level of knowledge and technical skills to be carried out. As a result, autonomous cave diving and scientific cave studies have developed hand in hand, especially during the second half of the 20th century. The development of **closed-circuit autonomous diving (rebreathers)** in military environments has, over the years, allowed this technology to reach a wider audience. Among all the characteristics of this system, it is important to highlight that it does not emit CO₂ bubbles into the environment. This substantially prevents damage that mats of different communities on the ceilings of completely flooded caves may suffer while monitoring tasks are being performed. Currently, different research teams use rebreathers for monitoring; however, their high cost, training requirements, and technical knowledge needed for use are some of the main drawbacks.

In recent years, **Autonomous Underwater Vehicles (AUVs)** have emerged as promising tools for marine cave monitoring, offering several advantages over traditional methods and Remote Operated Vehicles (ROVs). Unlike ROVs, AUVs operate independently without physical connections to operators, allowing for greater flexibility and access to challenging environments. These unmanned submersibles are equipped with a diverse array of instruments, including cameras, sensors, and sonar systems, enabling them to collect a wide range of data on cave environments. The use of AUVs in cave exploration and monitoring offers key benefits such as improved safety by accessing technically challenging or hazardous sections without risking human divers and potential standardization of monitoring practices. AUVs can be used in both marine and freshwater cave systems, expanding their applicability. However, the adoption of AUVs for cave monitoring faces some constraints, primarily related to cost and the need for technical expertise. Despite these limitations, as AUV technology continues to advance, we can expect potentially revolutionizing our understanding and monitoring of underwater cave systems in the future.

4 Guidelines to assess fragmentation at appropriate scales

Measuring fragmentation in marine caves is a complex process that requires a combination of methods to fully understand the intricate relationships within these unique ecosystems. Marine caves are naturally fragmented habitats characterized by a drastic change in environmental conditions compared to the outside surrounding environment (Muths et al., 2015). Connectivity between the marine communities and species that inhabit such areas is strongly influenced by hydrodynamics. Water movement both within and outside cave environments disperses propagules, as well as circulating nutrients and food. Unless they are directly connected, or in close proximity to each other through such water movement or the movement of species, there is a natural tendency towards limited exchange and/or interaction between the marine communities/species in different cave systems.

A multifaceted approach can allow researchers to examine fragmentation and connectivity at various levels.

At the genetic level, analyzing genetic markers offers valuable insights into gene flow between populations. This method can reveal connectivity patterns not only between different cave systems but also within a single cave or across various sections of a larger system. By examining genetic diversity and structure, researchers can infer the degree of isolation or connectivity between cave populations, offering a molecular perspective on fragmentation. At local scale, gene flow among caves was shown to be dependent on local hydrodynamic barriers in France (Lejeusne & Chevaldonné, 2006).

Community-level assessments provide another crucial dimension to understanding connectivity in marine caves. By examining species richness and composition across different cave sections, researchers can gain insights into habitat connectivity. The presence of rare or cave-exclusive species can serve as biological indicators of isolation. Comparing community structures between cave sections or systems can reveal patterns of fragmentation or connectivity.

Functional ecology approaches complement genetic and community-level analyses by focusing on the traits of cave organisms. Changes in community-weighted mean traits along cave gradients can indicate varying degrees of connectivity, while shifts in functional groups or ecological strategies in fragmented areas may reveal how fragmentation impacts ecosystem functioning.

There are few studies on the issue of connectivity/fragmentation in marine caves; however, this has been investigated for two species of marine cave-dwelling mysids in localities across the northwest Mediterranean. This study has revealed that, for these species, the caves have a mostly local recruitment and a restricted gene flow with other caves of their group (Lejeusne & Chevaldonné, 2006).

5 Next steps to address future needs

This document provided an analysis of the methodologies used for marine cave monitoring in the EU member states, comparing them with the ecological characteristics of caves that are most relevant to assess their condition, and proposed a methodology for the harmonisation of habitat monitoring across the EU. Although this proposal is based on extensive information and a review of experiences included in the national habitat monitoring manuals, it would be advisable to test it by national experts and practitioners in habitat monitoring to evaluate its feasibility and appropriateness in different EU member states and different contexts.

As regard the use of **typical species**, given the ecological and geographical variability of the Annex I habitat types, it is not realistic to have recommended lists of **typical species**, even for a biogeographical or marine region. 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. Furthermore, given the variability of habitat types across their range, even within a single biogeographical marine region, it is also very unlikely that all typical species will be present in all examples of the habitat. For this reason, this document only identifies potential groups from which to select typical species for monitoring marine caves. Further work is needed to identify the most relevant typical species for the assessment of habitat condition. This may take place at a national level but should also reflect any biogeographical and regional differences and ideally link to relevant existing monitoring programmes.

When making such a selection it should also be borne in mind that the priority is for good indicators of favourable structure and function and that as such, they may not be the most dominant species. Species selected also need to reflect the variety of biological communities/subtypes often found within marine caves given that they are often made up of a mosaic of marine communities rather than being uniform.

A summary of the **ecological characteristics and main variables** used to measure the habitat condition of marine caves is presented in this report together with a review and an analysis of variables which are specified in the national habitat monitoring manuals of EU Member States. There is much commonality but also potential to explore whether this can be standardized across Member States in at least some cases. Equally important is to make sure that there is consistency with variables being used for reporting of MSFD descriptors. Also relevant are the metrics which are used to monitor the different variables. Many considerations make it unlikely that the metrics could be standardized even for commonly agreed variables (e.g. due to the variations in this habitat across its range, practical considerations, measurement methods) but it is worth exploring whether there is any scope for intercalibration.

The **reference values and thresholds** applied by Member States to obtain condition indicators for caves are variously; very specific, based on trends, use indices, rely on expert judgement or any combination of these. Given the variability of habitat across its range, even within a single biogeographical marine region, it is unlikely that the same ranges and thresholds can be applied in all circumstances. The scope to have a common or favoured approach to setting reference values and thresholds for variables could usefully be investigated. Equally important is to make sure that there is consistency with ranges and thresholds being used for reporting of MSFD descriptors.

Finally, although there are many well established methods for **monitoring and sampling** caves new techniques are constantly being developed. It is particularly important to keep alert to these for harder to access locations (depth, conditions, nature of habitat etc.) which is where many advances may be made.

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