UNITED
NATIONS

EP

UNEP/MED WG.641/inf.3

Mediterranean
Action Plan
Barcelona
Convention

8 June 2026
Original: English

Back-to-back Meeting of the Ecosystem Approach Correspondence Groups on Monitoring (CORMON) Biodiversity and Fisheries and Economic and Social Analysis (COR ESA)

Videoconference, 10-11 June 2026

Agenda Item 3: Updated Guidance Factsheets for biodiversity (EO1)

3.1. Benthic Habitats (OE 1: Common Indicator 1 – habitat distributional range and Common Indicator 2 – condition of the habitat's typical species and communities)

Guidelines for inventorying and monitoring of dark habitats in the mediterranean sea

SPA/RAC
Tunis, 2026

Note by the Secretariat

1. The Action Plan for the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea (Dark Habitats Action Plan)[1] provide for improving inventories, location and characterization and enhancing national capacities and improve skills in taxonomy and monitoring methods.

2. In this context, SPA/RAC updated the Guidelines for inventorying and monitoring of dark habitats in the mediterranean sea to help countries in their efforts to conserve dark habitats

---

[1] CoP22 Decision IG.25/13, Annex II

GUIDELINES FOR INVENTORYING AND MONITORING OF DARK HABITATS IN THE MEDITERRANEAN SEA

SPA/RAC - 2025

Prepared by

Vasilis GEROVASILEIOU
Maïa FOURT

With contributions from

Markos DIGENIS
Torcuato PULIDO MANTAS
Antonietta ROSSO
Ricardo AGUILAR
Francesco ENRICHETTI
Marzia BO
Giovanni CHIMIENTI
Tatjana BAKRAN-PETRICIOLI
Vesna MAČIĆ
Leonardo TUNESI

Photographic material provided by

Vasilis GEROVASILEIOU
Ricardo AGUILAR / OCEANA
Michela ANGIOLILLO
Giovanni CHIMIENTI
Markos DIGENIS
Michela MAIORCA

Acknowledgements

Our special thanks go to all participants of the "Workshop on Inventory and Monitoring of Marine Cave Habitats", organized by SPA/RAC in collaboration with "Association Tunisienne de Taxonomie (ATUTAX)" in Tunisia (2024), for their constructive discussions and valuable feedback in updating the guidelines.

TABLE OF CONTENTS

CONTEXT AND AIMS

Within the framework of the Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD Protocol) under the Mediterranean Action Plan, the Contracting Parties to the Barcelona Convention adopted the "Dark Habitats Action Plan" (DHAP) for the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds, and chemosynthetic phenomena in the Mediterranean Sea (UNEP/MAP-SPA/RAC, 2021).

In the framework of the DHAP, dark habitats[1] are defined as ecosystems and species associated with underwater caves, seamounts and canyons, aphotic hard beds, and chemosynthetic phenomena (UNEP/MAP-SPA/RAC, 2021). These habitats are distributed throughout the Mediterranean Sea, ranging from the sea surface (e.g., underwater dark caves) to the deep sea (e.g., seamounts, canyons, and cold seeps) under aphotic conditions. Dark habitats encompass multiple complex ecosystems and a wide diversity of species that typically develop under stable environmental conditions.

As part of the DHAP implementation schedule, it is essential to establish and periodically update a set of *Guidelines for Inventorying and Monitoring of Dark Habitats in the Mediterranean Sea*. The first edition of these Guidelines was published in 2017 (SPA/RAC–UN Environment/MAP & OCEANA, 2017). Since then, notable advances in scientific research, survey technology and methodological approaches have improved our understanding of marine caves and deep-sea habitats, as well as the ecological communities they host. Building on these developments, the present document provides updated guidelines for the inventory, characterization, and monitoring of dark habitats in the Mediterranean Sea. By consolidating current best practices and promoting methodological standardisation across countries and research institutions, these guidelines aim to support a coordinated assessment and to strengthen the effectiveness of conservation and management measures for dark habitats and the vulnerable assemblages they support.

Despite the increase in scientific knowledge on Mediterranean dark habitats over recent decades, substantial knowledge gaps persist. The development of comprehensive inventorying initiatives and monitoring tools continues to be challenging due to: (1) the scarcity of data on the current state of these habitats (e.g., distribution and density of key species), mainly resulting from limited accessibility and high exploration costs; and (2) the limited historical datasets and long-term time series.

Furthermore, human activities and related pressures on marine habitats have intensified throughout the Mediterranean, including in deep-sea environments (e.g., destructive fishing practices such as bottom trawling, oil and gas exploration, and prospective deep-sea mining). Accordingly, an urgent need exists to establish a regional monitoring framework to support the implementation of the Ecosystem Approach (EcAp) and, in particular, the Integrated Monitoring and Assessment Programme (IMAP) at the regional level.

---

[1] Dark habitats are defined as environments where sunlight is either entirely absent or insufficient to support the development of photosynthetic plant communities. They encompass both shallow marine caves and deep-sea habitats, typically located at depths greater than 150–200 m.

I. HABITATS AND SPECIES ASSOCIATED WITH MARINE CAVES

Marine caves are defined as cavities entirely or partially occupied by seawater, accessible to humans, and exhibiting substantial horizontal and volumetric development; a possible criterion is that the ratio between the total volume (m³) and the entrance area (m²) should be greater than 1, and that the entrance width should not exceed the average internal width (Bianchi et al., 1996; Gerovasileiou & Bianchi, 2021). These habitats may be **fully submerged or semi-submerged**, displaying a wide range of morphologies, including blind-ended caves (cul-de-sacs), tunnels with single or multiple openings, vertical pits, and more complex morphologies (Gerovasileiou et al., 2016a).

A defining ecological feature of marine caves is the pronounced zonation of their communities, driven by **steep environmental gradients** that can occur over spatial scales of only a few meters. These gradients typically include rapid decrease of light, progressive hydrological confinement, and a decline in trophic inputs from the cave entrance towards the interior (Riedl, 1966; Harmelin et al., 1985; Bianchi & Morri, 1994). Marine caves host diverse sciaphilic communities organized into distinct biocoenotic zones. Up to six faunal and ecological zones have been described for Mediterranean marine caves (Pérès & Picard, 1964; Riedl, 1966; Bianchi & Morri, 1994). According to the most widely accepted bionomic model marine caves include **three main ecological zones (ecozones)**, associated with distinct communities (Pérès, 1967; Gerovasileiou & Bianchi, 2021):

- **Entrance zone:** The transitional area between the cave and the external environment, typically hosting a (pre-)coralligenous[2] community dominated by sciaphilic algae (mainly calcareous rhodophytes) and sciaphilic benthic invertebrates.
- **Semidark zone:** The twilight sector of the cave, dominated by sessile, filter-feeding invertebrates – mainly sponges and anthozoans – favoured by reduced light availability and the resulting absence of space-competing macroalgae. Several distinctive facies may develop on the walls and ceilings of semidark caves, typically dominated by sponges, hydroids, anthozoans (e.g., scleractinians, the red coral *Corallium rubrum*, and zoantharians), bryozoans, and ascidians (see Fig. 1a–b and Table 1).
- **Dark zone:** The innermost and most confined sector of the cave, characterized by markedly reduced biotic coverage, biomass, structural complexity, and species richness. A black mineral coating often develops on the bare rocky substrate. This zone is usually sparsely colonized by sponges, serpulid polychaetes, bryozoans, and brachiopods, while several cryptobiotic motile taxa (e.g., crustaceans and fishes), and species of bathyal origin frequently use these habitats as refuges (Figs. 1c–f; 2a–b).

The pronounced structural complexity and variable topography of marine caves contribute to substantial small-scale habitat heterogeneity and high species turnover, even within individual cave systems, an attribute often referred to as “**cave individuality**” (Bussotti et al., 2006; Gerovasileiou & Bianchi, 2021). This phenomenon results in unique ecological patterns and assemblage structures that are strongly influenced by local geomorphological and hydrological conditions. For instance, marine caves or those influenced by freshwater runoff often support distinctive communities and rock sponge formations (Table 1; Bianchi et al., 2021; Pisera & Gerovasileiou, 2021;). Specific assemblages, such as nodular and crest-like bryozoan formations, frequently develop within the transitional zone between the semidark and dark sectors of caves (Harmelin, 1985; Harmelin et al., 1985; Rosso et al., 2019).

---

[2] Coralligenous communities have been formally incorporated into the Action Plan for the Conservation of the Coralligenous and other Calcareous Bio-concretions in the Mediterranean Sea (UNEP/MAP-SPA/RAC, 2016).

Large bioconstructions formed primarily by aggregates of serpulid polychaetes (e.g., *Protula tubularia*), together with scleractinians, bryozoans, brachiopods, and foraminiferans, have been documented in several dark caves throughout the Mediterranean (Belmonte et al., 2009; Sanfilippo et al., 2015, 2017; Guido et al., 2017; Rosso et al., 2021). These structures, which may have developed over hundreds to thousands of years, represent long-term biogenic accretions of high ecological and paleoenvironmental value. Their discovery underscores the exceptional significance of cave-associated bioconstructions and highlights the urgent need for dedicated management and conservation measures to ensure their long-term protection.

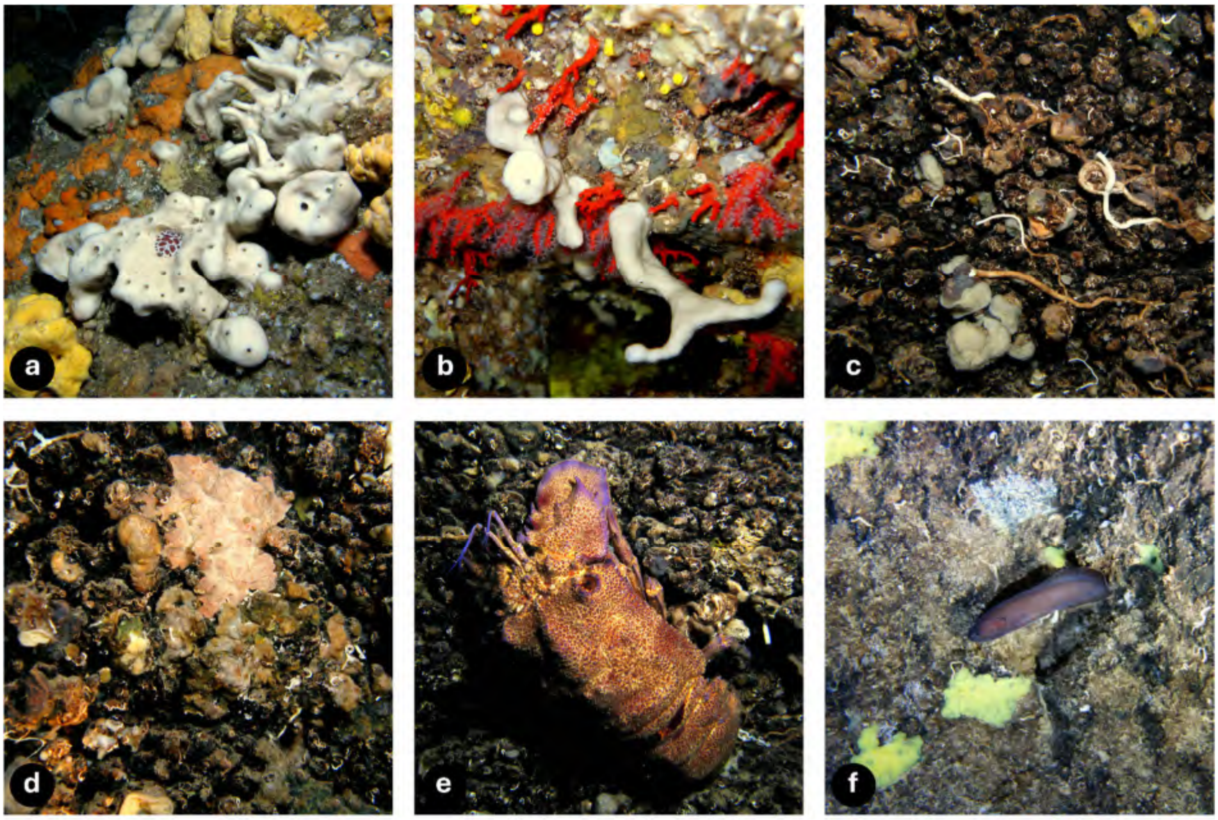


Figure 1. (a) Sponge-dominated and (b) anthozoan-dominated facies in the semidark zone of marine caves. The stony sponge *Petrosia ficiformis* (a, b) appears discoloured due to the absence of light and is preyed upon by the nudibranch *Peltodoris atromaculata*. In (b), the dominant anthozoans are the red coral *Corallium rubrum* and the yellow cup coral *Leptopsammia pruvoti*. (c, d) Dark-zone cave communities dominated by serpulid polychaetes, bryozoan nodules, encrusting sponges; the bare rocky substrate is covered by a thin black mineral coating. (e) The slipper lobster *Scyllarides latus* and (f) the speleophilic fish *Grammonus ater* in dark marine cave zones. Photos by Vasilis Gerovasileiou.

Cave sediments are predominantly muddy (Fig. 2a–c), resembling those of deep circalittoral or bathyal soft-bottom environments (Bianchi & Morri, 2003). However, they often contain detritic fragments, including skeletal remains, calcareous shells, and tubes of various taxa (e.g., molluscs, brachiopods, scleractinians, serpulids, and bryozoans) forming thanatocoenoses originating from the cave walls and ceilings (Fig. 2d; Monteiro-Marques, 1981; Rosso et al., 2013; Pino et al., 2020; Di Franco et al., 2021), or occasionally from external shallow-water habitats. However, soft-substrate communities inhabiting the floors of marine caves have been studied far less extensively than those on hard substrates, and information on their composition remains scarce (Gerovasileiou & Bianchi, 2021).

Although most bionomic classifications of marine cave communities focus on sessile taxa, these habitats also support diverse and abundant **motile assemblages** – primarily fishes (e.g., Figs. 1f; 2a,c), crustaceans (e.g., Figs. 1e; 2b) and molluscs (e.g., Fig. 1a) – which include species representing different ecological groups according to their degree of association with the cave environment (Kovačić et al., 2024; Digenis et al., 2025). For instance, diurnal reef-associated species may occur in caves, typically near the entrance, whereas the inner semidark and dark zones are inhabited by cryptic habitat dwellers, nocturnal shelter-seekers (e.g., mysids and the cardinal fish *Apogon imberbis*), deeper-water species, and speleophilic taxa (Digenis et al., 2025).

Syntheses of current knowledge on Mediterranean marine caves and their associated communities have been published in recent years (Ouerghi et al., 2019; SPA/RAC-UNEP/MAP, 2020; Gerovasileiou & Bianchi, 2021). Moreover, several reviews provide comprehensive checklists for specific taxonomic groups, such as sponges (Gerovasileiou & Voultsiadou, 2012; Grenier et al., 2017), crustaceans (Bianchi et al., 2022; Navarro-Barranco et al., 2023a), and fishes (Kovačić et al., 2024), as well as regional censuses (e.g., Gerovasileiou et al., 2015).

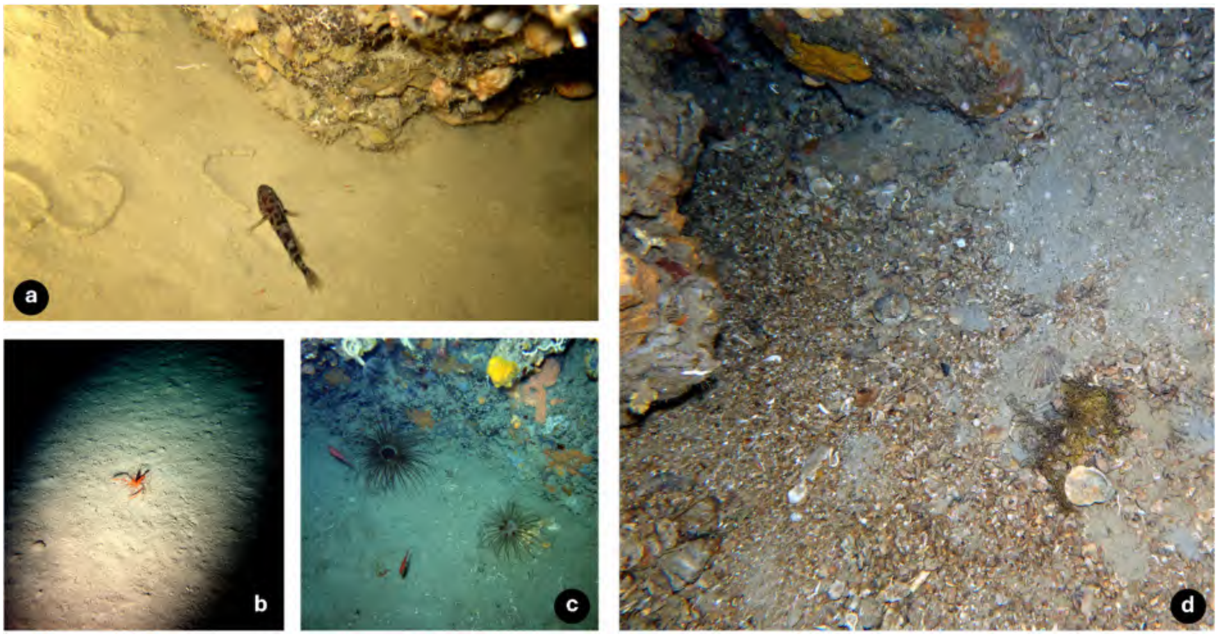


Figure 2. Cave sediment communities. (a) The cryptobenthic leopard-spotted goby *Thorogobius ephippiatus* on the muddy cave floor; (b) the shrimp *Stenopus spinosus* inhabiting the dark zone of a submerged cave; (c) tube anemones *Cerianthus membranaceus* and cardinal fish *Apogon imberbis* occurring on sediment near the cave side-wall margins; (d) thanatocoenoses composed of shells and broken calcareous fragments from various taxa, along with a piece of fishing net (bottom right). Photos by Vasilis Gerovasileiou (a–c) and Markos Digenis (d).

Despite their recognized importance, there remains a notable lack of quantitative data describing the environmental gradients (e.g., light levels, hydrodynamics, salinity, and nutrient availability) that shape community structure and function within marine caves (Gili et al., 1986; Bianchi et al., 1998). Although the DHAP fully covers dark marine caves[3] within its scope, inventorying and monitoring initiatives focusing on marine caves should explicitly adopt an ecosystem-based approach, considering the cave environment as an integrated system rather than as isolated habitat components. A comprehensive

---

[3] <0.01% of the light available at the sea surface according to Harmelin et al. (1985).

list of Mediterranean habitat types associated with marine caves is provided in Table 1, based on the updated classification system for marine habitats developed by SPA/RAC (Montefalcone et al., 2021).

Table 1. Reference list of Mediterranean habitat types associated with marine caves, based on the *Handbook for Interpreting Types of Marine Habitats for the Selection of Sites to be Included in the National Inventories of Natural Sites of Conservation Interest* (UNEP/MED WG.502/Inf.4, 2021), and the updated classification system for marine benthic habitats developed by SPA/RAC (Montefalcone et al., 2021).

LITTORAL

- MA1.52 Midlittoral caves[4]
    - MA1.521 Association with encrusting Corallinales or other Rhodophyta

INFRALITTORAL

- MB1.56 Semi-dark caves and overhangs (see sheet MC1.53)

CIRCALITTORAL

- MC1.53 Semi-dark caves and overhangs
    - MC1.53a Walls
        - MC1.531a Facies with sponges
        - MC1.532a Facies with Hydrozoa
        - MC1.533a Facies with *Corallium rubrum*
        - MC1.534a Facies with Scleractinia
        - MC1.535a Facies with Zoantharia
        - MC1.536a Facies with Bryozoa
        - MC1.537a Facies with Ascidiacea
    - MC1.53b Roof (see MC1.53a for examples of facies)
    - MC1.53c Detritic bottom (see MC3.51 for examples of facies)
    - MC1.53d Brackish water caves or caves subjected to freshwater runoff
        - MC1.531d Facies with lithistid sponges

UPPER BATHYAL

- ME1.52 Caves and ducts in total darkness

I.1. Inventorying marine caves

Inventorying marine cave communities involves two main levels:

- **Locating and characterizing the physical environment of marine caves**: This includes geo-referencing cave entrances, documenting topographic and geomorphological features, and producing detailed internal maps to describe cave morphology, spatial complexity, and environmental gradients.
- **Characterizing marine cave communities**: This involves assessing biodiversity, structure, and species composition of cave communities, as well as quantifying percent cover for sessile taxa and estimating abundance or density for motile taxa. Such data provide the basis for evaluating ecological patterns and detecting temporal changes.

---

[4] Midlittoral caves are usually not dark enough to be classified as "dark habitats", except in the case of long, semi-submerged caves or those with specific topographies that prevent sunlight from reaching their interiors.

I.1.a. Locating and characterizing the physical environment of marine caves

Diving is essential for the inventorying, exploration, and mapping of marine caves (see Box 1 for field safety and precautionary measures for marine cave inventorying), except in the case of shallow, semi-submerged caves, which can be detected and accessed directly from the sea surface by **snorkelling** (Fig. 3; see Dailianis et al., 2019; Furlani et al., 2023; and guidelines in Appendix 1). To some extent, preliminary information on the location, depth, and morphology of marine caves can be obtained through **local ecological knowledge (LEK)** and **citizen science** initiatives – for example, by engaging with local professional and recreational diving and fishing communities (see questionnaires in Dridi et al., 2019 and Quiles-Pons et al., 2025) – prior to undertaking dedicated mapping surveys. National legislation should be carefully considered in each country, particularly when cave exploration requires specific permits from the relevant authorities, and especially when such activities occur within marine protected areas (MPAs).

Most Mediterranean marine caves studied to date are semi-submerged or shallow, and few exceed 30 m in depth, reflecting logistical constraints associated with underwater work (Gerovasileiou & Bianchi, 2021). The inventorying of caves with deeper entrances or more complex internal systems requires **specialized expertise and advanced diving equipment**, such as Closed-Circuit Underwater Breathing Apparatus (CCUBA). These techniques, while enabling extended and deeper penetration, also entail greater operational risks compared to conventional SCUBA diving. The inventorying of deep-sea caves typically rely on Remotely Operated Vehicles (ROVs), although this approach has inherent limitations in terms of manoeuvrability and accessibility. Future assessments should also explore the potential of acoustic methods, particularly multibeam sonar, for detecting cave formations.

Caves are formed through a variety of processes in different rock types, including the dissolution of bedrock by water circulating through fissures and pores in karstic areas, fracturing and non-dissolution erosion, the formation of lava tubes in volcanic areas, or the development of talus caves among rock falls (Gerovasileiou & Bianchi, 2021). In marine environments, mechanical erosion caused by wave action can also play a significant role (Riedl, 1966). Consequently, various classification schemes and terminologies have been proposed based on cave origin and formation processes (speleogenesis) and host rock type, that could be possibly followed during inventorying initiatives (see definitions in Gerovasileiou et al., 2016a). Moreover, in some areas, local terms are used to describe specific cave types – for instance, “Vrulja” in Croatia refers to submarine springs or underground streams discharging below sea level (Surić et al., 2010).

A GPS device is necessary for **geo-referencing** the location of the access point to the surveyed marine cave at the sea surface level. It is also recommended to take geotagged photographs of the entrance of each marine cave before exploration (Fig. 3a). **Topography** plays a crucial role in structuring marine cave communities; therefore, recording key topographic features is essential for cave inventories, as well as for designing appropriate sampling schemes and monitoring protocols. A thorough understanding of a cave’s topography prior to underwater fieldwork is also vital for ensuring diver safety (Rastorgueff et al. 2015; see Box 1). The most important topographic parameters to consider during marine cave inventorying include (see definitions in Gerovasileiou et al., 2016a):

- **entrance** depth, orientation, and dimensions, as well as the entrance position (low or high relative to the overall development of the cave),
- **cave morphology** (e.g., blind-ended or tunnel-shaped caves with multiple openings),
- **submersion level** (semi-submerged or fully submerged),
- **maximum and minimum water depths** inside the cave,
- **total cave length**, and

- **other topographic features**, such as wall and floor inclination, the presence of sediment or exposed bedrock on the floor, and microhabitats that may support distinct communities or rare species (e.g., freshwater inflows, sulphur springs, bioconstructions, or internal beaches).

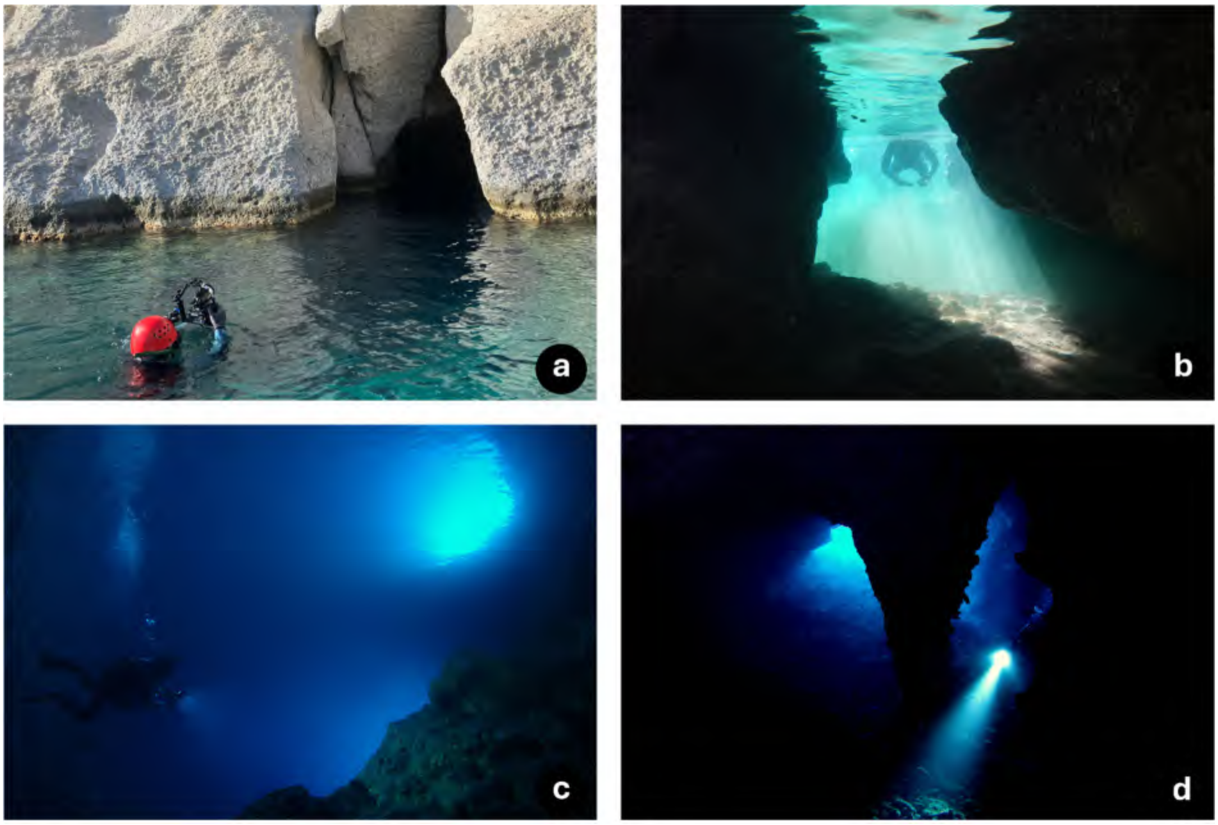


Figure 3. (a) Photographic documentation of the entrance to a semi-submerged cave and (b) entry by snorkelling. (c) Exploration of semidark and (d) dark cave zones using SCUBA diving. Photos by Vasilis Gerovasileiou (a, c, d) and Markos Digenis (b).

To date, a wide range of methodologies and equipment has been developed primarily for the mapping of terrestrial and groundwater caves, including remotely operated technologies, image-based techniques, and software platforms for three-dimensional (3D) cave modelling (e.g., Sellers & Chamberlain, 1998; Fairfield et al., 2007; Gallay et al., 2015; Alessandri et al., 2022). In the context of marine cave research in the Mediterranean Sea, Gerovasileiou et al. (2013) developed a rapid and cost-effective protocol for 3D mapping and visualization of fully and semi-submerged caves with simple, non-dendritic morphologies. The method can be implemented by two divers within one to two dives and enables the automatic generation of 3D cave morphology models using “Cavetopo” software. More recently, high-resolution 3D photogrammetry has been applied to map marine caves in the Parc National des Calanques (France), along the Adriatic coasts of Italy, and in the Balearic Islands (Spain), focusing on the quantitative assessment of benthic assemblages as a tool for ecological monitoring (Chemisky et al., 2015; Quiles-Pons et al., 2022; Pulido Mantas et al., 2023).

BOX1 – *Field safety and precautionary measures for marine cave inventorying*

Diving in marine caves, even in relatively shallow systems, is logistically demanding and requires strict adherence to **safety measures** in accordance with the **precautionary approach, even for experienced divers**. During storms, periods of strong swell, or intense wave activity, snorkelers and divers should avoid entering marine caves for safety reasons and, when necessary, use protective helmets (Fig. 3a). Cave floors are often covered by fine, silty sediments that can be easily disturbed, thereby reducing visibility and potentially obscuring the cave entrance. To mitigate these risks, divers should receive appropriate training tailored to their dive plan and the specific characteristics of each cave. They should carefully maintain buoyancy control and use a calibrated dive line (e.g., with distance markers every 1–5 m), in addition to standard SCUBA equipment such as short fins, multiple light sources, cutting tools, a dive computer, a magnetic compass, and a dive slate. Additional instruments are recommended for accurate distance and depth measurements, including a tape measure, portable echosounder, or waterproof distance-measuring device for semi-submerged caves. A practical protocol for inventorying semi-submerged caves which are used by Mediterranean monk seals (*Monachus monachus*) for resting or breeding is provided by Dendrinos et al. (2007). In areas inhabited by monk seals, additional precautionary measures should be implemented (see guidelines in Appendix 1), and cave exploration should ideally be scheduled during periods of low in-cave seal activity (late spring to early summer) to minimize potential disturbance.

I.1.b. Characterization of marine cave communities

Marine caves host highly diversified communities that develop across distinct ecological zones shaped by steep environmental gradients. Different assemblages typically occupy the entrance, semidark, and dark zones, while notable variations may occur within transitional areas between zones and between opposite cave walls and ceilings (Bussotti et al., 2006; Radolović et al., 2015; Gerovasileiou & Bianchi, 2021, and references therein). The characterization of marine cave communities relies on a wide variety of methods, which differ according to the study’s objectives, ecological or taxonomic focus (e.g., sessile or motile fauna, epi- or infauna, macro- or meiobenthos), and the type of substrate (e.g., hard cave walls and ceilings[5] versus soft sedimentary cave floors) (see guidelines in Appendix 2).

I.1.b.1. Characterization of hard substrate communities

Non-invasive techniques, such as **non-destructive photographic and visual methods (e.g., photoquadrats, video surveys, and visual censuses)**, are central to both current practices and recommended protocols for assessing biodiversity and ecological status in marine caves (Martí et al., 2004a; Bussotti et al., 2006; Parravicini et al., 2010; Gerovasileiou & Voultsiadou, 2016; Dimarchopoulou et al., 2018; Digenis et al., 2022; Derrien et al., 2025; Schiavo et al., 2024). These approaches are particularly valuable given the high sensitivity of benthic cave communities and the frequent occurrence of protected (e.g., *Corallium rubrum*) or rare species, as they minimize diver impact and reduce underwater exposure time [6] while still providing reference conditions for

---

[5] The general principles and methods used to characterize and monitor hard-substrate communities in marine caves are similar to those applied to coralligenous assemblages (UNEP/MAP-SPA/RAC, 2024).

[6] Fieldwork in marine cave environments presents additional logistical and safety challenges due to restricted visibility, confined spaces, and limited underwater working time (Gerovasileiou et al., 2013; Digenis et al., 2025).

monitoring (Bianchi et al., 2004). Whenever possible, high-quality **photoquadrats** (Fig. 4) should be preferred over direct visual assessments without photographic documentation, not only to reduce time underwater but, importantly, to facilitate subsequent species identification and data sharing. The use of laser pointers has also been suggested and applied in marine caves to avoid physical quadrats and direct contact with the benthos (Spaccavento et al., 2022).

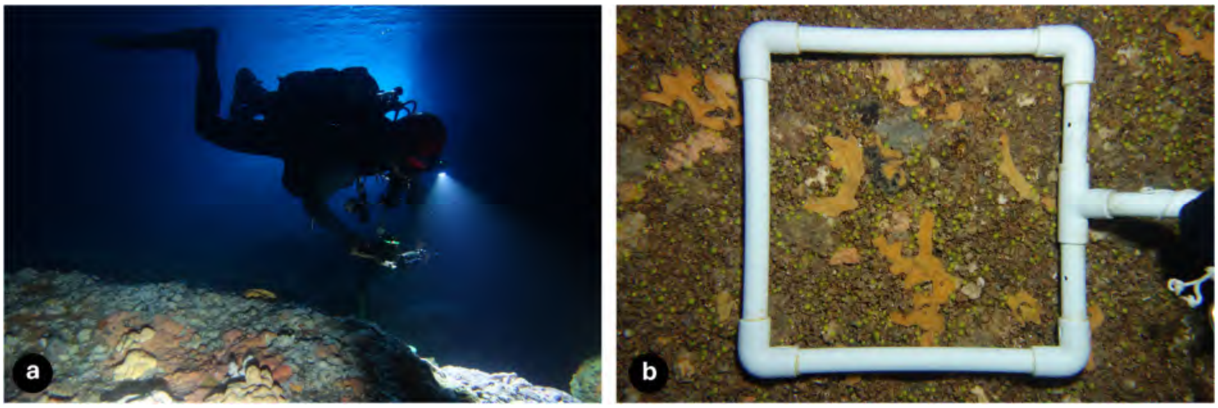


Figure 4. (a) A SCUBA diver photographing a quadrat placed on the rocky floor of a semi-submerged cave, and (b) a 25 × 25 cm photoquadrat from the vertical wall of a semidark cave, dominated by encrusting sponges and scleractinians. Photos by Vasilis Gerovasileiou.

Due to the pronounced spatial heterogeneity observed among marine caves – even those located in close proximity and exhibiting similar geomorphological characteristics – as well as between caves from different depths, areas, or distinct ecological zones within the same cave, establishing a threshold for the optimal sampling surface for benthic community monitoring remains highly challenging. Previous studies have proposed varying sampling requirements depending on cave community, target taxa and area (Table 2). The **density of photoquadrats** used in monitoring marine cave habitats largely depends on the objectives of the study, such as whether the aim is a detailed characterization and mapping of benthic assemblages or a rapid assessment for baseline evaluation and monitoring. In some cases, photoquadrats were taken at regular 5 m intervals along transects placed on different cave surfaces (e.g., ceiling and opposite side walls) following the entrance-to-interior axis, to allow fine-scale mapping and investigation of spatial community patterns (Gerovasileiou & Voultsiadou, 2016; Gerovasileiou et al., 2017; Sempere-Valverde et al., 2019). In other studies, a fixed number of quadrats (typically 10–20 per cave zone) was photographed to provide standardized coverage across ecological zones, encompassing both side walls and, when present, the rocky cave floor (Martí et al., 2004a; Dimarchopoulou et al., 2018; Digenis et al., 2022).

The recommended **photoquadrat size** also varies across studies, generally reflecting the scale of the habitat features and the target taxa. Most studies have employed **20 × 20 cm quadrats** (Gili et al., 1982; Bianchi et al., 2004; Kipson et al., 2011; Nepote et al., 2017; Sempere-Valverde et al., 2019; Pulido Mantas et al., 2023; Derrien et al., 2025; Table 2), whereas others have adopted slightly larger **25 × 25 cm quadrats** (Gerovasileiou & Voultsiadou, 2016; Gerovasileiou et al., 2017; Dimarchopoulou et al., 2018; Digenis et al., 2022; Lanza-Arroyo et al., 2024; Fig. 4b) or even **50 x 50 cm quadrats** (Pulido Mantas et al., 2023). Quadrat size selection inherently influences sampling effort and the likelihood of detecting uncommon species. Pulido Mantas et al. (2023) demonstrated that while 50 × 50 cm quadrats offer a more cost-effective solution compared to 20 × 20 cm quadrats, their larger dimensions present practical and ecological limitations. More specifically, in marine caves with narrow passages, manipulating large frames may be unfeasible or may risk damaging fragile sessile organisms, particularly species with brittle skeletons. Moreover, larger quadrats are better suited for assessing communities dominated by large-sized taxa (e.g., gorgonians – Kipson et al., 2011) but may underrepresent small-sized or rare organisms.

Table 2. Recommended minimum sampling area for the assessment of marine cave communities, derived from Mediterranean studies encompassing a range of taxonomic groups and geographic regions.

Study	Minimum sampling area	Taxonomic and geographic scope
Weinberg (1978)	4,000 cm² for dark caves and 20,000 cm² for open rocky surfaces outside caves (horizontal, sloping, or vertical)	Octocorals in the NW Mediterranean Sea (Banyuls-sur-Mer, south France)
Kipson et al. (2011)	2,500 cm²	*Corallium rubrum* assemblages on overhangs and cave entrances (14–20 m depth) in the NW Mediterranean Sea
Rastorgueff et al. (2015)	10,000–40,000 cm²	Recommended surface using a standardized photographic survey in the framework of the Cave Ecosystem-Based Quality Index (CavEBQI) for marine cave ecosystems in the NW Mediterranean Sea; benthic assemblages were classified as key functional groups: Passive Filter Feeders, Small Active Filter Feeders, and Large Active Filter Feeders
Grenier et al. (2017)	11,172–23,520 cm² (19–40 photoquadrats, each 21 × 28 cm) depending on the cave; however, in two caves where 40–50 quadrats were photographed, no asymptote was reached in the cumulative species–area curves	Sponge assemblages in the semidark zones of 13 marine caves (5–15 m depth) of the Marseille-Provence region (south France)
Lanza-Arroyo et al. (2024)	6,250 cm² (10 quadrats per cave – five from each opposite side wall, each 25 x 25 cm)	Semidark and dark zones of 17 caves in the Alboran Sea (5–45 m depth). Benthic assemblages were classified as key functional groups: Passive Filter Feeders, Small Active Filter Feeders, and Large Active Filter Feeders
Derrien et al. (2025)	9,200–11,600 cm² (23–29 photoquadrats, each 20 × 20 cm) in semidark zones and 5,600–10,800 cm² (14–27 photoquadrats, each 20 × 20 cm) in dark zones, although one dark cave exhibited a slight increase beyond the 32nd quadrat	Benthic communities in four marine caves (9–25 m depth) in the Marseille region (south France)

In conclusion, **the selection of photoquadrat size should achieve an appropriate balance between spatial representativeness, logistical feasibility, and the ecological characteristics of the target community**. In all the abovementioned studies, quadrat deployments on hard substrates were performed **randomly**, covering distinct ecological zones within caves and various positions within each zone (e.g., opposite side walls, ceilings, and rocky cave floors, when present). Consequently, even in cases where an optimal sampling surface cannot be determined or area–species accumulation curves cannot be readily generated (e.g., during rapid assessments of multiple marine caves with differing characteristics), it is advisable to photograph a **standardized number of quadrats** within the ranges previously reported (Table 2) from **each ecological cave zone** (e.g., entrance, semidark, and dark zone), ensuring that both opposite walls and the ceiling or rocky floor (when present) are adequately represented (see detailed guidelines in Appendix 2).

Underwater photogrammetry has recently emerged as a powerful, non-invasive method for documenting benthic assemblages and offers a highly accurate and promising approach for improving monitoring efforts and long-term ecological assessments of marine cave communities. Beyond enabling the calculation of spatial cover or seascape indices, this technique provides a comprehensive 3D representation of the spatial structure of hard-substrate communities. However, its application in marine caves remains limited (Chemisky et al., 2015; Marroni et al., 2022; Quiles-Pons et al., 2022; Pulido Mantas et al., 2023), largely because of the substantial field and analytical demands involved. Photogrammetric surveys typically require **longer bottom times, specialized imaging systems, additional artificial lighting, and considerable computational capacity**, particularly when processing large or high-resolution datasets (Pulido Mantas et al., 2023).

Both photoquadrat sampling and effective photogrammetric work in marine cave environments require **careful operational planning and appropriate diving skills**. Operators must maintain precise buoyancy control, avoid disturbing fragile substrates, and follow systematic image-acquisition patterns while keeping a consistent distance from cave surfaces (Di Camillo et al., 2025). Uniform illumination, suitable camera settings, and the inclusion of metric reference scales are essential to ensure image quality, complete spatial coverage, and accurate model reconstruction (see Appendix 3 for practical considerations to implement photogrammetric surveys in marine caves). The potential contribution of experienced volunteer divers is also being explored through **citizen science initiatives**, offering opportunities to support data collection while adhering to strict safety and quality protocols (Marroni et al., 2022).

However, it should be noted that underwater photogrammetry is still in its early stages of development, and examples may often be drawn from other marine habitats, organisms, or regions. For instance, in the Mediterranean Sea, it has been applied to the monitoring of *Posidonia oceanica* seagrass meadows, biogenic reefs, red coral and sponges on rocky substrates (e.g., Royer et al., 2018; Abadie et al., 2022; Richaume et al., 2021; Ventura et al., 2021; Pulido Mantas et al., 2024). Numerous additional applications are documented across diverse marine environments worldwide, reflecting the technique's broad and growing potential.

Quantitative analysis of underwater images and video can be conducted using a wide range of tools, methods, and strategies, including **advanced processing software** and methodological approaches for annotations and surface coverage calculations (see Box 2). However, visual identification has inherent limitations for certain benthic taxa, such as sponges, bryozoans, and ascidians, and **supplementary qualitative sampling** is often necessary to fully characterize hard-substrate communities. When such sampling is required, only small tissue fragments from modular or colonial invertebrates should be collected to minimize disturbance and allow natural regeneration or recovery of the remaining tissues/specimens. All sampled organisms should ideally be **photographed and tagged in the field** – preferably underwater, but alternatively on the vessel or on shore immediately after collection – as many sessile taxa undergo changes in colour and morphology following preservation. Sampling activities must **comply fully with national legislation** and be conducted only under appropriate permits issued by the competent authorities, particularly when involving protected or threatened species or sites located within MPAs. It is also strongly recommended that all collected materials (e.g., photographs, completed field protocols, physical samples, and associated metadata) be systematically organized and archived on the same day of sampling to ensure that no information or material is lost or overlooked. In addition, adequate laboratory time for expert taxonomic determination must be factored into project planning.

BOX2 – *Tools and methods for image and video analysis*

Advanced image processing software dedicated to marine biological research integrate a variety of tools and methods that enable accurate extraction of species coverage, abundance, as well as morphometric information from photoquadrats (e.g., Kohler & Gill, 2006; Teixidó et al., 2011; Trygonis & Sini, 2012). Several approaches can be applied to estimate the surface cover of sessile taxa, including grid cell counts, point counts (using random or stratified points overlaid on photoquadrats), delineation of outlines around specific image features (e.g., individual species), and image segmentation (i.e., partitioning an image into multiple segments that share common characteristics such as colour) (Trygonis & Sini, 2012). The selection of the most appropriate method, grid cell size, or number of points depends on the objectives and resolution of the study. In addition to manual analyses, automated or semi-automated image segmentation and annotation techniques – based on deep learning algorithms – can greatly accelerate processing and provide powerful tools for monitoring marine biodiversity (Beijbom et al., 2015; Sierra et al., 2023). However, such approaches require substantial effort and time to train the automated classifiers, and annotations are typically limited to the most common and larger taxa, higher taxonomic levels, morpho-functional groups, or broad substrate categories.

Photographic guides (e.g., Baldacconi & Trainito, 2013; 2016; André et al., 2014; Le Granché et al., 2018; Bay-Nouailhat & Bay-Nouailhat, 2020), **online databases** (e.g., DORIS: https://doris.ffessm.fr/; World Register of marine Cave Species – WoRCS: https://www.marinespecies.org/worcs/), and scientific publications – combined with laboratory examination of samples collected and photographed *in situ* – should be used for the identification of benthic taxa in photoquadrats and video material. However, given the taxonomic constraints associated with identifying benthic taxa to species level based solely on visual data, the use of **biological surrogates** (e.g., growth forms, morpho-functional groups, or other trait-based functional categories such as trophic guilds) is necessary. This surrogate-based approach has been widely applied in studies of Mediterranean marine caves. For example, sponges can be classified into several **morphological groups**, including massive, erect, encrusting, boring, and repent forms (Gerovasileiou & Voultsiadou, 2016; Schiavo et al., 2024). Other studies have categorized benthic taxa according to **growth forms**, considering the ratio between height (h) and radius (r) of sessile organisms as well as their overall body shape (Fig. 5). In this framework, organisms are typically grouped into: prostrate sheets and runners (encrusting two-dimensional forms), flattened mounds ($h < r$), hemispherical mounds ($h \approx r$), domed mounds ($h > r$), and foliaceous or branched erect forms (3D structures) that contribute to higher habitat complexity (Parravicini et al., 2010; Montefalcone et al., 2018, 2023; Sempere-Valverde et al., 2019). Similarly, **trophic guilds** commonly used for benthic taxa are defined according to feeding strategies and include: autotrophs (e.g., macroalgae); passive filter feeders (e.g., cnidarians); active ciliary feeders with lophophores (e.g., bryozoans and brachiopods); active ciliary feeders without lophophores (e.g., serpulid polychaetes); muscular active pumping feeders (e.g., ascidians and bivalves); active pumping sponges; and mixotrophic sponges hosting phototrophic endosymbionts, such as the stony sponge *Petrosia ficiformis* (Nepote et al., 2017; Montefalcone et al., 2018, 2023).

Even when benthic taxa are identified to species or genus levels, several morpho-functional categories or operational taxonomic units (OTUs) are often used for specimens that cannot be reliably identified. These may include encrusting rhodophytes, turf-forming algae, encrusting sponges (often further distinguished by colour), encrusting bryozoans, erect bryozoans, and tube-dwelling polychaetes (e.g., those of the family Serpulidae – although it is often impossible to determine whether the tubes are inhabited or belong to dead specimens). Abiotic substrate categories are also commonly recorded, such as non-living biogenic structures, bare substrate, holes, and sediment (Gerovasileiou et al., 2017; Nepote et al., 2017; Digenis et al., 2022).

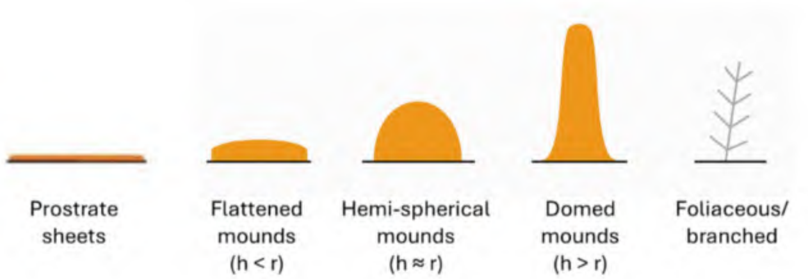


Figure 5. Classification of sessile taxa in marine caves according to the ratio between height (h) and radius (r) and their overall body shape (modified from Sempere-Valverde *et al*., 2019).

I.1.b.2. Characterization of soft substrate communities

The collection of **sediment samples** by SCUBA divers is essential for the characterization of soft-substrate communities inhabiting cave floors[7]. Sediment samples can be collected manually and stored in containers of standard volume, obtained either from the superficial layer (e.g., upper 0–3 cm) or from deeper layers (e.g., down to 10 cm), using hand-held corers (Todaro et al., 2006; Navarro-Barranco et al., 2012, 2013, 2014; Janssen et al., 2013; Ape et al., 2016; Romano et al., 2018, 2023; Bergamin et al., 2020). The required sample volume should be determined according to the objectives of the study.

Sampling should be performed at multiple stations along the cave's horizontal axis, covering the different ecological zones (e.g., entrance, semidark, and dark zones), and replicate samples should be collected at each station to ensure statistical robustness. Post-sampling procedures should follow standard protocols for soft-substrate benthic analyses. Sediment samples are typically washed through sieves of standardized mesh sizes (e.g., 0.5 mm for macrobenthos and 0.1 mm for meiobenthos), stained with rose Bengal to enhance the visibility of animal tissues, and the infauna is subsequently sorted, identified, and counted under stereoscopic (binocular) microscopes.

In addition to the samples used for community composition analyses, additional aliquots should be taken for granulometric (grain-size) analysis and for determining relevant biogeochemical parameters, such as phytopigment concentrations, biochemical composition of organic matter, and total nitrogen content.

---

[7] Cave floors are often covered by fine, silty sediments that can be easily disturbed during sampling, reducing visibility and potentially obscuring the cave entrance. To minimize risks, divers should be appropriately trained and maintain precise buoyancy control, avoid unnecessary fin movements near the substrate, and ensure constant orientation and visual contact with exit references. When visibility may be compromised, the use of calibrated guideline reels securely fixed at the cave entrance is necessary to ensure a safe return path, even for experienced divers.

I.1.b.3. Characterization of motile communities

Visual census methods are widely used to study the structure and composition of motile communities in marine caves. Motile fauna (mainly fishes and decapod crustaceans) is typically recorded using a modified **underwater visual census transect method** while SCUBA diving (Harmelin-Vivien et al., 1985), adapted to the specific conditions of cave habitats (Bussotti et al., 2002, 2006, 2015, 2018; Denitto et al., 2009; Bussotti & Guidetti, 2009). In this approach, the transect width is maintained constant (2–3 m), while the transect length is adjusted (e.g., 5–25 m) according to the cave's shape, size, and morphological discontinuities. Because the area covered by each transect may vary, counts of individuals must be standardized to density values (i.e., number of individuals per surveyed surface). Abundance values should be recorded by the diver on underwater slates, with counts noted per species during each transect. In cases where motile species (e.g., *Apogon imberbis*, *Atherina* spp., *Plesionika narval*) form large schools, their abundance may be recorded in size classes, following a geometric progression scale (e.g., 1, 3–5, 6–10, 31–50, 51–100, 101–200, 201–500, and >500 individuals) (Bussotti et al., 2002, 2003). Transects should be conducted across different sub-habitats (e.g., walls, ceilings, and floor) and the various ecological zones within the cave (e.g., entrance, semidark, and dark zones). The number of transects per cave should be determined based on the cave's extent, internal heterogeneity, and accessibility.

According to the protocol of the only existing ecological index for marine cave communities (CavEBQI), a **visual census covering the entire cave** is required to assess species richness and density (expressed as the number of individuals per 5 minutes per species) of motile taxa, as well as the semi-quantitative abundance of cave-dwelling mysids (classified as absent, few, or swarm). This assessment should be conducted during a 20–30-minute dive by two trained scientific divers (Rastorgueff et al., 2015). A recent modification of this methodology, developed for marine caves of the Alboran Sea, proposed the use of a single diver performing a 20–25-minute dive to record species richness and abundance of selected motile taxa (from a reference list of 66 taxa potentially occurring in caves) and to semi-quantitatively record the presence of mysids only in the semidark and dark zones of the cave, excluding the entrance area (Lanza-Arroyo et al., 2024). For schooling species (e.g., *A. imberbis*), the maximum count was set at 50 individuals to minimize estimation errors associated with larger aggregations.

Recently, Digenis et al. (2025) developed a **rapid visual census protocol** for the assessment of motile faunal communities in marine caves. According to this protocol, a single diving scientist records abundance per species for all observed motile taxa during a **3-minute visual survey transect conducted within each of the three ecological cave zones** (i.e., entrance, semi-dark, and dark). Within each zone, motile organisms are recorded across all available sub-habitats, including walls, crevices, overhangs, the cave floor, and ceiling (when present in fully submerged caves). This protocol is designed for non-decompression, single-dive surveys in caves up to 30 m depth, taking into account the logistical constraints of limited bottom time at such depths (typically 16–20 minutes maximum). The method ensures a safe and efficient exit from the innermost cave zones while maintaining a standardized research effort across marine caves with differing topography (i.e., depth, size, and extent of ecological zones). Overall, the protocol requires a maximum of nine minutes per cave survey (three minutes per ecological zone), with shorter duration (six minutes) applicable in caves lacking a dark zone.

Nevertheless, **visual census protocols in marine caves typically do not allow for a complete assessment of motile cave biota**, particularly in caves with extensive chambers or high structural complexity containing numerous microhabitats (e.g., fissures and overhangs) (Digenis et al., 2025). To address these limitations, **supplementary photographic or video documentation** conducted outside the time-limited transects, combined with **qualitative sampling** (especially for small-sized taxa such as gastropods), can capture a broader diversity of motile species. However, to accurately assess the distribution and abundance of cryptobenthic fishes in marine caves, including those occupying "cave-within-cave" microhabitats, more targeted methodologies may be required, such as the use of anaesthetic agents and quadrat-based sampling (see methodology in Kovačić et al., 2021).

Temporal variability also plays a significant role, with annual, seasonal, and diurnal variations influencing the structure of motile communities in marine caves (Digenis et al., 2025). For example, some species (e.g., *A. imberbis* and mysids) exhibit nychthemeral (diel) migrations, sheltering inside caves during the day and moving outside to feed at night (Bussotti et al., 2002, 2018; Rastorgueff et al., 2015). Therefore, **multiple surveys by experienced divers are often necessary**, even within the same cave, to adequately record highly mobile and cryptobenthic taxa (Kovačić et al., 2024). To ensure consistency and comparability across sites, it is also crucial to standardize the sampling period and time of day during visual censuses. In addition, because the involvement of multiple observers can introduce variability, it is recommended that the same trained diver consistently applies the visual census protocol across all surveyed caves to minimize observer bias (Digenis et al., 2025).

Apart from the larger motile taxa, numerous small motile species (primarily benthic invertebrates) inhabit marine caves as **epifauna** (i.e., living on other organisms such as sponges, hydrozoans, scleractinians, and bryozoans), or as **infauna** (e.g., residing within sponge canals and cavities). To study and characterize these associated communities, the invertebrate hosts should be gently enclosed with a plastic bag or a 0.5 mm mesh nylon net prior to detachment from the substrate, in order to prevent the escape of highly motile macrofaunal organisms (e.g., small crustaceans) (Navarro-Barranco et al., 2014, 2016; Gerovasileiou et al., 2016b). After collection, invertebrate hosts should be carefully dissected into small pieces using a scalpel (e.g., along sponge canals or internal cavities). The associated fauna should then be sorted, identified, and counted according to standard procedures for benthic sample processing. Because invertebrate hosts can vary substantially in shape and size, their volume should be measured using the water displacement method to ensure data standardization and enable comparisons among samples. Any additional organisms recovered from the water used during rinsing or water displacement should also be collected, identified, and processed using the same procedures. To facilitate comparisons among hosts of different sizes, the abundance of each associated taxon should be standardized to the number of individuals per litre of host volume.

I.2. Monitoring marine cave habitats according to the recommendations of the Integrated Monitoring and Assessment Programme (IMAP)

According to IMAP recommendations, an appropriate set of Ecological Objectives (EOs) and Common Indicators (CIs) should be selected for monitoring marine cave habitats (see Appendix 4). Further details on these indicators are provided below:

Ecological Objective 1 (EO1) – Biodiversity

CI1: Habitat distributional range

Within the IMAP framework, CI1 of EO1 considers **habitat extent** as a key attribute. This indicator provides information on the geographic area in which a benthic habitat occurs. The main outputs of monitoring under this indicator are spatial maps illustrating habitat presence and distributional range. Regularly updated and comprehensive maps are essential for effective management and conservation planning, while enabling the detection of significant changes in habitat distribution patterns, the assessment of spatial dynamics over time, and the evaluation of deviations from baseline conditions, potentially resulting from environmental pressures or human impacts.

Marine cave formations occur along rocky coastlines throughout the Mediterranean Sea. According to recent assessments, more than 3,000 marine caves have been documented across the region (Giakoumi et al., 2013; Gerovasileiou & Bianchi, 2021), with the majority located along the northern shores. These areas, characterized by **extensive carbonate coastlines**, have also been subject to more intensive survey efforts. Marine caves are densely concentrated around islands and rocky peninsulas, particularly along the eastern Adriatic, Aegean, Tyrrhenian, Provençal, and Ionian coasts. Despite this broad coverage, the actual number of marine caves is likely underestimated, even in well-studied regions. This underestimation stems from the geomorphological complexity of Mediterranean coastlines and a prevailing survey bias toward shallow, semi-submerged caves that are easier to detect and access. Significant data gaps remain for deeper caves (below approximately 30 m), where research is constrained by the technical challenges of underwater operations, as well as in the southern and eastern Mediterranean, where field surveys, habitat mapping, and data-sharing initiatives remain limited. However, in many of these regions, extensive coastlines are composed of sedimentary shores where caves are absent, or of rocky areas whose geological characteristics may not have favoured cave formation.

As the **processes of speleogenesis** (e.g., bedrock dissolution and mechanical erosion by wave action) **and natural cave degradation are generally very slow**, changes in the habitat distributional range of marine caves are unlikely to occur within typical monitoring timescales. Therefore, any apparent changes are more likely to reflect variations in research effort (e.g., new mapping initiatives) or methodological differences. The principal methods for inventorying and mapping marine caves are described in detail in Section I.1.a ("Locating and characterizing the physical environment of marine caves"), while step-by-step guidelines for marine cave detection and documentation are provided in the Appendix 1.

Consequently, this parameter is expected to remain relatively stable over time, except in rare cases of cave collapse or physical destruction. Conversely, **temporal changes** are more likely to occur in the structure and composition of benthic communities within marine caves. Such changes are better detected and assessed under CI2 (see below). Nonetheless, the lack of quantitative data and the scarcity of long-term monitoring series for marine cave ecosystems across most Mediterranean subregions remain major obstacles to robust temporal assessments.

CI2: Condition of the habitat's typical species and communities

CI2 assesses the **ecological status of benthic habitats**. This indicator focuses on evaluating habitat condition through the analysis of **typical or target species** that serve as ecological indicators, as well as through assessments of **community structure and composition**. By tracking these biological components, the indicator enables the detection of significant changes in habitat condition and supports the assessment of progress toward Good Environmental Status (GES). The establishment of consistent, long-term datasets is essential to identify trajectories of change and to inform adaptive management and conservation planning.

Marine caves exhibit a **high degree of environmental and biological heterogeneity** across multiple spatial scales. As a result, the "typical species" that characterize marine cave communities may vary substantially among ecological zones within a single cave (e.g., entrance, semidark, dark, or intermediate transitional zones) and across different Mediterranean ecoregions (Gerovasileiou & Voultsiadou, 2012; Bussotti et al., 2015; Gerovasileiou & Bianchi, 2021). For instance, species traditionally considered characteristic of marine caves in the western Mediterranean – such as *C.*

rubrum – may be rare or absent in the eastern basin, and vice versa. Furthermore, species composition differs according to substrate type, with distinct assemblages typically developing on hard surfaces (e.g., ceilings and side walls) compared to those occurring on soft sediments covering cave floors (see Section I: "Habitats and species associated with marine caves").

Non-exhaustive lists of **species commonly recorded in Mediterranean marine caves**, along with **typical cave-dwelling species** and **functional (trophic) groups** relevant for monitoring initiatives, are provided in Appendices 5 and 6. These lists are based on recent syntheses and regional inventories of marine cave fauna (Gerovasileiou & Voultsiadou, 2012, 2014; Gerovasileiou et al., 2015; Rastorgueff et al., 2015; Mačić et al., 2018a; Öztürk *et al.*, 2019; Gerovasileiou & Bianchi, 2021; Bianchi et al., 2022; Kovačić et al., 2024; Lanza-Arroyo et al., 2024; Mačić et al., 2024; Digenis et al., 2025) and consultation with experts. In addition to recording the typical species present in marine caves, it is recommended to document the occurrence of **threatened and protected species**. Particular attention should be given to taxa listed under international conservation frameworks, including the IUCN Red List of Threatened Species, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the Annexes of the Bern Convention, and the SPA/BD Protocol of the Barcelona Convention. Recording the presence and/or abundance of these species will facilitate the monitoring of their conservation status.

At present, the limited availability of **quantitative data and long-term time-series** from marine caves (and their typical species) across most Mediterranean sub-regions constitutes a major obstacle to assessing changes in their ecological condition. Despite these data gaps, an increasing number of studies have reported ecological alterations in marine cave communities resulting from climate change (e.g., necrosis associated with marine heatwaves; Fig. 6a), local anthropogenic disturbances (e.g., coastal construction, tourist boat visits, and unregulated SCUBA diving; Fig. 6b), pollution (e.g., marine litter; Fig. 6c–d), illegal coral harvesting and spearfishing, and biological invasions, suggesting a gradual and often unrecognized decline in habitat quality (Parravicini et al., 2010; Gerovasileiou et al., 2016c, 2022; Nepote et al., 2017; Costa et al., 2018; Mačić et al., 2018b; Montefalcone et al., 2018, Rosso et al., 2018; Montefalcone et al., 2023; Gerovasileiou & Bianchi, 2021; Digenis et al., 2022; Mačić et al., 2024; Quiles-Pons et al., 2025; Savva et al., 2026). Detailed information about the major drivers of alterations identified in Mediterranean marine caves is described in Appendix 7.

Evaluating and monitoring **threats and pressures** is essential for assessing their potential **impact** on marine cave habitats and communities (Navarro-Barranco et al., 2023b). Detailed species identification is not always necessary to detect ecological changes; a range of **visual indicators** can be used to assess the condition of sessile assemblages. Such indicators include:

- **Tissue necrosis:** The percentage of partial or total tissue necrosis (Fig. 6a), estimated visually or from photoquadrats, can be used to quantify the impact and intensity of mortality events associated with marine heatwaves and temperature anomalies.
- **Changes in community structure:** Variations in the relative surface cover of morphological and functional descriptors, such as growth forms and trophic groups, can indicate community-level changes. Examples include the loss of specific growth forms (e.g., massive or erect invertebrates) or trophic groups (e.g., active suspension feeders with or without lophophores, such as bryozoans and polychaetes, respectively), coupled with an increase in two-dimensional growth forms (e.g., encrusting sponges), turf-forming species, or sediment accumulation. Such changes are often linked to temperature anomalies and/or local anthropogenic disturbances (Parravicini et al., 2010; Montefalcone et al., 2018, 2023).
- **Mechanical damage:** Visible signs of mechanical damage to benthic organisms can signal negative impacts from human activities. For example, the disappearance of fragile erect taxa (e.g., bryozoans of the genera *Adeonella*, *Smittina*, and *Reteporella*) from cave ceilings and

walls, along with the accumulation of broken fragments on cave floors, or the presence of necrotic substrate patches on ceilings caused by exhaled air bubbles (Fig. 6b), can indicate unregulated diving impacts. Marine litter is also frequently observed entangled in sessile cave fauna, with organisms growing over and partially covering it (Fig. 6c). If not handled properly, the removal of such litter can itself cause additional mechanical damage.

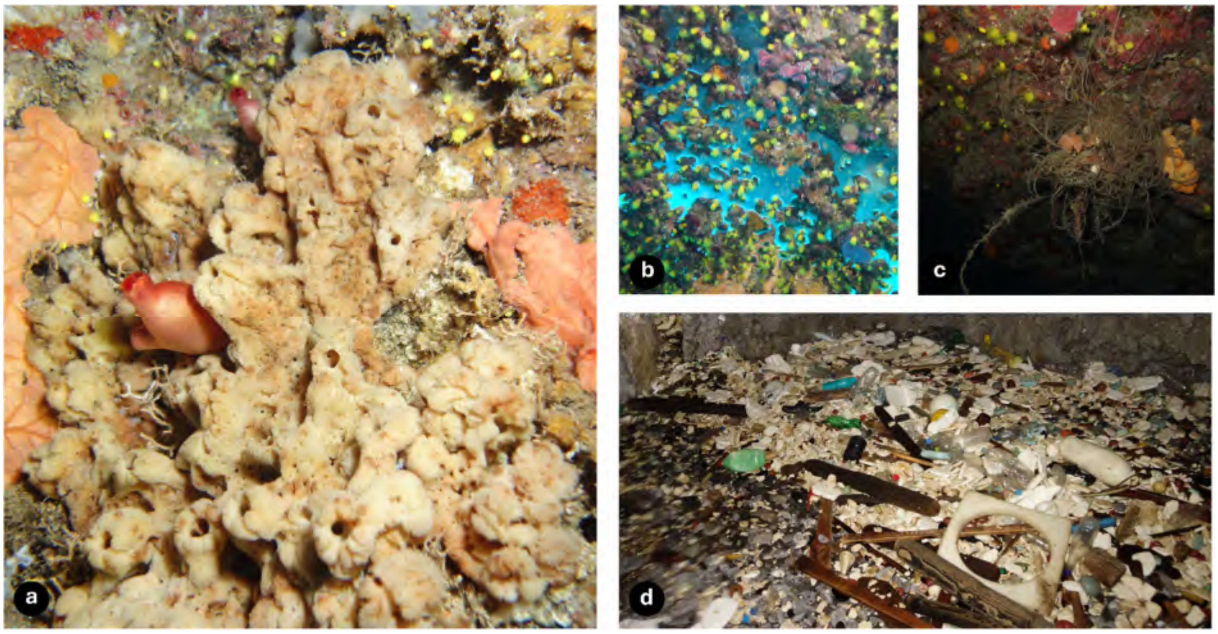


Figure 6. (a) Necrosed massive sponge *Agelas oroides* at the entrance of a marine cave; (b) exhaled air bubbles from SCUBA divers accumulating on a cave ceiling, affecting the scleractinian *Leptopsammia pruvoti*; (c) fishing line entangled on sessile benthic biota on the ceiling of a submerged cave; (d) litter accumulated on the internal beach of a semi-submerged marine cave. Photos by Vasilis Gerovasileiou.

Quantification of the percent coverage of taxonomic, morphological, and functional descriptors should be conducted through photoquadrat analysis (see Box 2), whereas motile fauna should be assessed using visual censuses (see Section "I.1.b.3. Characterization of motile communities"). When quantitative analysis is not feasible due to logistical constraints, visual estimations of surface cover (e.g., 0–25%, 25–50%, 50–75%, or >75%) by morpho-functional or trophic groups, and/or semi-quantitative assessments (e.g., present, rare, common, abundant) obtained through visual censuses by trained divers, can still provide valuable information on community structure and ecological condition. Nevertheless, high-quality photoquadrats are preferred, both to minimize underwater time and, importantly, to facilitate subsequent species identification and data sharing.

Underwater photogrammetry is also a powerful tool for monitoring sessile communities within marine caves, as it allows the detection and quantification of structural changes over time. By generating high-resolution, 3D spatial models, this approach enables precise assessments of species cover, growth dynamics, and habitat complexity (Quiles-Pons et al., 2022; Pulido Mantas et al., 2023).

In marine caves frequented by SCUBA divers and boat visitors, recording the number of divers and the frequency of boat visits provides valuable data for evaluating pressure–impact relationships (Quiles-Pons et al., 2025). Such information can support the establishment of management thresholds aimed at ensuring sustainable use and effective protection of marine cave environments.

Before undertaking any baseline survey or monitoring activity, diving scientists should prepare **annotated slates or data sheets** listing the attributes to be recorded underwater (see guidelines in

Appendix 2). These should include identifiable species or higher taxa, characteristic morpho-functional groups, and any visible signs of environmental pressures or disturbances (e.g., necrosis, trapped air bubbles, broken bryozoans, or litter). To ensure standardized data collection and promote comparability across studies and monitoring programmes, a field recording data sheet has been developed and is provided in Appendix 8. This form serves as a template for documenting:

- Basic topographic and morphological features of the surveyed cave;
- Characteristic taxa representing different functional components of the ecosystem;
- Protected and threatened species; and
- Observed pressures and threats affecting the cave environment.

Assessing the ecological status of marine cave ecosystems

An ecosystem-based index, the **Cave Ecosystem-Based Quality Index (CavEBQI)**, was developed to assess the ecological quality status of marine cave ecosystems in the northwestern Mediterranean (Rastorgueff et al., 2015). The index has since been applied and refined in other Mediterranean regions, including the Aegean Sea (Digenis et al., 2022) and the Alboran Sea (Lanza-Arroyo et al., 2024), demonstrating its adaptability to different regional contexts, although specific thresholds and methodological parameters may vary among regions. The CavEBQI integrates multiple ecological components to provide an evaluation of cave ecosystem health status, including the:

- **Percent coverage of key functional groups of sessile fauna:** Passive Filter Feeders (e.g., hydrozoans and anthozoans), Small Active Filter Feeders (e.g., encrusting sponges, serpulids, small bryozoans, and brachiopods), and Large Active Filter Feeders (e.g., massive sponges, bivalves, large bryozoans, and ascidians);
- **Vertical stratification of the sessile community**, serving as an indicator of habitat condition and food availability for erect growth forms;
- **Presence and abundance of cave-dwelling mysids** (categorized as absent, few, or swarm); and
- **Species richness and abundance of motile fauna**, grouped into three trophic categories: Detritus Feeders and Omnivores, Characteristic Cave Carnivores, and Associate Carnivores.

Each component is evaluated on a scale from 0 to 4, where 4 indicates the highest ecological quality. The methodological reliability of each assessment is evaluated using a confidence index, also ranging from 0 to 4, with 4 representing the most robust and recommended methodological approach. The final ecological status and confidence index for each cave are derived as weighted averages of all assessed components. A detailed description of the original methodology, along with examples of representative species within each category, is provided in the original publication by Rastorgueff et al. (2015). Regional considerations, adaptations of threshold values, and methodological modifications have subsequently been reported by Digenis et al. (2022) and Lanza-Arroyo et al. (2024).

According to the abovementioned theoretical model, high ecological quality status is typically indicated by a high spatial coverage of suspension feeders with 3D growth forms (e.g., red coral) and large active filter feeders (e.g., massive sponges), in combination with the presence of large mysid swarms and a diverse and abundant assemblage of omnivorous and carnivorous fishes and decapod crustaceans. However, it is important to note that several shallower or semi-submerged caves, particularly those located in more oligotrophic regions (e.g., eastern Mediterranean and insular archipelagos), may naturally exhibit lower surface cover of sessile invertebrates, reduced vertical stratification (e.g., absence of large passive suspension feeders such as gorgonians), and smaller populations of motile taxa compared to deeper caves or those in the northwestern Mediterranean. These differences may be the result of **natural environmental constraints** – such as stronger hydrodynamic regimes caused by wave action – or reflect the **oligotrophic and biogeographically distinct conditions** of different sub-regions, rather than a decline in ecological quality (Digenis et al., 2022). On the other hand, in such cases, interpretation of low or moderate ecological status should be approached with caution, as shallower marine caves are more exposed to sea surface temperature change, anthropogenic pressures (e.g., tourism), and the establishment of non-indigenous species (Digenis et al., 2022; Gerovasileiou et al., 2022).

Data collection to assess the ecological status of marine cave ecosystems typically requires **multiple surveys** conducted by experienced scientific divers and/or coordinated diving teams with clearly defined roles. This ensures the collection of data for both sessile and motile communities and helps capture potential temporal variability in motile fauna (see Section "I.1.b.3. Characterization of motile communities").

Monitoring soft substrate communities in marine caves

Although a variety of **ecological indices** have been developed to assess the status of soft-substrate benthic communities, their application within marine cave environments remains limited. Navarro-Barranco et al. (2014) applied the AMBI (AZTI Marine Biotic Index; Borja et al., 2000) and BENTIX (Simboura and Zenetos, 2002) indices to evaluate soft-substrate assemblages in marine caves along the Mediterranean coast of southern Spain. In this study, amphipod species were categorized according to their **tolerance to environmental stressors**, classified as sensitive, indifferent, or tolerant species for AMBI, and sensitive or tolerant species for BENTIX. The results indicated a high abundance of sensitive species in both internal and external sampling stations (each one approximately 10 m from the cave entrance), suggesting that the studied soft-bottom habitats were of good ecological quality. However, the applicability and reliability of these biotic indices in cave environments remain uncertain. Future research should aim to test these indices across caves exhibiting a wider gradient of anthropogenic or natural stress, which would help refine species tolerance classifications and improve the accuracy of ecological status assessments.

Benthic Foraminifera can also serve as **valuable proxies for (palaeo)ecological characterization** of marine cave habitats, as their assemblages respond sensitively to a range of environmental conditions and reflect fine-scale habitat partitioning (Romano et al., 2022). Studies conducted in Italy and Spain have shown that yearly monitoring of benthic Foraminifera can provide insights into seasonal patterns and short-term climatic variability (Romano et al., 2018, 2022, 2023; Bergamin et al., 2020). For a comprehensive overview of the ecological significance and methodological applications of foraminiferans in marine cave research, see the review by Romano et al. (2022).

The study of **thanatocoenoses on sedimentary cave floors** (i.e., assemblages composed of skeletal remains, calcareous shells, and tubes of sessile invertebrates) can yield valuable information on past environmental conditions and long-term ecological changes within marine caves (Monteiro-Marques, 1981; Rosso et al., 2013; Pino et al., 2020; Di Franco et al., 2021). These assemblages can serve as complementary evidence for reconstructing historical community composition or identifying past disturbance events. However, for monitoring and ecological status assessments, only living communities should be considered. The inclusion of dead remains can bias interpretations of current ecological conditions and may not accurately reflect present-day environmental quality. Thanatocoenoses, therefore, are best used as a palaeoecological or supplementary tool, rather than as a primary indicator in contemporary monitoring frameworks.

Periodicity of monitoring of marine cave ecosystems

Defining the **periodicity** of monitoring for marine caves remains challenging, as the temporal variability of their communities, even at seasonal scales, has been rarely investigated and appears to exhibit inconsistent patterns among sites (e.g., Martí et al., 2004b; Bussotti et al., 2006). In contrast, long-term datasets have revealed clear patterns of change over decadal timescales (Parravicini et al., 2010; Montefalcone et al., 2018, 2023; Costa et al., 2018; Sempere-Valverde et al., 2019). Colonization and community succession processes in marine caves generally occur over decades rather than years, particularly in the semidark and dark cave sectors, where environmental conditions are more stable and recruitment is limited (Harmelin, 1980; Denitto et al., 2007). Therefore, according to Rastorgueff et al. (2015), periods exceeding ten years can be regarded as representative of the "past" for marine cave communities, whereas intervals shorter than ten years reflect more "recent" conditions.

Given the accelerating environmental changes affecting the Mediterranean – driven by climate change, anthropogenic pressures, and biological invasions – it is recommended that monitoring schemes include **repeated investigations in marine caves approximately twice per decade (every 5–6 years)**. This frequency aligns with the reporting cycles of IMAP and the European Union's Marine Strategy Framework Directive (MSFD), ensuring consistency and comparability across regional and international monitoring frameworks. Nevertheless, shorter monitoring intervals may be necessary for certain taxa or communities, depending on their life-history traits, ecological sensitivity, or exposure to site-specific pressures. Such adaptive scheduling will enhance the effectiveness and responsiveness of monitoring programs in detecting ecological change and supporting timely conservation actions.

Ecological Objective 2 (EO2) – Non-Indigenous Species

CI6: Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species

Non-indigenous species (NIS) – also referred to as alien, exotic, non-native, or allochthonous species – are species, subspecies, or lower taxa introduced, intentionally or unintentionally, outside their natural range (past or present) and beyond their natural dispersal potential. Invasive alien species (IAS) constitute a subset of NIS that have established self-sustaining populations, are spreading or have demonstrated potential to spread, and exert measurable impacts on biodiversity, ecosystem functioning, socio-economic values, and/or human health. Such impacts typically result from competition with, and sometimes replacement of, native species.

In the Mediterranean Sea, NIS monitoring is conducted primarily as trend monitoring, aiming to establish reliable, long-term datasets as a foundation for detecting temporal and spatial changes. Following a risk-based approach, monitoring efforts should focus on IAS and their major introduction hotspots, such as ports, marinas, and aquaculture installations. In addition, areas of special ecological importance, including MPAs and particular habitats such as marine caves, should be selected for monitoring on a case-by-case basis, especially when located near introduction hotspots.

Recent studies have documented a **growing number of NIS, particularly in marine caves of the eastern and southern Mediterranean** (Gerovasileiou et al., 2016c, 2022; Digenis et al., 2022, 2025). As of December 2022, a total of 126 introduced species belonging to 12 phyla have been reported from Mediterranean marine caves, including 107 NIS, 15 cryptogenic species (of uncertain native or introduced origin), two crypto-expanding species (with unclear dispersal mode), and two species with questionable status (Gerovasileiou et al., 2022). This represents an increase of approximately 225%

over the previous checklist within eight years, although part of this rise may reflect increased research effort.

Most introduced species have been recorded in the entrance (60 species) and semidark (52 species) zones of marine caves, while only 19 species have been reported from the dark zone. These distributions suggest that most NIS colonize caves from adjacent habitats unaided, although secondary vectors such as tourist boats visiting semi-submerged caves cannot be excluded. In addition, 14 NIS molluscs have been reported as empty shells (thanatocoenoses) in cave sediments, but their living presence within the caves cannot be confirmed (their shells may have been transported by currents or benthic fauna such as hermit crabs or predators) (Di Franco et al., 2021; Gerovasileiou et al., 2022).

The majority of NIS found in Mediterranean marine caves (approximately 61%) have entered the basin through the Suez Canal as Lessepsian migrants (pathway: Corridors), while 35% are associated with vessel-mediated transfer (pathway: Transport–Stowaway). In addition, 35 of these species are classified as high-impact based on European Alien Species Information Network (EASIN) data and nine of the ten most invasive species in the Mediterranean Sea – as ranked by Tsirintanis et al. (2022) according to their biodiversity impact scores – have been recorded in marine caves. Six of these species have been found only at cave entrances (five are macroalgae), two only in semidark zones (*Brachidontes pharaonis* and *Spondylus spinosus*), while *Siganus rivulatus* has been found in both zones.

In recent years, three highly invasive Indo-Pacific fish species have become increasingly common – and often abundant – in marine caves across the southeastern Mediterranean Sea, representing a potential ecological threat that requires further investigation and monitoring. These are the lionfish *Pterois miles* (Fig. 7a), the sweeper fish *Pempheris rhomboidea* (Fig. 7b), and the red squirrelfish *Sargocentron rubrum* (Fig. 7c). *Pterois miles* is an opportunistic predator capable of altering food webs by preying on a variety of fish and invertebrates and competing with native consumers (Tsirintanis et al., 2022 and references therein). *Pempheris rhomboidea* and *S. rubrum*, as well as *P. miles*, shelter inside caves and seabed crevices during the daytime and move out at night to feed. These diel movements, particularly in large schools of *P. rhomboidea*, may influence the oligotrophic cave ecosystem by increasing the flow of organic matter from external environments, in a manner similar to that of the native cardinalfish *Apogon imberbis* (Gerovasileiou et al., 2016c; 2022; Bussotti et al., 2018). Visual surveys in marine caves of the South Aegean Sea (Greece) confirmed these three species as the most frequently encountered NIS, with *P. rhomboidea* comprising approximately 53% of total fish abundance in the southeasternmost studied cave (Digenis et al., 2025).

Currently, there is no quantitative data on the ecological impacts of IAS within marine caves (Nicolosi & Gerovasileiou, 2024). The lack of historical data series on the past ecological state of most Mediterranean marine caves, particularly in the understudied eastern basin, hinders assessments of long-term change (Gerovasileiou et al., 2016c). Nevertheless, the high diversity of NIS and the population explosion of some non-native fishes in the eastern Mediterranean highlight the urgent need for systematic and continuous inventorying and monitoring.

Marine caves should therefore be integrated into national and regional monitoring networks to assess their current ecological status (see EO1) and establish baselines for future comparisons. Monitoring should aim to identify NIS, using standardized methods. **NIS can be visually identified and quantified through *in situ* baseline and monitoring surveys, using visual census techniques and photoquadrat analyses (for motile and sessile taxa, respectively)**. The **total number of NIS (and IAS)**, as well as the abundance **ratio between non-native and native species** (especially for fishes), represent useful metrics for evaluating this indicator (Digenis et al., 2025). A non-exhaustive list of motile and sessile introduced species to be recorded during monitoring initiatives in Mediterranean marine caves (mainly in the Eastern and Central Mediterranean basins) is provided in Appendix 9.

The adoption of **environmental DNA (eDNA) approaches**, which has rarely been implemented in cave ecosystems (Saccò et al., 2024), should also be considered as an early warning tool, particularly for inconspicuous or cryptic taxa. Furthermore, the development of experimental approaches and comparative analyses between invaded and non-invaded caves (Digenis et al., 2025) are recommended to assess the potential effects of NIS on marine cave ecosystems and their unique biodiversity.

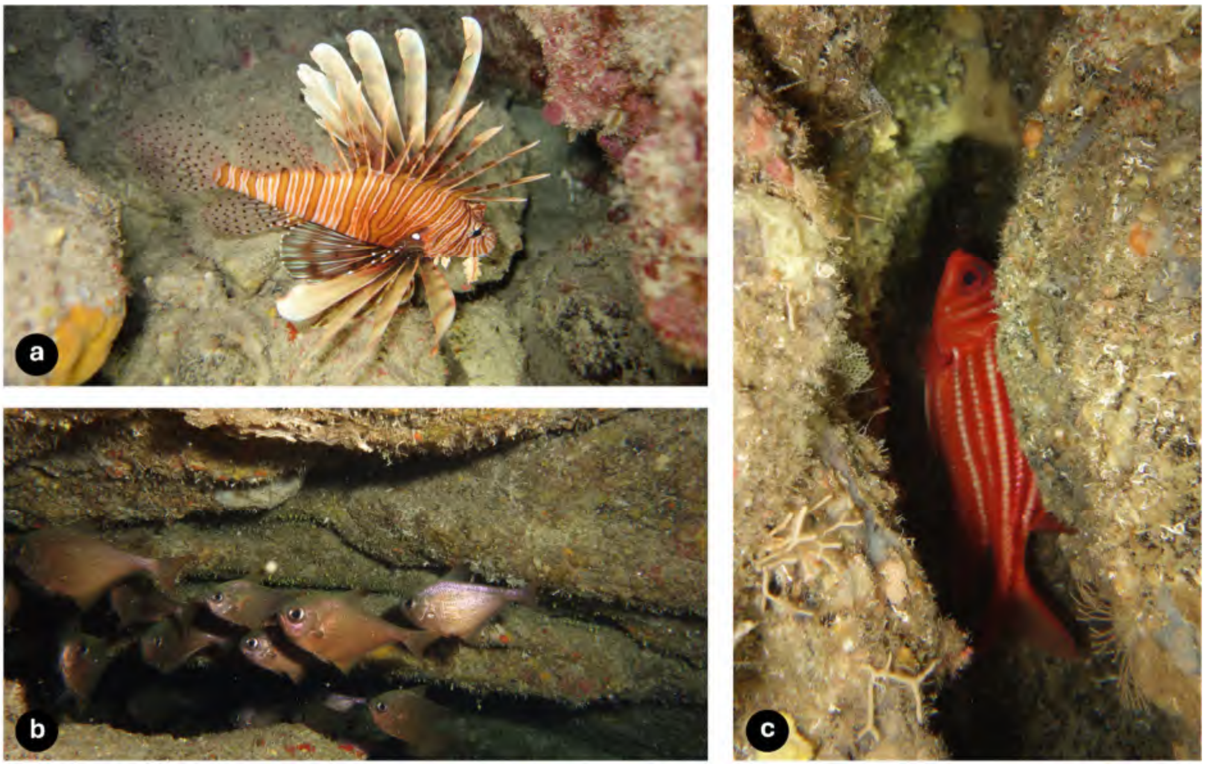


Figure 7. (a) The lionfish *Pterois miles*; (b) the sweeper fish *Pempheris rhomboidea*; and (c) the red squirrelfish *Sargocentron rubrum* in the semidark zone of Eastern Mediterranean marine caves. Photos by Vasilis Gerovasileiou.

Other relevant IMAP Ecological Objectives and Common Indicators

In addition to indicators of the Ecological Objectives 1 and 2, several other IMAP indicators may be considered on a supplementary basis for marine cave habitats, particularly in areas assessed as high-risk or where interactions between multiple ecological objectives are expected. The following IMAP EOs and CIs may be relevant, depending on site-specific conditions and monitoring objectives:

- **EO4 – Marine food webs:**
  The development of CIs for EO4 is currently in progress.
  The objective is to ensure that human-induced changes to marine food webs do not result in long-term negative impacts.

- **EO5 – Eutrophication:**
  CI-13: Concentration of key nutrients in the water column.
  These indicators can help detect nutrient enrichment and potential eutrophication processes that may influence sensitive cave ecosystems.

- **EO6 – Sea-floor integrity:**
  Proposed CI-37. Extent of physical loss of natural habitat.
  Proposed CI-38. Extent of adverse effects on benthic habitat.
  EO6 is intended to have a broad scope, covering all seabed habitats across the Mediterranean from the littoral zone to the deep-sea floor (see UNEP/MED WG.630/4).

- **EO7 – Hydrography:**
  CI-15: Location and extent of habitats directly impacted by hydrographic alterations.
  Changes in hydrodynamics, such as reduced water exchange, altered circulation and modifications in freshwater inflow can significantly affect marine cave biota and habitat stability.

- **EO8 – Coastal Ecosystems and Landscapes:**
  CI-16: Length of coastline subject to physical disturbance due to the influence of man-made structures.
  This indicator supports the assessment of coastal modifications (e.g., coastal construction, harbour expansion, and beach nourishment) that may alter the environmental conditions of marine caves.

- **EO9 – Pollution:**
  CI-19: Occurrence, origin (where possible), and extent of acute pollution events (e.g., oil slicks or discharges of hazardous substances), and their impacts on affected biota.
  Monitoring of pollution events (e.g., oil slicks) is critical for evaluating potential contamination pathways and assessing cumulative impacts on sensitive marine cave ecosystems.

- **EO10 Marine litter:**
  CI-22: Trends in the amount of litter washed ashore and/or deposited on coastlines (including analysis of its composition, spatial distribution and, where possible, source).
  CI-23: Trends in the amount of litter in the water column including microplastics and on the seafloor.
  Marine litter within marine caves (Fig. 6c–d), when present – particularly in semi-submerged caves, or in dark zones with limited water circulation (e.g., ceilings of submerged caves) – should be systematically recorded, quantified, and categorized according to:
  - **Material type:** Artificial polymer materials / plastics, paper and cardboard, wood (processed or not), metal, glass and ceramics, cloth / textiles, rubber, and miscellaneous / other.
  - **Probable source or usage**: tourism and recreational activities, fisheries and aquaculture, shipping, sanitary and sewage-related sources, and illegal dumping (fly-tipping).

  This classification enhances the identification of pollution sources and supports the assessment of the extent and impact of marine litter on marine cave habitats and associated biota (Savva et al., 2026). The resulting data can inform targeted management actions, mitigation strategies, and public awareness initiatives aimed at reducing litter input and accumulation in these sensitive coastal ecosystems.

II. HABITATS AND SPECIES ASSOCIATED WITH SEAMOUNTS, CANYONS, APHOTIC HARD (AND SOFT) BEDS AND CHEMOSYNTHETIC PHENOMENA (DEEP-SEA HABITATS)

Dark deep-sea habitats are those in which no sunlight arrives, or where the light that does reach the seafloor is insufficient to support plant or algal communities. These aphytal habitats, typically located below 150–200 m (although they may begin as shallow as 100 m), include the *Offshore circalittoral*, *Upper* and *Lower bathyal*, and *Abyssal* depth zones (see Table 3) (Montefalcone et al., 2021).

For the purposes of this document, "deep-sea habitats" refer exclusively to aphytal deep habitats, thereby excluding mesophotic zones and rhodolith/maerl beds. However, certain geoformations that typically host dark habitats may extend into the photic zone due to their wide bathymetric range. For example, the summits of some seamounts may rise to depths shallow enough to support phytal communities. In such cases, and in line with the need to maintain ecological integrity, the entire habitat is considered within the classification of dark habitats (UNEP/MED WG.502/Inf.4, 2021).

The diversity of deep-sea habitats reflects the heterogeneity of underwater geoformations and the range of environmental conditions shaped by substrate type, slope, prevailing currents, sedimentation rates, and the availability of organic matter in the overlying water column. Offshore rocks and reefs arising from the continental shelf, canyon heads and flanks, seamounts, aphotic hard and soft bottoms, and cold seeps all provide distinct environmental settings that support specific assemblages.

Offshore circalittoral formations (rocks and reefs) may occur at the outer margins of large continental shelves and lie entirely within aphotic conditions. These structures typically host dense assemblages of anthozoans and sponges, surrounded by soft-bottom areas that support few or no erect species. Such formations act as oases for sessile hard-substrate communities, creating structurally complex habitats that attract a variety of motile fauna. Examples include fishes such as *Conger conger*, *Capros aper*, *Scorpaena elongata*, and *Macroramphosus scolopax*, as well as crustaceans (e.g., *Palinurus mauritanicus*), cephalopods, and echinoderms such as *Cidaris cidaris*, which feed on the associated sponge and anthozoan fauna.

Soft-bottom substrates on the **outer continental shelf and upper continental slope** within the upper bathyal zone can host dense populations of *Leptometra phalangium*, forming a characteristic facies that supports juveniles of several commercially important species (e.g., *Parapenaeus longirostris*, *Eledone cirrhosa*, *Micromesistius poutassou*, *Trisopterus capelanus*) (Colloca et al., 2004; Millot et al., 2024). Detrital and sandy bottoms at the **shelf edge** may also sustain populations of *Dendrophyllia* spp. and their associated fauna. In the Western Mediterranean, *D. cornigera* (Fig. 8) may occur as loose colonies in association with anthozoans, sponges, and various vagile species (e.g., cephalopods, *Scorpaena elongata*, *Conger conger*) (Fourt et al., 2017; Enrichetti et al., 2023). In contrast, in parts of the Eastern Mediterranean, the dominant species is often *D. ramea* (Orejas et al., 2019).

The origin of **submarine canyons** is diverse, and their morphology and substrate composition can vary substantially both between canyons and within individual canyon systems. This geomorphological variability (Harris & Whiteway, 2011; Würtz, 2012), together with heterogeneous current regimes, creates a wide range of environmental contexts that support diverse sessile assemblages. Within the bathyal zone, cnidarians represent some of the most important habitat-structuring taxa. Key groups include octocorals [e.g., *Callogorgia verticillata* (Fig. 9) and *Viminella flagellum*], antipatharians [e.g., *Antipathes dichotoma*, *Parantipathes larix* (Fig. 10) and *Leiopathes glaberrima* (Fig. 11)], and scleractinians, such as the white corals *Madrepora oculata* (Fig. 12) and *Desmophyllum pertusum*, which often occur in association with sponges. These assemblages support a diverse associated fauna, including several species of economic importance (e.g., *Conger conger*, *Lophius* spp., *Merluccius merluccius*, *Phycis blennoides*, *Helicolenus dactylopterus*, *Scorpaena* spp., *Pagellus* spp., and *Polyprion americanus*).

On **soft-bottom substrates**, erect anthozoans such as *Isidella elongata* (Fig. 13) and *Funiculina quadrangularis*, as well as sponges such as *Thenea muricata*, can form three-dimensional facies that rise above the sediment surface and enhance local habitat complexity. These assemblages support a range of associated species, including several of commercial relevance (e.g., *Galeus melastomus*, *Merluccius merluccius*, *Nephrops norvegicus*, *Palinurus mauritanicus*, *Parapenaeus longirostris*, and *Phycis blennoides*). Some benthic facies on soft bottoms occur across multiple sediment types. While certain species may be more abundant in specific substrates (e.g., detritic, sand, mud), many are not strictly restricted to a single sediment type and can be found across different soft-bottom habitats. For instance, Pennatulacea are commonly observed not only on mud but also on other soft sediments.

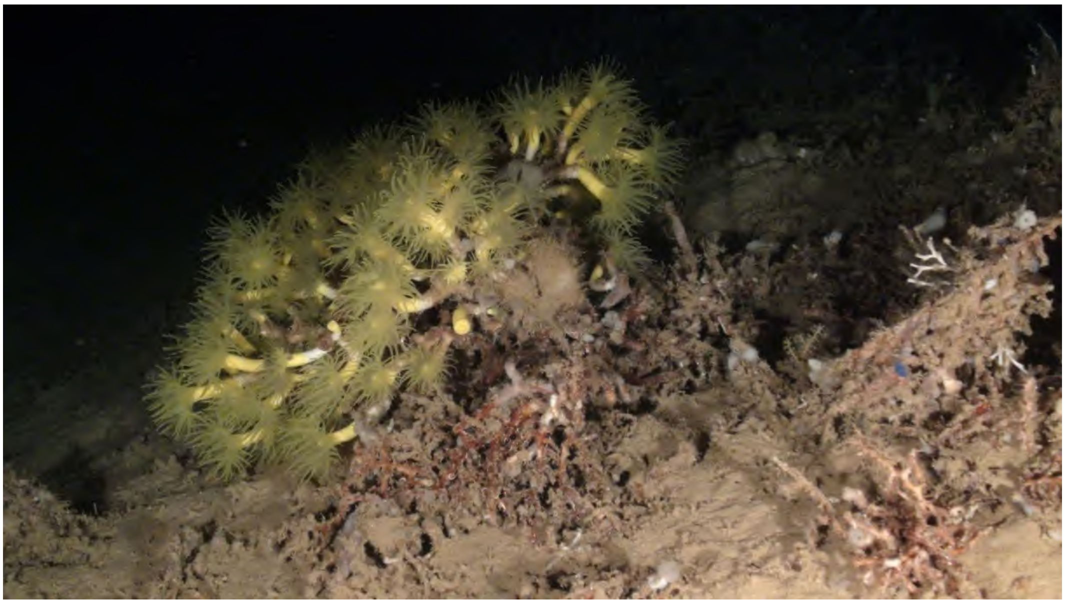


Figure 8. The bright yellow hard coral *Dendrophyllia cornigera* at Catifas Bank. ROV photo by © OCEANA.

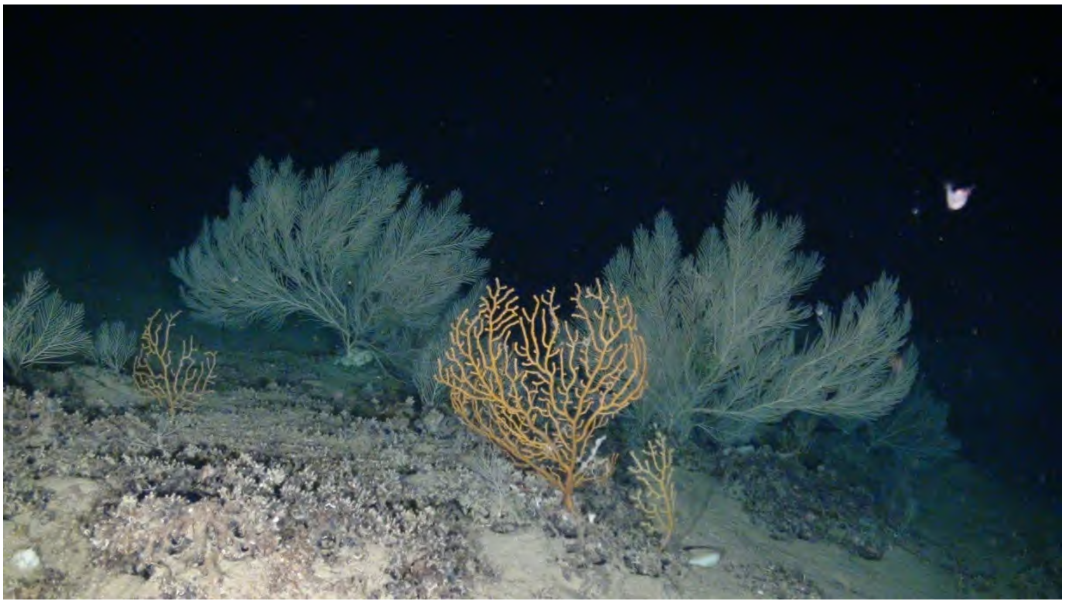


Figure 9. Fan-shaped gorgonians *Callogorgia verticillata* and *Placogorgia* sp. at Ses Olives Seamount. ROV photo by © OCEANA.

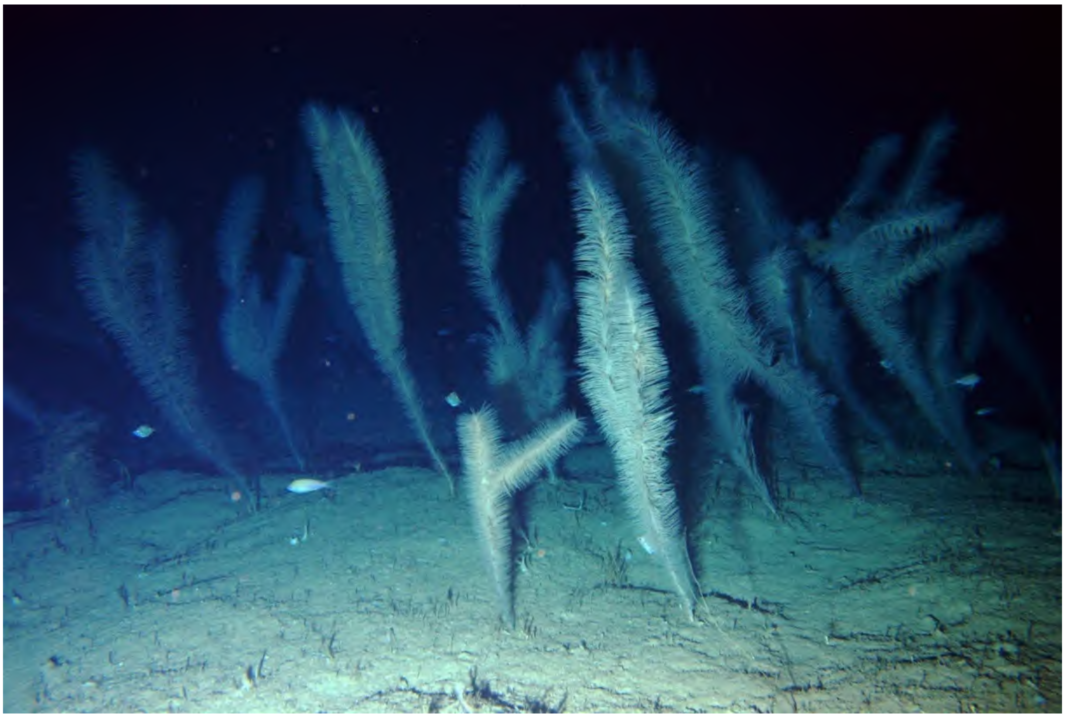


Figure 10. Black corals *Parantipathes larix* at Montecristo Island (Tuscan Archipelago, Italy). ROV photo by © ISPRA.

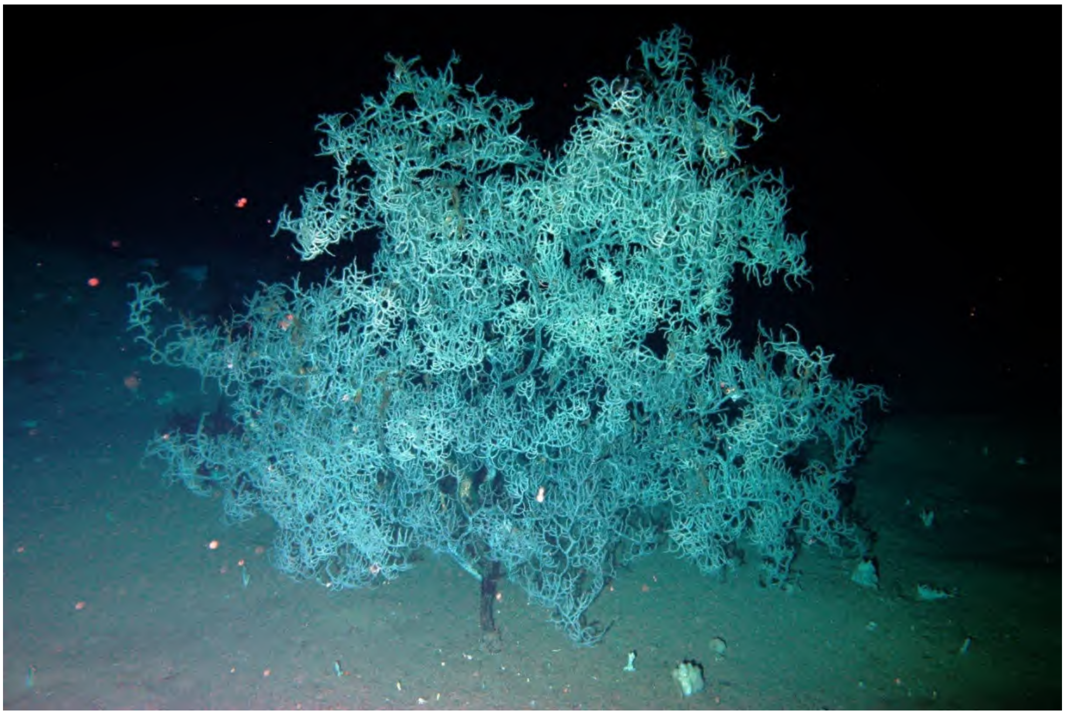


Figure 11. Black coral *Leiopathes glaberrima* at SW Sardinia, Italy. ROV photo by Michela Angiolillo. © ISPRA.

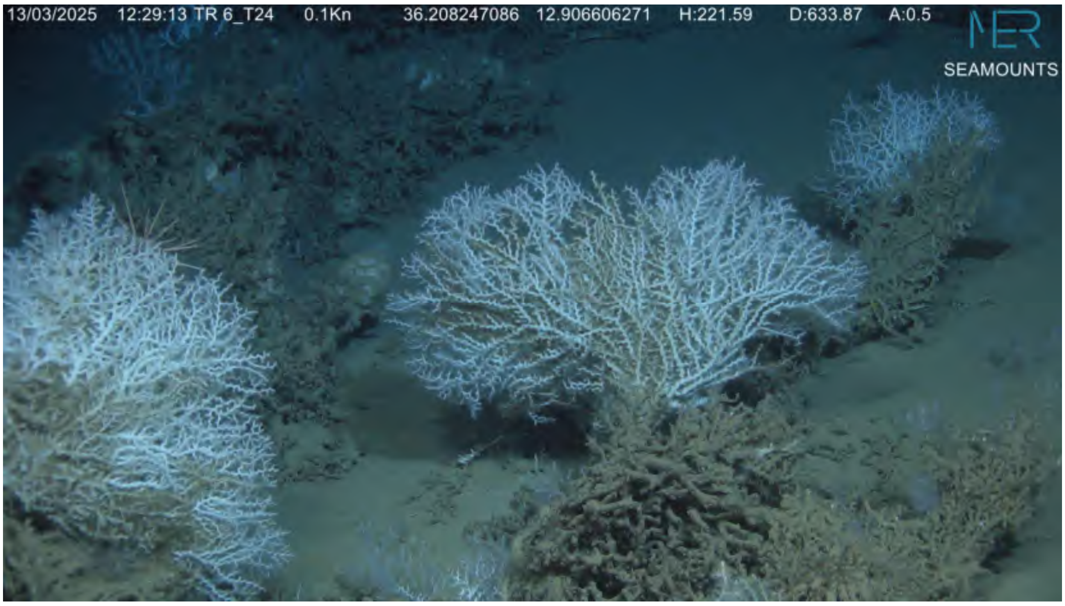


Figure 12. Colonies of the white coral *Madrepora oculata* at Linosa III seamount (Sicily Channel, Italy). ROV photo by Michela Angiolillo. © NextGenerationEU, MASE, ISPRA – PNRR MER – Marine Ecosystem Restoration project – Seamounts (A13-A15).

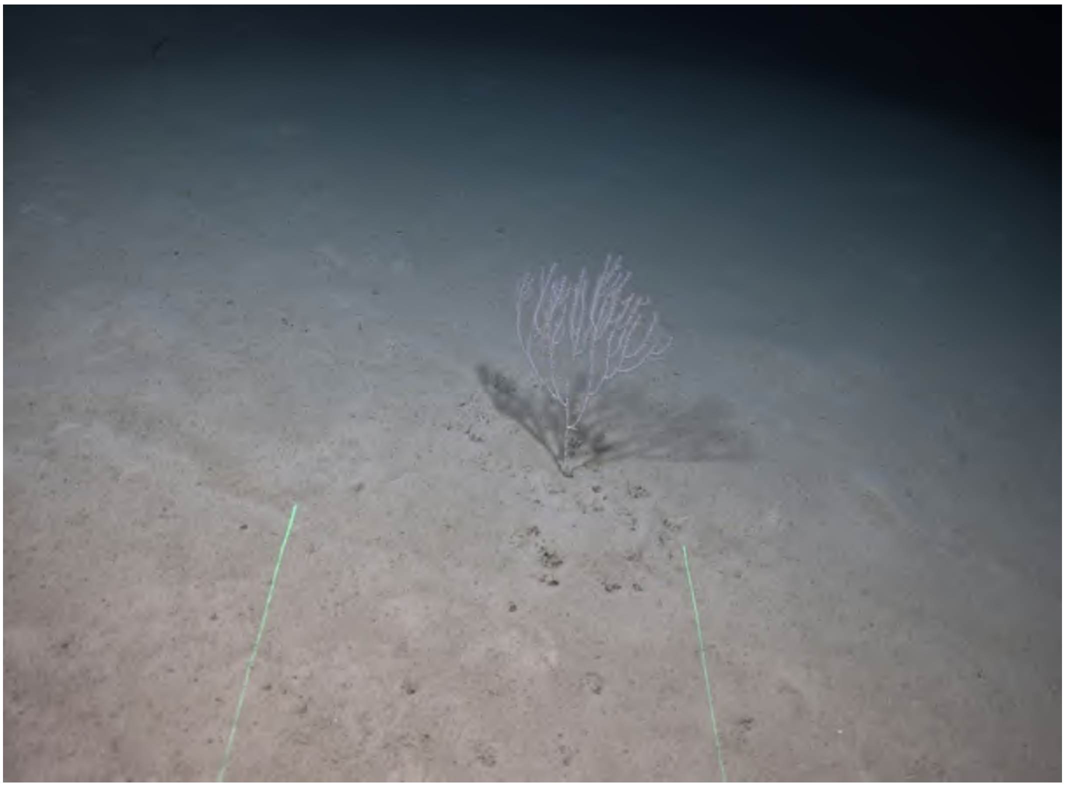


Figure 13. Bamboo corals at Pantelleria central bank (Sicily Channel, Italy). Parallel points of scale lasers are spaced 60 cm apart. ROV photo by Michela Angiolillo. © NextGenerationEU, MASE, ISPRA – PNRR MER – Marine Ecosystem Restoration project – Seamounts (A13-A15).

Although **deep-sea soft-bottom habitats** are not explicitly referenced in the title of the DHAP, they are considered here as an integral component of dark habitats. This inclusion is justified by the vulnerability of soft-substrate deep-sea facies, – such as *Isidella elongata* (Fig. 13), *Thenea muricata*, and *Leptometra phalangium* (Fig. 14) – to disturbance and degradation.

Figure 14. Facies of *Leptometra phalangium* at Algarrobo Bank. ROV photo by © OCEANA.

Seamounts are particularly abundant in the Alboran and Tyrrhenian Seas, but they are present throughout the Mediterranean (Würtz & Rovere, 2015; Sakellariou et al., 2022). Rising from soft-bottom substrates, these geological features support rich benthic assemblages and attract a variety of fishes and marine mammals (Würtz & Rovere, 2015).

In the Mediterranean, knowledge of **deep chemosynthetic habitats** remains limited. These include a few hydrothermal vents, sometimes associated with seamounts (e.g., Marsili Seamount), as well as more numerous cold seeps, such as mud volcanoes and pockmark fields (Taviani, 2014).

A comprehensive list of deep-sea habitats is provided in Table 3. This typology should be applied wherever possible for the identification and characterization of deep-sea habitats. However, many facies may occur in **mixed assemblages** (e.g., biogenic reefs combining oysters, cirripeds, and corals, or "forests" of sponges and gorgonians), and this should be taken into account. It is also important to recognize that habitats and sediment types frequently occur as **mosaics within the seascape**. Such spatial heterogeneity should be carefully documented in research data and analyses to avoid information loss and to support a comprehensive understanding of deep-sea ecosystem structure and function.

Table 3. Reference list of Mediterranean habitat types associated with seamounts, canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea, based on the *Handbook for Interpreting Types of Marine Habitats for the Selection of Sites to be Included in the National Inventories of Natural Sites of Conservation Interest* (UNEP/MED WG.502/Inf.4, 2021), and the updated classification system for marine benthic habitats developed by SPA/RAC (Montefalcone et al., 2021).

OFFSHORE CIRCALITTORAL near the shelf edge or even close to the beginning of the bathyal zone, limited by the low light conditions and the high silting rates, with no coralligenous bioconstruction and usually situated at about 120–200 m depth.
MD1.5 Offshore circalittoral rock
MD1.51 Offshore circalittoral rock invertebrate-dominated

    - MD1.511 Facies with small sponges
    - MD1.512 Facies with large and erect sponges
    - MD1.513 Facies with Alcyonacea
    - MD1.514 Facies with Antipatharia
    - MD1.515 Facies with Scleractinia
    - MD1.516 Facies with Ceriantharia
    - MD1.517 Facies with Zoantharia
    - MD1.518 Facies with Polychaeta
    - MD1.519 Facies with Bivalvia
    - MD1.51A Facies with Brachiopoda
    - MD1.51B Facies with Bryozoa
  - MD1.52 Offshore circalittoral rock invertebrate-dominated covered by sediment (see MD1.51 for examples of facies)
  - MD1.53 Deep offshore circalittoral banks
    - MD1.531 Facies with Antipatharia
    - MD1.532 Facies with Alcyonacea
    - MD1.533 Facies with Scleractinia
- MD2.5 Offshore circalittoral biogenic habitat
  - MD2.51 Offshore reefs
    - MD2.511 Facies with Vermetidae and/or Serpulidae
  - MD2.52 Thanatocoenosis of corals, or Brachiopoda, or Bivalvia (see MD1.51 for examples of facies)
- MD3.5 Offshore circalittoral coarse sediment
  - MD3.51 Offshore circalittoral detritic bottoms
    - MD3.511 Facies with Bivalvia
    - MD3.512 Facies with Brachiopoda
    - MD3.513 Facies with Polychaeta
    - MD3.514 Facies with Crinoidea
    - MD3.515 Facies with Ophiuroidea
    - MD3.516 Facies with Echinoidea
- MD4.5 Offshore circalittoral mixed sediment
  - MD4.51 Offshore circalittoral detritic bottoms (see MD3.51 for examples of facies)
- MD5.5 Offshore circalittoral sand
  - MD5.51 Offshore circalittoral sand (see MD3.51 for examples of facies)
- MD6.5 Offshore circalittoral mud
  - MD6.51 Offshore terrigenous sticky mud
    - MD6.511 Facies with Pennatulacea
    - MD6.512 Facies with Polychaeta
    - MD6.513 Facies with Bivalvia
    - MD6.514 Facies with Brachiopoda
    - MD6.515 Facies with Ceriantharia

UPPER BATHYAL ranging from about 200 m to 500 m depth and embracing most canyon heads and the most extensive deep biogenic habitats.

- ME1.5 Upper bathyal rock
  - ME1.51 Upper bathyal rock invertebrate-dominated
    - ME1.511 Facies with small sponges
    - ME1.512 Facies with large and erect sponges
    - ME1.513 Facies with Antipatharia
    - ME1.514 Facies with Alcyonacea
    - ME1.515 Facies with Scleractinia
    - ME1.516 Facies with Cirripedia
    - ME1.517 Facies with Crinoidea
    - ME1.518 Facies with Bivalvia

    - ME1.519 Facies with Brachiopoda
  - ME1.52 Caves and ducts in total darkness
- ME2.5 Upper bathyal biogenic habitat
  - ME2.51 Upper bathyal reefs
    - ME2.511 Facies with small sponges
    - ME2.512 Facies with large and erect sponges
    - ME2.513 Facies with Scleractinia
    - ME2.514 Facies with Bivalvia
    - ME2.515 Facies with Serpulidae
    - ME2.516 Facies with Brachiopoda
  - ME2.52 Thanatocoenosis of corals, or Brachiopoda, or Bivalvia, or sponges (see ME1.51 for examples of facies)
- ME3.5 Upper bathyal coarse sediment
  - ME3.51 Upper bathyal coarse sediment
    - ME3.511 Facies with Alcyonacea
- ME4.5 Upper bathyal mixed sediment
  - ME4.51 Upper bathyal mixed sediment
    - ME4.511 Facies with Bivalvia
    - ME4.512 Facies with Brachiopoda
- ME5.5 Upper bathyal sand
  - ME5.51Upper bathyal detritic sand
    - ME5.511 Facies with small sponges
    - ME5.512 Facies with Pennatulacea
    - ME5.513 Facies with Crinoidea
    - ME5.514 Facies with Echinoidea
    - ME5.515 Facies with Bivalvia
    - ME5.516 Facies with Brachiopoda
    - ME5.517 Facies with Bryozoa
    - ME5.518 Facies with Scleractinia
- ME6.5 Upper bathyal mud
  - ME6.51 Upper bathyal mud
    - ME6.511 Facies with small sponges
    - ME6.512 Facies with Pennatulacea
    - ME6.513 Facies with Alcyonacea
    - ME6.514 Facies with Scleractinia
    - ME6.515 Facies with Crustacea Decapoda
    - ME6.516 Facies with Crinoidea
    - ME6.517 Facies with Echinoidea
    - ME6.518 Facies with Bivalvia
    - ME6.519 Facies with Brachiopoda
    - ME6.51A Facies with Ceriantharia
    - ME6.51B Facies with Bryozoa
    - ME6.51C Facies with giant Foraminifera

LOWER BATHYAL located between 500 m and 2500-3000 m depth, and including some deep canyons, seamounts, chemosynthetic habitats, and the deep bathyal plains.

- MF1.5 Lower bathyal rock
  - MF1.51 Lower bathyal rock
    - MF1.511 Facies with small sponges
    - MF1.512 Facies with Alcyonacea
    - MF1.513 Facies with Scleractinia
    - MF1.514 Facies with chemosynthetic benthic species
- MF2.5 Lower bathyal biogenic habitat

- MF2.51 Lower bathyal reefs
  - MF2.511 Facies with Scleractinia
- MF2.52 Thanatocoenosis of corals, or Brachiopoda, or Bivalvia, or sponges (see MF1.51 for examples of facies)

MF6.5 Lower bathyal mud
- MF6.51 Sandy mud
  - MF6.511 Facies with small sponges
  - MF6.512 Facies with Alcyonacea
  - MF6.513 Facies with Echinoidea
  - MF6.514 Facies with Pennatulacea
  - MF6.515 Facies with bioturbations

ABYSSAL (>3000 m), whose existence, widely debated, is here accepted and refers to the deepest and least explored regions of the Mediterranean Sea, hosting the Deep Hypersaline Anoxic Basins (DHABs).

MG1.5 Abyssal rock
- MG1.51 Abyssal rock
  - MG1.511 Facies with small sponges
  - MG1.512 Facies with Alcyonacea
  - MG1.513 Facies with Polychaeta
  - MG1.514 Facies with Crustacea (Amphipoda, Isopoda, Tanaidacea)

MG6.5 Abyssal mud
- MG6.51 Abyssal mud
  - MG6.511 Facies with small sponges
  - MG6.512 Facies with Alcyonacea
  - MG6.513 Facies with Polychaeta
  - MG6.514 Facies with Crustacea (Amphipoda, Isopoda, Tanaidacea)
  - MG6.515 Facies with bioturbations

II.1. Inventorying of deep-sea habitats

The most characteristic habitat-forming species of aphotic zones are sponges and anthozoans. However, other phyla and classes – such as mollusks, polychaete tube worms, bryozoans, and cirripede crustaceans – can also play a dominant role in certain habitats or form an integral part of mixed assemblages, contributing to complex bioconstructions or extensive communities that provide three-dimensional habitat structures.

The inventory of deep-sea habitats involves two main levels:

- Locating the specific deep-sea habitat of interest.
- Characterizing the communities it supports.

II.1.a Locating deep-sea habitats

In the Mediterranean Sea, approximately 80% of the seabed lies deeper than 200 m, providing extensive habitat for deep-sea communities. Globally, the identification and mapping of specific deep-sea habitats – such as submarine canyons, seamounts, and chemosynthetic assemblages – have advanced considerably. Within the Mediterranean, more than 500 large submarine canyons (Harris & Whiteway, 2011) and around 240 seamount-like structures (Würtz & Rovere, 2015) have been documented, along with approximately twenty confirmed sites of deep-water chemosynthetic assemblages (Taviani, 2014).

At the Mediterranean scale, the most recent distribution maps of canyons, seamounts, and known chemosynthetic assemblages are those presented in the previous *Guidelines for Inventorying and*

Monitoring of Dark Habitats in the Mediterranean Sea (SPA/RAC–UN Environment/MAP & OCEANA, 2017; see Appendix 10). Additional information on the spatial distribution and biodiversity of deep-sea habitats (e.g., canyons and seamounts) in the Eastern Mediterranean basin is provided in the *Deep-sea Atlas of the Eastern Mediterranean Sea* (Otero & Mytilineou, 2022 and chapters therein).

Methodologies for studying deep-sea habitats involve a wide variety of technologies and equipment, typically including a combination of acoustic, visual, and extractive techniques to obtain accurate and comprehensive information (De Clippele et al., 2025). Tools such as multibeam sonar, side-scan sonar, and acoustic ground discrimination systems provide an overview of geological formations, seascapes, and seabed structure, enabling the identification and localization of seamounts, canyons, mud volcanoes, pockmarks, carbonate mounds, reefs, and other features. These approaches also help detect areas likely to host other, more cryptic deep-sea habitats.

To localize specific deep-sea habitats in vast and little-known areas, it is necessary to narrow the search area by: (i) compiling and analysing existing data to focus the study area, and/or (ii) using seafloor mapping tools capable of surveying large areas efficiently as a first step.

(i) Compiling and analysing existing data to focus the study area

Indications to narrow the exploration area (step i) can be obtained primarily from the following sources (e.g., Salomidi et al., 2022; Smith et al., 2022; Bo et al., 2023):

- Past historical scientific explorations, which often relied on grab sampling, dredging, and trawling on soft sediments.
- Existing data from industrial activities conducted in deep-sea environments (e.g., submarine cable or pipeline installation).
- Deep-sea surveys carried out for other purposes, such as geological or geophysical explorations.
- Trawling activities, both scientific (e.g., MEDITS – the MEDiterranean International Trawl Survey) and commercial, which can provide information on vagile species and by-catch of sessile taxa characteristic of particular habitats.
- FAO databases reporting Vulnerable Marine Ecosystems (VMEs).
- Local ecological knowledge (LEK) from fishers; Fishers using nets or longlines at depths down to 200 m or more can offer valuable insights, particularly for offshore circalittoral and upper bathyal habitats.

(ii) Using seafloor mapping tools able to cover large surfaces

Remote mapping tools (step ii) (see also Table 4) include:

- Side-scan sonar, which detects and images objects on the seafloor. Image quality depends on the resolution used. Because it does not measure bathymetry, it should be employed in combination with single-beam or multibeam sonar. Side-scan systems are typically mounted on a towfish, an ROV (Remotely Operated Vehicle), or an AUV (Autonomous Underwater Vehicle).
- Multibeam sonar, which provides detailed 3D bathymetric maps of surveyed areas. These systems are generally hull-mounted on research vessels to cover large areas but can also be mounted on ROVs or AUVs to obtain higher-resolution data over smaller surfaces.
- AGDS (Acoustic Ground Discrimination Systems), which measure the geophysical properties of the seafloor. These systems are relatively inexpensive and easy to use (Brown, 2007). Depending on the resolution, AGDS data can provide valuable indications of habitat type.

Instruments can sometimes be mounted on the hull of vessels (e.g., side-scan sonar), but are more frequently deployed on AUVs or ROVs launched from a support ship. AUVs equipped with multibeam echosounders (side-scan sonar), and high-resolution cameras are widely used to explore and map extensive areas of deep-sea environments. Although the cost of these platforms often limits their use by smaller research institutions, the large volumes of data acquired and the broad spatial coverage they provide make them highly advantageous compared to extended operations with large research vessels.

Collaboration among Contracting Parties, particularly for surveys in areas beyond national jurisdiction, is essential and enables the use of advanced exploration technologies. For example, within the RAMOGE Agreement (France, Monaco, and Italy), three major deep-sea exploration campaigns have been conducted by pooling resources, expertise, and operational costs (see RAMOGE deep-sea campaigns here and Fourt et al., 2015; Rouanet et al., 2019; Schon et al., 2023).

II.1.b Characterization of deep-sea habitats

To characterize a deep-sea habitat, it is recommended to collect data and information that allow assessment of the following variables:

- **Geolocation** of the habitat.
- **Spatial extent**, where it can be estimated.
- **Physical environmental conditions** (e.g., depth, surrounding seawater temperature, prevailing currents, the presence of upwelling or downwelling phenomena, and seafloor slope).
- **Geomorphological context**, identifying the specific feature in which the habitat occurs (e.g., canyon head or flank, continental shelf, seamount, cold seep).
- **Substrate type**, such as hard rock, biogenic formations, or muddy or sandy soft bottoms, along with an evaluation of siltation.
- **Structuring (habitat-forming) species** and other key species.
- Broader **biological assemblages**, identifying as many associated species as possible, including motile fauna and species of commercial interest (Fig. 15).
- **Condition of the assemblages**, including assessment of visible physical damage to erect species and the presence of marine litter, such as abandoned, lost or otherwise discarded fishing gear (ALDFG).

Deep-sea expeditions are costly and must therefore be meticulously planned to ensure the acquisition of the maximum amount of usable data and information within a limited timeframe. This requires thorough pre-expedition organization. All tools and materials that may be needed should be identified in advance, including equipment for data backup and materials for properly labelling samples. Likewise, a standardized nomenclature for ROV dives, samples, video footage, and photographs should be established prior to departure to ensure consistency and efficient data management.

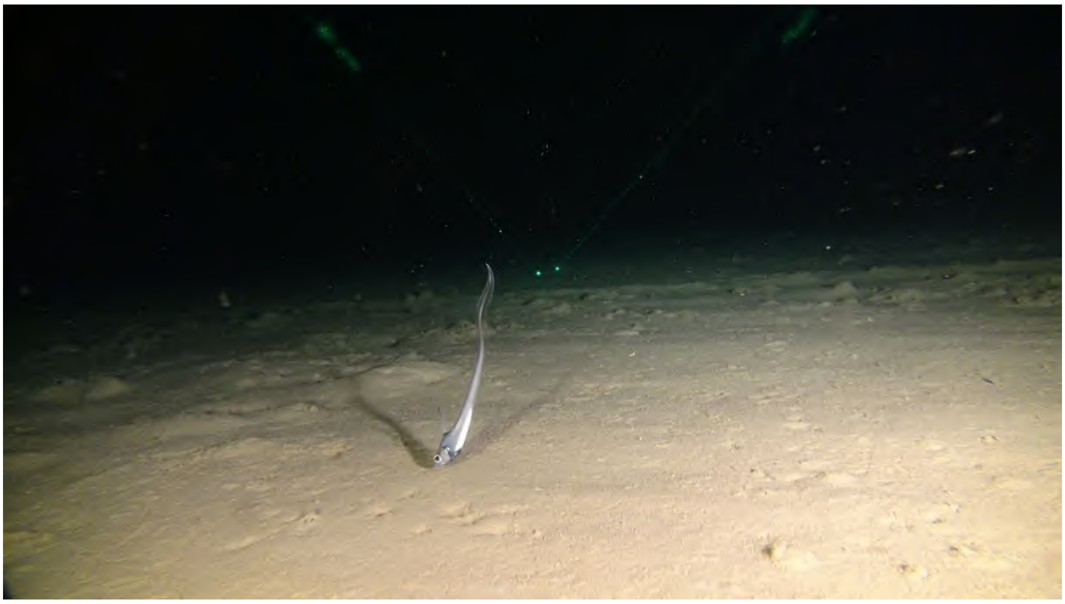


Figure 15. The bluntsnout grenadier *Nezumia sclerorhynchus*. ROV photo by Giovanni Chimienti.

Recommendations on data quality, backup procedures, and sample fixation

The characterization of deep-sea habitats and the identification of species rely primarily on topographic and seabed mapping outputs, as well as visual data, including video footage and still photographs. It is therefore essential to determine, in advance, the appropriate resolution and technical settings for each data type to ensure adequate spatial coverage and sufficient detail for the identification of habitats and species. In particular, image quality is crucial, not only for scientific identification but also for producing high-quality photographs to support communication and outreach. To achieve optimal image quality, it is also important that the camera is equipped with adequate lighting and operated at a slow, constant speed, while maintaining a stable and consistent distance from the seabed.

The use of **scaling lasers** with predefined distances between points (Fig. 13) is highly recommended for measuring spatial features or estimating organism size (Chimienti et al., 2018; 2026). The number and spacing of laser points vary in the scientific literature; typically, systems deploy two parallel points spaced 6–25 cm apart, although three-point configurations are also used. While lasers can generally be activated or deactivated during a dive, their intended use should be planned in advance.

Appropriate arrangements for **data storage** and **sample preservation** must be made prior to the expedition. This includes ensuring the availability of sufficient digital storage capacity (e.g., external hard drives) for data and backups, as well as materials for sample preservation, such as 95% ethanol, freezing capabilities, or filters for eDNA water samples. Consequently, the types of samples to be collected must be defined beforehand so that all necessary preservation materials can be brought onboard.

All exploration activities must take place in authorized areas, and any planned sampling must comply with relevant national and international regulations. In most cases, **sampling authorizations** must be obtained from the competent national authority, and some countries also require authorization for image acquisition. For scientific publications, journals now frequently request evidence of the appropriate sampling permits.

Data acquisition tools

A wide range of instruments is available for acquiring data and information on deep-sea habitats. Several of the most commonly used tools are described below and summarized in Table 4.

For **hard-substrate habitats, blind sampling methods should generally be avoided**, as they tend to be destructive and are usually inefficient. In contrast, grabs and corers are appropriate for soft-bottom environments, where they provide essential information on infaunal communities and small organisms that cannot be detected or identified through visual techniques.

Tools such as AUVs, ROVs, bathyscaphes, submarines, landers, and dropped or towed camera systems are indispensable for deep-sea exploration. These platforms generate **georeferenced visual data** on benthic communities and are vital for confirming information obtained through acoustic or mapping tools, as well as for verifying the identity and structure of benthic habitats and species.

In the Mediterranean Sea, deep-sea assemblages on hard substrates are most commonly characterized using **photographs and video footage obtained during ROV surveys** (Fig. 16), while other visual tools such as towed cameras or drop-down cameras are used less frequently. ROVs enable the observation of species *in situ*, including their natural colouration and certain behavioural traits. They also provide the means to assess distribution patterns, species densities, biological associations, and other ecological features. Moreover, ROVs can conduct controlled transects and collect selective samples (e.g., Enrichetti et al., 2025a), greatly facilitating reliable species identification.

Table 4. Survey tools for locating, mapping, and assessing biodiversity in deep-sea habitats (adapted from UNEP, 2016, and SPA/RAC–UN Environment/MAP & OCEANA, 2017).

Data acquisition tools	HABITAT		Litter
	Bathyal hard substrate	Detritic and other soft substrate	Litter on the seafloor
Remote tools			
Side scan sonar [8]	Location and extent	Location and extent	Only large objects can be detected
Multibeam bathymetry [8]	Location and extent of geomorphological formations	Location and extent of geomorphological formations	Not relevant
AGDS [8] **(Acoustic Ground Discrimination Systems)**	Location and extent of different types of substrate and benthic communities	Location and extent of different types of substrate and benthic communities	Not relevant
Direct sampling or observation methods			
Grab/core sampling	Not recommended	Biodiversity especially endofauna and substrate	Not relevant
Towed video	Not recommended	Extent of facies of erect species	Quantitative assessment of litter density

---

[8] For all remote sensing methods, the ability to distinguish habitats from each other and from the surrounding seabed depends on the resolution of the survey. Higher-resolution data provide more detailed information, enabling more accurate habitat differentiation, but cover smaller areas and are generally more time-consuming and costly to collect and process compared to lower-resolution data.

Drop-down camera	Biodiversity, habitat and monitoring for a limited area	Biodiversity, habitat and monitoring of facies of erect species for a limited area	Quantitative assessment of litter density for a limited area
Baited lander with video (e.g., Nalmpanti et al., 2023)	Motile biodiversity (mainly fish and crustaceans) Characterization and monitoring	Motile biodiversity (mainly fish and crustaceans) Characterization and monitoring	Not relevant
ROV or HOV with cameras (e.g., Enrichetti et al., 2025a)	Biodiversity, habitat and monitoring. Can give indications on extent of habitats	Can help evaluate the extent of habitats dominated by e.g., *Leptometra phalangium* and erect anthozoans	Quantitative and qualitative assessment of litter density (see e.g., Hanke et al., 2025)
eDNA metabarcoding (e.g., La Torre et al., 2024)	Biodiversity	Biodiversity	Not relevant
Epibenthic trawls / dredges	Not recommended	Not recommended	Not recommended

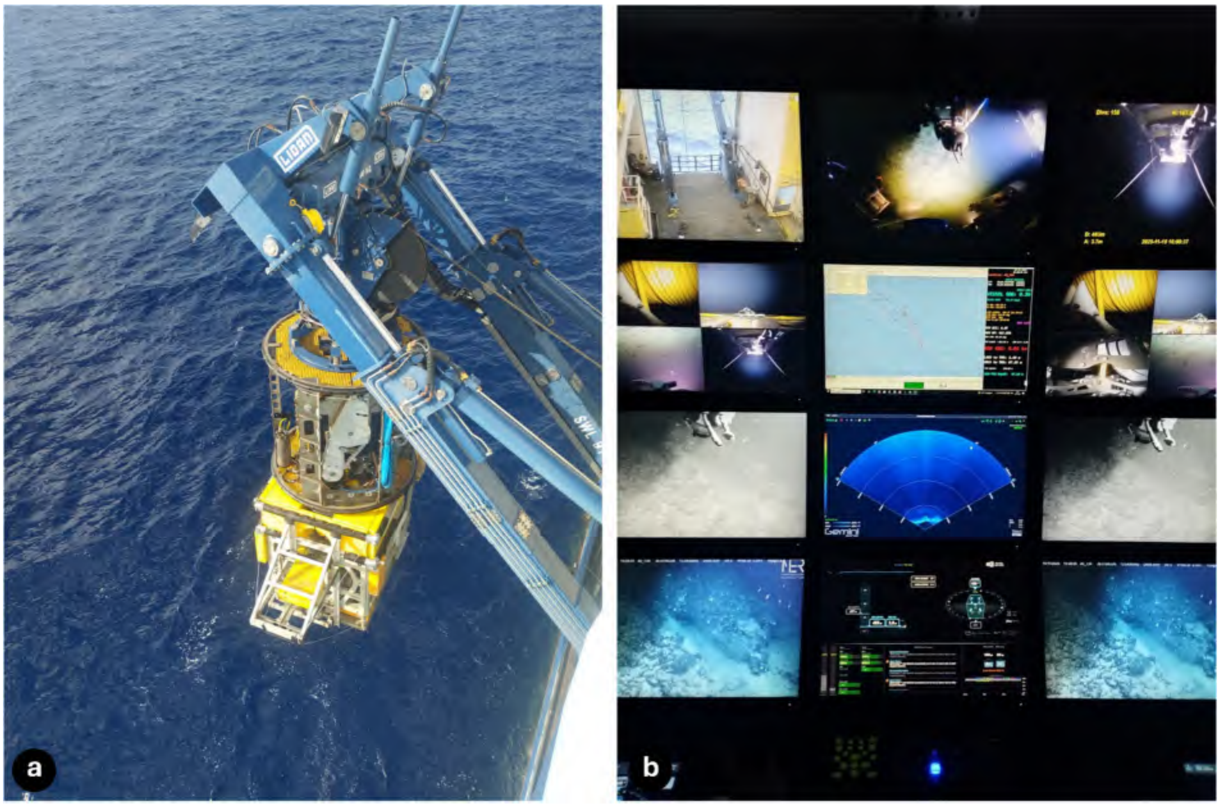


Figure 16. (a) Deployment of the Remotely Operated Vehicle (ROV Schilling HD23) used for underwater exploration. b) ROV control room showing the multiscreen layout with real-time video feeds, navigation telemetry, and positioning instruments used during seafloor surveys. Photos by Michela Maiorca.

Advances in visual analysis now allow **3D reconstructions** of deep-sea video footage, providing detailed morphometric data for large, conspicuous species, such as the glass sponge *Asconema setubalense* (Fig. 17; Prado et al., 2021; Heres et al., 2024). These models enable the assessment of temporal changes in populations, habitat condition, and conservation status, offering a non-invasive tool for long-term monitoring. More broadly, **deep-sea photogrammetry** is applied to map seafloor habitats and quantify spatial distribution of benthic organisms like sea pens and corals (Rodríguez-Basalo et al., 2022; Susini et al., 2025). While challenges such as low light, turbidity, and navigation uncertainty remain, 3D models significantly enhance our ability to detect changes, measure growth, and evaluate the structural complexity of deep-sea ecosystems.

Figure 17. Felt vase glass sponge sponges *Asconema setubalense* at Chella Bank. ROV photo by © OCEANA.

Recently, **small ROVs**, more accessible in terms of cost, have become available on the market. Although they can be very useful for shallow habitats down to the circalittoral zone under certain conditions, they are not suitable for deep-sea habitats due to their limited maximum operating depth and technical constraints, including difficulties in navigating against currents. They are therefore not recommended for deep-sea applications. However, they can be effective for identifying the deepest occurrences of photo-dependent species and habitats, and for examining the transition zone between these communities and true deep-sea habitats.

Baited and unbaited landers offer a non-destructive method for collecting information on motile species, particularly fish. These autonomous devices capture images and can also register additional environmental data (e.g., Linley et al., 2017; D'Onghia et al., 2018; Bo et al., 2024).

Biological and water sampling can be conducted in various ways depending on the objectives of the expedition and the available equipment. Some ROVs are equipped to collect water, biological material, and even substrate or sediment. However, apart from water sampling – which is generally a simple manoeuvre – dedicated ROV dives are required for biological sampling, separate from exploratory or survey dives. Because ROV-based sampling is time-consuming, it should be restricted to cases where collection is strictly necessary. ROVs equipped with retrieval tools, such as robotic arms, are valuable for identifying key or habitat-forming species. Once organisms have been documented through imagery, these tools allow selective sampling of specimens essential for subsequent taxonomic verification, without the need for additional collection efforts if the species is encountered again.

The use of **CTDs** and other instruments for water and current analysis provides complementary information on water masses, currents, and physicochemical parameters, all of which significantly enhance understanding of deep-sea ecosystem functioning.

New approaches such as **eDNA** analysis can provide insights into the presence of species and populations (e.g., La Torre et al., 2024; Galli et al., 2025). They may even reveal species inhabiting an area that were not detected using other methods, and in some cases can offer indications of species abundance. This technique relies on the detection of genetic material from different matrices, including water samples, enabling non-invasive species identification. However, at present, eDNA cannot be used as a stand-alone method for habitat characterization, although results are promising.

Data labelling and management

Proper data labelling is critical but often underestimated. Mislabelled or inconsistently labelled data can lead to confusion, errors, or even render collected data unusable. Given the high cost and effort of deep-sea expeditions, such errors must be avoided. To ensure no loss of information, all data should follow a codified nomenclature that allows clear identification of:

- Expedition name
- Acquisition tool (e.g., AUV, ROV, CTD) or specific tool name (e.g., ROV Super Achille)
- Location (e.g., canyon name)
- ROV/AUV dive number
- Date and the time
- Depth

An example of a standardized labelling system for a hypothetical expedition is provided in Appendix 11. In addition, the time and effort required for post-sampling processing and analysis should be carefully considered during project planning.

Data processing

Data obtained from side-scan sonar (if available) and bathymetric acquisition tools (e.g., multibeam sonar) should be processed to generate georeferenced bathymetric maps of the seafloor at the highest possible resolution. These maps provide a framework onto which ROV tracks can be overlaid for precise spatial analysis.

All events observed along an ROV track should be linked to a georeferenced point, time, and depth, and stored in a dedicated database. Video footage can be analysed in various ways depending on the objectives, ranging from full annotation of all events – such as species sightings, litter, or behaviours – to recording only major habitat or substrate changes. While detailed event logging is time-consuming, it provides highly informative data. Initial entry can occur during the ROV dive, but post-mission analysis of the video footage is generally required to complete the dataset.

To reduce processing time, different **image sampling** strategies have been applied in the literature (including studies on coralligenous habitats using ROV transects; Fig. 18):

- Fixed-length transects, e.g., 200 or 50 m, at constant speed (Enrichetti et al., 2019; 2025b).
- Timed transects, e.g., 10 minutes at constant speed.
- Frame extraction from video, e.g., photos every 10 seconds or randomly selected images (Ferrigno et al., 2017).
- Automated species recognition, using deep learning on images (e.g., Mahmood et al., 2016).

The choice of image sampling strategy should be driven by the objectives of the study. Exhaustive annotation of every observation is not always necessary and analysis can be streamlined to focus on information required to serve only the purpose of the study.

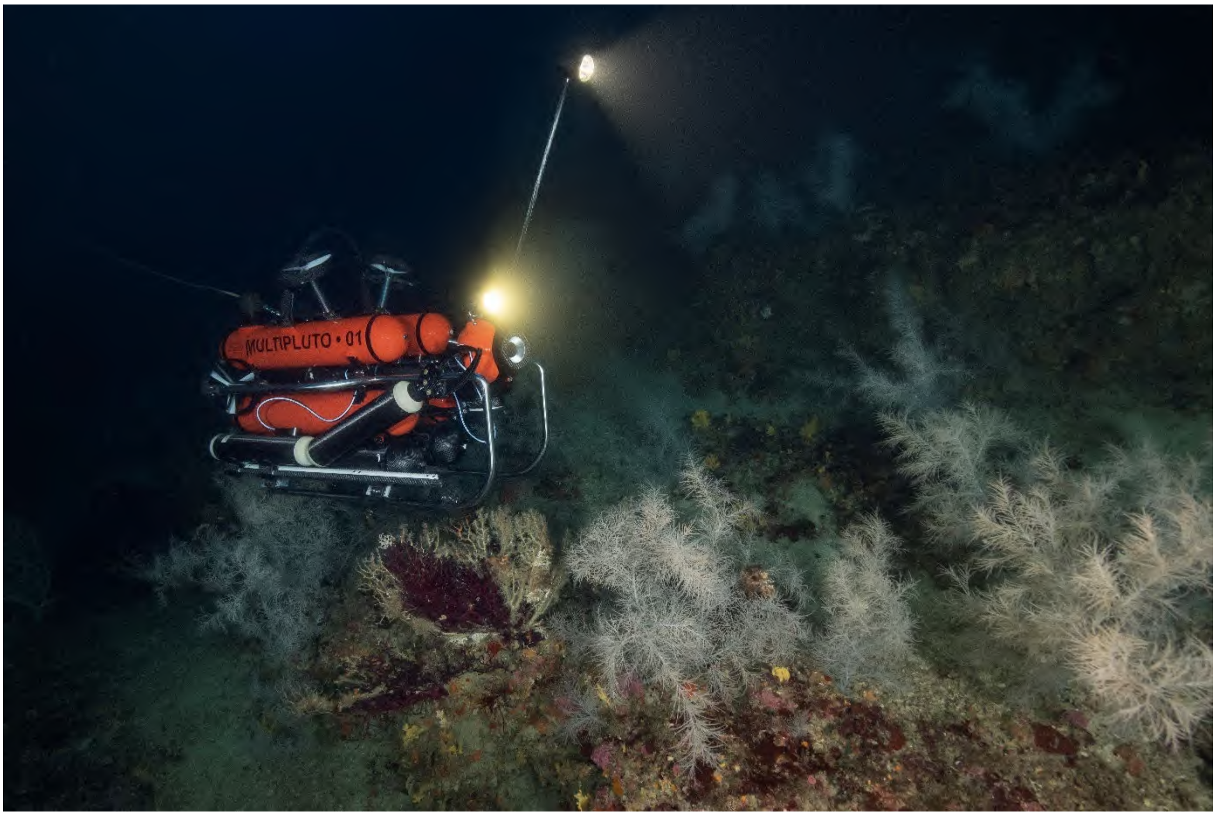


Figure 18. ROV transect in front of a forest of the black coral *Antipathella subpinnata*. Photo by Filippo Borghi, courtesy of Giovanni Chimienti.

Useful identification guides

To facilitate the **identification of deep-sea species**, it is recommended to have several identification guides on board during expeditions. General guides specific to the Mediterranean Sea include:

- Otero, M.M., Serena, F., Gerovasileiou, V., Barone, M., Bo, M., Arcos, J.M., Vulcano, A., Xavier, J. (2019). ***Identification guide of vulnerable species incidentally caught in Mediterranean fisheries***. IUCN, Málaga, Spain, 204 pp.
  Link: https://portals.iucn.org/library/sites/library/files/documents/2019-050-En.pdf
  This guide includes identification information for marine mammals, marine reptiles, chondrichthyans, seabirds, large anthozoans, and sponges. Photos of benthic species are not *in situ*, as the guide is designed for fisheries bycatch monitoring.

- Fourt, M., Goujard, A., Pérez, T., Chevaldonné, P. (2017). ***Guide de la faune profonde de la mer Méditerranée. Exploration des roches et canyons sous-marins des côtes françaises***. Museum national d'Histoire naturelle, Paris, 184 pp.
  This guide contains numerous *in situ* photos of species observed by ROVs. While not exhaustive, it provides a practical reference for species in the Western Mediterranean.

To **categorize marine macro-litter** on the deep-sea seafloor, items can be assigned to the following categories (adapted from EU MSFD guidance):

- Artificial polymer materials / plastics,
- Paper and cardboard,
- Wood (processed or not),
- Metal,
- Glass and ceramics,
- Cloth / textiles,
- Rubber
- Miscellaneous / other.

These categories are a selection for the deep-sea seafloor, of the category types proposed for EU MSFD. These categories are further refined into subtypes in Fleet et al. (2021) and in the EU online catalogue of the Joint List of Litter Categories (Link). In deep-sea hard-substrate habitats, discarded fishing gear is among the most commonly observed litter (Giusti et al., 2019).

Recommended references for litter assessment in deep-sea habitats include:

- Galgani, F., Ruiz, O.S.P.L., Ronchi, F., Tallec, K., Fischer, E., Matiddi, M. et al. (2023). ***Guidance on the monitoring of marine litter in European seas***. JRC Publications Repository. https://doi.org/10.2760/59137
  The document provides guidelines for assessing seafloor litter through **image-based surveys and trawling**.

- Fleet, D., Vlachogianni, Th., Hanke, G. (2021). ***Joint list of litter categories for marine macro-litter monitoring: Manual for the application of the classification system*.** Publications Office of the European Union. https://data.europa.eu/doi/10.2760/127473
  This document details a hierarchical classification system for marine litter under the MSFD framework

- The **online Photo Catalogue of the Joint List of Litter Categories** (Link) is a useful and user-friendly online database illustrating litter types and subtypes, supporting consistent identification during fieldwork.

Indices to characterise the state of conservation of hard substrate assemblages

Several indices originally applied to mesophotic assemblages using ROV video footage can be adapted for deep-sea habitats on hard substrates. These indices were developed to fulfil MSFD requirements for assessing the state of conservation of hard-substrate communities. More recently, a new multiparametric index has been specifically created to assess the ecological status of Mediterranean cold-water scleractinian frameworks. A summary of these indices is provided in Table 5, which focuses on monitoring deep-sea habitats using Common Indicator 2 (CI2) of the Integrated Monitoring and Assessment Programme (IMAP).

II.2. Monitoring deep-sea habitats according to the recommendations of the Integrated Monitoring and Assessment Programme (IMAP)

In line with the overarching principles of the IMAP (see Appendix 4), a set of indicators has been selected for monitoring deep-sea habitats (Table 5). These were drawn from the initial list of common and candidate indicators agreed upon as the core of IMAP. As recommended by IMAP, selected indicators must be reliable, reproducible, and, as far as possible, comparable across operators throughout the Mediterranean Sea (or within subregions). The selection was based on relevance and applicability to deep-sea habitats. General considerations and assessment guidelines for the selected Common Indicators are provided below, following IMAP Guidance.

Table 5. Monitoring guidelines for deep-sea habitats (based on IMAP UNEP(DEPI)/MED IG.22/Inf.7, modified from SPA/RAC–UN Environment/MAP & OCEANA, 2017).

EO1 [Biological diversity]

COMMON INDICATOR 1. *Habitat distributional range to also consider habitat extent as a relevant attribute*

Operational objective: Ensure that key deep-sea habitats are not lost.

- **Parameter/metric:** Surface area of habitat lost, quantified for each habitat type, ideally derived from mapping products.
- **Target:** The allowable loss per habitat type should not exceed an acceptable percentage of the baseline value, as defined by the relevant Contracting Party.

GENERAL CONSIDERATIONS

CI1. *Habitat distributional range to also consider habitat extent as a relevant attribute*

Constraints for monitoring:

- **Availability of high-resolution bathymetric maps:** Tools such as multibeam echosounders are extremely useful for locating and assessing the extent of deep-sea habitats; however, such data are often not readily available.
- **Resource limitations:** Obtaining new data can be constrained by the high costs of research vessels (R/Vs) and the specialized technology required for deep-sea exploration.
- **Baseline requirements:** Monitoring this indicator requires the establishment of an initial state, including defining baseline values and thresholds for acceptable loss.

Sampling

- **Methods**: Hard-substrate seafloor mapping is preferably conducted using optical, non-destructive approaches, such as underwater video or ROV surveys. Collection of living specimens can be performed using an ROV manipulator arm. Infaunal communities on soft sediments can be sampled with standardized grabs or corers. All sampling requires appropriate authorizations from national or international authorities.
- **Design:** Sampling and monitoring should be carried out in accordance with national or international guidelines, prioritizing non-invasive methods or modelling approaches that reduce effort, cost, and environmental impact over the long term.

ASSESSMENT GUIDELINES

CI1. *Habitat distributional range to also consider habitat extent as a relevant attribute*

Selection of sites

A risk-based approach is recommended to prioritize monitoring efforts and ensure cost-effectiveness. Site selection can be based on the following considerations:

- Essential habitats for
    (1) Early developmental stages of predominantly motile fauna (e.g., spawning or feeding grounds).
    (2) Benthic assemblages considered key components of deep-sea ecosystems, including habitat-engineering species and other species critical to ecosystem functioning. Full monitoring programs are recommended for habitats under protective regulations or whose key species are categorized as

threatened (e.g., IUCN Red List).
- Marine Protected Areas (MPAs).
- Vulnerable Marine Ecosystems (VMEs). Knowledge of VME indicators can guide the identification of vulnerable deep-sea ecosystems (see Appendices 12 and 13).
- Geomorphological predictions: The potential location of a habitat type can be inferred from substrate type (muddy, rocky, sandy) and physical parameters (depth, currents), as these influence the expected biological communities.

Possible data sources

- Existing multibeam bathymetric maps, side-scan sonar, and AGDS (see Table 4) to identify potential areas for ROV exploration or grab sampling; however, these data are rare.
- Past historical scientific explorations.
- Industrial datasets (e.g., from submarine cable or pipeline installations).
- Deep-sea explorations in other domains (e.g., geological surveys).
- Scientific and commercial trawl surveys, including MEDITS, which can provide information on motile species and bycatch of sessile organisms (e.g., Stamouli et al., 2023).
- FAO database on reported VMEs.
- Local ecological knowledge (LEK) from fishers, particularly for offshore circalittoral and upper bathyal zones (nets and longlines deployed down to 200 m or deeper).

Data acquisition and monitoring methods

- Hard-substrate habitats: Georeferenced images (photos and/or video) collected primarily using ROVs.
- Soft-substrate habitats: Sampling using grabs or data from existing scientific trawls (e.g., MEDITS). However, these methods do not provide detailed information on the composition and spatial distribution of specific benthic facies.
- Remote mapping tools (e.g., multibeam bathymetry) are used to define habitat extent.
- Modelling approaches (e.g., Species Distribution Models – SDMs) can also serve as valuable tools for predicting and mapping habitat suitability for VME indicator species across wide geographic regions under current environmental conditions, as well as for projecting potential changes under future climate scenarios (Georges et al., 2024; Millot et al., 2024).

The UNEP/MED WG.606/05 document provides methodology, criteria, and threshold values for IMAP CI1 (Habitat Distributional Range), which considers habitat extent as a relevant attribute, although it is not specific to deep-sea habitats.

Periodicity

Monitoring should be conducted every six years to allow data analysis for the Mediterranean Quality Status Report (MED QSR) (see UNEP/MED WG.606/05, 2025).

EO1 [Biological diversity]

COMMON INDICATOR 2 . ***Condition of the habitat's typical species and communities***

Operational Objective: Ensure that key habitats remain in their natural condition, maintaining both structure and ecological functions.

- **Target:** Achieve a ratio of typical and/or characteristic species comparable to baseline conditions for all considered communities.
- **Calculation:** Compare the presence and abundance of typical and/or characteristic species per habitat and sub-region against baseline data for the same communities.

GENERAL CONSIDERATIONS

CI2. *Condition of the habitat's typical species and communities*

Typical species

The concept of typical species is grounded in the conservation status of natural habitats, focusing on their long-term distribution, structure, and functions, as well as the persistence of characteristic species within a given territory. For a habitat to be considered in natural condition, its typical species composition should closely reflect natural conditions.

Reference list of habitats

For guidance on habitat types, see Table 3 of this document, based on the *Handbook for Interpreting Types* *of Marine Habitats for the Selection of Sites to be Included in the National Inventories of Natural Sites of Conservation Interest* (UNEP/MED WG.502/Inf.4, 2021), and the updated classification system for marine benthic habitats developed by SPA/RAC (Montefalcone et al., 2021).

Methods

- The selection of monitoring methods depends on the habitat type and the target species. Large attached epibenthic species on hard substrates are preferably monitored using optical, non-destructive techniques, such as underwater video. Endobenthic communities are sampled using standardized grabs or corers to obtain representative specimens from the sediment, while motile fauna associated with specific deep-sea habitats can be effectively monitored using baited landers.
- Regarding indices for habitat condition, most existing indices were originally developed for mesophotic assemblages, particularly in the North-Western Mediterranean. Some, such as the MACS index, have been tested in the Eastern Mediterranean and have shown potential for broader application. However, these indices may require adaptation to be fully applicable to deep-sea habitats in the southern Mediterranean, where environmental conditions and species composition can differ significantly.

General remarks on potential sources of bias

- Surveys must be designed to avoid focusing solely on rare or endangered species, as this can overlook more common species that are critical to ecosystem functioning.
- Likewise, after topographic or geomorphological data are collected, surveys often tend to prioritize rocky bottoms, canyons, seamounts, and other prominent features. This focus can result in under-sampling of flat, soft-bottom areas, where information on habitat distribution is typically limited but equally important for a comprehensive understanding of the ecosystem.

ASSESSMENT GUIDELINES

CI2. *Condition of the habitat's typical species and communities*

Typical species lists

Typical species lists should be defined for each sub-region or bioregion to enable consistent assessment of the state or condition of deep-sea habitats. These lists should include long-lived species and those with high structuring or functional value for the community. At the same time, small and short-lived species that are characteristic of the habitat under natural conditions should also be included, as they can play critical functional roles. The lists should reflect the state of conservation of the habitat, incorporating endangered and threatened species, such as those listed in Annex II of the SPA/BD Protocol (Decision IG.26/4 – see here). Several deep-sea cnidarians are included in these lists. Anthozoans categorized as Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), or Data Deficient (DD) should also be monitored when present (see Otero et al., 2017 available here).

Data acquisition and monitoring methods

For hard-substrate habitats, the condition of typical species is primarily assessed using high-quality georeferenced images (photos or video), most often obtained with ROVs. For soft substrates, images may be used to monitor erect species, such as facies of *Isidella elongata*, but sampling with grabs is necessary to assess endofauna. The UNEP/MED WG.606/05 document provides methodology, criteria, and threshold values for IMAP CI2 – *Condition of the habitat's typical species and communities*, although it is not specific to deep-sea habitats. Protocols developed for mesophotic habitats in environmental impact assessments (UNEP/MAP-SPA/RAC, 2024) can also be useful for monitoring deep-sea hard-substrate habitats. Additionally, GFCM Fisheries Restricted Areas (FRAs) designated to protect VMEs can serve as a baseline for monitoring relevant habitats (see Appendices 12 and 13). For molecular approaches, eDNA methodologies (Giraud et al., 2024) may be applied, although these guides are not specific to deep-sea ecosystems.

Periodicity: Ideally, the condition of habitats should be assessed every six years (UNEP/MED WG.606/05).

Indices

The **Mesophotic Assemblages Ecological Status (MAES)**, including its simplified form q-MAES (Cánovas Molina et al., 2016), uses ROV photos and videos to evaluate three parameters: the structure of the assemblage, the condition of erect species, and visible anthropogenic impacts.

The **Mesophotic Assemblage Conservation Status (MACS)** (Enrichetti et al., 2019) was initially applied to mesophotic reefs of the Ligurian, Tyrrhenian, and Sardinian Seas (Pierdomenico et al., 2021; Moccia et al.,

2022) and was recently tested in the Eastern Mediterranean (Salomidi et al., 2025). MACS comprises two components: an Index on Status, including six metrics targeting associated biodiversity, biogenic substrate, and canopy complexity; and an Index of Impact, with six metrics targeting siltation levels, canopy condition, and marine litter occurrence.

The **BAthyal coral Reef Conservation Status (BARCS)** (Enrichetti et al., 2025b) assesses the ecological condition of Mediterranean cold-water coral reefs and has been tested across the Gulf of Lions and the Ligurian, Tyrrhenian, Ionian, and Adriatic seas. The BARCS index integrates two components: a Structure Index, which assesses reef complexity, demographic parameters of habitat-forming scleractinians, and associated species richness; and an Impact Index, which quantifies anthropogenic pressure based on sedimentation, entanglement, and marine litter.

Resources required

- Research vessels, capable of operating in bathyal zones (below 150–200 m depth).
- Adequate equipment (box core samplers, grabs, underwater camera systems, etc.) for sample collection.
- Laboratory infrastructure to analyse samples (e.g., microscopes and weighing scales).
- Qualified personnel for data processing, analysis, and interpretation.
- Taxonomic expertise to ensure accurate species identification; post-expedition support can be used when high-quality images are available.

EO2 [Non-indigenous species]

COMMON INDICATOR 6. *Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas*

GENERAL CONSIDERATIONS

CI6. *Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas*

Although deep-sea ecosystems appear to be minimally impacted by Non-Indigenous Species (NIS), their presence in deep-sea habitats is an emerging concern. Monitoring of NIS should be conducted concurrently with the assessment of the conservation status of deep-sea habitats (CI2) to ensure efficient and integrated data collection.

ASSESSMENT GUIDELINES

CI6. *Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas*

Selection of sites

Sites that are potentially susceptible to hosting NIS can be incorporated into the broader monitoring of deep-sea habitats. The likelihood of NIS occurrence can serve as a criterion for prioritizing site selection within a deep-sea monitoring strategy.

Data acquisition and monitoring methods

Monitoring for NIS can be conducted alongside regular surveys of selected deep-sea habitats. Additionally, existing datasets – such as those from MEDITS campaigns – can provide valuable information on species presence (e.g., Stamouli et al., 2022; Certain et al., 2025 database available here). In addition, eDNA analysis of water samples collected near the habitats can also help detect NIS, offering a non-invasive method to complement visual and trawl-based surveys.

EO3 [Harvest of Commercially exploited fish and shellfish]

COMMON INDICATOR 12. *Bycatch of vulnerable and non-target species (EO1 and EO3)*

Operational objective: incidental catches of vulnerable species are minimized.

GENERAL CONSIDERATIONS

CI12. *Bycatch of vulnerable and non-target species (EO1 and EO3)*

Standardized information on bycatch in deep-sea fisheries is currently limited in the Mediterranean Sea. Available data are primarily derived from reporting obligations under the GFCM regulatory framework, which focus on the bycatch of vulnerable and non-target species. While bycatch monitoring has traditionally emphasized pelagic species such as cetaceans and turtles, it has been progressively extended to include other vulnerable species, reflecting the realities and conservation needs of Mediterranean fisheries.

ASSESSMENT GUIDELINES

CI12. *Bycatch of vulnerable and non-target species (EO1 and EO3)*

The **GFCM reporting system**, which entered into force in 2016, requires reporting through the Data Collection Reference Framework (DCRF). The DCRF aims to quantify incidental catches by fleet segment and assess the impact of fisheries on species of conservation concern. Task 3 specifically addresses the reporting of incidental catches, including benthic species.

Data acquisition and monitoring methods

To monitor incidental catches of vulnerable species, several approaches can be applied:

Harmonized mandatory data: Utilize the GFCM reporting system on incidental catches of vulnerable species, including outputs from initiatives such as the Medbycatch project and Carpentieri et al. (2021).

Direct observations:

- ✓ On-board observers monitoring commercial catches. Due to limited current data, GFCM is developing a regional pioneer sampling program with observers.
- ✓ Dedicated surveys designed to quantify bycatch.
- ✓ Fishers sampling their own bycatch, following standardized protocols.
- ✓ Questionnaires and interviews with fishers to collect qualitative data, complementing on-board observations and providing an integrated management perspective.

Useful documents

- ✓ FAO. (2019a). *Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries : Methodology for data collection* (FAO Fisheries and Aquaculture Technical Paper No. 640). FAO. https://spa-rac.org/en/publication/download/1011/monitoring-the-incidental-catch-of-vulnerable-species-in-mediterranean-and-black-sea-fisheries-methodology-for-data-collection
- ✓ Otero, M. M., Serena, F., Gerovasileiou, V., Barone, M., Bo, M., Arcos, J. M., Vulcano, A., Xavier, J. (2019). ***Identification guide of vulnerable species incidentally caught in Mediterranean fisheries***. IUCN, Málaga, Spain. 204 pp. https://portals.iucn.org/library/sites/library/files/documents/2019-050-En.pdf
- ✓ FAO. (2017a). *Deep-Sea Corals of the Mediterranean Sea*. https://openknowledge.fao.org/items/c958c805-d416-45e0-a2a0-e1073b507067
- ✓ FAO. (2017b). *Deep-sea Sponges of the Mediterranean Sea*. https://openknowledge.fao.org/items/9a45ed26-16d1-4ac0-9af2-6ca264d84812

EO6 [Sea-floor integrity]

Proposed COMMON INDICATOR 37. Extent of physical loss of natural habitat

Proposed COMMON INDICATOR 38. Extent of adverse effects on benthic habitat

These two Common Indicators were submitted for approval at COP 24 (1–5 December 2025). Their development should take place in close collaboration with other Ecological Objectives (EO1, EO3, and EO8). EO6 is intended to have a broad scope, covering all seabed habitats across the Mediterranean – from the littoral zone to the deep-sea floor (see UNEP/MED WG.630/4).

EO10 [Marine litter]

COMMON INDICATOR 23. *Trends in the amount of litter in the water column, including microplastics, and on the seafloor*

Target: The sampling strategy should provide high-quality, detailed data that allow identification of likely sources, assessment of trends over time, and evaluation of the effectiveness of management measures.

GENERAL CONSIDERATIONS

CI23. *Trends in the amount of litter in the water column, including microplastics, and on the seafloor*

Sampling

- The most common and cost-efficient method for evaluating seafloor litter distribution is opportunistic sampling.
- Procedures for assessing seafloor litter are similar to those used in benthic and biodiversity surveys. In deep-sea areas, this can be performed using submersibles or ROVs, although such equipment is expensive.

- Sampling is often conducted in conjunction with fisheries surveys, professional bottom trawling operations, monitoring in MPAs, offshore platforms, or regular biodiversity monitoring programs.
- Sampling units should be stratified based on potential litter sources (urban, rural, riverine inputs) and impacted offshore areas (major currents, shipping lanes, fisheries areas). Emphasis should be placed on the abundance and type of items (e.g., bags, bottles, plastic fragments) rather than their mass.

Data acquisition and monitoring methods

Monitoring programs for demersal fish stocks (e.g., MEDITS) operate at a large regional scale, mainly on the continental shelf, using harmonized protocols. These programs can provide consistent data to support regional-scale seafloor litter monitoring on a regular basis. Despite this, trawling is generally not recommended as a sampling strategy due to its destructive impact. Sampling during ROV-based habitat surveys is likely the most suitable approach for deep-sea habitats, allowing non-destructive, high-resolution data collection on litter distribution.

ASSESSMENT GUIDELINES

CI23. *Trends in the amount of litter in the water column, including microplastics, and on the seafloor*

Strategy

- The strategy for monitoring deep-sea marine litter should be determined nationally by each Contracting Party. Large-scale evaluations are currently limited due to resource constraints for deep-sea data collection.
- Monitoring efforts should focus on the most affected areas, with coastal canyons being a priority, as litter tends to accumulate there. Because litter degrades slowly in deep-sea waters, a multiyear assessment is generally sufficient to detect trends.

Data acquisition and monitoring methods

- Existing data should be leveraged whenever possible, including trawling surveys (e.g., MEDITS) and ROV images obtained during habitat characterization or monitoring.
- Methods are derived from the MEDITS protocol (MEDITS Working Group, 2017).

Templates for data recording are included in the MEDITS Manuals and FAO technical guidelines for trawl surveys (Carpentieri et al., 2020).

Marine litter items **can be categorized** for deep-sea seafloor monitoring as follows:

- Artificial polymer materials / plastics,
- Paper and cardboard,
- Wood (processed or not),
- Metal,
- Glass and ceramics,
- Cloth (textile),
- Rubber
- Other miscellaneous items.

These categories are adapted from the EU MSFD list and further refined in Fleet et al. (2021) and the EU online catalogue of the Joint List of Litter Categories (Link). In deep-sea hard substrate habitats, fishing gear is among the most commonly observed litter items (Giusti et al., 2019).

The following documents can be useful to assess litter in deep-sea habitats including on soft bottoms. Useful guidance for monitoring litter in deep-sea can be searched for in:

- Galgani, F., Ruiz, O.S.P.L., Ronchi, F., Tallec, K., Fischer, E., Matiddi, M. et al. (2023). *Guidance on the monitoring of marine litter in European seas*. JRC Publications Repository. https://doi.org/10.2760/59137
- Fleet, D., Vlachogianni, Th., Hanke, G. (2021). *Joint list of litter categories for marine macro-litter monitoring: Manual for the application of the classification system*. Publications Office of the European Union. https://data.europa.eu/doi/10.2760/127473
- Carpentieri, P., Bonanno, A., Scarcella, G. (2020). *Technical guidelines for scientific surveys in the Mediterranean and the Black Sea* (FAO Fisheries and Aquaculture Technical Papers No. 641; p. 108). FAO. https://archimer.ifremer.fr/doc/00710/82198/87002.pdf

III. RECOMMENDATIONS

Marine caves and deep-sea habitats are among the most ecologically unique and sensitive environments in the Mediterranean. Historically, these “dark habitats” have been largely overlooked due to their remoteness and the technical challenges associated with their study. In recent years, scientific understanding has grown, but significant knowledge gaps remain, particularly in the southern and eastern Mediterranean. These habitats face multiple threats and pressures, including extraction of living and non-living resources, pollution, climate change, biological invasions, and unregulated human visitation (for marine caves), making coordinated research and conservation efforts a pressing priority.

The following recommendations provide guidance for scientists, policymakers, and managers working to protect these habitats.

Strategic planning and policy development

Countries should develop national action plans tailored to the conservation of dark habitats, taking into account local ecological and socio-economic conditions. Policies should ensure that human activities such as coastal development, fishing, and tourism are carefully assessed for their impacts on these vulnerable ecosystems. Enforcement of existing regulations is essential, particularly those aimed at reducing destructive fishing practices such as bottom trawling. At the same time, sustainable fishing practices should be promoted in areas where ecosystem engineers – such as corals, sponges, and other habitat-forming species – occur, including the use of modified gear or alternative fishing methods.

In addition, MPAs and no-take zones should be established to conserve sites of high ecological value. Management plans for these areas should integrate habitat sensitivity and species protection, considering restoration or mitigation measures where habitats have been degraded.

Inventory, monitoring and methodological advances

Effective conservation requires robust and coordinated mapping and monitoring programs. Efforts should prioritize underrepresented regions and include both historical and contemporary data to allow long-term trend analyses. Baseline surveys are critical to identify the most sensitive areas and to guide management decisions.

Monitoring should employ standardized methods to ensure spatial and temporal comparability. In marine caves, this includes photoquadrats, visual and video surveys, and the use of photogrammetry and 3D habitat modelling to capture spatial structure, species distributions, and temporal changes. For deep-sea habitats, the combination of ROVs, AUVs, and standardized benthic sampling allows the assessment of community composition and environmental pressures over large scales.

Accurate species identification is fundamental. Investments in taxonomic expertise are needed, and the development of field guides for caves and deep-sea habitats is strongly recommended. Monitoring programs should record not only species presence but also functional traits (e.g., morphology), life history characteristics, and ecological interactions.

Although soft-bottom habitats are not explicitly referenced in the title of the DHAP, specific habitats – such as bathyal mud and sand hosting crinoids, alcyonaceans, and sponges – should also be considered. These environments support species that are highly vulnerable to mechanical disturbance, including impacts from bottom-trawling activities.

Assessing threats

Both marine caves and deep-sea habitats are exposed to a wide range of pressures. These threats should be systematically documented and quantified to support risk assessments and inform management actions. In deep-sea habitats, measures to reduce bycatch, such as limiting soak times of fishing gear, avoiding areas with VMEs, and improving gear design, can help minimize impacts. Where feasible, active restoration techniques should be explored.

Data management and collaboration

Data collected through inventories and monitoring programmes should be stored in centralized and accessible databases to facilitate information sharing and long-term use. Mediterranean countries are encouraged to establish collaborative data networks and to promote open data publication (e.g., recent releases of RAMOGE deep-sea exploration data here – Schohn et al., 2024). Such initiatives enable scientists, managers, and policymakers to share methodologies, exchange best practices, and integrate findings across the region.

Capacity building is also essential. Training workshops, thematic symposia, and inter-institutional collaborations can improve species identification skills, strengthen research networks, and promote consistent monitoring protocols.

Awareness, education, and stakeholder engagement

Public awareness is critical for the protection of dark habitats. Educational initiatives should target local communities, youth, and the general public, emphasizing the ecological importance of marine caves and deep-sea ecosystems and the threats they face. Engaging stakeholders – including the fishers, tourism operators, and local authorities – can help reduce impacts and encourage sustainable practices.

Long-term research and conservation priorities

Despite recent advances, knowledge gaps remain. Research should focus on underrepresented regions, and understudied habitats and species. Long-term, large-scale monitoring programs are needed to detect changes in biodiversity, habitat condition, and anthropogenic pressures on dark habitats over time.

Given the high costs and technical challenges of deep-sea research, international cooperation and interdisciplinary collaboration are essential. Joint projects and shared funding can improve spatial coverage, reduce duplication of effort, and facilitate more effective conservation outcomes.

Summary of key actions

- Develop national and regional action plans for dark habitats.
- Conduct systematic mapping and baseline inventories of marine caves and deep-sea habitats.
- Strengthen legislation and enforcement to reduce harmful human impacts.
- Establish standardized monitoring protocols and adopt new technologies.
- Build taxonomic expertise and publish identification guides.
- Document and quantify threats, including marine litter, invasive species, and fishing impacts.

- Integrate data into shared platforms and promote international cooperation.
- Engage stakeholders and raise public awareness through dedicated education and communication campaigns.
- Implement restoration measures and adaptive management strategies where needed.
- Support long-term, interdisciplinary research and coordinated monitoring programs.

APPENDICES

APPENDIX 1 – Basic guidelines for locating and characterizing the physical environment of marine caves

1. **Collect existing information:** Gather available data on cave location and internal morphology from existing literature, web-based sources, local ecological knowledge (e.g., fishers, recreational and professional divers), or citizen science initiatives.
2. **Locate caves:** Identify potential cave areas from the sea surface by following the coastline by boat, maintaining close proximity to the rocky shore and observing geological features that may indicate the presence of partially or fully submerged caves (e.g., openings, ridges, fractures, or overhangs near sea level). Marine caves are more likely to occur in karstic areas. Fully submerged caves can be located through direct visual surveys of rock walls and the seabed conducted by divers or with the use of Remotely Operated Vehicles (ROVs). Diver Propulsion Vehicles (DPVs) may also be employed to extend the exploratory range and facilitate the scanning of larger areas.
3. **Document entrance location:** Take geotagged photographs of each cave entrance and record surface coordinates using a GPS device.
4. **Ensure diver and snorkeler safety:** Enter caves cautiously, following a precautionary approach. During storms, periods of strong swell, or intense wave activity, snorkelers and divers should avoid entering marine caves for safety reasons and, when necessary, use protective helmets. For submerged caves, divers should adjust buoyancy, use a calibrated dive line, carry additional lights and cutting tools, and ensure that only individuals with specific training in cavern or cave exploration conduct such dives.
5. **Minimize disturbance to marine fauna:** In areas inhabited by Mediterranean monk seals, maintain a position close to the cave walls to allow the animal free passage. Leave the cave immediately if a seal is encountered. Ideally, cave exploration should be scheduled during periods of low in-cave seal activity to minimize potential disturbance.
6. **Record topographic and visual data:** In each cave, record at minimum the key topographic parameters (e.g., entrance depth and dimensions, cave morphology, submersion level, maximum water depth, and length) and collect comprehensive photo and video documentation to support visual records of cave structure and habitat features.

APPENDIX 2 – Basic guidelines for the characterization of marine caves

1. Select appropriate characterization methods

Choose methods suited to the cave type (e.g., semi- or fully submerged), target community or species (e.g., sessile hard-substrate assemblages, infaunal soft-sediment organisms, or motile fauna), and substrate (i.e., hard cave walls and ceilings vs. soft sedimentary floors).

- Use non-invasive techniques (photoquadrats, video surveys, photogrammetry, visual censuses) for hard-substrate and motile communities, which support several fragile and protected species.
- Apply destructive sampling only when necessary (e.g., taxonomic verification and epi- or infaunal communities), collecting minimal material to avoid habitat disturbance.
- Consider all ecological cave zones (entrance, semidark, and dark) and adapt methods accordingly.

2. Design a robust and safe sampling strategy

Plan sampling to capture environmental gradients and spatial heterogeneity.

- Cover both horizontal (entrance–interior) and vertical (walls, ceiling, floor) variation.
- For hard substrates, photograph 1–4 m^2 per cave, using 20 x 20 or 25 x 25 cm quadrats and at least 10–30 replicates per zone.
- For soft substrates, collect replicate sediment cores (0–10 cm depth) along the entrance–interior cave axis for community and sediment analyses.
- For motile fauna, use standardized visual transects (2–3 m wide, 5–25 m long) or time-transects (3 minutes each) across all zones and sub-habitats.
- All designs should incorporate replication, zonal coverage, and temporal consistency, considering diel and seasonal variability in cave fauna.
- Always balance scientific objectives with diver safety, minimizing bottom time and following a precautionary approach.

3. Prepare and check equipment

Before each survey, test and prepare all diving and sampling equipment, including:

- underwater lights and cameras,
- quadrat frames of appropriate dimensions,
- measuring tapes or calibrated reels,
- data slates or tablets,
- containers and labels for biological samples,
- spare equipment and backups for lights, reels, and batteries.

4. Obtain required permits and authorizations

Obtain all required research and sampling permits from relevant authorities or MPA managers. Permits must cover all intended activities, especially when working with protected species or within MPAs.

5. Assess environmental and safety conditions

Prior to any fieldwork:

- Review weather forecasts, sea state (e.g., waves or swell), and visibility.
- Notify relevant authorities and colleagues of the planned fieldwork dates, dive times and locations, names and contact details of participating divers.
- Prioritize diver safety over sampling completeness. All cave dives must follow established scientific diving and safety protocols under a precautionary approach. Maintain continuous visual contact between team members, use guide ropes or calibrated reels for navigation, and ensure a surface contact person is informed before entry and immediately after exit from each dive.

6. Manage samples and data immediately

After fieldwork:

- Label, organize, and archive all materials (photos, samples, field sheets, metadata).
- Back up digital files and store specimens properly to prevent degradation.
- Photograph specimens before preservation to document colour and morphology.
- Use image-processing tools for cover and abundance estimates; classify taxa by morpho-functional or trophic groups when species-level identification is not possible.

7. Ensure ethical and scientific standards

All research should follow minimal-impact, reproducible, and transparent practices.

- Standardize methods to allow comparison between sites and times.
- Avoid unnecessary disturbance of fragile species and habitats.
- Archive and share data in accessible repositories to support long-term monitoring.

APPENDIX 3 – Practical considerations for implementing photogrammetric surveys in marine caves

1. Camera system

Selection of the camera system – whether an action camera or a higher-performance compact, reflex, or mirrorless camera – should be guided by the required final output resolution, available budget, and operational constraints. In narrow or confined spaces, the physical size of underwater housings may limit manoeuvrability and hinder use in tight passages.

2. Camera settings

Because contemporary Structure-from-Motion (SfM) workflows rely on still images, time-lapse mode is generally preferred. Depending on the operator's swimming speed, a shooting interval of 0.5–2 secs is recommended to maintain 60–80% overlap between consecutive frames. ISO and shutter-speed settings should be adjusted according to ambient light, the artificial lighting system used, and the operator's ability to maintain stability during image capture. To ensure high-quality 3D reconstruction, images must be sharp and free of motion blur.

3. Illumination system

Strobes and/or continuous video lights may be used to illuminate the field of view. The chosen lighting system must provide homogeneous illumination to minimize shadows, hotspots, and other artefacts that could degrade the final 3D model.

4. Image acquisition pattern

During image acquisition, the operator should maintain a relatively constant distance from the substrate, typically 0.3–1 m, depending on the desired resolution of the 3D model or orthomosaic. Contact with the cave floor, walls, ceiling, or benthic biota must be avoided to prevent sediment resuspension and ecological disturbance. The survey path should be adapted to the cave's topography, but a combined vertical-horizontal zigzag (boustrophedonic) pattern is commonly employed to ensure complete coverage and adequate vertical and horizontal image overlap.

5. Diving skills

Operators must have sufficient diving skills to ensure both personal safety and the protection of the cave environment and its benthic communities throughout image acquisition.

6. Scaling of 3D reconstructions

Accurate scaling of the resulting 3D models requires the inclusion of objects of known dimensions within the scene. At least five flat metric reference scales (e.g., rulers, quadrats, triaxial scales) should be positioned within the cave prior to imaging and must remain fixed for the duration of the survey.

APPENDIX 4 – IMAP Ecological Objectives (EO) and Common Indicators (CI) selected (highlighted in blue) for the monitoring of marine cave and deep-sea habitats

Ecological Objective (EO)	COMMON INDICATORS (CI)	Marine caves	Deep-sea habitats
EO1	**1. Habitat distributional range (EO1) to also consider habitat extent as a relevant attribute**	**Yes**	**Yes**
	2. Condition of the habitat's typical species and communities (EO1)	**Yes**	**Yes**
	3. Species distributional range (EO1 related to marine mammals, seabirds, marine reptiles)		
	4. Population abundance of selected species (EO1, related to marine mammals, seabirds, marine reptiles)		
	5. Population demographic characteristics (EO1, e.g., body size or age class structure, sex ratio, fecundity rates, survival/mortality rates related to marine mammals, seabirds, marine reptiles)		
EO2	**6. Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas (EO2, in relation to the main vectors and pathways of spreading of such species)**	**Yes**	**Yes**
EO3	7. Spawning stock Biomass (EO3)		
	8. Total landings (EO3)		
	9. Fishing Mortality (EO3)		
	10. Fishing effort (EO3)		
	11. Catch per unit of effort (CPUE) or Landing per unit of effort (LPUE) as a proxy (EO3)		
	12. Bycatch of vulnerable and non-target species (EO1 and EO3)		**Yes**
EO4	*The development of CIs for EO4 is currently in progress.*	Potentially	
EO5	13. Concentration of key nutrients in water column (EO5)	Potentially	
	14. Chlorophyll-a concentration in water column (EO5)		
EO6	**37. Extent of physical loss of natural habitat ***	**Yes**	**Yes**
	38. Extent of adverse effects on benthic habitats *	**Yes**	**Yes**
EO7	15. Location and extent of the habitats impacted directly by hydrographic alterations (EO7) to also feed the assessment of EO1 on habitat extent	Potentially	
EO8	16. Length of coastline subject to physical disturbance due to the influence of man-made structures (EO8) to also feed the assessment of EO1 on habitat extent	Potentially	
EO9	17. Concentration of key harmful contaminants measured in the relevant matrix (EO9, related to biota, sediment, seawater)		
	18. Level of pollution effects of key contaminants where a cause-and-effect relationship has been established (EO9)		

	19. Occurrence, origin (where possible), and extent of acute pollution events (e.g., slicks from oil, oil products and hazardous substances) and their impact on biota affected by this pollution (EO9)	Potentially	
	20. Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels in commonly consumed seafood (EO9)		
	21. Percentage of intestinal enterococci concentration measurements within established standards (EO9)		
EO10	22. Trends in the amount of litter washed ashore and/or deposited on coastlines (including analysis of its composition, spatial distribution and, where possible, source) (EO10)	Potentially	
	23. Trends in the amount of litter in the water column (including microplastics) and on the seafloor (EO10)	**Yes**	**Yes**
CANDIDATE INDICATORS			
EO10	24. Candidate Indicator: Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds and marine turtles (EO10)		
EO8	25. Candidate Indicator: Land use change (EO8)		
EO11	26. Candidate indicator: Proportion of days and geographical distribution where loud, low, and mid-frequency impulsive sounds exceed levels that are likely to entail significant impact on marine animals (EO11)		
	27. Candidate Indicator: Levels of continuous low frequency sounds with the use of models as appropriate (EO11)		

* These CIs were proposed for validation at COP 24 (December 2025).

APPENDIX 5 – List of common and typical species in Mediterranean marine caves

* Species listed in Annex II of the SPA/BD Protocol – List of endangered or threatened species
** Species listed in Annex III of the SPA/BD Protocol – List of species whose exploitation is regulated
‡ Species that may be uncommon or present at low densities yet are nonetheless characteristic of marine cave habitats

FORAMINIFERA
Miniacina miniacea (Pallas, 1766)

PORIFERA
Aaptos aaptos (Schmidt, 1864)
Acanthella acuta Schmidt, 1862
Agelas oroides (Schmidt, 1864)
Aplysilla sulfurea Schulze, 1878
Aplysina cavernicola (Vacelet, 1959) * ‡ – more common in Western Mediterranean caves
Axinella damicornis (Esper, 1794)
Axinella verrucosa (Esper, 1794)
Chondrosia reniformis Nardo, 1847 – often discoloured
Clathrina spp.
Cliona spp.
Crambe crambe (Schmidt, 1862)
Dendroxea lenis (Topsent, 1892)
Diplastrella bistellata (Schmidt, 1862)
Erylus discophorus (Schmidt, 1862)
Haliclona (Halichoclona) fulva (Topsent, 1893)
Haliclona (Rhizoniera) sarai (Pulitzer-Finali, 1969)
Haliclona (Soestella) mucosa (Griessinger, 1971)
Hexadella pruvoti Topsent, 1896 ‡
Ircinia oros (Schmidt, 1864)
Ircinia variabilis (Schmidt, 1862)
Merlia normani Kirkpatrick, 1908
Myrmekioderma spelaeum (Pulitzer-Finali, 1983) ‡
Neophrissospongia spp. ‡
Oscarella spp.
Penares spp.
Petrobiona massiliana Vacelet & Lévi, 1958 * ‡
Petrosia (Petrosia) ficiformis (Poiret, 1789) – often discoloured
Phorbas tenacior (Topsent, 1925)
Plakina spp.
Pleraplysilla spinifera (Schulze, 1879)
Spirastrella cunctatrix Schmidt, 1868
Spongia (Spongia) officinalis Linnaeus, 1759 **
Spongia (Spongia) virgultosa (Schmidt, 1868)
Terpios gelatinosa (Bowerbank, 1866)
Thymosiopsis spp. ‡

CNIDARIA
Astroides calycularis (Pallas, 1766) * – in southern areas of the Western Mediterranean
Caryophyllia (Caryophyllia) inornata (Duncan, 1878)
Cerianthus membranaceus (Spallanzani, 1784)
Clytia spp.
Corallium rubrum (Linnaeus, 1758) ** ‡
Eudendrium spp.
Eunicella cavolini (Koch, 1887) – more common at the entrance of Western Mediterranean caves
Halecium spp.
Hoplangia durotrix Gosse 1860
Leptopsammia pruvoti Lacaze-Duthiers 1897
Madracis pharensis (Heller, 1868)
Obelia dichotoma (Linnaeus, 1758)
Paramuricea clavata (Risso, 1827) – more common at the entrance of Western Mediterranean caves
Parazoanthus axinellae (Schmidt, 1862)
Phyllangia americana mouchezii (Lacaze-Duthiers, 1897)
Polycyathus muellerae (Abel, 1959)

ANNELIDA
Bonellia viridis Rolando, 1822
Filograna implexa Berkeley, 1835
Filogranula spp.
Hermodice carunculata (Pallas, 1766)
Josephella marenzelleri Caullery & Mesnil, 1896
Protula tubularia (Montagu, 1803)
Semivermilia crenata (O. G. Costa, 1861)
Serpula cavernicola Fassari & Mollica, 1991 ‡
Serpula vermicularis Linnaeus, 1767
Spiraserpula massiliensis (Zibrowius, 1968) ‡
Vermiliopsis labiata (O. G. Costa, 1861)
Vermiliopsis monodiscus Zibrowius, 1968 ‡

CRUSTACEA
Athanas nitescens (Leach, 1814)
Brachycarpus biunguiculatus (Lucas, 1846) ‡
Dardanus spp.
Dromia personata (Linnaeus, 1758)
Eualus occultus (Lebour, 1936)
Galathea strigosa (Linnaeus, 1761)
Hemimysis spp.
Herbstia condyliata (Fabricius, 1787)
Lysmata nilita Dohrn & Holthuis, 1950
Lysmata seticaudata (Risso, 1816)
Palaemon serratus (Pennant, 1777)
Palinurus elephas (Fabricius, 1787) **
Plesionika narval (Fabricius, 1787) – more common in Eastern Mediterranean caves
Scyllarides latus (Latreille, 1802) **

Scyllarus arctus (Linnaeus, 1758) **
Siriella gracilipes Nouvel, 1942
Stenopus spinosus Risso, 1826

MOLLUSCA
Barbatia barbata (Linnaeus, 1758)
Clanculus spp.
Homalopoma sanguineum (Linnaeus, 1758) ‡
Lima lima (Linnaeus, 1758)
Lithophaga lithophaga (Linnaeus, 1758) *
Luria lurida (Linnaeus, 1758) *
Muricopsis cristata (Brocchi, 1814)
Naria spurca (Linnaeus, 1758) *
Neopycnodonte cochlear (Poli, 1795)
Peltodoris atromaculata Bergh, 1880
Rocellaria dubia Pennant, 1777

BRACHIOPODA
Argyrotheca spp.
Joania cordata (Risso, 1826)
Megathiris detruncata (Gmelin, 1790)
Novocrania spp.
Tethyrhynchia mediterranea Logan & Zibrowius, 1994 ‡

BRYOZOA
Erect rigid:
Adeonella spp.
Myriapora truncata (Pallas, 1766)
Reteporella spp.
Schizoretepora spp.
Schizotheca fissa (Busk, 1856)
Erect flexible:
Caberea boryi (Audouin, 1826)
Candidae spp.
Chlidonia pyriformis (Bertoloni, 1810)
Crisia spp.
Encrusting – laminar (uni- or multi-laminar) and celleporiform (elevate colonies with disorderly arranged zooids):
Celleporina caminata (Waters, 1879)
Cribrilinidae – including *Cribrilaria* spp. and *Glabrilaria* spp.
Hippaliosina depressa (Busk, 1854) – restricted in Eastern Mediterranean caves
Onychocella spp.
Schizomavella spp.
Setosella spp.
Turbicellepora spp.

ECHINODERMATA

Arbacia lixula (Linnaeus, 1758)
Centrostephanus longispinus (Philippi, 1845) *
Holothuria spp.
Ophioderma longicauda (Bruzelius, 1805)
Ophiothrix fragilis (Abildgaard, 1789)
Paracentrotus lividus (de Lamarck, 1816) **
Ophidiaster ophidianus (Lamarck, 1816) *

ASCIDIACEA

Aplidium spp.
Didemnum spp.
Halocynthia papillosa (Linnaeus, 1767)
Microcosmus spp.
Pyura spp.

TELEOSTEI

Anthias anthias (Linnaeus, 1758)
Apogon (Apogon) imberbis (Linnaeus, 1758)
Atherina spp.
Chromis chromis (Linnaeus, 1758)
Conger conger (Linnaeus, 1758)
Corcyrogobius liechtensteini (Kolombatovic, 1891)
Diplodus spp.
Epinephelus costae (Steindachner, 1878)
Epinephelus marginatus (Lowe, 1834)
Gammogobius steinitzi Bath, 1971 ‡
Grammonus ater (Risso, 1810) ‡
Marcelogobius splechtnai (Ahnelt & Patzner, 1995) ‡
Oblada melanura (Linnaeus, 1758)
Phycis phycis (Linnaeus, 1766)
Sciaena umbra Linnaeus, 1758 *
Scorpaena spp.
Serranus cabrilla (Linnaeus, 1758)
Serranus scriba (Linnaeus, 1758
Thorogobius ephippiatus (Lowe, 1839)
Tripterygion spp.

APPENDIX 6 – List of principal functional groups for monitoring in marine caves

Passive filter feeders (cnidarians)
Hydrozoans, e.g.: *Clytia* spp., *Eudendrium* spp., *Halecium* spp., *Obelia dichotoma*
Anthozoans, e.g.: *Astroides calycularis*, *Caryophyllia inornata*, *Corallium rubrum*, *Eunicella cavolini*, *Hoplangia durotrix*, *Leptopsammia pruvoti*, *Paramuricea clavata*, *Parazooanthus axinellae*
Large active filter feeders (e.g., massive sponges, bivalves, erect bryozoans, ascidians)
Sponges (massive and large forms), e.g.: *Agelas oroides*, *Chondrosia reniformis*, *Haliclona* spp.
Bivalve molluscs, e.g.: *Lithophaga lithophaga*, *Neopycnodonte cochlear*
Bryozoans (large and erect forms), e.g.: *Adeonella* spp., *Myriapora truncata*, *Reteporella* spp.
Large ascidians, e.g.: *Aplidium* spp., *Halocynthia papillosa*, *Microcosmus* spp., *Pyura* spp.
Small Active Filter Feeders
Encrusting and small-sized sponges, e.g.: *Crambe crambe*, *Dendroxea lenis*, *Diplastrella bistellata*, *Petrobiona massiliana*, *Spirastrella cunctatrix*
Polychaetes, e.g.: Serpulidae
Brachiopods, e.g.: *Argyrotheca* spp., *Joania* spp., *Novocrania* spp.
Encrusting and small bryozoans, e.g.: *Celleporina caminata*, *Cribrilaria* spp., *Glabrilaria* spp., *Hippaliosina depressa*, *Onychocella marioni*, *Setosella* spp.
Small ascidians, e.g.: *Didemnum* spp.
Detritus feeders and omnivores
Crustaceans, e.g.: *Dardanus* spp., *Dromia personata*, *Galathea strigosa*, *Herbstia condyliata*, *Scyllarus arctus*
Echinodermata, e.g.: *Holothuria* spp., ophiurids, sea urchins (e.g., *Arbacia lixula*, *Paracentrotus lividus*)
Annelids, e.g.: *Bonellia viridis*, *Hermodice carunculata*
Cave-dwelling mysids
e.g., *Hemimysis* spp. *Siriella gracilipes* – to be classified as absent, few, or swarm
Characteristic carnivores
Fishes, e.g.: *Apogon imberbis*, *Gammogobius steinitzi*, *Grammonus ater*, *Scorpaena notata*, *S. maderensis*, *Thorogobius ephippiatus*
Crustaceans, e.g.: *Lysmata nilita*, *L. seticaudata*, *Palaemon serratus*, *Plesionika narval*, *Stenopus spinosus*
Associate carnivores
Fishes, e.g.: *Anthias anthias*, *Chromis chromis*, *Conger conger*, *Corcyrogobius liechtensteini*, *Marcelogobius plechtnai*, *Diplodus* spp., *Epinephelus* spp., *Gobius* spp., *Oblada melanura*, *Phycis phycis*, *Sciaena umbra*, *Scorpaena scrofa*, *Serranus* spp.
Crustaceans, e.g.: *Palinurus elephas*, *Homarus gammarus*, *Scyllarides latus*,
Anemones, e.g.: *Cerianthus membranaceus*

APPENDIX 7 – Major drivers of alterations identified in Mediterranean marine caves

The major drivers of alterations identified in Mediterranean marine caves are described below:

- **Climate change:**
  Marine heatwaves and temperature anomalies have been identified as key drivers of change in both motile and sessile cave communities. Thermal stress has led to the replacement of typical cave-dwelling mysids, which form large swarms, by thermotolerant congeners which form smaller groups, thereby altering trophic dynamics (Chevaldonné & Lejeusne, 2003). Heat-related mortality (i.e., partial or total necrosis) has also been recorded in sensitive sessile taxa such as rhodophytes (at the cave entrance zone), sponges, anthozoans, and bryozoans, with limited recovery potential and cascading effects on ecosystem functioning (Digenis et al., 2022; Garrabou *et al.*, 2022; Carlot *et al.*, 2025).
- **Local human pressures:**
  In several caves coastal construction, harbour expansion, and beach nourishment can contribute to habitat modification and increased sedimentation in adjacent marine caves. These activities – combined with temperature anomalies due to climate change – drive the structural and functional homogenization of cave assemblages, such as the replacement of three-dimensional growth forms with two-dimensional ones (e.g., encrusting taxa) and increased turf and sediment cover (Nepote *et al.*, 2017; Costa *et al.*, 2018; Montefalcone *et al.*, 2018, 2023; Sempere-Valverde *et al.*, 2019).
- **Pollution and marine litter:**
  Marine caves often accumulate debris on internal beaches, particularly in semi-submerged caves, or in confined dark zones (e.g., ceilings of submerged caves) with limited water circulation. This trapped litter poses entanglement, smothering, and chemical-pollution risks to benthic organisms (Mačić *et al.*, 2018b; Öztürk *et al.*, 2019; Gerovasileiou & Bianchi, 2021; Digenis *et al.*, 2022). Additionally, the inner sectors of karstic marine caves may be vulnerable to nutrient enrichment associated with groundwater discharges (Sempere-Valverde et al., 2019; Navarro-Barranco et al., 2023b).
- **Direct physical disturbance:**
  Fragile, erect, or calcareous benthic species are highly susceptible to mechanical damage caused by unregulated visits by SCUBA divers and tourist boats. In addition, associated pressures include sediment resuspension and air-bubble accumulation under cave ceilings, which further degrade sensitive microhabitats (Milazzo *et al.*, 2002; Di Franco *et al.*, 2010; Guarnieri *et al.*, 2012; Di Camillo et al., 2025; Quiles-Pons et al., 2025).
- **Extraction of living resources:**
  Illegal harvesting of the red coral (*Corallium rubrum*) and spearfishing (e.g., of *Sciaena umbra* and *Phycis phycis)* continue to affect some unprotected caves, contributing to local biodiversity loss (Ouerghi et al., 2019; Navarro-Barranco et al., 2023b)
- **Non-indigenous species:**
  The introduction and establishment of non-indigenous species (NIS) – and particularly invasives – represent an emerging, though insufficiently studied, pressure that can alter both motile and sessile community structure of marine caves, especially in the eastern Mediterranean basin (Gerovasileiou *et al.*, 2016c, 2022; Digenis *et al.*, 2022, 2025).

For more detailed information on threats and pressures affecting dark habitats, see Navarro-Barranco et al. (2023b).

APPENDIX 8 – Example of a field data sheet for marine cave monitoring

Area: **Date:** **Observer:**

Latitude: **Longitude:**

Submersion level: Submerged / Semi-submerged **Cave morphology:** Blind / Tunnel - No. entrances:

Total length: **Max depth:** **Minimum depth:**

Entrance A’ – Max depth (m): Height (m): Width (m): Orientation:

Entrance B’ – Max depth (m): Height (m): Width (m): Orientation:

Other topographic features:

Motile taxa (number of individuals observed per ecological zone)

Taxa	**ENTRANCE**	**SEMIDARK**	**DARK**
Mysids	0	few	swarm
Plesionika narval	0	few	swarm

Cerianthus membranaceus **(No. individuals):**	**Other anemones (No. individuals):**	
Other typical and/or protected species	**Pressures & threats**	
	Boat visits	
	Litter	
	Air bubbles	
	Broken bryozoans	
	Mortality	
	Alien species	
	Other	
	Comments:	

APPENDIX 9 – Introduced species to be considered in monitoring initiatives for marine caves

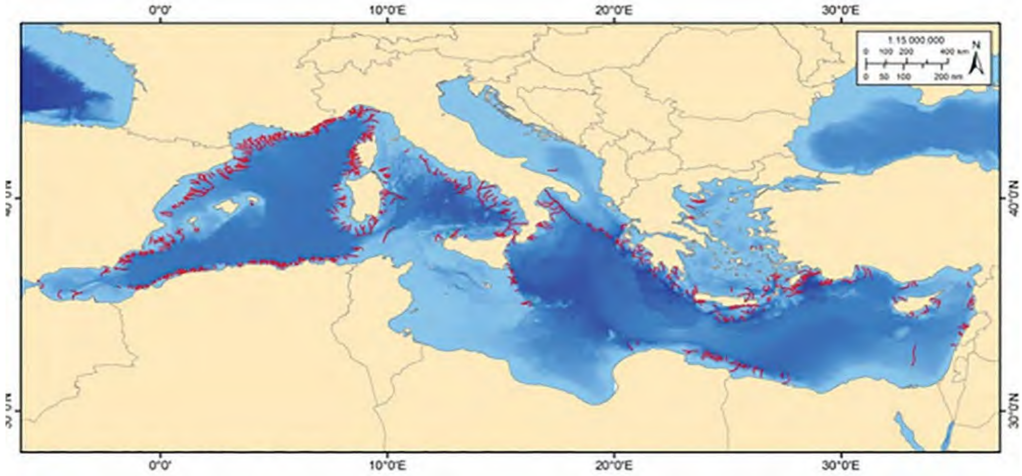
Motile taxa

Diadema setosum (Leske, 1778)
Enchelycore anatina (Lowe, 1838)
Pempheris rhomboidea Kossmann & Räuber, 1877
Percnon gibbesi (H. Milne Edwards, 1853)
Pterois miles (Bennett, 1828)
Sargocentron rubrum (Forsskål, 1775)
Siganus spp. – mostly at the entrance zone of marine caves
Torquigener flavimaculosus Hardy & Randall, 1983
Urocaridella pulchella Yokes & Galil, 2006

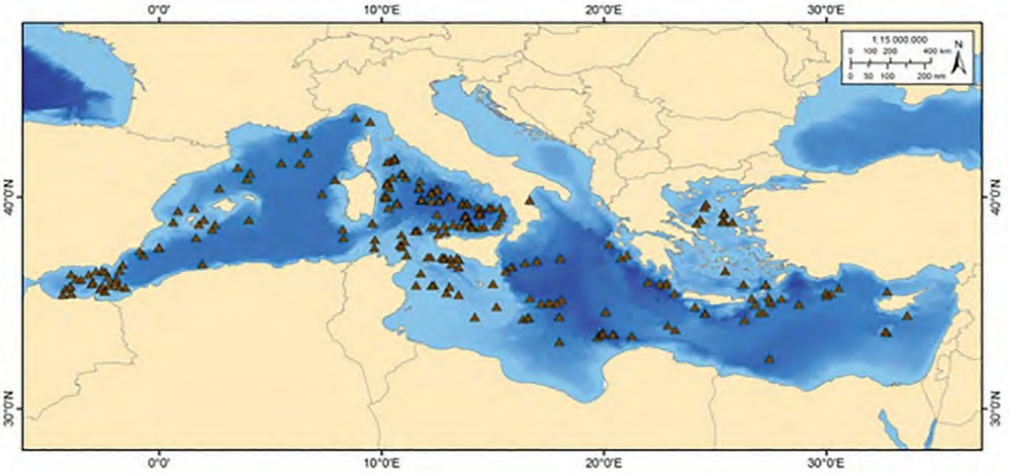
Sessile taxa

Brachidontes pharaonis (P. Fischer, 1870)
Chama pacifica Broderip, 1835
Clytia linearis (Thorneley, 1900)
Dendostrea cf. *folium* (Linnaeus, 1758)
Herdmania momus (Savigny, 1816)
Isognomon spp.
Malleus regula (Forsskål in Niebuhr, 1775)
Septifer cumingii Récluz, 1849
Spondylus spinosus Schreibers, 1793

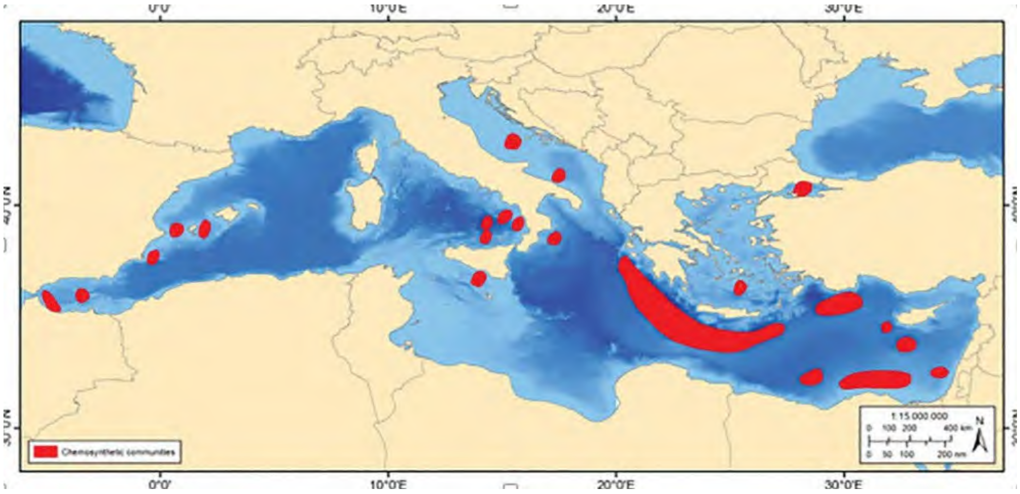
APPENDIX 10 – Distribution maps of known mediterranean submarine canyons, seamounts and chemosynthetic assemblages (from SPA/RAC–UN Environment/MAP & OCEANA, 2017)



Distribution of Mediterranean known submarine canyons.



Distribution of Mediterranean known seamounts.



Distribution of Mediterranean known areas with chemosynthetic assemblages.

APPENDIX 11 – Example of labelling for a fictive expedition

Labelling for a fictive expedition named Altaïr to explore the cold-water corals in the Lacaze-Duthier canyon between 10/10/2035 and 13/10/2035 using a Super Achile ROV

Expedition code	ALT
Canyon code	LD
ROV code	ACH
Dive	DXX, number from 01 to 99 starting at 01 for each new area (e.g., new canyon)

Example of diving table

Date	Dives	Sampling
10/10/2035	D01, D02, D03 vertical to head of canyon, D04 eastern flank near the head	High quality video Photos Water sampling at head of canyon
11/10/2035	No dive, observation of seabirds and mammals	No sampling
12/10/2035	D05 eastern flank for sampling D06 aborted because of technical problems D07, D08 western flank	High quality video Photos Sampling of biota cnidarians and sponges Water sampling with ROV
13/10/2035	D09 again in head of canyon finishing on continental shelf	High quality video Photos No sampling

Example of labelling nomenclature

Type of data	Nomenclature	Example
Dive	ALT_LD_ACH_DXX	ALT_LD_ACH_D01 (first dive)
Videos	ALT_LD_ACH_DXX_XXX	ALT_LD_ACH_D01_025 (25th video of first dive with ROV in the Lacaze-Duthier canyon during the Altaïr expedition). Videos should be good quality, preferably displaying time and depth as well as name. Laser beams can be useful.
Photos	ALT_LD_ACH_DXX_XXXX_HHMMSS	ALT_LD_ACH_D07_0090_101530 90th photo taken at 10:15:30 during dive 7 (the 12/10/35). The time is not necessary in the labelling if the time is included in the photo file and is the same as the ROV track file.
Sample	ALT_LD_ACH_D05_SXX_depth_type	ALT_LD_ACH_D05_S10_350m_cnidaria Name of photos of the sample can be associated
		ALT_LD_ACH_D05_S01_400m_water sample

APPENDIX 12 – Vulnerable Marine Ecosystem (VME) indicators (features, habitats and taxa) as indicated by the General Fisheries Commission for the Mediterranean (GFCM) (from FAO, 2019b)

(a) Mediterranean VME indicator features *The following features potentially support VMEs:*		
✓ Seamounts and volcanic ridges ✓ Canyons and trenches ✓ Steep slopes ✓ Submarine reliefs (slumped blocks, ridges, cobble fields, etc.) ✓ Cold seeps (pockmarks, mud volcanoes, reducing sediment, anoxic pools, methanogenetic hard bottoms) ✓ Hydrothermal vents		
(b) Mediterranean VME indicator habitats *The following habitats potentially support VMEs:*		
Cold-water coral reefs Coral gardens - Hard-bottom coral garden - Soft-bottom coral gardens Sea pen fields	Deep-sea sponge aggregations - “Ostur” sponge aggregations - Hard-bottom sponge gardens - Glass sponge communities - Soft-bottom sponge gardens	Tube-dwelling anemone patches Crinoid fields Oyster reefs and other giant bivalves Seep and vent communities Other dense emergent fauna
(c) Mediterranean VME indicator taxa		
Phylum	**Class**	**Subclass (Order)**
Cnidaria	Anthozoa	Hexacorallia (Antipatharia, Scleractinia) Octocorallia (Alcyonacea, Pennatulacea) Ceriantharia
	Hydrozoa	Hydroidolina
Porifera (sponges)	Demospongiae	
	Hexactinellida	Amphidiscophora Hexasterophora
Bryozoa	Gymnolaemata	Cheilostomatida
	Stenolaemata	Cyclostomatida
Echinodermata	Crinoidea	Articulata
Mollusca	Bivalvia	Gryphaeidae (*Neopycnodonte cochlear, N. zibrowii*) Heterodonta* (Lucinoida) (e.g., *Lucinoma kazani*) Pteriomorphia* (Mytiloida) (e.g., *Idas modiolaeformis*)
Annelida*	Polychaeta	Sedentaria (Canalipalpata) (e.g., *Lamellibrachia anaximandri*, *Siboglinum* spp.)
Arthropoda*	Malacostraca	Eumalacostraca (Amphipoda) (e.g., *Haploops* spp.)

* only chemosynthetic species that indicate the presence of a cold seep or hydrothermal vent are considered

APPENDIX 13 – VME encounter reporting in the GFCM area of application (from FAO, 2019b)

Separate forms to be completed for each deployment of the fishing gear (haul/set) in which VME Indicator Taxa are caught.

A. Fishing Trip Information	
Country:	
Vessel name:	
Captain (name and last name):	
Date of encounter (dd/mm/yyyy):	
B. Fleet and gear information*	
Fleet segment:	
Fishing gear:	
C. VME Encounter coordinates	
GSA:	**Statistical grid:**
Point 1 (Start)	**Point 2 (End)**
Latitude:	Latitude:
Longitude:	Longitude:
Fishing depth (average or range, m):	
VME Feature and/or Habitat	
D. VME Indicator Taxa catch information	
Total live weight of **corals** in the haul/set (kg):	
Total live weight of **sponges** in the haul/set (kg):	
Total live weight of **other vulnerable benthic taxa** in the haul/set (kg):	
Identify VME Taxa to lowest taxonomic level (species if possible) and provide comments.	
F. Pictures of VME Indicator Taxa (by fishers and/or observers on board)	
Take pictures of the different VME Indicator Taxa and submit them as an attachment to the current form.	

* Refer to: GFCM, 2018. GFCM Data Collection Reference Framework (DCRF). Version 25.2. (http://www.fao.org/gfcm/data/dcrf/en/)

REFERENCES

Abadie A., Viala C., Marre G., Boissery P., 2022. *La photogrammétrie sous-marine. Application au suivi des herbiers de posidonie*. Publications scientifiques – Seaviews, 108 pp.

Alessandri, L., Baiocchi, V., Del Pizzo, S., Di Ciaccio, F., Onori, M., Rolfo, M.F., Troisi, S., 2022. A flexible and swift approach for 3D image–based survey in a cave. *Applied Geomatics*, 14 (Suppl 1), 5-19.

André, F., Corolla, J.P., Lanza, B., Rochefort, G., 2014. *Bryozoaires d'Europe. Les carnets du plongeur*. Editions Neptune Plongée, Gargas, 255 pp.

Ape, F., Arigo, C., Gristina, M., Genovese, L., Di Franco, A., Di Lorenzo, M., Baiata, P., Aglieri, G., Milisenda, G., Mirto, S., 2016. Meiofaunal diversity and nematode assemblages in two submarine caves of a Mediterranean Marine Protected Area. *Mediterranean Marine Science*, 17, 202-215.

Baldacconi, R., Trainito, E., 2013. *Spugne del Mediterraneo*. Il Castello, 128 p.

Baldacconi, R., Trainito, E., 2016. *Coralli del Mediterraneo*. Il Castello, 176 pp.

Bay-Nouailhat, A., Bay-Nouailhat, W., 2020. *Guide des Tuniciers de l'Europe de l'Ouest*. Mer et Littoral – M&L éditions, Melgven, 240 pp.

Beijbom, O., Edmunds, P.J., Roelfsema, C., Smith, J., Kline, D.I., Neal, B.P., Dunlap, M.J., Moriarty, V., Fan, T-Y., Tan, C-J., Chan, S., Treibitz, T., Gamst, A., Mitchell, B.G., Kriegman, D., 2015. Towards Automated Annotation of Benthic Survey Images: Variability of Human Experts and Operational Modes of Automation. *PLoS ONE*, 10(7), e0130312.

Belmonte, G., Ingrosso, G., Poto, M., Quarta, G., D'Elia, M., Onorato, R., Calcagnile, L., 2009. Biogenic stalactites in submarine caves at the Cape of Otranto (SE Italy): Dating and hypothesis on their formation. *Marine Ecology*, 30, 376-382.

Bergamin, L., Taddei Ruggiero, E., Pierfranceschi, G., Andres, B., Constantino, R., Crovato, C., d'Ambrosi, A., Marassich, A., Romano, E., 2020. Benthic foraminifera and brachiopods from a marine cave in Spain: Environmental significance. *Mediterranean Marine Science*, 21, 506-518.

Bianchi, C.N., Abbiati, M., Airoldi, L., Alvisi, M., Benedetti-Cecchi, L., Cappelletti, A., Cinelli, F., Colantoni, P., Dando, P.R., Morri, C., Niccolai, I., Picco, P., Southward, A., Southward, E., 1998. Hydrology and water budget of a submarine cave with sulphur water springs: the Grotta Azzurra of Capo Palinuro (Southern Italy). *Proceedings of the Italian Association of Oceanology and Limnology*, 12, 285-301.

Bianchi, C.N., Cattaneo-Vietti, R., Cinelli, F. , Morri, C., Pansini, M., 1996. Lo studio biologico delle grotte sottomarine: Conoscenze attuali e prospettive. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova*, 60-61, 41-69.

Bianchi, C.N., Gerovasileiou, V., Morri, C., Froglia, C., 2022. Distribution and Ecology of Decapod Crustaceans in Mediterranean Marine Caves: A Review. *Diversity*, 14, 176.

Bianchi, C.N., Gerovasileiou, V., Morri, C., Sanfilippo, R., 2021. MC1.53d Brackish water caves or caves subjected to freshwater runoff. p. 223-226. In: Handbook for interpreting types of marine habitats for the selection of sites to be included in the national inventories of natural sites of conservation interest. Montefalcone, M., Bianchi, C.N., Bo, M., Piazzi, L. (Eds), UNEP-MAP/SPA-RAC, Tunis.

Bianchi, C.N., Morri, C., 1994. Studio bionomico comparativo di alcune grotte marine sommerse; definizione di una scala di confinamento. *Memorie dell'Istituto Italiano di Speleologia serie II*, 6, 107-123.

Bianchi, C.N., Morri, C., 2003. Comunità dell'infauna. In: Grotte marine: cinquant'anni di ricerca in Italia (Cicogna, F., Bianchi, C.N., Ferrari, G., Forti, P., Eds). Ministero dell'Ambiente e della Tutela del Territorio, Roma: 267-272.

Bianchi, C.N., Pronzato, R., Cattaneo-Vietti, R., Benedetti-Cecchi, L., Morri, C., Pansini, M., Chemello, R., Milazzo, M., Fraschetti, S., Terlizzi, A., Peirano, A., Salvati, E., Benzoni, F., Calcinai, B., Cerrano, C., Bavestrello, G., 2004. Mediterranean marine benthos: a manual of methods for its sampling and study. Hard bottoms. *Biologia Marina Mediterranea*, 11, 185-215.

Bo, M., Costa, A., Coppari, M., Enrichetti, F., Bavestrello, G., Di Caro, A., Canese, S., Betti, F. (2024). Diel rhythms of fish frequentation in a temperate mesophotic antipatharian forest and sleeping behaviour of the red swallowtail perch *Anthias anthias* (Linnaeus, 1758). *Marine Biology*, 171, 166.

Bo, M., Enrichetti, F., Betti, F., Gay, G., Quarta, G., Calcagnile, L., Bavestrello, G., 2023. The cold-water coral province of the eastern Ligurian Sea (NW Mediterranean Sea): historical and novel evidences. *Frontiers in Marine Science*, 10, 1114417.

Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin*, 40, 1100-1114.

Brown, C., 2007. Seafloor imagery, remote sensing and bathymetry: Acoustic Ground Discrimination Systems (AGDS). *Special Paper - Geological Association of Canada*, 3-10.

Bussotti, S., Denitto, F., Guidetti, P., Belmonte, G., 2002. Fish assemblages in shallow marine caves of the Salento Peninsula (Southern Apulia, SE Italy). *Marine Ecology*, 23, 11-20.

Bussotti, S., Di Franco, A., Bianchi, C.N., Chevaldonne, P., Egea, L., Fanelli, E., Lejeusne, C., Musco, L., Navarro-Barranco, C., Pey, A., Planes, S., Vieux-Ingrassia, J.V., Guidetti, P., 2018. Fish mitigate trophic depletion in marine cave ecosystems. *Scientific Reports*, 8, 9193.

Bussotti, S., Di Franco, A., Francour, P., Guidetti, P., 2015. Fish Assemblages of Mediterranean Marine Caves. *PLoS ONE*, 10(4), e0122632.

Bussotti, S., Guidetti, P., 2009. Do Mediterranean fish assemblages associated with marine caves and rocky cliffs differ? *Estuarine, Coastal and Shelf Science*, 81, 65-73.

Bussotti, S., Terlizzi, A., Fraschetti, S., Belmonte, G., Boero, F., 2006. Spatial and temporal variability of sessile benthos in shallow Mediterranean marine caves. *Marine Ecology Progress Series*, 325, 109-119.

Cánovas-Molina, A., Montefalcone, M., Bavestrello, G., Cau, A., Bianchi, C.N., Morri, C., Canese, S., Bo, M., 2016. A new ecological index for the status of mesophotic megabenthic assemblages in the mediterranean based on ROV photography and video footage. *Continental Shelf Research, 121,* 13-20.

Carlot, J., Galobart, C., Gómez-Gras, D., Santamaria, J., Golo, R., Sini, M., Cebrian, E., Gerovasileiou, V., Ponti, M., Turicchia, E., Comeau, S., Rilov, G., Tamburello, L., Pulido Mantas, T., Cerrano, C., Ledoux, J.B., Gattuso, J.P., Ramirez-Calero, S., Millán Agudo, L., Montefalcone, M., Katsanevakis, S., Bensoussan, N., Garrabou, J., Teixidó, N., 2025. Trait diversity vulnerability across the Mediterranean Sea following Mass Mortality Events. *Nature Communications*, 16, 1571.

Carpentieri, P., Bonanno, A., Scarcella, G., 2020. *Technical guidelines for scientific surveys in the Mediterranean and the Black Sea*. FAO Fisheries and Aquaculture Technical Papers No. 641, Rome, FAO, 108 pp. https://archimer.ifremer.fr/doc/00710/82198/87002.pdf

Carpentieri, P., Natsasi, A., Sessa, M., Srour, A., 2021. *Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries : A review*. GFCM Studies and Reviews No. 101, Rome, FAO, 317 pp. https://www.fao.org/gfcm/publications/studies-reviews/101/en/

Certain, G., Jadaud, A., Vaz, S., Metral, L., Cheret, I., Billet, N., Schull, Q., Tessier, E., Cornou, A.S., Souplet, A., 2025. *MEDITS catch data : Bentho-demersal assemblages of the Gulf of Lion and eastern Corsica (North-western Mediterranean) from 2016 to present* [Jeu de données]. SEANOE. https://doi.org/10.17882/99052

Chemisky, B., Seguin, E., Goujard, A., Fourt, M., Senturier, J., Chevaldonné, P., Pérez, T., Daniel, B., Accornero-Picon, A., 2015. Les fonds marins accessibles à tous avec la restitution tridimensionnelle haute résolution. Colloque merIGéo - De la côte à l'océan : l'information géographique en mouvement - Brest, 24-26 novembre 2015, p. 57-60.

Chevaldonné, P., Lejeusne, C., 2003. Regional warming-induced species shift in north-west Mediterranean marine caves. *Ecology Letters*, 6, 371-379.

Chimienti, G., Angeletti, L., Rizzo, L., Tursi, A., Mastrototaro, F., 2018. ROV vs trawling approaches in the study of benthic communities: the case of *Pennatula rubra* (Cnidaria: Pennatulacea). *Journal of the Marine Biological Association of the United Kingdom*, 98, 1859-1869.

Chimienti, G., Ferrigno, F., Tarantini, R., Tursi, A., Russo, G.F., Sandulli, R., Mastrototaro, F., 2026. Recovery capability of the tall sea pen *Funiculina quadrangularis* after fishing ban in the Adriatic Sea. *Marine Pollution Bulletin*, 222, 118906.

Colloca, F., Carpentieri, P., Balestri, E., Ardizzone, G., 2004. A critical habitat for Mediterranean fish resources : Shelf-break areas with *Leptometra phalangium* (Echinodermata: Crinoidea). *Marine Biology*, 145, 1129-1142.

Costa, G., Betti, F., Nepote, E., Cattaneo-Vietti, R., Pansini, M., Bavestrello, G., Bertolino, M., 2018. Sponge community variations within two semi-submerged caves of the Ligurian Sea (Mediterranean Sea) over a half-century time span. *The European Zoological Journal*, 85, 382–392.

D'Onghia, G., Capezzuto, F., Carlucci, R., Carluccio, A., Maiorano, P., Panza, M., Ricci, P., Sion, L., Tursi, A., 2018. Using a benthic lander to explore and monitor vulnerable ecosystems in the Mediterranean Sea. *Acta IMEKO*, 7(2), 45-49.

Dailianis, T., Gerovasileiou, V., Chatzigeorgiou, G., Vernadou, E., Glampedakis, I., Arvanitidis, C., Kargiolaki, H., 2022. Exploratory baseline survey of cave habitats within a marine protected area in the Aegean Sea. Proceedings of the 3rd Mediterranean Symposium on the Conservation of Dark Habitats, p. 19-24.

De Clippele L.H., Aguilar R., Canals M., Chimienti, G., Martín-García, L., Kotsev, I., Prodanov, B., Poursanidis, D., Vinha, B., 2025. Tools and approaches for mapping Marine Animal Forests: A practical overview for researchers and conservationists [version 1; peer review: 2 approved with reservations]. *Open Research Europe*, 5, 272.

Dendrinos, P., Karamanlidis, A.A., Kotomatas, S., Legakis, A., Tounta, E., Matthiopoulos, J., 2007. Pupping habitat use in the Mediterranean monk seal: a long-term study. *Marine Mammal Science*, 23, 615-628.

Denitto, F., Moscatello, S., Belmonte, G., 2009. Occurrence and distribution pattern of Palaemon spp. shrimps in a shallow submarine cave environment: a study case in South-eastern Italy. *Marine Ecology*, 30, 416-424.

Denitto, F., Terlizzi, A., Belmonte, G., 2007. Settlement and primary succession in a shallow submarine cave: Spatial and temporal benthic assemblage distinctness. *Marine Ecology*, 28, 35-46.

Derrien, M., Chevaldonné , P., Pérez, T., 2024. Patterns of benthic diversity in marine underwater caves of the Marseille Region (France, North-Western Mediterranean Sea). *Mediterranean Marine Science,* 25(3), 666-681.

Di Camillo, C.G., Turicchia, E., Roveta, C., Quadrini, G., Storari, A., Scarpa, C., Coppari, M., Marrocco, T., Torsani, F., Gregorin, C., Pulido Mantas. T., Cerrano, C., Ponti, M., 2025. Towards blue diving: analysis and solutions to prevent scuba diving impact in the Mediterranean sea. *Ocean & Coastal Management*, 269, 107833.

Di Franco, A., Ferruzza, G., Baiata, P., Chemello, R., Milazzo, M., 2010. Can recreational scuba divers alter natural gross sedimentation rate? A case study from a Mediterranean deep cave. *ICES Journal of Marine Science*, 67, 871-874.

Di Franco, D., Jimenez, C., Albano, P.G., 2021. Unexpected high molluscan diversity in a submarine cave in the Eastern Mediterranean. *Marine Biodiversity*, 51, 85.

Digenis, M., Arvanitidis, C., Dailianis, T., Gerovasileiou, V., 2022. Comparative study of marine cave communities in a Protected Area of the South-Eastern Aegean Sea, Greece. *Journal of Marine Science and Engineering*, 10, 660.

Digenis, M., Ragkousis, M., Dimitriadis, C., Katsanevakis, S., Gerovasileiou, V., 2025. Assessing the motile fauna of eastern Mediterranean marine caves. *Fishes*, 10, 383.

Dimarchopoulou, D., Gerovasileiou, V., Voultsiadou, E., 2018. Spatial variability of sessile benthos in a semi-submerged marine cave of a remote Aegean Island (eastern Mediterranean Sea). *Regional Studies in Marine Science*, 17, 102–111.

Dridi, A., Zribi, I., Mnasri, I., Achouri, M.S., Zakhama-Sraieb, R., 2019. Preliminary data on the distribution of marine caves along the Tunisian coast. Proceedings of the 2nd Mediterranean Symposium on the Conservation of Dark Habitats, p. 41-46.

Enrichetti, F., Bavestrello, G., Bo, M., 2025a. Deep-sea octocorals from bathyal canyons and seamounts of the Ligurian Sea (NW Mediterranean Sea). *Coral Reefs*. https://doi.org/10.1007/s00338-025-02744-4

Enrichetti, F., Bo, M., Morri, C., Montefalcone, M., Toma, M., Bavestrello, G., Tunesi, L., Canese, S., Giusti, M., Salvati, E., Bertolotto, R.M., Bianchi, C.N., 2019. Assessing the environmental status of temperate mesophotic reefs : A new, integrated methodological approach. *Ecological Indicators*, 102, 218-229.

Enrichetti, F., Toma, M., Bavestrello, G., Betti, F., Giusti, M., Canese, S., Moccia, D., Quarta, G., Calcagnile, L., Andaloro, F., Greco, S., Bo, M., 2023. Facies created by the yellow coral *Dendrophyllia cornigera* (Lamarck, 1816) : Origin, substrate preferences and habitat complexity. *Deep Sea Research Part I: Oceanographic Research Papers*, 195, 104000.

Enrichetti, F., Toma, M., Bo, M., Bavestrello, G., Bianchi, C. N., Di Lorenzo, B., Giusti, M., Michez, N, Morri, C., Rende, S.F., Tunesi, L., Angiolillo, M., 2025b. Assessing the environmental status of cold-water coral reefs: BARCS, a novel multi-parametric index. *Ecological Indicators*, 181, 114466.

Fairfield, N., Kantor, G., Wettergreen, D., 2007. Real-time SLAM with octree evidence grids for exploration in underwater tunnels. *Journal of Field Robotics*, 24, 3-21.

FAO, 2017a. *Deep-Sea Corals of the Mediterranean Sea*. https://openknowledge.fao.org/server/api/core/bitstreams/5a3b3f31-4b7d-492b-90bd-1e5a750d22ad/content

FAO, 2017b. *Deep-sea Sponges of the Mediterranean Sea*. https://openknowledge.fao.org/items/c958c805-d416-45e0-a2a0-e1073b507067

FAO, 2019a. *Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries : Methodology for data collection*. FAO Fisheries and Aquaculture Technical Paper No. 640, Rome, FAO. https://spa-rac.org/en/publication/download/1011/monitoring-the-incidental-catch-of-vulnerable-species-in-mediterranean-and-black-sea-fisheries-methodology-for-data-collection

FAO, 2019b. *Report of the forty-second session of the General Fisheries Commission for the Mediterranean (GFCM) (22-26 October 2018)*. GFCM Report No. 42, Rome, 146 pp. https://openknowledge.fao.org/server/api/core/bitstreams/edd16e17-cca7-4193-960c-b64e2efc91c5/content

Ferrigno, F., Russo, G.F., Sandulli, R., 2017. Coralligenous Bioconstructions Quality Index (CBQI) : A synthetic indicator to assess the status of different types of coralligenous habitats. *Ecological Indicators*, 82, 271-279.

Fleet, D., Vlachogianni, Th., Hanke, G., 2021. *Joint list of litter categories for marine macro-litter monitoring : Manual for the application of the classification system*. Publications Office of the European Union. https://data.europa.eu/doi/10.2760/127473

Fourt, M., Goujard, A., Canese, S., Salvati, E., Tunesi, L., Daniel, B., Vissio, A., 2015. *Rapport de la campagne océanographique « RAMOGE Exploration canyons et roches profondes 2015 »* (p. 80) [Accord Ramoge - Agence des aires marines protégées]. http://www.ramoge.org/documents/Rapport%20Ramoge%20DSI_2016_complet_final.pdf

Fourt, M., Goujard, A., Pérez, T., Chevaldonné, P., 2017. *Guide de la faune profonde de la mer Méditerranée. Exploration des roches et canyons sous-marins des côtes françaises*. Museum national d'Histoire naturelle, Paris, 184 pp.

Furlani, S., Antonioli, F., Colica, E., D'Amico, S., Devoto, S., Grego, P., Gambin, T., 2023. Sea Caves and Other Landforms of the Coastal Scenery on Gozo Island (Malta): Inventory and New Data on Their Formation. *Geosciences*, 13, 164.

Galgani, F., Ruiz-Orejón, L.F., Ronchi, F., Tallec, K., Fischer, E.K., Matiddi, M., Anastasopoulou, A., Andresmaa, E., Angiolillo, M., Bakker Paiva, M., Booth, A.M., Buhhalko, N., Cadiou, B., Clarò, F., Consoli, P., Darmon, G., Deudero, S., Fleet, D., Fortibuoni, T., Fossi, M.C., Gago, J., Gérigny, O., Giorgetti, A., González-Fernández, D., Guse, N., Haseler, M., Ioakeimidis, C., Kammann, U., Kühn, S., Lacroix, C., Lips, I., Loza, A.L., Molina Jack, M.E., Norén, K., Papadoyannakis, M., Pragnel-Raasch, H., Rindorf, A., Ruiz, M., Setälä, O., Schulz, M., Schultze, M., Silvestri, C., Soederberg, L., Stoica, E., Storr-Paulsen, M., Strand, J., Valente, T., van Franeker, J., van Loon, W.M.G.M., Vighi, M., Vinci, M., Vlachogianni, T., Volckaert, A., Weiel, S., Wenneker, B., Werner, S., Zeri, C., Zorzo, P., Hanke, G., 2023. *Guidance on the Monitoring of Marine Litter in European Seas An update to improve the harmonised monitoring of marine litter under the Marine Strategy Framework Directive*. EUR 31539 EN, Publications Office of the European Union, Luxembourg, 2023, ISBN 978-92-68-04093-5, doi:10.2760/59137, JRC133594. https://doi.org/10.2760/59137

Gallay, M., Kaňuk, J., Hochmuth, Z., Meneely, J.D., Hofierka, J., Sedlák, V., 2015. Large-scale and high-resolution 3-D cave mapping by terrestrial laser scanning: a case study of the Domica Cave, Slovakia. *International Journal of Speleology*, 44, 277-291.

Galli, S., Maiello, G., Marinchel, N., Carpentieri, P., Chatzispyrou, A., Damalas, D., Charalampous, K., Stamouli, C., Mariani, S., Russo, T., Sbrana, A., 2025. *Harnessing Environmental DNA Metabarcoding for the Detection and Mapping of Vulnerable Marine Ecosystems in the Mediterranean Sea*. 21 August 2025, PREPRINT (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-6907089/v1

Garrabou, J., Gómez-Gras, D., Medrano, A., Cerrano, C., Ponti, M., Schlegel, R., Bensoussan, N., Turicchia, E., Sini, M., Gerovasileiou, V., Teixido, N., Mirasole, A., Tamburello, L., Cebrian, E., Rilov, G., Ledoux, J.B., Ben Souissi, J., Khamassi, F., Ghanem, R., Benabdi, M., Grimes, S., Ocaña, O., Bazairi, H., Hereu, B., Linares, C., Kersting, D.K., Rovira, G., Ortega, J., Casals, D., Pagès-Escolà, M., Margarit, N., Capdevila, P., Verdura, J., Ramos, A., Izquierdo, A., Barbera, C., Rubio-Portillo, E., Anton, I., López-Sendino, P., Díaz, D., Vázquez-Luis, M., Duarte, C., Marbà, N., Aspillaga, E., Espinosa, F., Grech, D., Guala, I., Azzurro, E., Farina, S., Gambi, M.C., Chimienti, G., Montefalcone, M., Azzola, A., Pulido Mantas, T., Fraschetti, S., Ceccherelli, G., Kipson, S., Bakran-Petricioli, T., Petricioli, D., Jimenez, C., Katsanevakis, S., Tuney Kizilkaya, I., Kizilkaya, Z., Sartoretto, S., Rouanet, E., Ruitton, S., Comeau, S., Gattuso, J.P., Harmelin, J.G., 2022. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. *Global Change Biology*, 28, 5708-5725.

Georges, V., Vaz, S., Carbonara, P., Fabri, M.-C., Fanelli, E., Follesa, M.C., Garofalo, G., Gerovasileiou, V., Jadaud, A., Maiorano, P., Marin, P., Mytilineou, C., Orejas, C., Otero, M., Smith, C.J., Thasitis, I., Lauria, V., 2024. Mapping the habitat refugia of *Isidella elongata* under climate change and trawling impacts to preserve Vulnerable Marine Ecosystems in the Mediterranean. *Scientific Reports*, 14, 6246.

Gerovasileiou, V., Bancila, R.I., Katsanevakis, S., Zenetos, A., 2022. Introduced species in Mediterranean marine caves: an increasing but neglected threat. *Mediterranean Marine Science*, 23, 995-1005.

Gerovasileiou, V., Bianchi, C.N., 2021. Mediterranean marine caves: a synthesis of current knowledge. *Oceanography and Marine Biology : An Annual Review*, 59, 1-88.

Gerovasileiou, V., Chintiroglou, C., Vafidis, D., Koutsoubas, D., Sini, M., Dailianis, T., Issaris, Y., Akritopoulou, E., Dimarchopoulou, D., Voultsiadou, E., 2015. Census of biodiversity in marine caves of the Eastern Mediterranean Sea. *Mediterranean Marine Science*, 16, 245-265.

Gerovasileiou, V., Chintiroglou, C.C., Konstantinou, D., Voultsiadou, E., 2016b. Sponges as 'living hotels' in Mediterranean marine caves. *Scientia Marina*, 80, 279-289.

Gerovasileiou, V., Dimitriadis, C., Arvanitidis, C., Voultsiadou, E., 2017. Taxonomic and functional surrogates of sessile benthic diversity in Mediterranean marine caves. *PLoS ONE*, 12, e0183707.

Gerovasileiou, V., Martínez, A., Álvarez, F., Boxshall, G., Humphreys, W.F., Jaume, D., Becking, L.E., Muricy, G., van Hengstum, P.J., Dekeyzer, S., Decock, W., Vanhoorne, B., Vandepitte, L., Bailly, N., Iliffe, T.M. 2016a. World Register of marine Cave Species (WoRCS): a new thematic species database for marine and anchialine cave biodiversity. *Research Ideas and Outcomes*, 2, e10451.

Gerovasileiou, V., Trygonis, V., Sini, M., Koutsoubas, D., Voultsiadou, E., 2013. Three-dimensional mapping of marine caves using a handheld echosounder. *Marine Ecology Progress Series*, 486, 13–22.

Gerovasileiou, V., Voultsiadou, E., 2012. Marine caves of the Mediterranean Sea: a sponge biodiversity reservoir within a biodiversity hotspot. *PLoS ONE*, 7, e39873.

Gerovasileiou, V., Voultsiadou, E., 2014. Mediterranean marine caves as biodiversity reservoirs: a preliminary overview. In Symposia on the conservation of Mediterranean marine key habitats, Portorož, 27-31 October 2014. Tunis: RAC/SPA, pp. 45-50.

Gerovasileiou, V., Voultsiadou, E., 2016. Sponge diversity gradients in marine caves of the eastern Mediterranean. *Journal of the Marine Biological Association of the United Kingdom*, 96, 407–416.

Gerovasileiou, V., Voultsiadou, E., Issaris, Y., Zenetos, A., 2016c. Alien biodiversity in Mediterranean marine caves. *Marine Ecology*, 37, 239-256.

GFCM, 2018. *GFCM Data Collection Reference Framework (DCRF)*. Version 25.2, 200 pp. https://www.fao.org/gfcm/data/dcrf/en/

Giakoumi, S., Sini, M., Gerovasileiou, V., Mazor, T., Beher, J., Possingham, H.P., Abdulla, A., Çinar, M.E., Dendrinos, P., Gucu, A.C., Karamanlidis, A.A., Rodic, P., Panayotidis, P., Taskin, E., Jaklin, A., Voultsiadou, E., Webster, Ch., Zenetos, A., Katsanevakis, S., 2013. Ecoregion-based conservation planning in the Mediterranean: dealing with large-scale heterogeneity. *PLoS ONE*, 8, e76449.

Gili, J.M., Olivella, I., Zabala, M., Ros, J.D., 1982. Primera contribución al conocimiento del poblamiento de las cuevas submarinas del litoral catalán. p. 818-836. In: Niell, F.X., Ros, J.D. (Eds) Actas del Ier simposio Ibérico de estudios del bentos marino, San Sebastián.

Gili, J.M., Riera, T., Zabala, M., 1986. Physical and biological gradients in a submarine cave on the Western Mediterranean coast (north-east Spain). *Marine Biology*, 90, 291-297.

Giraud, C., Boissery, P., Dalongeville, A., Dejean, T., Deter, J., Lacoeuilhe, A., Mouillot, D., 2024. *User guide to environmental DNA in marine environments. Discovering, understanding and using eDNA to protect marine biodiversity*. 88 pp. https://medtrix.fr/wp-content/uploads/2025/02/Guide_marin_anglais_VF_BD.pdf

Giusti, M., Canese, S., Fourt, M., Bo, M., Innocenti, C., Goujard, A., Daniel, B., Angeletti, L., Taviani, M., Aquilina, L., Tunesi, L., 2019. Coral forests and derelict fishing gears in submarine canyon systems of the Ligurian Sea. *Progress in Oceanography*, 178, 102186.

Grenier, M., Ruiz, C., Fourt, M., Santonja, M., Dubois, M., Klautau, M., Vacelet, J., Boury-Esnault, N., Pérez, T., 2018. Sponge inventory of the French Mediterranean waters, with an emphasis on cave-dwelling species. Zootaxa, 4466, 205-228.

Guarnieri, G., Terlizzi, A., Bevilacqua, S., Fraschetti, S., 2012. Increasing heterogeneity of sensitive assemblages as a consequence of human impact in submarine caves. *Marine Biology*, 159, 1155-1164.

Guido, A., Jimenez, C., Achilleos, K., Rosso, A., Sanfilippo, R., Hadjioannou, L., Petrou, A., Russo, F., Mastandrea, A., 2017. Cryptic serpulid-microbialite bioconstructions in the Kakoskali submarine cave (Cyprus, Eastern Mediterranean). *Facies*, 63, 21.

Hanke, G., Canals, M., Vescovo, V., MacDonald, T., Martini, E., Ruiz-Orejón, L.F., Galgani, F., Palma, M., Papatheodorou, G., Ioakeimidis, C., Sakellariou, D., Drakopoulou, P., Fakiris, E., 2025. Marine litter in the deepest site of the Mediterranean Sea. *Marine Pollution Bulletin*, 213, 117610.

Harmelin, J.G., 1985. Bryozoan dominated assemblages in Mediterranean cryptic environments. p. 135-143. In: Nielsen C. & G.P. Larwood (Eds) Bryozoa: Ordovician to recent. Olsen & Olsen, Fredensborg.

Harmelin, J.G., Vacelet, J., Vasseur, P., 1985. Les grottes sous-marines obscures: un milieu extrême et un remarquable biotope refuge. *Téthys*, 11, 214-229.

Harmelin-Vivien, M.L., Harmelin, J.G., Chauvet, C., Duval, C., Galzin, R., Lejeune, P., Barnabe, G., Blanc, F., Chevalier, R., Duclerc, J., Lasserre, G., 1985. Evaluation des peuplements et populations de poissons. Méthodes et problemes. *Revue Ecologie (Terre Vie)*, 40, 467-539.

Harris, P.T., Whiteway, T., 2011. Global distribution of large submarine canyons : Geomorphic differences between active and passive continental margins. *Marine Geology*, 285(1), 69-86.

Heres, P., Rios, P., Cristobo, J., Abad-Uribarren, A., Rodríguez-Basalo, A., Prado, E., 2024. Characterization of deep-sea sponge ground (*Asconema setubalense*) using structure from motion methodology. *Journal of Sea Research*, 200, 102511.

Janssen, A., Chevaldonné, P., Martínez Arbizu, P., 2013. Meiobenthic copepod fauna of a marine cave (NW Mediterranean) closely resembles that of deep-sea communities. *Marine Ecology Progress Series*, 479, 99-113.

Kipson, S., Fourt, M., Teixidó, N., Cebrian, E., Casas, E., Ballesteros, E., Zabala, M., Garrabou, J., 2011. Rapid biodiversity assessment and monitoring method for highly diverse benthic communities: A case study of Mediterranean coralligenous outcrops. *PLoS ONE*, 6, e27103.

Kohler, K.E., Gill, S.M., 2006. Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Computers and Geosciences*, 32(9), 1259-1269.

Kovačić, M., Gerovasileiou, V., Patzner, R.A., 2024. Fishes in Marine Caves. *Fishes*, 9, 243.

Kovačić, M., Glavičić, I., Paliska, D.; Valić, Z., 2021. A first qualitative and quantitative study of marine cave fish assemblages of intracave cavities. *Estuarine, Coastal and Shelf Science*, 263, 107624

La Torre, M., Cussigh, A., Crobe, V., Spiga, M., Ferrari, A., Cariani, A., Piattoni, F., Costantini, F., Franzellitti, S., Pallavicini, A., Stanković, D., Stefanni, S., 2024. Environmental DNA Metabarcoding of Cephalopod Diversity in the Tyrrhenian Deep Sea. *Journal of Marine Science and Engineering*, 12(11), 1897.

Lanza-Arroyo, P., Sempere-Valverde, J., Digenis, M., Remón, J.M., Moreno, D., Barrajón, A., de la Linde, A., Arroyo, M.C., Fernández-Casado, M., Mallofret, E., Sánchez-Tocino, L., Gerovasileiou, V., Navarro-Barranco, C., 2024. Baseline for marine cave monitoring strategies in the Alboran Sea using modified Cave Ecosystem Based Quality Index (CavEBQI). *Marine Pollution Bulletin*, 209, 117065.

Le Granché, P., André, F., Rochefort, G., 2018. *Spongiaires de France – Les carnets du plongeur*. Editions Neptune Plongée, Gargas, 256 pp.

Linley, T.D., Lavaleye, M., Maiorano, P., Bergman, M., Capezzuto, F., Cousins, N.J., D'Onghia, G., Duineveld, G., Shields, M.A., Sion, L., Tursi, A., Priede, I.G., 2017. Effects of cold-water corals on fish diversity and density (European continental margin : Arctic, NE Atlantic and Mediterranean Sea): Data from three baited lander systems. *Deep Sea Research Part II: Topical Studies in Oceanography*, 145, 8-21.

Mačić, V., Dorđević, N., Petović, S., Malovrazić, N., Bajković, M., 2018b. Typology of marine litter in 'Papuča' (Slipper) cave. *Studia Marina*, 31, 38–43.

Mačić, V., Panou, A., Bundone, L., Varda, D., Pavićević, M., 2018a. First Inventory of the Semi-Submerged Marine Caves in South Dinarides Karst (Adriatic Coast) and Preliminary List of Species. *Turkish Journal of Fisheries and Aquatic Sciences*, 19, 765–774.

Mačić, V., Petović, S., Đorđević, N., Varda, D., Panou, A., 2024. *Marine Caves—Biodiversity and Threats*. G. Barovic (ed.), Speleology of Montenegro, Cave and Karst Systems of the World. Springer Nature Switzerland.

Mahmood, A., Bennamoun, M., An, S., Sohel, F., Boussaid, F., Hovey, R., Kendrick, G., Fisher, R.B., 2016. Automatic annotation of coral reefs using deep learning. *OCEANS 2016 MTS/IEEE Monterey*, 1-5. https://doi.org/10.1109/OCEANS.2016.7761105

Marroni, L., Brandt, P., Gaertner, P., Marassich, A., (2022). Citizen science and 3D modeling to study and protect Mediterranean marine caves: a real application in the caves of the Gulf of Orosei (Sardinia, Italy). EGU General Assembly 2022, Vienna, Austria, 23–27 May 2022, EGU22-3817, https://doi.org/10.5194/egusphere-egu22-3817.

Martí, R., Uriz, M.J., Ballesteros, E., Turón, X., 2004a. Benthic assemblages in two Mediterranean caves: Species diversity and coverage as a function of abiotic parameters and geographic distance. *Journal of the Marine Biological Association of the United Kingdom*, 84, 557-572.

Martí, R., Uriz, M.J., Ballesteros, E., Turon, X., 2004b. Temporal variation of several structure descriptors in animal dominated benthic communities in two Mediterranean caves. *Journal of the Marine Biological Association of the United Kingdom*, 84, 573-580.

MEDITS Working Group, 2017. *MEDITS-Handbook*. Version n. 9. *MEDITS Project*, 106 pp.

Milazzo, M., Chemello, R., Badalamenti, F., Camarda, R., Riggio, S., 2002. The impact of human recreational activities in Marine Protected Areas: What lessons should be learnt in the Mediterranean Sea? *Marine Ecology*, 23, 280–290.

Millot, J., Georges, V., Lauria, V., Hattab, T., Dominguez-Carrió, C., Gerovasileiou, V., Smith, C.J., Mytilineou, C., Teresa Farriols, M., Fabri, M.-C., Carbonara, P., Massi, D., Rinelli, P., Profeta, A., Chimienti, G., Jadaud, A., Thasitis, I., Camilleri, K., Mifsud, J., Vaz, S., 2024. Habitat shifts of the vulnerable crinoid *Leptometra phalangium* under climate change scenarios. *Progress in Oceanography*, 229, 103355.

Moccia, D., Carugati, L., Follesa, M.C., Cannas, R., Carbonara, P., Pusceddu, A., Cau, A., 2022. Environmental Status and Geomorphological Characterisation of Seven Black Coral Forests on the Sardinian Continental Shelf (NW Mediterranean Sea). *Biology*, 11, 732.

Montefalcone, M., De Falco, G., Nepote, E., Canessa, M., Bertolino, M., Bavestrello, G., Morri, C., Bianchi, C.N., 2018. Thirty year ecosystem trajectories in a submerged marine cave under changing pressure regime. *Marine Environmental Research*, 137, 98-110.

Montefalcone, M., Ferraro, V., Barbieri, F., Morri, C., Bianchi, C.N., 2023. Ecological gradients in a marine cave revisited 26 years after. *Estuarine, Coastal and Shelf Science*, 293, 108517.

Montefalcone, M., Tunesi, L., Ouerghi, A., 2021. A review of the classification systems for marine benthic habitats and the new updated Barcelona Convention classification for the Mediterranean. *Marine Environmental Research*, 169, 105387.

Monteiro-Marques, V., 1981. Peuplements des planchers envasés de trois grottes sous-marines de la région de Marseille. Étude préliminaire. *Téthys*, 10, 89-96.

Nalmpanti, M., Chrysafi, A., Meeuwig, J., Tsikliras, A., 2023. Monitoring marine fishes using underwater video techniques in the Mediterranean Sea. *Reviews in Fish Biology and Fisheries*, 33, 1-20.

Navarro-Barranco, C., Ambroso, S., Gerovasileiou, V., Gómez-Gras, D., Grinyó, J., Montseny, M., Santín, A., 2023b. *Conservation of dark habitats*. Chapter 6. p. 147-170. In: Espinosa F (Ed) Coastal Habitat Conservation New Perspectives and Sustainable Development of Biodiversity in the Anthropocene. Academic Press, 276 pp. https://doi.org/10.1016/B978-0-323-85613-3.00005-0

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., Florido, M., García-Gómez, J.C., 2016. Amphipod community associated with invertebrate hosts in a Mediterranean marine cave. *Marine Biodiversity*, 46, 105-112.

Navarro-Barranco, C., Guerra-Garcia, J.M., Sanchez-Tocino, L., Garcia-Gomez, J.C., 2014. Mobile epifaunal community in marine caves: Does it differ from open habitats? *Aquatic Biology*, 20, 101-109.

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., García-Gómez, J.C., 2012. Soft-bottom crustacean assemblages in Mediterranean marine caves: the cave of Cerro-Gordo (Granada, Spain) as case study. *Helgoland Marine Research*, 6, 567-576.

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., García-Gómez, J.C., 2014. Amphipods from marine cave sediments of the southern Iberian Peninsula: diversity and ecological distribution. *Scientia Marina*, 78, 415-424.

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., Jiménez Parada, P., Cea Sánchez, S., García-Gómez, J.C. 2013a. Soft-bottom diversity patterns in marine caves: Lessons from crustacean community. *Journal of Experimental Marine Biology and Ecology*, 446, 22-28.

Navarro-Barranco, C., Martínez, A., Sempere-Valverde, J., Chebaane, S., Digenis, M., Plaitis, W., Voultsiadou, E., Gerovasileiou, V., 2023. Amphipods in Mediterranean Marine and Anchialine Caves: New Data and Overview of Existing Knowledge. *Diversity*, 15, 1180.

Nepote, E., Bianchi, C.N., Morri, C., Ferrari, M., Montefalcone, M., 2017. Impact of a harbour construction on the benthic community of two shallow marine caves. *Marine Pollution Bulletin*, 114, 35-45.

Nicolosi, G., Gerovasileiou, V., 2024. Towards invasion ecology for subterranean ecosystems. *Biodiversity and Conservation*, 33, 1561-1569.

Orejas, C., Gori, A., Jiménez, C., Rivera, J., Kamidis, N., Alhaija, R.A., Iacono, C.L., 2019. Occurrence and distribution of the coral *Dendrophyllia ramea* in Cyprus insular shelf : Environmental setting and anthropogenic impacts. *Deep Sea Research Part II: Topical Studies in Oceanography*, 164, 190-205.

Otero, M., Mytilineou, C. (Ed.), 2022. *Deep-sea Atlas of the Eastern Mediterranean Sea*. IUCN-HCMR DeepEastMed Project. Publisher, IUCN Gland, Malaga, 371pp.

Otero, M.M., Numa, C., Bo, M., Orejas, C., Garrabou, J., Cerrano, C., Kružić, P., Antoniadou, C., Aguilar, R., Kipson, S., Linares, C., Terrón-Sigler, A., Brossard, J., Kersting, D., Casado-Amezúa, P., García, S., Goffredo, S., Ocaña, O., Caroselli, E., Maldonado, M., Bavestrello, G., Cattaneo-Vietti, R., Özalp, B., 2017. *Overview of the conservation status of Mediterranean anthozoans*. IUCN, Malaga, Spain. x + 73 pp.

Otero, M.M., Serena, F., Gerovasileiou, V., Barone, M., Bo, M., Arcos, J.M., Vulcano, A., Xavier, J., 2019. *Identification guide of vulnerable species incidentally caught in Mediterranean fisheries*. IUCN, Málaga, Spain, 204 pp. https://portals.iucn.org/library/sites/library/files/documents/2019-050-En.pdf

Ouerghi, A., Gerovasileiou, V., Bianchi, C.N., 2019. Mediterranean marine caves: a synthesis of current knowledge and the Mediterranean Action Plan for the conservation of "dark habitats". p. 1-13. In:

Marine Caves of the Eastern Mediterranean Sea. Biodiversity, Threats and Conservation. Öztürk B. (Ed.). Turkish Marine Research Foundation (TUDAV) Publication no: 53, Istanbul, Turkey.

Öztürk, B. (ed.), 2019. Marine Caves of the Eastern Mediterranean Sea. Biodiversity, Threats and Conservation. Istanbul: Turkish Marine Research Foundation (TUDAV) Publication no. 53.

Parravicini, V., Guidetti, P., Morri, C., Montefalcone, M., Donato, M., Bianchi, C.N., 2010. Consequences of sea water temperature anomalies on a Mediterranean submarine cave ecosystem. *Estuarine and Coastal Marine Science*, 86, 276-282.

Pérès J.M., 1967. Mediterranean Benthos. *Oceanography and Marine Biology - An Annual Review*, 5, 449-533.

Pérès, J.M., Picard, J., 1949. Notes sommaires sur le peuplement des grottes sous-marines de la région de Marseille. *Compte Rendu Sommaire des Séances de la Société de Biogéographie*, 227, 42-45.

Pierdomenico, M., Bonifazi, A., Argenti, L., Ingrassia, M., Casalbore, D., Aguzzi, L., Viaggiu, E., Le Foche, M., Chiocci, F.L., 2021. Geomorphological characterization, spatial distribution and environmental status assessment of coralligenous reefs along the Latium continental shelf. *Ecological indicators*, 131, 108219.

Pisera, A., Gerovasileiou, V., 2021. Lithistid demosponges of deep-water origin in marine caves of the north-eastern Mediterranean Sea. *Frontiers in Marine Science*, 8, 630900.

Prado, E., Cristobo, J., Rodríguez-Basalo, A., Ríos, P., Rodríguez-Cabello, C., Sánchez, F., 2021. *In situ* Growth Rate Assessment of the Hexactinellid Sponge *Asconema setubalense* Using 3D Photogrammetric Reconstruction. *Frontiers in Marine Science*, 8, 612613.

Pulido Mantas, T., Roveta, C., Calcinai, B., Benelli, F., Coppari, M., Di Camillo, C. G., Pantaleo, U., Puce, S., Cerrano, C., 2024. Structure from Motion Photogrammetry as an Effective Nondestructive Technique to Monitor Morphological Plasticity in Benthic Organisms: The Case Study of *Sarcotragus foetidus* Schmidt, 1862 (Porifera, Demospongiae) in the Portofino MPA. *Diversity*, 16(3), 175.

Pulido Mantas, T., Roveta, C., Calcinai, B., Coppari, M., Di Camillo, C.G., Marchesi, V., Montagna, M., Musco, L., Palma, M., Pica, D., Ponti, M., Cerrano, C., 2023. Photogrammetry as a promising tool to unveil marine caves' benthic assemblages. *Scientific Reports*, 13(1), 7587.

Quiles-Pons, C., Baena, I., Calvo-Manazza, M., de la Ballina, N.R., Díez, S., Goñi, R., Mallol, S., Maresca, F., Morató, M., Muñoz, A., Prado, E., Real, E., Sánchez, F., Díaz, D., 2022. Monitoring the complex benthic habitat on semi-dark underwater marine caves using photogrammetry-based 3D reconstructions. Proceedings of the 3rd Mediterranean Symposium on the conservation of Dark Habitats, p. 113-114.

Quiles-Pons, C., Maresca, F., De La Ballina, N.R., Real, E., Baena-Vega, I., Muñoz, A., Morató, M., Díez, S., Mallol, S., Díaz, D., 2025. Ask the audience: Characterization of diving pressure on marine caves using diving operators' knowledge. *Mediterranean Marine Science*, 26(4), 1005-1016.

Radolović, M., Bakran-Petricioli, T., Petricioli, D., Surić, M., Perica, D., 2015. Biological response to geochemical and hydrological processes in a shallow submarine cave. *Mediterranean Marine Science*, 16 (2), 305-324

Rastorgueff, P.A., Bellan-Santini, D., Bianchi, C.N., Bussotti, S., Chevaldonné, P., Guidetti, P., Harmelin, J.G., Montefalcone, M., Morri, C., Pérez, T., Ruitton, S., Vacelet, J., Personnic, S., 2015. An ecosystem-based approach to evaluate the ecological quality of Mediterranean undersea caves. *Ecological Indicators*, 54, 137-152.

Richaume, J., Cheminée, A., Drap, P., Bonhomme, P., Cadene, F., Ferrari, B., Hartmann, V., Michez, N., Bianchimani, O., 2021. 3D Photogrammetry Modeling Highlights Efficient Reserve Effect Apparition After 5 Years and Stillness After 40 for Red Coral (*Corallium rubrum*) Conservation in French MPAs. *Frontiers in Marine Science*, 8, 639334.

Riedl, R., 1966. *Biologie der Meereshöhlen*. Paul Parey, Hamburg, 636 pp.

Rodríguez-Basalo, A., Ríos, P., Arrese, B., Abad-Uribarren, A., Cristobo, J., Ibarrola, T.P., Gómez-Ballesteros, M., Prado, E., Sánchez, F., 2022. Mapping the habitats of a complex circalittoral rocky shelf in the Cantabrian Sea (south Bay of Biscay). *Estuarine, Coastal and Shelf Science*, 273, 107912.

Romano, E., Bergamin, L., Berto, D., Chiocci, F.L., Miccadei, E., Paglia, G., Piattelli, V., Pierfranceschi, G., Rampazzo, F., Sorci, A., Taddei Ruggiero, E., 2023. Geomorphological, sedimentological, and ecological characterization of marine caves from Capraia Island (Tremiti Archipelago, southern Adriatic Sea, Italy): An integrated approach. Marine Geology, 455, 106952,

Romano, E., Bergamin, L., Parise, M., 2022. Benthic Foraminifera as Environmental Indicators in Mediterranean Marine Caves: A Review. *Geosciences*, 12, 42.

Romano, E., Bergamin, L., Pierfranceschi, G., Provenzani, C., Marassich, A., 2018. The distribution of benthic foraminifera in Bel Torrente submarine cave (Sardinia, Italy) and their environmental significance. *Marine Environmental Research*, 133, 114-127.

Rosso, A., Di Martino, E., Pica, D., Galanti, L., Cerrano, C., Novosel, M., 2018. Non-indigenous bryozoan species from natural and artificial substrata of Mediterranean submarine caves. *Marine Biodiversity*, 48, 1345-1355.

Rosso, A., Di Martino, E., Sanfilippo, R., Di Martino, V., 2013. Bryozoan communities and thanatocoenoses from submarine caves in the Plemmirio Marine Protected Area (SE Sicily). In: Bryozoan Studies 2010, Lecture Notes in Earth System Sciences, vol. 143, A. Ernst (e d.). Berlin, Heidelberg: Springer, 251-269.

Rosso, A., Gerovasileiou, V. , Sanfilippo, R., Guido, A., 2019. Bryozoan assemblages from two submarine caves in the Aegean Sea (Eastern Mediterranean). *Marine Biodiversity*, 49, 707-726.

Rosso, A., Sanfilippo, R., Guido, A., Gerovasileiou, V., Taddei Ruggiero, E., Belmonte, G., 2021. Colonisers of the dark: Biostalactite-associated metazoans from “lu Lampiùne” submarine cave (Apulia, Mediterranean Sea). *Marine Ecology*, 42, e12634.

Rouanet, E., Schohn, T., et l’ensemble des scientifiques de la campagne, 2019. *Campagne océanographique d’exploration de canyons et monts sous-marins de la zone de l’Accord RAMOGE « RAMOGE EXPLO 2018 » Rapport final*. Accord RAMOGE – Agence Française pour la Biodiversité & GIS Posidonie. 51 p + 5 annexes. https://ramoge.org/wp-content/uploads/2022/01/RAMOGE_EXPLO_2018_Rapport_final.pdf

Royer, J.-P., Nawaf, M. M., Merad, D., Saccone, M., Bianchimani, O., Garrabou, J., Ledoux, J.-B., Lopez-Sanz, A., Drap, P., 2018. Photogrammetric Surveys and Geometric Processes to Analyse and Monitor Red Coral Colonies. *Journal of Marine Science and Engineering*, 6(2), 42.

Saccò, M., Elmasri, A., Tawal, M., Alther, R., Altermatt, F., Martínez, A., Mammola, S., Zagmajster, M., Saenz-Agudelo, P., Takahashi, M., Thornhill, J., Andouche, A., Baratti, M., Bilal, M., Bilandžija, H., Bolpagni, R., Boulamail, S., Celico, F., Chauveau, C.A., Chavez, E., Collard, O., Cooper, S.J.B., Couton, M., Cozzoli, F., Dawkins, K., Di Lorenzo, T., Dubinsky, X., Hahn, H.J., Flot, J.-F., Glatting, F., Griebler, C., Guareschi, S., Guy-Haim, T., Herbert, S., Hose, G., Humphreys, W.F., Hutchins, B., Iepure, S., Johns, T., Karwautz, C., Kirschner, D., Kolodny, O., Konec, M., Manenti, R., Meziane, T., Mori, N., Pazhenkova, E., Piggott, M.P., Prieto-Davó, A., Raphalen, Z., Rau, H., Reboleira, A.S., Recknagel, H., Robertson, A., Royaux, C., Schwartz, B., Schwenk, K., Simoes, N., Stanković, D., Stoch, F., Taylor, J.D., Van Der Heyde, M., Vehovar, Ž., Zakšek, V., White, N.E., Allentoft, M.E., Nevill, P., Korbel, K.L., Guzik, M.T., 2025. Advancing subterranean conservation through Global Research on eDNA in Groundwaters (GReG). *Subterranean Biology*, 53, 31-40.

Sakellariou, D., Drakopoulou, V., Rousakis, G., Livanos, I., Loukaidi, V., Kyriakidou, Ch., Morfis, I., Panagiotopoulos, I., Tsampouraki-Kraounaki, K., Manta, K., 2022. *Geomorphological Features*. p. 19-121. In: Otero M, Mytilineou C (Eds), Deep-sea Atlas of the Eastern Mediterranean Sea. IUCN-

HCMR DeepEastMed Project, IUCN, Gland and Malaga. https://uicnmed.org/docs/deep-sea-eastern-med/GEOMORPHOLOGICAL-FEATURES.pdf

Salomidi, M., Gerovasileiou, V., Stamouli, C., Drakopoulou, V., Otero, M.M., Jimenez, C., Kiparissis, S., Mytilineou, Ch., Papadopoulou, N., Smith, C.J., Thasitis, I., Anastasiadou, Ch., Lefkaditou, E., Makovsky, Y., Schüler, M., 2022. *Deep-sea vulnerable benthic fauna*. Chapter 3. p. 123-144. In: Otero M, Mytilineou C (Eds), Deep-sea Atlas of the Eastern Mediterranean Sea. IUCN-HCMR DeepEastMed Project, IUCN, Gland and Malaga. https://uicnmed.org/docs/deep-sea-eastern-med/DEEP-SEA-VULNERABLE-BENTHIC-FAUNA.pdf

Salomidi, M., Marchiò, A., Issaris, Y., Gerakaris, V., Dailianis, T., Gerovasileiou, V., 2025. Advancing knowledge on red coral *Corallium rubrum* (Linnaeus, 1758) populations and associated mesophotic communities in the Aegean Sea, Eastern Mediterranean. *Mediterranean Marine Science*, 26(2), 378-392.

Sanfilippo, R., Rosso, A., Guido, A., Gerovasileiou, V., 2017. Serpulid communities from two marine caves in the Aegean Sea, eastern Mediterranean. Journal of the Marine Biological Association of the United Kingdom, 97, 1059-1068.

Sanfilippo, R., Rosso, A., Guido, A., Mastandrea, A., Russo, F., Ryding, R., Taddei Ruggero, E., 2015. Metazooan/microbial biostalactites from modern submarine caves in the Mediterranean Sea. *Marine Ecology*, 36, 1277-1293.

Savva, I., Cai, L.L., Marcou, M., Angel, D., 2026. The untold story of Sea Caves: Lost and found anthropogenic debris in confined marine environments. *Marine Pollution Bulletin*, 223, 119065.

Schiavo, A., Oddenino, M., Giménez, G., Longo, C., 2024. First Survey of the Sponge Community of a Semi-Submerged Marine Cave along the Adriatic Apulian Coast. *Journal of Marine Science and Engineering*, 12(4), 682.

Schohn, T., Belloni, B., Rouanet, E., Aquilina, L., Angiolillo, M., Caro, A., Champion, F., Fourt, M., Gerigny, O., Giusti, M., Meinesz, C., Pazzini, A., Poydenot, F., Rossi, L., Tambutté, E., Vissio, A., Tunesi, L., 2023. *Campagne océanographique d'exploration des zones sous-marines profondes de la zone de l'Accord RAMOGE - «RAMOGE EXPLO 2022» - Rapport final*. Accord RAMOGE. GIS Posidonie publ., Marseille, Fr. : 68 p. + Annexes. https://ramoge.org/wp-content/uploads/2024/10/RAMOGE_EXPLO_2022_Rapport_final_valide.pdf

Schohn, T., Belloni, B., Tunesi, L., Caro, A., Aquilina, L., Champion, F., 2024. *Marine exploration dataset of the 2022 RAMOGE Exploration : Description of observations made during Remotely Operated Vehicle Dives in the RAMOGE agreement area* [Jeu de données]. SEANOE. https://doi.org/10.17882/100656

Sellers, W.I., Chamberlain, A.T., 1998. Ultrasonic cave mapping. Journal of Archaeological Science, 25, 867-873.

Sempere-Valverde, J., Sabino Lorenzo, A., Espinosa, F., Gerovasileiou, V., Sanchez-Tocino, L., Navarro-Barranco, C., 2019. Taxonomic and morphological descriptors reveal high benthic temporal variability in a Mediterranean marine submerged cave over a decade. *Hydrobiologia*, 839, 177-194.

Sierra, S., Prado, E., Rodríguez-Cobo, L., Quiles-Pons, C., Roldán-Varona, P., Díaz-Viñolas, D., Anuarbe-Cortés, P., Cobo, A., Sánchez, F., 2023. Density estimation of the main structuring sessile species in underwater marine caves with a deep learning approach. *Journal of Geography and Cartography*, 6(1), 1980.

Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. *Mediterranean Marine Science*, 3(2), 77-111.

Smith, C.J., Gerovasileiou, V., Mytilineou, Ch., Jimenez, C., Papadopoulou, K., Salomidi, M., Sakellariou, D., Drakopoulou, V., Otero, M., 2022. *Revisiting underwater surveys to uncover sites of conservation* *interest*. Chapter 4. p. 146-171. In: Otero M, Mytilineou C (Eds), Deep-sea Atlas of the Eastern Mediterranean Sea. IUCN-HCMR DeepEastMed Project, IUCN, Gland and Malaga. https://uicnmed.org/docs/deep-sea-eastern-med/REVISITING-UNDERWATER-SURVEYS.pdf

SPA/RAC–UN Environment/MAP & OCEANA, 2017. *Guidelines for inventorying and monitoring of dark habitats in the Mediterranean Sea*. By Gerovasileiou, V., Aguilar, R., Marin, P. SPA/RAC - Deep Sea Lebanon Project, Tunis, 44 pp. + Annexes.

SPA/RAC-UNEP/MAP, 2020. *Mediterranean marine caves: remarkable habitats in need of protection*. By Gerovasileiou, V., Bianchi, C.N. Ed. SPA/RAC, Tunis: 80 pp.

Spaccavento, M., Mastrototaro, F., Tursi, A., Montesanto, F., Bottalico, A., Longo, C., Chimienti., G. 2022. A non-invasive monitoring method to assess the composition of megabenthic communities in semi-submerged marine caves. IEEE International Workshop on Metrology for the Sea; Learning to Measure Sea Health Parameters (MetroSea), Milazzo, Italy, 2022, pp. 257-261.

Stamouli, C., Gerovasileiou, V., Voultsiadou, E., 2023. Sponge Community Patterns in Mesophotic and Deep-Sea Habitats in the Aegean and Ionian Seas. *Journal of Marine Science and Engineering*, 11, 2204.

Stamouli, C., Zenetos, A., Kallianiotis, A., Voultsiadou, E., 2022. Megabenthic invertebrate diversity in Mediterranean trawlable soft bottoms: a synthesis of current knowledge. *Mediterranean Marine Science*, 23(3), 447-459.

Surić, M., Lončarić, R., Lončar, N., 2010. Submerged caves of Croatia: Distribution, classification and origin. *Environmental Earth Sciences*, 61, 1473-1480.

Susini, I., Van Audenhaege, L., Price, D.M., Pearman, T.R., Mitchell, E.G., Huvenne, V.A., 2025. Fine-scale spatial organisation of deep-sea sea pens in a NE atlantic submarine canyon conservation area. *Scientific Reports*, 15(1), 29332.

Taviani, M., 2014. *Marine Chemosynthesis in the Mediterranean Sea*. In: Goffredo, S., Dubinsky, Z. (eds) The Mediterranean Sea. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6704-1_5

Teixidó, N., Albajes-Eizagirre, A., Bolbo, D., Le Hir, E., Demestre, M., Garrabou, J., Guigues, L., Gili, J.M., Piera, J., Prelot, T., Soria-Frisch, A., 2011. Hierarchical segmentation-based software for cover classification analyses of seabed images (Seascape). *Marine Ecology Progress Series*, 431, 45-53.

Todaro, M.A., Leasi, F., Bizzarri, N., Tongiorgi, P., 2006. Meiofauna densities and gastrotrich community composition in a Mediterranean sea cave. *Marine Biology*, 149, 1079-1091.

Trygonis, V., Sini, M., 2012. photoQuad: a dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. *Journal of Experimental Marine Biology and Ecology*, 424-425, 99-108.

Tsirintanis, K., Azzurro, E., Crocetta, F., Dimiza, M., Froglia, C., Gerovasileiou, V., Langeneck, J., Mancinelli, G., Rosso, A., Stern, N., Triantaphyllou, M., Tsiamis, K., Turon, X., Verlaque, M., Zenetos, A., Katsanevakis, S., 2022. Bioinvasion impacts on biodiversity, ecosystem services, and human health in the Mediterranean Sea. *Aquatic Invasions*, 17, 308-352.

UNEP, 2016. *Integrated Monitoring and Assessment Guidance*. UNEP(DEPI)/MED IG.22/Inf.7.

UNEP/MAP-SPA/RAC, 2016. Action Plan for the conservation of the Coralligenous and Other Calcareous Bio-concretions in the Mediterranean Sea. Ed. SPA/RAC, Tunis: 28 pp + Annex.

UNEP/MAP-SPA/RAC, 2021. Action Plan for the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea. Ed. SPA/RAC, 32 pp.

UNEP/MAP-SPA/RAC, 2024. Guidelines for the Assessment of Environmental Impact on Coralligenous and Maërl Assemblages. Ed. SPA/RAC, Tunis. 55 pp. ISBN 978-9938-79-370-3

UNEP/MED WG.502/Inf.4, 2021. Agenda item 5: Conservation of Species and Habitats Interpretation Manual of Marine Habitat Types in the Mediterranean Sea, SPA/RAC, Tunis. https://www.rac-spa.org/meetings/nfp15/nfp_docs/inf/21wg502_inf04_en.pdf

Ventura, D., Dubois, S.F., Bonifazi, A., Jona Lasinio, G., Seminara, M., Gravina, M.F., Ardizzone, G., 2021. Integration of close-range underwater photogrammetry with inspection and mesh processing software: a novel approach for quantifying ecological dynamics of temperate biogenic reefs. *Remote Sensing in Ecology and Conservation*, 7, 169-186.

Weinberg, S., 1978. The minimal area problem in invertebrate communities of Mediterranean rocky substrata. *Marine Biology*, 49, 33-40.

Würtz, M. (Éd.)., 2012. *Mediterranean submarine canyons : Ecology and governance*. IUCN, Gland, Switzerland and Malaga, Spain, 216 pp.

Würtz, M., Rovere, M. (Éds.), 2015. *Atlas of the Mediterranean seamounts and seamount-like structures*. IUCN, Gland, Switzerland and Malaga, Spain, 276 pp.