

## Mediterranean Marine Science

Vol 25, No 3 (2024)

Mediterranean Marine Science



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doi: [10.12681/mms.38566](https://doi.org/10.12681/mms.38566)

### To cite this article:

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. <https://doi.org/10.12681/mms.38566>

## Patterns of benthic diversity in marine underwater caves of the Marseille Region (France, North-Western Mediterranean Sea)

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Contributing Editor: Vasilis GEROVASILEIOU

Received: 02 August 2024; Accepted: 02 October 2024; Published online: 30 October 2024

### Abstract

Underwater caves are remarkable habitats of the Mediterranean Sea. In the present study, we compare the geomorphology of four underwater caves of the Marseille area and the associated sessile assemblages across two contrasted communities (Semi-Dark and Dark-cave communities). Using a non-destructive method of sampling, photoquadrats of the walls of the caves were performed in 2020 to assess the biodiversity and the structure of these communities. In addition, taking advantage of available reference data from 2015, we evaluate the changes in sponge assemblages for the period 2015-2020, focusing on observations of the Semi-Dark cave community. Our results illustrate how the diversity of environmental settings and cave morphologies shape the benthic community composition of the four caves. In all four caves, the Semi-Dark community is the more diverse and harbours the higher number of species when compared to the Dark community. Each cave presents a different species assemblage with in most cases, distinct dominant species which highlight the singularity of each cave. Moreover, our temporal change assessment demonstrates a critical decrease in sponge species richness for all caves, possibly related to the increasing frequency of marine heatwaves that have occurred in the last decade. Although some species (mostly in the Keratosa subclass) seem to be particularly affected, other species such as *Chondrosia reniformis* appear to have benefitted from the situation. Our findings underline the importance of biodiversity monitoring in a context of a rapidly changing environment, in particular in poorly resilient marine ecosystems such as underwater caves.

**Keywords:** Benthic biodiversity; Porifera; Photo survey; Underwater cave; Mediterranean Sea.

### Introduction

The Mediterranean Sea is a global biodiversity hotspot, harbouring about 10% of the marine species acknowledged in the world ocean (Bianchi & Morri, 2000; Coll *et al.*, 2010). Among the remarkable ecosystems distributed across this miniature ocean, underwater caves represent genuine reservoirs of biodiversity, displaying poorly resilient communities with more than 2300 taxa reported thus far (Gerovasileiou & Bianchi, 2021) and a considerable number of putative taxa remaining to be described. To date, 3000 underwater caves have been recorded in the Mediterranean Sea (Giakoumi *et al.*, 2013) but only a very few have been subjected to extensive investigation. The exploration of these caves began with the popularization of SCUBA diving. Pérès & Picard (1949) provided the first description of cave biocoenoses, and since then several studies have unveiled a unique and teeming biodiversity, frequently composed of rare, exclusive and/or deep-sea species (Harmelin *et al.*, 1985; Vacelet *et al.*, 1995; 1996).

From the entrance to the back of a marine underwater cave, the decrease in both light and water movement leads to a decrease in food supply, which is responsible for a confinement gradient (Harmelin *et al.*, 1985; Bussotti *et al.*, 2006). These conditions explain a benthic biodiversity gradient where the numerous erect growth forms at the entrance of a cave are progressively replaced by more scarce encrusting species as one penetrates towards the back (Harmelin *et al.*, 1985). At both ends of the confinement gradient, two distinct communities can be acknowledged: the Semi-Dark cave community near the entrance, and the Dark cave community at the back (Pérès & Picard, 1964). The boundary between these two communities is often difficult to define. The Semi-Dark cave community (SD) is characterized by the rare occurrence of macroalgae and the dominance of sponges, anthozoans and bryozoans (Harmelin *et al.*, 1985; Laborel & Vacelet, 1958; Grenier *et al.*, 2018), and by a benthic coverage of 100%. In the Dark cave community (D), the benthic coverage can be less than 10%, and the sessile

biodiversity is usually restricted to encrusting sponges and serpulid worms (Pérès 1967; Laborel & Vacelet, 1959; Vacelet *et al.*, 1976). Although the SD biodiversity is rather well described due to its accessibility, the most remote D community remains poorly known.

Sponges are essential members of these communities. As powerful active filter feeders, they are components of short food-webs, feeding on a wide range of microscopic particles and recycling organic materials of various origins (e.g., Topçu *et al.*, 2010). In caves, where most of the food comes from adjacent ecosystems, and primarily from the pelagic system of the open ocean, sponges are considered as keystone participants in benthic-pelagic coupling (Bell, 2008).

Underwater caves figure among the remarkable habitats listed by the EU Habitats Directive (92/43/EEC, Habitat type 8330). Their biodiversity and ecosystem functioning are, however, threatened by global change, with climate warming and the increasing occurrence of marine heatwaves (MHWs) representing the main environmental hazards (Parravicini *et al.*, 2010; Garrabou *et al.*, 2022). These phenomena trigger mass mortality events impacting the main benthic components of the SD community: sponges, anthozoans, bryozoans and also ascidians (see for instance Pérez *et al.*, 2000, 2003; Garrabou *et al.*, 2009, 2022; Lejeusne *et al.*, 2010; Grenier *et al.*, 2023). For example, the sponges *Petrosia ficiformis*, *Ircinia* spp., *Dysideidae* spp., *Spongia officinalis* and the red coral *Corallium rubrum* count among the SD cave dwellers regularly affected by MHWs (Pérez *et al.*, 2000; Garrabou *et al.*, 2009; Grenier *et al.*, 2023). In the D cave community, such negative impacts also occur, but have so far been thoroughly documented for mobile species only (Chevaldonné & Lejeusne, 2003).

The aim of the present work is to study the patterns

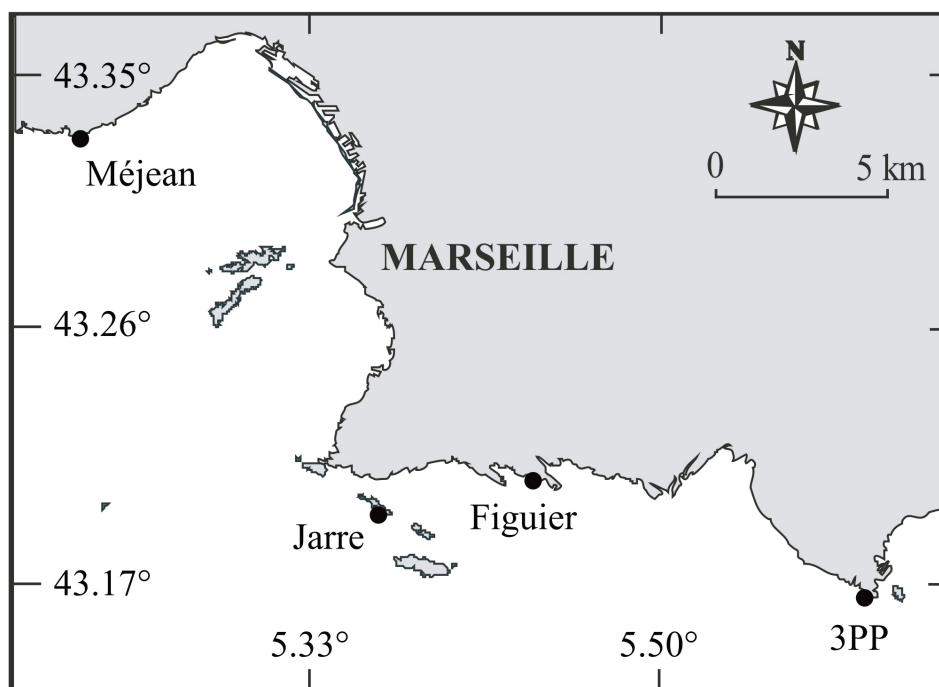
of benthic diversity in the SD and D cave communities in the Marseille area using a rapid assessment method. We describe and compare the composition of SD and D communities of four underwater caves of the Marseille area presenting contrasted environmental conditions. Moreover, benefiting from reference data for the SD communities in four caves (Grenier *et al.*, 2018), we also assess temporal changes in biodiversity over a 5-year time frame from 2015 to 2020, which corresponds to the warmest period ever recorded in the NW Mediterranean Sea (Garrabou *et al.*, 2022).

## Material and Methods

### Study sites & sampling

The four underwater caves studied here, Méjean, Jarre, Figuier and 3PP are located in the Western Mediterranean Sea, in the Marseille region, from the North of the city to the Eastern extremity of the Calanques National Park (Fig. 1). These caves display variable environmental characteristics which shape rather distinct species assemblages.

Sampling was carried out by SCUBA diving using a non-destructive method. Photoquadrats of the walls of the caves were obtained in winter 2020 following the method deployed by Grenier *et al.* (2018). A total of 236 photoquadrats were taken randomly around predetermined points in the SD and D communities of the four caves. Figuier cave benefits from two entrances so sampling of its SD communities was performed in the main entrance, which is the deepest and largest (Table 1). Photoquadrat number varied slightly between sampling stations according to cave geomorphology, particularly in case of narrowness (Table 1).



**Fig. 1:** Location of the four studied caves in the Marseille area (Mediterranean Sea, France) (modified from Chevaldonné & Lejeusne, 2003).

**Table 1.** Locations of the studied underwater caves and topography of the sampling stations. For each cave, the numbers of photoquadrats is given per station. SD: Semi-Dark community, D: Dark community. \* refers to the distance from the main entrance of Figuier, where SD photoquadrats were taken.

Caves	Coordinates	SD			D		
		Depth (m)	Distance from entrance (m)	Number of photoquadrats	Depth (m)	Distance from entrance (m)	Number of photoquadrats
Méjean	43.32800° N, 5.22100° E	15	1-2	24	9	18	33
Jarre	43.19600° N, 5.36570° E	17	1-2	30	18	50	33
Figuier	43.20500° N, 5.44700° E	22	2-5	30	10	45*	32
3PP	43.16300° N, 5.60000° E	15	2-4	33	25	50	21

### Photoquadrats analysis

Independently from their taxonomic identification (see below), species present in the photoquadrats were recorded using PhotoQuad, a software dedicated to the analysis of benthic communities (Trygonis & Sini, 2012) and previously used in underwater caves (Gerovasileiou & Voultsiadou, 2016; Digenis *et al.*, 2022). Each photoquadrat was processed by overlaying a virtual quadrat of 20 cm x 20 cm in order to keep the same sampling surface. One hundred points were then randomly distributed in 100 sub-cells of the redefined surface, with 1 point attributed per cell (stratified and random projection method). Each point was assigned either to a taxonomic level (from species to phylum), to a morpho-functional category (e.g., encrusting red algae, epibiont) or to a non-living category (e.g., substrate, mud). An automatic community matrix was generated by PhotoQuad to present the number of points associated to the categories per photo and per sampling station. Of the 123 recorded taxonomic units, 52 were identified to species, genus or family level (Table 2). After photo processing, we obtained 123 distinct taxonomic units that comprise:

- the identified taxa (to species, genus and family) with  $n = 52$  (Table 2);
- the unidentified taxa (e.g., Porifera sp.1, Porifera sp.2, small orange Bryozoa) with  $n = 63$ ;
- the morpho-functional categories with undetermined taxa (e.g., other non-identified organisms such as other sponges, epibiontic organisms) with  $n = 8$ .

In cases where the taxonomic identification of sponges was difficult, additional close-up photos and specimen collection were conducted. Taxonomic analyses were carried out using ad-hoc methods for the Porifera phylum (e.g., Boury-Esnault & Rützler, 1997) to determine a family identification and most often a species name.

### Biodiversity measurements

#### Composition of the communities

All data were analysed with R (v.3.4.0). The matrix exported from PhotoQuad was first cleaned by removing the points associated with non-living categories and by normalizing to 100 the points associated with living categories. The R ‘vegan’ package (v. 2.5-7, Oksanen *et al.*, 2018) was used to explore the diversity of the communities. The community matrix, with the 123 recorded taxonomic units, was transformed into a Bray-Curtis dissimilarity matrix by using the ‘vegdist’ function. A PERMANOVA (Adonis2 function, 999 permutations) was performed to test the influence of the degree of confinement (SD vs. D stations) and of the location, as well as their interaction on the community composition.

Calculation of biodiversity indices was performed on the 115 clearly distinct taxonomic units by using the number of points recorded for each. Cumulative species richness was calculated for each station by using the “specaccum” function that determines the taxonomic unit accumulation curves per station by adding the number of new taxonomic unit records quadrat after quadrat. At the end of this process, the total number of “species” (here: taxonomic units or taxa) in a station is obtained. The shape of the cumulative species richness curve gives an indication of the efficiency of the sampling effort, but also an overall picture of the benthic diversity. For instance, in addition to the species richness of the sampling site, the number of quadrats required to reach the asymptote of the curve also gives an indication of the diversity of the benthic community: the fewer the quadrats needed, the faster the sampling saturation, and thus the poorer the diversity of the benthic community. Additionally, the mean species richness (S) (function “spenumber”), the Shannon (H) diversity index (function “diversity”) and Pielou’s evenness ( $H / \log(S)$ ) were also assessed for each station.



**Table 2.** Inventory of the 52 identified taxa (species, genus, family) at the study sites and their distribution in the Semi-Dark (SD) and Dark (D) communities. Species in bold are common to the four caves. \* distinct morphotypes with unconfirmed species names.

	Méjean		Jarre		Figuier		3PP	
	SD	D	SD	D	SD	D	SD	D
<b>Porifera (38)</b>								
<i>Acanthella acuta</i> Schmidt, 1862					X		X	
<b><i>Agelas oroides</i></b> (Schmidt, 1864)	X		X		X		X	
<i>Aplysina cavernicola</i> (Vacelet, 1959)			X				X	
<i>Axinella damicornis</i> (Esper, 1794)	X		X		X			
<i>Chondrosia reniformis</i> Nardo, 1847	X				X		X	
<i>Clathrina clathrus</i> (Schmidt, 1864)			X	X			X	
<i>Crella pulvinar</i> (Schmidt, 1868)	X				X	X	X	
<b><i>Dendroxea lenis</i></b> (Topsent, 1892)	X		X	X	X	X	X	
<b><i>Diplastrella bistellata</i></b> (Schmidt, 1862)	X	X	X	X	X	X	X	
<b>Dysideidae spp.</b>	X		X		X	X	X	
<i>Erylus</i> sp.					X		X	
<i>Eurypon</i> sp.	X		X				X	
<i>Haliclona fulva</i> (Topsent, 1893)			X		X			
<b><i>Haliclona mucosa</i></b> (Griessinger, 1971)	X	X	X	X	X	X	X	
<i>Haliclona</i> sp.						X		
Chalinidae sp.		X		X				
<i>Hippospongia communis</i> (Lamarck, 1814)			X				X	
Hymedesmiidae sp.								X
<i>Ircinia dendroides</i> (Schmidt, 1862)			X				X	
<i>Ircinia oros</i> (Schmidt, 1864)			X					
<b><i>Ircinia variabilis</i></b> (Schmidt, 1862)	X		X		X		X	
<i>Lycopodina hypogea</i> (Vacelet & Boury-Esnault, 1996)								X
<i>Merlia deficiens</i> Vacelet, 1980							X	
<i>Myceliospongia araneosa</i> Vacelet & Pérez, 1998				X		X		
<i>Oscarella balibaloï</i> Pérez, Ivanisevic, Dubois, Pedel, Thomas, Tokina & Ereskovsky, 2011			X				X	
<i>Oscarella microlobata</i> Muricy, Boury-Esnault, Bézac & Vacelet, 1996	X							
<b><i>Oscarella</i> spp. (<i>O. tuberculata</i> or <i>O. lobularis</i>)</b>	X	X	X	X	X		X	
<i>Penares helleri</i> (Schmidt, 1864)		X		X				
<i>Pleraplysilla spinifera</i> (Schulze, 1879)			X		X		X	
<b><i>Petrobiona massiliana</i></b> (Vacelet & Lévi, 1958)		X		X		X	X	
<b><i>Petrosia ficiformis</i></b> (Poiret, 1789)	X		X	X	X		X	
<i>Phorbates tenacior</i> (Topsent, 1925)	X		X			X		
<i>Plakina jani</i> Muricy, Boury-Esnault, Bézac & Vacelet, 1998				X	X		X	
<i>Raspaciona aculeata</i> (Johnston, 1842)					X			
<i>Rhabderemia</i> sp.		X						
<i>Terpios gelatinosus</i> (Bowerbank, 1866)					X	X	X	
<i>Thymosiopsis</i> spp. ( <i>T. cuticulatus</i> or <i>T. conglomerans</i> )	X							
<i>Timea</i> sp.								X
<b>Ascidacea (5)</b>								
<i>Botrylloides crystallinus</i> Bay-Nouailhat, Bay-Nouailhat, Gasparini & Brunetti, 2020	X							

*Continued*

Table 2 continued

	Méjean		Jarre		Figuier		3PP	
	SD	D	SD	D	SD	D	SD	D
<i>Clavelina lepadiformis</i> (Müller, 1776)	X							
<i>Halocynthia papillosa</i> (Linnaeus, 1767)	X							
<i>Lissoclinum perforatum</i> (Giard, 1872)	X			X		X		
Polyclinidae sp.	X							
<b>Cnidaria (3)</b>								
<i>Caryophyllia inornata</i> (Duncan, 1878)	X		X		X			
<i>Corallium rubrum</i> (Linnaeus, 1758)			X		X			
<b><i>Leptopsammia pruvoti</i></b> Lacaze-Duthiers, 1897	X		X		X	X	X	
<b>Bryozoa (3)</b>								
<i>Reteporella</i> sp.			X		X			
<i>Crassimarginatella solidula</i> * (Hincks, 1860)						X		
<i>Dentiporella sardonica</i> * (Waters, 1879)			X		X			
<b>Mollusca (2)</b>								
<i>Chama gryphoides</i> Linnaeus, 1758							X	
<i>Neopycnodonte cochlear</i> (Poli, 1795)							X	
<b>Foraminifera (1)</b>								
<b><i>Miniacina miniacea</i></b> (Pallas, 1766)	X		X			X	X	

Significant differences between stations were determined according to ANOVA tests followed by post hoc Tukey's test when the conditions of normality of residuals and homogeneity of variances were respected. Otherwise, a Kruskal-Wallis test followed by a post-hoc Dunn's test was conducted.

We then assessed the differences in biodiversity composition between communities. Based on the large matrix with the 123 taxonomic units, the proportion of each living category per station was calculated by adding up the number of points belonging to each category. We term this "abundance" in the Results, where it is represented in stacked bar charts. Additionally, based on the 115 taxonomic units, a Principal Coordinates Analysis (PCoA) representing the community structure per station was conducted using the R script available in Gauff *et al.* (2022). The 'envfit' function of the vegan package was used to overlay vectors of taxa significantly correlated with the ordination ( $p$ -value < 0.05,  $R^2$  > 0.2; Oksanen *et al.*, 2018). To identify taxa contributing the most to the distinction between stations, a multipattern analysis was performed with the 'indicspecies' R package (De Cáceres *et al.*, 2011). Parameters were set to 999 permutations while testing for station.

### Temporal change in sponge diversity

The assessment of temporal change in sponge diversity was based on surveys undertaken in 2015 with the same sampling method, but only at SD stations (Grenier *et al.*, 2018). The 5-year comparison of the sponge assemblages was based on the list of SD cave representative sponge species proposed for the Marseille area (Grenier *et al.*, 2018). The cumulative and mean species richness was de-

termined and  $t$ -tests (or Mann-Whitney when normality and homoscedasticity conditions were not met) were performed to assess the difference of mean specific richness between 2015 and 2020 for each SD station.

Additionally, for each photoquadrat, species occurrences were calculated as follows:

$$\text{Species occurrence} = \frac{\text{number of photos recording a given species}}{\text{total number of photos per cave}}$$

These values were then compared to those from Grenier *et al.* (2018) by measuring the rate of change between species occurrences in 2020 and 2015, and thus enabled to evaluate their stability or variability in the studied communities.

## Results

### General description of the four studied caves

The four caves studied here are all entirely submerged but display variable geomorphological and environmental characteristics, as described below:

The Méjean cave (see Millet, 1977) has a karstic origin and is located in the Côte Bleue Marine Park (a marine protected area – MPA) in the northwestern part of the Bay of Marseille, east of Méjean village. The cave is rather shallow with an angled shape and ascending profile (49 m long, 15 m depth at the entrance and ascending to the surface). The entrance is fairly small and the overall size is moderate (48 m long, up to 2 m wide and 3 m high). The Semi-Dark zone is ca. 6 m long and 1.5 m wide. The Dark zone begins 18 m from the entrance after a bend to the left, at 9 m depth where the cave is ca. 2 m wide. Outflow of fresh water frequently occurs in the Dark zone

of the cave. The floor of the cave is without silt and is mostly covered with pebbles or composed of bedrock, testifying to significant occasional water movement.

Jarre cave (see Fichez, 1989) is located on the southern side of Jarre Island, Riou Archipelago, in the Calanques National Park (CNP), south-east of Marseille and is of karstic origin. The cave has a tubular shape (130 m long, up to 6 m wide and 2 m high on average) and presents a mostly horizontal profile (17-18 m for most of the cave) allowing water homogenization with the outside, with scattered deeper recesses. The far end of the cave beyond 80 m is very narrow and ascends almost to the surface, but was not explored during this study. The Jarre cave is one of the largest caves in the area. The Semi-Dark zone is ca. 20 m long and 6 m wide. The Dark zone begins after a mound, 50 m from the entrance. Silt covers the floor of the studied zones of the cave, indicative of low water movement in those areas.

The Figuier cave (see Pouliquen, 1972) is located west of the Morgiou Cape, in the heart of the CNP and is of karstic origin. This cave has a curved shape (65 m long) with an entrance at each extremity: the largest is located between 18 and 27 m depth and the second is between 10 and 12 m depth. These two connected entrances ensure that this cave benefits from a significant water renewal rate. Sampling in the SD zone was carried out in the deepest and largest entrance of the cave. Figuier cave displays the largest SD zone of the four caves (28 m long and 25 m wide). The Dark zone studied here is fairly small (4 m<sup>2</sup>) and is located 45 m from the larger entrance and 25 m from the smaller one, at 10 m depth. This cave presents other D zones that could not be included in this study. Silty sand and pebbles cover the floor of the larger entrance and are gradually replaced by boulders and bedrock elsewhere. Water movement in the cave obviously derives from the two entrances.

The 3PP cave (see Vacelet *et al.*, 1994) is located below the “Bec de l’Aigle” Cape in the eastern part of the CNP. Unlike the other caves studied here, the rock is an Upper Cretaceous “Poudingue” (Puddingstone conglomerate) rather than limestone. The cave has a tubular shape (120 m long, 3-10 m wide and up to 8 m high) and is one of the largest caves of the area. The floor has a descending profile (15 m depth at the entrance to 25 m depth at the end). This peculiar topography leads to thermal stratification of the water in summer, when cold water is trapped in the inner cave chamber due to its density. For example, while the outside water temperature varies throughout the year (from 13 to 24°C on average), the water temperature of the inner chamber remains roughly constant (at about 14°C on average). Water renewal inside the cave is thus reduced, but the presence of cold water represents an opportunity for deep water taxa. The SD zone is ca. 20 m long and 3 m wide. The Dark zone begins at the end of the slope, 25 m from the entrance and at 22 m depth. The floor of the cave is covered by very fine silty sediment with traces of biogenic activity.

## Description of the communities

### Global overview

In total, 123 distinct taxonomic units were recorded: 82 Porifera, 16 Bryozoa, 5 Ascidiacea, 4 Cnidaria, 3 Algae, 2 Mollusca, 2 Annelida and 1 Foraminifera. Eight units correspond to epibiontic and other non-identified organisms. The total number of distinct taxonomic units varied between caves (3PP (57), Figuier (54), Méjean (46) and Jarre (44)) according to the confinement level, with 95 distinct taxonomic units found in the SD community and 40 found in the D community. The SD community is characterized by colourful, erect and massive specimens belonging to several taxonomic groups: sponges, cnidarians, ascidians, bryozoans and sometimes encrusting red algae (Fig. 2). The D community is primarily represented by encrusting sponges and bryozoans, serpulid polychaetes and a few scleractinian corals and foraminiferans (Fig. 3). In the SD zone, on average 83% of points were assigned to a living category, which provides an estimate of the benthic cover. In the D zones, only 33% could be assigned to such a category.

Of the reported taxonomic units, 52 were identified at the species, genus or family levels (Table 2). Eleven were commonly found in the 4 caves. Three of them (sponges) were restricted to the SD community: *Agelas oroides*, *Ircinia dendroides*, and *Ircinia variabilis* (Table 2, Fig. 2). The sponges *Dendroxea lenis*, *Diplastrella bistellata*, *Haliclona mucosa*, *Oscarella* spp., *P. ficiformis*, the coral *Leptopsammia pruvoti*, and the foraminiferan *Miniacina miniacina* were predominantly found in the SD community, but were also identified in the D community in some caves (Table 2, Fig. 2 and 3). *Petrobiona massiliensis*, habitually reported as a D cave dweller, as in Méjean, Jarre, and Figuier, was also found in the SD cave community of 3PP (Table 2).

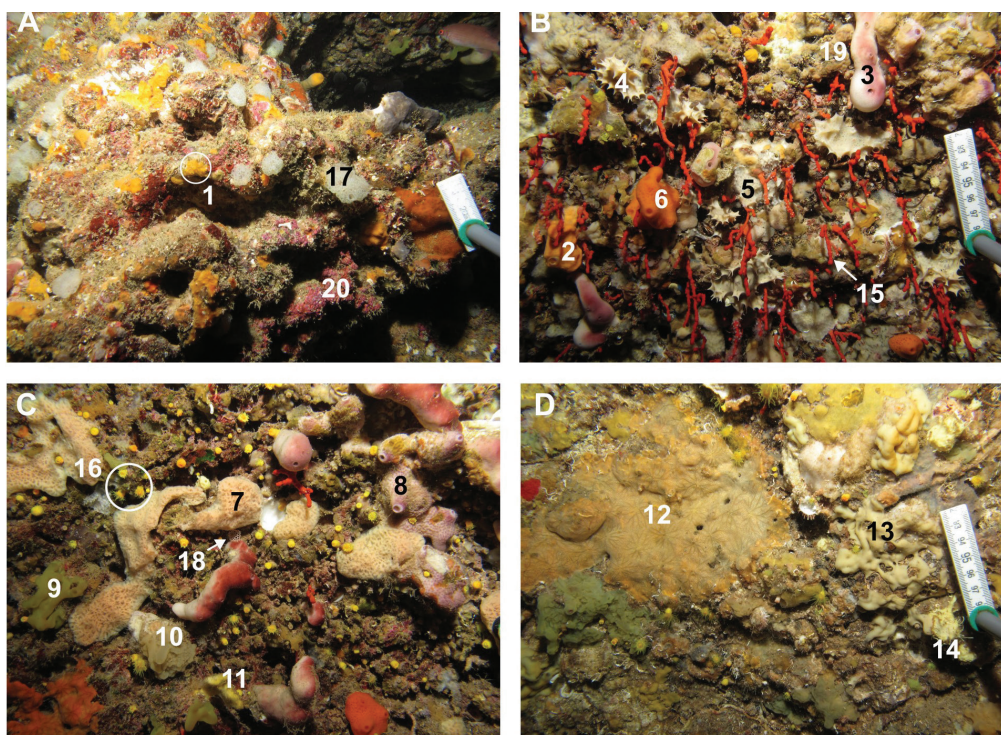
### SD vs. D cave communities

Examination of the matrix with the 123 taxonomic units revealed significant differences in biodiversity composition between caves and between the two types of communities (PERMANOVA, p-value = 0.001). The ratio between the variance of each group and the total variance (F) indicates a five-fold greater difference between the SD and the D communities across caves (F = 85) than between the caves (across communities) (F = 17).

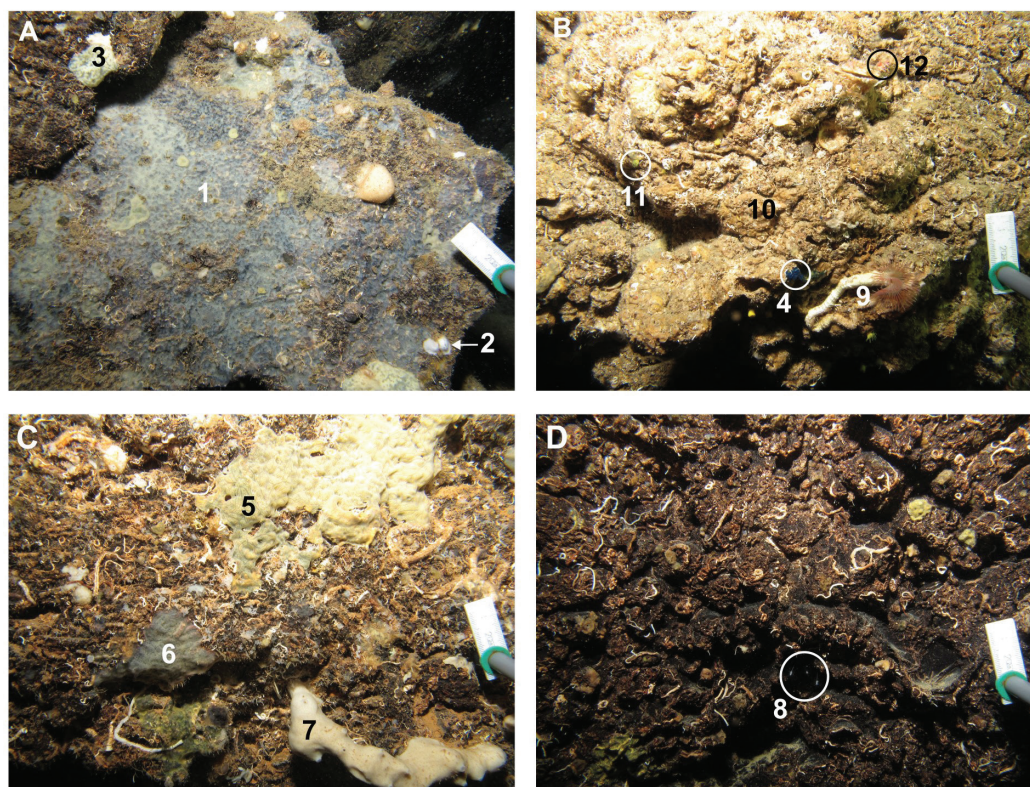
- Semi-Dark cave community

Of the 115 taxonomic units (here excluding the 8 undetermined taxa), the SD communities of 3PP and Figuier display the highest total number of taxonomic units with 46 and 42 respectively, followed by Méjean (37) and Jarre (35) (Fig. 4A). The cumulative curve reached a plateau for Méjean after the 23<sup>rd</sup> photoquadrat, for Jarre after the 29<sup>th</sup>, for Figuier after the 27<sup>th</sup> and for 3PP after the 26<sup>th</sup>. Mean species richness and the Shannon (H) index





**Fig. 2:** *In situ* photographs of the benthic diversity in the Semi-Dark cave community of (A) Méjean, (B) Figuier, (C) Jarre, and (D) 3PP caves. Each cave harbours a specific assemblage including: sponges – (1) *Crella pulvinar*, (2) *Agelas oroides*\*, (3) *Petrosia ficiformis*\*, (4) *Pleraplysilla spinifera*, (5) *Dysideidae* sp., (6) *Haliclona fulva*, (7) *Haliclona mucosa*\*, (8) *Ircinia variabilis*\*, (9) *Oscarella* sp.\*, (10) *Oscarella balibalo*, (11) *Axinella damicornis*, (12) *Diplastrella bistellata*\*, (13) *Plakina jani*, (14) *Aplysina cavernicola*; cnidarians – (15) *Corallium rubrum*, (16) *Leptopsammia pruvoti*\*; ascidians – (17) *Botrylloides crystallinus*; bryozoans – (18) *Reteporella* sp., (19) *Bryozoa* sp.15; and an encrusting red alga in (20). \*Species/morphotypes common to the Semi-Dark zones of the four caves.



**Fig. 3:** *In situ* photographs of the benthic diversity in the Dark cave community of (A) Méjean, (B) Figuier, (C) Jarre, and (D) 3PP caves. Each cave harbours a specific assemblage including: sponges – (1) *Rhabderemia* sp., (2) *Petrobiona massiliana*, (3) *Haliclona mucosa*, (4) *Terpios gelatinosus*, (5) *Diplastrella bistellata*, (6) *Dendroxea lenis*, (7) *Petrosia ficiformis*, (8) *Lycopodina hypogea*; serpulid polychaetes (9); bryozoans – (10) *Crassimarginatella solidula* (unconfirmed identification); cnidarians – (11) *Leptopsammia pruvoti*; and foraminiferans – (12) *Miniacina miniacea*.

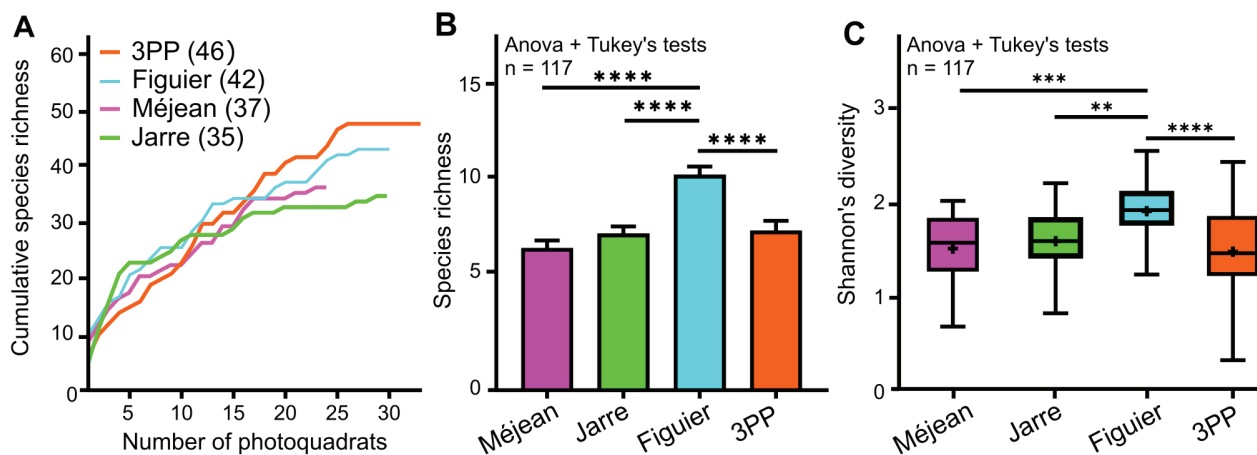


indicate that the SD community in Figuier is the most diverse (p-value < 0.01), with about 10 taxonomic units per photoquadrat and an H index of 1.92 (Fig. 4B and 4C). The other caves present 6 to 7 taxonomic units per photoquadrat on average and an H index between 1.50 and 1.61 (Fig. 4B and 4C). Pielou's evenness varies slightly but not significantly: from 0.77 at 3PP to 0.85 at Figuier.

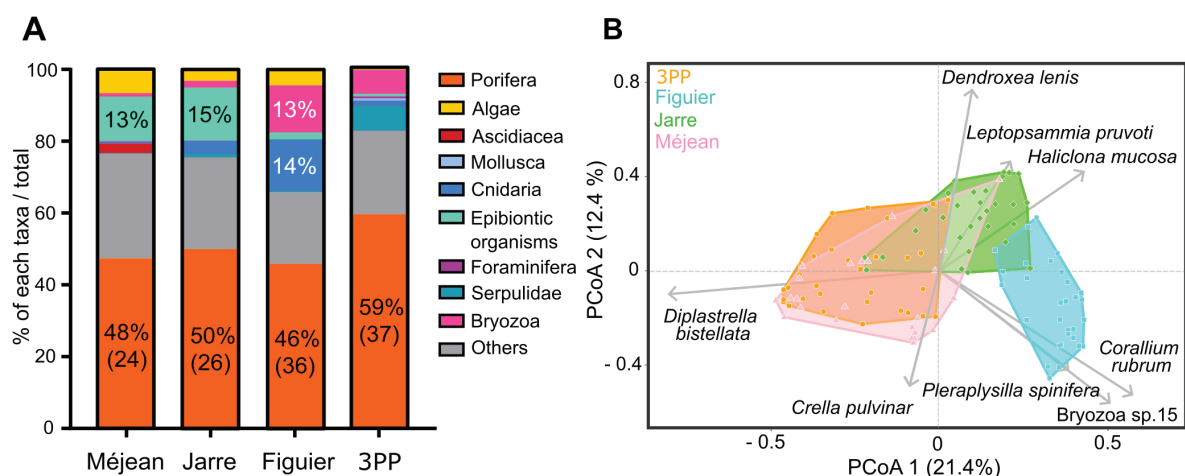
Of the number of points associated to each of the 123 categories, sponges represent nearly 50% (in abundance) of the species assemblage (Fig. 5A). Each cave has its own particularities: the SD community of the 3PP cave presents the highest number of sponge taxa (37, constituting 59% of the assemblage). Figuier's SD community differs from the others by a higher abundance of bryozoans (13%) and cnidarians (14%). Méjean's SD community presents encrusting red algae (6%) and ascidians

(3%), while Jarre's SD community is characterized by the presence of cnidarians (4%) and the highest proportion of epibiontic non-identified organisms (15%) (Fig. 5A).

Of the 115 taxonomic units, 33.8% of the variability in community composition can be explained by the PCoA (Fig. 5B). Taxa abundances, positively correlated with ordination, are significantly associated to the different sites (p-value < 0.01) (Fig. 5B.). For instance, Figuier's SD community is distinguished by the higher abundance of the red coral *C. rubrum* (22%), the sponge *Pleraplysilla spinifera* (5%) and a non-identified bryozoan (Bryozoa sp.15; 15%) (Fig. 5B, Fig. 2B.). Another sponge, *H. mucosa*, is highly represented in this community (13%) as well as in Jarre (19%) (Fig. 5B, Fig. 2C.). Jarre's SD community is characterized by the abundance of the sponge *D. lenis* (17 %) and the coral *L. pruvoti* (7%) (Fig. 5B,



**Fig. 4:** Biodiversity indices in the Semi-Dark cave community at the four study sites. (A) Cumulative richness curves. Numbers in parentheses represent the total number of taxa per station. (B) Mean species richness per quadrat and standard errors. (C) The Shannon index with boxes indicating first to last quartile, and whiskers representing maximum and minimum values. Crosses correspond to the mean values. Significant pairwise differences (Dunn's or Tukey's post hoc tests) are indicated by brackets. \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001; \*\*\*\*: p < 0.0001; no brackets: non-significant.



**Fig. 5:** Biodiversity composition of the studied Semi-Dark cave community. (A) Benthic composition with the relative percentage of each taxon per station. Numbers in parentheses represent the total number of sponge species. (B) Principal Coordinate Analysis (PCoA) plot of the community structure. Vector overlay indicates species having a positive correlation (p < 0.05; R<sup>2</sup> > 0.2) with the cave community projections.



Fig. 2C.). The SD community at 3PP and Méjean share the same dominant sponge species, *D. bistellata* (31% and 22%), but Méjean differs by the abundance of the sponge *Crella pulvinar* (8.5%) and the tunicate *Botrylloides crystallinus* (4.5%) (Fig. 5B, Fig. 2A.).

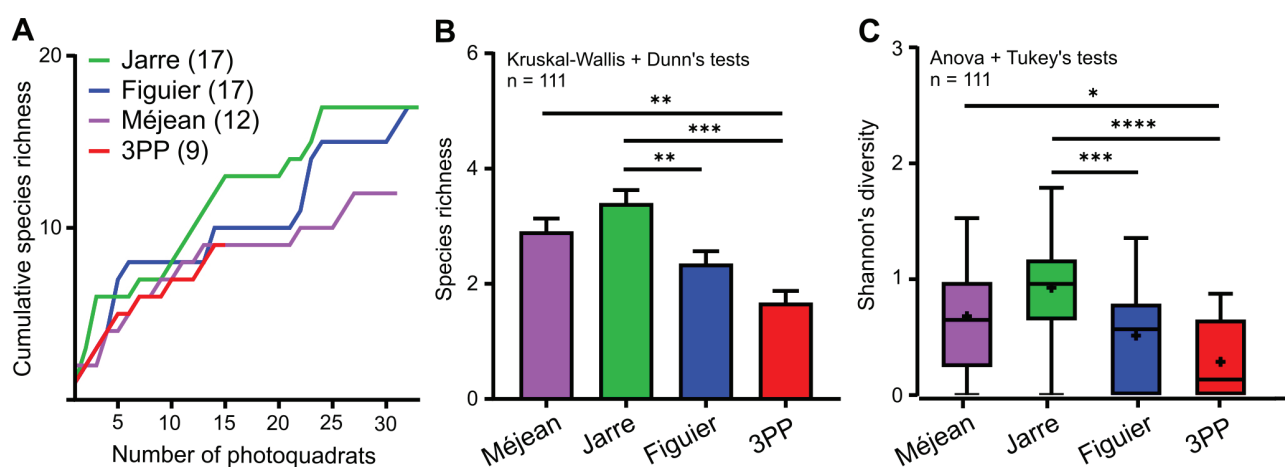
The SD community can also be distinguished by the uniqueness of some encountered species (Table 2). For instance, *Raspaciona aculeata* appears exclusive to Figuier, and *Merlia deficiens* was restricted to 3PP. Additionally, four of the five recorded tunicates were found only in Méjean.

- Dark cave community

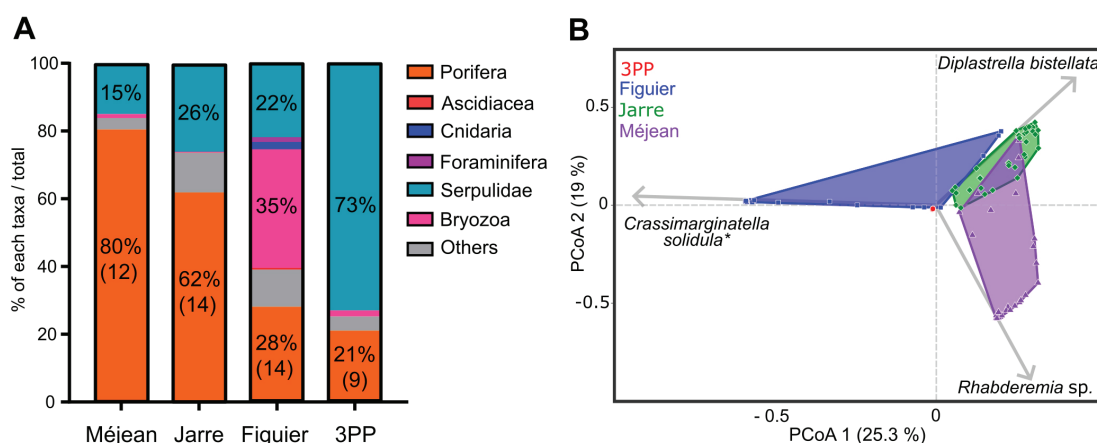
Of the 115 taxonomic units, the D communities at Jarre and Figuier present the highest total number of dis-

tinct taxonomic units (17), followed by Méjean (12) and 3PP (9) (Fig. 6A). The cumulative curve reached a plateau for all the stations. No taxonomic units were recorded after the 27<sup>th</sup> photoquadrat for Méjean SD, the 24<sup>th</sup> for Jarre SD and the 14<sup>th</sup> for 3PP SD. For Figuier, the number of taxonomic units is shown to slightly increase after the 32<sup>nd</sup>. The mean species richness (S) and the Shannon (H) index show that the D community at Jarre is significantly more diverse than those observed at Figuier and 3PP (p-value < 0.01), with approximately 3.4 taxonomic units per photoquadrat and an H index of 0.91 (Figs. 6B, 6C). The 3PP D community is the least diverse, with mean values of S (1.7), H (0.28) and a significantly lower Pielou evenness (0.36) than for Jarre and Méjean (p-value < 0.05).

Of the number of points associated to each of the 123



**Fig. 6:** Biodiversity indices in the Dark cave community at the four study sites. (A) Cumulative richness curves. Numbers in parentheses represent the total number of species per station. (B) Mean species richness per quadrat and standard error. (C) The Shannon index, with boxes showing first to last quartile, and whiskers representing maximum and minimum values. Crosses correspond to the mean values. Significant pairwise differences (Dunn's or Tukey's post hoc tests) are indicated by brackets. \*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001; \*\*\*\*: p < 0.0001; no bracket: non-significant.



**Fig. 7:** Biodiversity composition of the studied Dark cave community. (A) Benthic composition with the relative percentage of each taxon per station. Numbers in parentheses represent the total number of sponge species. (B) Principal Coordinate Analysis (PCoA) plot of the community structure. Vector overlay indicates species having a positive correlation (p < 0.05; R<sup>2</sup> > 0.2) with the cave community projections. \*distinct morphotype with unconfirmed species name.

categories, sponges account for the largest proportion of the living organisms in this community at Méjean (80%) and Jarre (62%) (Fig. 7A). In Figuier's D community, sponges (28%) share the substrate with bryozoans (35%) and serpulid worms (22%). In the 3PP D community, serpulid worms (73%) dominate and sponges represent only 21% (Fig. 7A). However, it must be noted that on photoquadrats it is rarely possible to ascertain whether serpulid worms are living or not.

Of the 115 taxonomic units, 44.3% of the variability in species composition is explained by the PCoA (Fig. 7B). As the D community of 3PP was particularly represented by non-identified polychaetes (alive or dead) and the PCoA constructed solely with distinguishable taxa, only a few photoquadrats were considered and appeared to be superimposed on the PCoA. However, two taxa were reported exclusively in the 3PP D community: the carnivorous sponge *Lycopodina hypogea* and *Timea* sp. (Table 2).

The taxa which correlate positively with ordination are also significantly associated with the different sites based on their abundance in the D communities (p-value = 0.001) (Fig. 7B). Figuier's D community is characterized by the dominance of an encrusted bryozoan (38%), potentially *Crassimarginatella solidula* (Harmelin *et al.*, 2000). Jarre's D community is defined by the presence of the sponge species *D. bistellata* (13 %), whereas the D community in Méjean is mostly dominated by another sponge, *Rhabderemia* sp. (50%), which is found there exclusively (Fig. 7B, Fig. 3A and C).

### Temporal change in the sponge assemblage

Compared to 2015, the total number of representative sponge species (listed in Grenier *et al.*, 2018) decreased for all SD sites (Table 3). Therefore, the mean species richness per site significantly declined for Méjean, Jarre and 3PP (p-value < 0.001, Table 3).

Variations in the occurrence of sponge taxa (listed in Grenier *et al.*, 2018) can be classified into five groups (Fig. 8). The first group includes 13 uncommon species

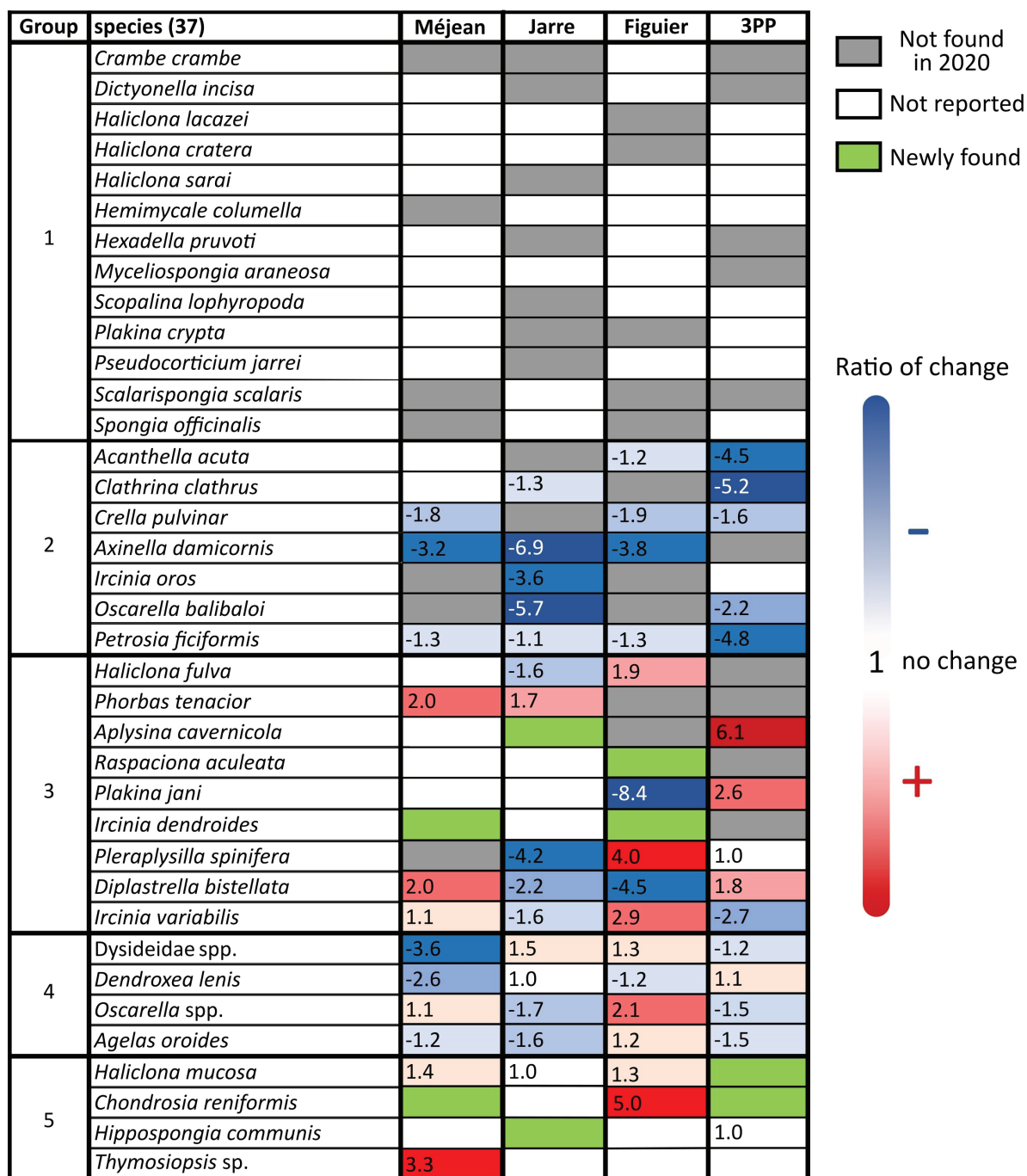
initially reported to occur in fewer than 19% of the 2015 photoquadrats but which were no longer found in the 2020 photoquadrats. The second group consists of seven species whose occurrence declined in all the sites where they were initially recorded in 2015. Three of these species were initially well represented: *A. acuta* was recorded in 54% of Jarre's photoquadrats, *Axinella damicornis* in 63% of the 3PP photoquadrats, and *Oscarella balibai* in 43% of Méjean's photoquadrats (unpublished data from Grenier *et al.*, 2018). The third group includes species with occurrences which have either increased or decreased in photoquadrats, depending on the site. Among the most contrasted variations between sites is the occurrence of *P. spinifera* which was four times higher in the Figuier SD community in 2020 (63%) compared to 2015 (16%), and 4.2 times lower in Jarre in 2020 (13%) compared to 2015 (56%). Similarly, *Plakina jani* was 2.6 times higher in the 3PP community but 8.4 times lower in the Figuier community in 2020. Interestingly, *Aplysina cavernicola* was not recorded in Figuier's SD community in 2020, despite being present in the 2015 photoquadrats. However, its occurrence increased in the 2020 photoquadrats of the SD community at 3PP and Jarre due to some small specimens of an unusual growth form. The fourth group presents taxa with moderate variations in their occurrence, except for *D. lenis* and Dysideidae spp., which appear to have been 2.6 to 3.6 times less represented in 2020 in Méjean compared to 2015. The last group includes taxa whose presence seems to have increased in all sites. For example, *Chondrosia reniformis* was newly reported in the SD community at 3PP and Méjean, and its occurrence was five times higher in Figuier in 2020 than in 2015.

### Discussion

Marine caves around the world are recognized as biodiversity hotspots (Gerovasileiou & Bianchi, 2021; Pérez *et al.*, 2016) and they harbour original benthic species assemblages essentially described in the North-Western and Eastern Mediterranean and. Sponges are the main

**Table 3.** Total and Mean Species richness per quadrat (with standard errors) of the Semi-Dark Cave community of the four studied sites in 2015 and 2020. This comparison takes into account only the listed sponge species of this community as proposed by Grenier *et al.* (2018). Differences between 2015 and 2020 were tested with a Student/Kruskal-Wallis (K-W) test. p-value (p) < 0.01; \*\*\* : p < 0.001; \*\*\*\*, ns: non-significant.

	Total Species richness		Mean Species richness / quadrat		Student/ K-W test
	2015	2020	2015	2020	
Méjean	20	14	5.4 +/- 0.29	3.8 +/- 0.24	***
Jarre	25	18	8.6 +/- 0.3	5.1 +/- 0.26	****
Figuier	23	16	6.2 +/- 0.25	5.5 +/- 0.24	ns
3PP	25	18	6.3 +/- 0.24	3.8 +/- 0.25	****



**Fig. 8:** Heatmap presenting the rates of change for each sponge taxa occurrence between 2015 and 2020 in the Semi-Dark Cave community (based on the species list from Grenier *et al.*, 2018). Group 1: 13 sponge taxa initially reported to occur in fewer than 20% of the 2015 photoquadrats but which were no longer found in the 2020 photoquadrats. Group 2: 7 sponge taxa whose occurrence decreased in all the sites where they were initially recorded in 2015. Group 3: sponge taxa with occurrences in photoquadrats that have increased or decreased, depending on the site. Group 4: sponge taxa with quite moderate variations in their occurrence. Group 5: sponge taxa whose presence seems to have increased in most sites.

representative taxa of cave communities and have been particularly well studied (Sarà, 1968; Pouliquen, 1972; Bibiloni *et al.*, 1989; Corriero *et al.*, 2000; Bell, 2002; Gerovasileiou & Voultsiadou, 2012, 2016; Grenier *et al.*, 2018; Longo *et al.*, 2023). Bryozoans and cnidarians are also well represented (Harmelin, 1969, 1985, 2000; Pérès, 1967; Zibrowius, 1978, 1980; Rosso *et al.*, 2019). However, few studies have qualitatively and quantitatively compared the benthic composition and the biodiversity

indices between Semi-Dark and Dark communities, and between caves (Martí *et al.*, 2004; Bussotti *et al.*, 2006; Gerovasileiou *et al.*, 2017). In the present study, we assess how the environmental settings and the geomorphology of caves participate in structuring the SD and D communities, thus highlighting the singularities of each cave. We also assess short-term changes in sponge biodiversity, possibly resulting from climate change effects, for the first-time.

## Comparison of abiotic factors between caves

The geomorphology and the environment render our four cave habitats very distinct and thus likely to host different benthic assemblages. The three caves of the CNP are bathed by the Northern Mediterranean Current, while Méjean is mostly influenced by the Rhone River plume and other more local currents which are induced by the dominant NW wind (Mistral) of the Marseille area (Castelbon, 1972). In addition, Jarre, Figuier and Méjean caves were formed from the karstic dissolution of limestone which often results in a great number of micro- to macro-crevices and holes. The non-karstic ‘poudingue’ nature of 3PP has resulted in a more friable rocky substrate, smoothed by marine currents, with many fewer crevicular habitats. The morphology of a cave (depth and size of the entrance, profile of the cave) determines the degree of light penetration as well as the circulation of water within, therefore controlling the supply of nutrients and/or colonists (Poulliquen, 1972; Harmelin *et al.*, 1985; Martí *et al.*, 2004; Radolović *et al.*, 2015). For instance, Méjean and 3PP present very contrasted configurations. Méjean is the smallest, narrowest and shallowest cave, with an ascending profile, favouring water renewal. Conversely, 3PP has the largest volume and its descending profile is responsible for trapping cold-water within the cave. This leads to a constant water temperature in the Dark part of the cave (Vacelet *et al.*, 1994, Vacelet, 1996) and to a water density barrier which limits food/colonist supply during most of the year. Figuier benefits from two entrances, one of which is very wide with a low ceiling, ensuring better light penetration into the Semi-Dark part of the cave and great water renewal (Poulliquen, 1972). The geomorphology of a cave thus determines the gradients of numerous environmental parameters in the cave habitat and shapes the detailed composition of the benthic biodiversity (Martí *et al.*, 2004; Bussotti *et al.*, 2006; Dimarchopoulou *et al.*, 2018). As a result, each cave harbours a distinct species assemblage, which partly explains the variation in numbers of taxa between caves, including the presence of unique species.

## General comparison of benthic biodiversity

In all four studied caves there is a clear change in biodiversity between the Semi-Dark and Dark zones. Erect, colourful and massive sponges, corals and bryozoans of the SD community are replaced by encrusted and less colourful specimens of sponges, bryozoans and serpulids in the D community. This pattern of biodiversity was first highlighted in pioneer studies of the Marseille area (Laborel & Vacelet, 1958; Harmelin *et al.*, 1985) and again more recently in other Mediterranean and tropical caves (Vasseur, 1984; Bianchi & Morri, 1994; Harmelin *et al.*, 2003; Pérez *et al.*, 2016; Dimarchopoulou *et al.*, 2018), testifying that the confinement gradient, resulting from reduced light, hydrodynamics and nutrient availability, modulates the biodiversity distribution along a horizontal zonation. Of the 52 identified taxa, eleven species (see

section 2.1 of the results) are common to all four caves and are recognized as characteristic species of Mediterranean caves (Pérès & Picard, 1964; Manconi *et al.*, 2013; Grenier *et al.*, 2018; Gerovasileiou & Bianchi, 2021; Longo *et al.*, 2023). Other taxa are found exclusively in the SD community (*A. oroides*, *Ircinia* spp., *C. rubrum*) or in D communities (e.g., *Rhabderemia* sp.) and are considered as characteristic taxa of the underwater cave ecosystem (Harmelin *et al.*, 2003; Grenier *et al.*, 2018). However, taxa distribution is not always exclusive to one of these two cave communities. The sponges *D. lenis*, *D. bistellata*, *H. mucosa*, *Oscarella* sp., *P. ficiformis*, *P. massiliana*, the coral *L. pruvoti* and the foraminiferan *Miniacina miniacea* have been reported in both communities, as demonstrated in previous studies (Vacelet *et al.*, 1959; Pérès, 1967; Balduzzi & Cattaneo, 1985; Gerovasileiou & Voultsiadou, 2016; Longo *et al.*, 2023), suggesting a rather patchy distribution depending on their affinity with the environmental conditions and the absence of sessile competitors (Harmelin *et al.*, 1985; Corriero *et al.*, 2000; Manconi *et al.*, 2009). Moreover, a good number of taxa are shared between SD caves and adjacent habitats of the littoral zone, and some D cave dwellers may also be found under boulders in shallow waters or in deep-sea ecosystems (see for instance Pérez *et al.*, 2017; Cárdenas *et al.*, 2018 or Grenier *et al.*, 2018 for sponges).

The cumulative richness curves demonstrate that the sampling effort per station was satisfactory, except for the Figuier D community. For this station, a few more photoquadrats might reveal higher species richness, a point which should be considered for any future sampling.

## SD vs. D cave communities

In the SD community, sponges represent nearly 50% (in abundance) of the assemblage, a percentage in line with previous studies (Corriero *et al.*, 2000; Digenis *et al.*, 2022). Each cave harbours a specific assemblage which may be related to the cave-specific environmental conditions. The Figuier SD community is the most diverse and the most heterogeneous of the four caves, offering an extremely colourful seascape. The depth of the entrance, the height and large development of the ceiling, and the absence of competitors such as algae provide ideal conditions for the establishment of *C. rubrum*, which is particularly well represented and thus develops one of the main facies of this community (Laborel, 1960; Poulliquen, 1972). In addition, the sponges *P. spinifera* and *Haliclona fulva* are also well represented, which appears to be in line with their listing among the species frequently associated with the *C. rubrum* facies (Michez *et al.*, 2017). The Méjean SD community is very different from the other three, which is probably linked to its geographical location in the north of our study area where it is under the influence of the Rhone River plume. In this area, the Northern Current plays only a minor role, compared to in the three other sites. The Côte Bleue water mass has been shown to be somehow distinct from that of the Calanques Coast, the two being separated by a noteworthy barrier



to gene flow (Lejeusne & Chevaldonné, 2006). This may explain the greater diversity of ascidians and the abundance of some sponge species such as *C. pulvinar* in the Méjean SD community. In the D cave communities the contrast between caves is even more pronounced. Jarre's D community appears to be the most diverse, marked by the presence of sponges such as *D. bistellata* and *P. ficiformis* which are usually found in abundance in the SD community. The high values of benthic diversity of the Jarre D cave community suggests a regular water circulation (Chevaldonné & Lejeusne, 2003; Rastorgueff *et al.*, 2014), ensuring a sufficient food supply (Fichez, 1991; M. Derrien, unpublished data) for the establishment of these species. Furthermore, the presence of a dense population of mysids (*Hemimysis margalefi*) in the back of the cave may also contribute to mitigating the oligotrophy of the D community (Fichez, 1991; Rastorgueff *et al.*, 2011) by performing daily migrations between the Dark zone and the outside. Conversely, the peculiar environmental conditions of the 3PP cave are responsible for the low species diversity recorded in the Dark zone, and also of the occurrence of cold-water species usually found in deep-sea ecosystems (Vacelet *et al.*, 1994, Vacelet & Boury-Esnault, 1995; Harmelin & Vacelet, 1997).

### Temporal change in the sponge assemblage

Significant changes have occurred in the sponge assemblage of the SD community since 2015. In each cave, species richness has decreased and some sponge taxa, initially well represented in 2015, were rarely or not recorded in 2020. Changes in Mediterranean sponge assemblages have been reported in previous studies and related to climate change and anthropogenic disturbances (Goodwin *et al.*, 2013; Bertolino *et al.*, 2016; Nepote *et al.*, 2017; Costa *et al.*, 2018). Garrabou *et al.* (2022) have notably shown that during the 2015-2019 period, the Mediterranean Sea experienced exceptional thermal conditions (+ 0.7°C on average in the Marseille area compared to 1984), resulting in five consecutive years of widespread mass mortality events (MMEs) across the basin and affecting sponge species down to 45 m depth. Sponges of the subclass Keratosa (e.g., Dysideidae, Irciniidae and Spongiidae) have been reported as particularly affected by MMEs (Pérez *et al.*, 2000; Garrabou *et al.*, 2009; Costa *et al.*, 2019). Six of the 37 species considered in Figure 8 showed reduced occurrence in 2020 in all the caves where they were initially recorded: *Crambe crambe*, *Scalarispongia scalaris*, *S. officinalis*, *A. acuta*, *A. damicornis*, *Clathrina clathrus*, *Ircinia oros* and *P. ficiformis*. The sensitivity of a great number of species to marine heat waves has been documented in several previous studies (Pérez *et al.*, 2000; Garrabou *et al.*, 2009; Bertolino *et al.*, 2016; Gómez-Gras *et al.*, 2019; Verdura *et al.*, 2019; Grenier *et al.*, 2023) and is likely to explain the observed variations in occurrence between 2015 and 2020. Moreover, the decrease in abundance of some of these species might impact the survival of other species. For example, Pérez *et al.* (2011) reported that *O.*

*balibalo* usually overgrows other invertebrate species, with *S. officinalis* and *I. oros* being among its preferred biological supports. The observed decrease in the occurrence of these two species may thus also partly explain the reduced occurrence of *O. balibalo* in our studied caves. Our temporal change assessment has also highlighted taxa for which occurrence increased in at least some caves in 2020. *Chondrosia reniformis* appears to be among the species whose occurrence has increased since 2015, particularly in the SD community of Figuier and has been newly reported in the SD communities of Méjean and 3PP. The ecological success and the resistance of *C. reniformis* when faced with diverse disturbances (including temperature increase) has been reported (Garrabou & Zabala, 2001; Idan *et al.*, 2020), suggesting that this species likely benefits from the availability of space after the death of other species.

Given the extent of the changes recorded between 2015 and 2020, and considering the devastating marine heat waves which occurred in summer 2022 (Grenier *et al.*, 2023), it is crucial to maintain surveys of the benthic diversity in underwater caves. These surveys should include the D zones, and monitoring frequency should perhaps be increased to target other biological compartments (e.g., mobile fauna, soft bottom benthic fauna). We strongly emphasize herein the importance of biodiversity monitoring in the context of a rapidly changing environment, and in poorly resilient marine ecosystems in particular. More frequent monitoring of this kind should make it possible to measure more accurately the direct impact of climate change on marine species richness and to better evaluate the putative impacts on marine ecosystem functioning. Given the likelihood that any change in species composition and/or abundance will impact biotic interactions, there is a clear need for new methods to more accurately monitor ecosystem functioning and eventually the services provided to human societies.

### Acknowledgements

The authors are grateful to the Biodiversa + project SPONBIODIV (ANR-22-EBIP-0004-06) and to the AMIDEX project MARRIO (AMX-22-IN1-46) for funding our research. We thank Dorian Guillemain and the diving service of OSU Institut Pythéas, the captains and crews of R/V Antedon 2 and Astroides, and Sacha Molinari for their help in the field. We thank Robin Gauff and Christophe Lejeusne for discussions about photoquadrat treatments. We are also grateful to Jean Vacelet, Nicole Boury-Esnault, Jean-Georges Harmelin and Marie Grenier for providing their precious help in the taxonomic identification of some species.

**Author contribution statement:** TP took the photoquadrats. TP, MD, PC sampled the specimens needed for identification. MD analysed the data. MD, PC and TP wrote the manuscript, all authors revised and approved the final text.



**Conflict of interest:** The authors declare that they have no conflict of interest.

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