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Spatial variability of sessile benthos in a semi-submerged marine cave of a remote Aegean Island (eastern Mediterranean Sea)

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ABSTRACT

The spatial heterogeneity of sessile benthos was investigated, for the first time in the eastern Mediterranean, in a semi-submerged cave of a NATURA 2000 Special Protection Area in the North Aegean Sea. The use of a non-destructive photographic method and advanced image analysis revealed the presence of 46 taxa of Chlorophyta, Ochrophyta, Rhodophyta, Foraminifera, Porifera, Anthozoa, Polychaeta, Bryozoa, and Ascidiacea, including new records of rare species. Sponges and macroalgae were the dominant groups, in terms of substrate coverage and number of taxa. Sponges were found in all cave sectors covering considerable part of the substrate, whereas macroalgae dominated the entrances and were not recorded further than the middle part of the cave. Different patterns were observed between the walls and the floor with regard to both the biotic coverage, which decreased from the entrances towards the inner part of the cave, and diversity, possibly due to the higher sedimentation rate on the floor. Both the distance from the entrances and the position within the cave, as well as the combination of these factors, had a statistically significant effect on the observed patterns. Resemblance analysis separated the floors at the luminous entrances from the rest of the tunnel, revealing groups that roughly corresponded to the sciaphilic algal-dominated entrance zone and the intermediate semi-dark cave sectors, where sessile invertebrates dominated. The present study, which is unique in the eastern Mediterranean basin, can be utilized for comparative studies and can serve as a solid basis for future monitoring.

1. Introduction

More than half of the Mediterranean coastline is covered with rocky substrata encompassing a high number of cave formations (Riedl, 1966; Cicogna et al., 2003; Giakoumi et al., 2013). Marine caves constitute biodiversity reservoirs, supporting species-rich communities, endemic taxa and protected species (Gerovasileiou and Voultsiadou, 2012). One of the most emblematic protected species, the endangered Mediterranean monk seal *Monachus monachus*, uses semi-submerged caves with internal beaches to rest and give birth (Dendrinos et al., 2007). Therefore, marine caves are protected by the European and Mediterranean legislation (European Community, 1992; UNEP-MAP-RAC/SPA, 2015).

Marine cave formations present diverse geomorphology and submersion levels, including entirely or semi-submerged blind caves, tunnels and pits, more or less topographically complex (Bianchi et al., 1986; Ugolini et al., 2003). This diversity is reflected on their benthic community structure, which is influenced by gradients of abiotic parameters, such as illumination and water circulation, generated by the cave-specific topography (Balduzzi et al., 1989; Morri et al., 1994; Bussotti et al., 2006; Radolović et al., 2015). Thus, semi-submerged caves may present different spatial community gradients than submerged ones (Pouliquen, 1972; Martí et al., 2004). Semi-submerged caves, therefore, merit further investigation and monitoring as they are easy to access and prone to impacts related to visitation, coastal and marine infrastructure construction, and pollution (Giakoumi et al., 2013; Nepote et al., 2017). In addition, the illuminated zones of semi-submerged caves and tunnels, which are also exposed to high hydrodynamic regimes, are more vulnerable to biological invasions (Gerovasileiou et al., 2016); the latter are highly important in the eastern Mediterranean where the number of alien species is dramatically increasing (Raitsos et al., 2010; Bianchi et al., 2014; Katsanevakis et al., 2014).

The majority of studies on marine cave assemblages have focused on shallow caves of the northwest and central Mediterranean Sea (Balduzzi et al., 1989; Corriero et al., 2000; Martí et al., 2004; Bussotti et al., 2006; Rosso et al., 2013; Radolović et al., 2015). Although one fourth of the recorded Mediterranean caves are located in the eastern basin (Giakoumi et al., 2013), only recently has a first assessment of the eastern Mediterranean marine

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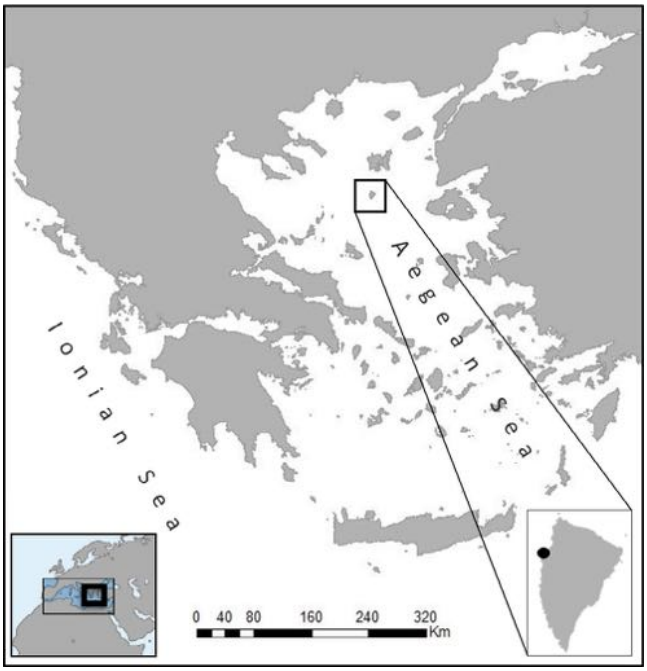

Fig. 1. Location of the studied cave (Trypia Spilia) on Agios Efstratios Island in the North Aegean Sea, Greece.

cave biota been conducted (Gerovasileiou et al., 2015) and only two submerged caves in the Aegean Sea have so far been quantitatively studied regarding their benthic community structure and diversity gradients (Gerovasileiou and Voultsiadou, 2016; Gerovasileiou et al., 2017; Sanfilippo et al., 2017).

Recently, the availability of baseline data on the benthic composition of marine caves in the western Mediterranean has enabled the detection of shifts in community structure due to summer heat waves and harbour construction (Parravicini et al., 2010; Nepote et al., 2017). Nevertheless, the absence of quantitative data on the community structure of vulnerable semi-submerged caves from the eastern Mediterranean inhibits monitoring of marine cave communities and estimation of possible alterations through time due to various pressures and impacts.

Within this context, the aim of the present work was to quantitatively describe, for the first time, the benthic community zonation in a semi-submerged marine cave of the eastern Mediterranean (North Aegean Sea), within a remote NATURA 2000 site, thus providing a basis for future monitoring.

2. Materials and methods

2.1. Study site

The Greek island Agios Efstratios is an isolated Aegean island, standing in the centre of the North Aegean Sea (Fig. 1), approximately 30 km from the nearest island, Lemnos, and surrounded by waters deeper than 200 m. The island with its surrounding marine area constitutes a Special Protection Area (GR4110014) of the Greek NATURA 2000 network, encompassing extensive *Posidonia oceanica* meadows, rocky reefs, steep sea cliffs, and numerous cave formations, including important shelters for the Mediterranean monk seal *Monachus monachus*. Nevertheless, the area does not benefit from any management plan and only scattered information is available on its marine biodiversity (e.g. Akritopoulou et al., 2013).

The studied cave, named Trypia Spilia (meaning hollow cave), is located on the western coasts of Agios Efstratios Island (39° 32' 5.94'' N, 24° 58' 39.72'' E), in proximity (800 m) to the only port of the island, which was significantly enlarged in 2016. It is one of the most easily accessible and iconic marine caves of the island, frequently visited by recreational fishers and tourists.

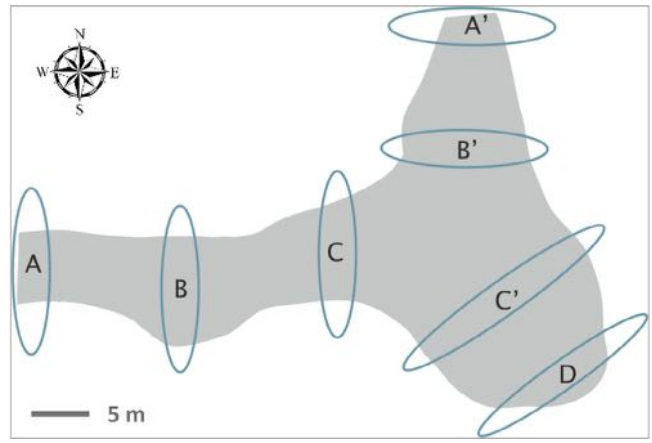

Fig. 2. Plan view of the studied cave, designed with the software *cavetopo* (Gerovasileiou et al., 2013), on which the sampling sectors are depicted (A and A′ are entrances to the cave).

Trypia Spilia is a large semi-submerged, throughout its entire ground plan, cave (Fig. 2) characterized by complex geomorphology; it has two entrances leading to two elongated tunnels: a 45 m long, west oriented tunnel (sectors A, B, C) and a 25 m long, north oriented tunnel (sectors A′, B′, C′). The two tunnels join to form an inner, 30 m long chamber (sector D) giving a "ϒ" shape to the cave. Two dark blind tunnels start from the inner edge of this chamber, one of which leads to an internal beach; these tunnels were not mapped or investigated quantitatively within the present study, due to their shallow depth and narrow width. The depth at the two entrances is 3 (sector A) and 5 m (sector A′), whereas the inner chamber is 1–2 m deep. The biggest part of the cave floor is covered with rocks lying on a sandy substrate, thus creating micro-habitats for benthic taxa with varying degrees of shading.

2.2. Sampling scheme

Sampling took place in August 2009 by means of SCUBA diving. For the quantitative analysis of benthic communities, we used a non-destructive photographic method, as used before for the study of submerged caves in the Aegean Sea (Gerovasileiou and Voultsiadou, 2016; Gerovasileiou et al., 2017). Along with the photographic samples, we performed extensive qualitative sampling of the species depicted in the quadrats (approximately 50 samples) in order to ensure the maximum possible accuracy in their taxonomic identification. Despite limitations regarding species identification, photographic methods are highly recommended for protected hard substrate habitats, such as marine caves, due to their non-destructive character as well as to the fact that they provide ease in underwater sampling and offer the possibility for future monitoring (Bianchi et al., 2004).

For the needs of the present study, we photographed quadrats from seven distinctive sectors along the two tunnels from the entrances to the inner part of the cave (A–D and A′–D) (Figs. 2 and 3). Cave mapping and visualization was performed with the specialized 3D cave imaging software *cavetopo* using the suggested mapping protocol and required equipment for semi-submerged caves (Gerovasileiou et al., 2013). A total of 63 quadrats were photographed at 3 different positions within each sector (3 replicate samples on each wall, left and right, and 3 on the cave floor). Sectors A and A′ were each composed of the nine quadrats of the

respective cave entrance. Sectors B and C were composed of the quadrats of the mid and inner part of the west-facing tunnel (15 and 30 m from the respective entrance), sector B′ comprised the quadrats of the inner part of the north-facing tunnel (10 m from the respective entrance) and sector C′ the inner part of the tunnels (at 30–45 m from the entrances). Lastly, sector D included the inner part of the chamber (at 70 and 50 m from the two entrances, respectively).

2.3. Image processing and data analysis

For the calculation of substrate coverage with sessile organisms, we used photoQuad, a specialized image processing software dedicated to ecological applications (Trygonis and Sini, 2012), which has been recently used in other cave studies in the area (Gerovasileiou and Voultsiadou, 2016; Gerovasileiou et al., 2017). The methods employed for the extraction of percentage coverage were those of image segmentation-based regions (SG), in which the software divides the photoquadrat into pixel groups that share common features such as colour and light intensity, and freehand regions (FH), in which the researcher manually draws the outline of the areas of interest (Trygonis and Sini, 2012). Species identification was performed using voucher specimens collected with the qualitative samples.

For the quantitative description of the sessile benthic communities three diversity indices were calculated, using substrate coverage data: (i) species richness (S); (ii) Shannon–Wiener diversity index (H′) and (iii) Pielou's species evenness index (J′).

Statistical analyses were conducted with PRIMER-E 6, including PERMANOVA+, and with IBM SPSS Statistics 22. Variability of index values at the different sectors and positions of the cave was statistically evaluated using non-parametric analysis of variance (PERANOVA) (Anderson et al., 2008). Variability was tested against two factors: (i) cave sector (Se) with seven fixed levels (different distances from the entrances: A, B, C, D, C′, B′, A′) and (ii) position within each sector (Po) with three fixed levels (F: floor, L: left wall, R: right wall).

Also, in order to investigate spatial patterns of the sessile community structure we used hierarchical clustering (CLUSTER) and multidimensional scaling (MDS). For these analyses, the data were double square root transformed, thus mitigating contribution of the very abundant species, and a triangular similarity matrix was created based on the Bray–Curtis similarity index (Clarke and Warwick, 2001). The impact of sector, position, as well as their combination, on the observed grouping patterns was examined with two-way PERMANOVA test, following the same design that was used for PERANOVA (Anderson et al., 2008). Lastly, the contribution of each taxon to the observed similarities and dissimilarities between the groups of quadrats was estimated with SIMPER analysis (Clarke and Warwick, 2001).

3. Results

3.1. Faunal and floral composition of benthic communities

In total, 46 taxa belonging to 9 higher taxonomic groups were identified to the lowest possible taxonomic level (2 Chlorophyta, 2 Ochrophyta, 5 Rhodophyta, 1 Foraminifera, 26 Porifera, 5 Anthozoa, 1 Polychaeta, 3 Bryozoa, and 1 Ascidiacea) (Table 1). Most taxa were identified to the species level but when visual identification was not possible, groups of common morphological characteristics, such as "turf-forming algae", "non-calcified Bryozoa" and "encrusting Bryozoa" were formed. The dominant organisms in terms of biotic coverage were sponges that covered more than half (54%) of the examined surface, followed by rhodophytes (28%) and bryozoans (8%). The red alga *Peyssonnelia* sp. and the sponges *Spirastrella cunctatrix*, *Agelas oroides*, and *Ircinia paucifilamentosa* were the species that contributed half (51%) of the biotic coverage (Table 1).

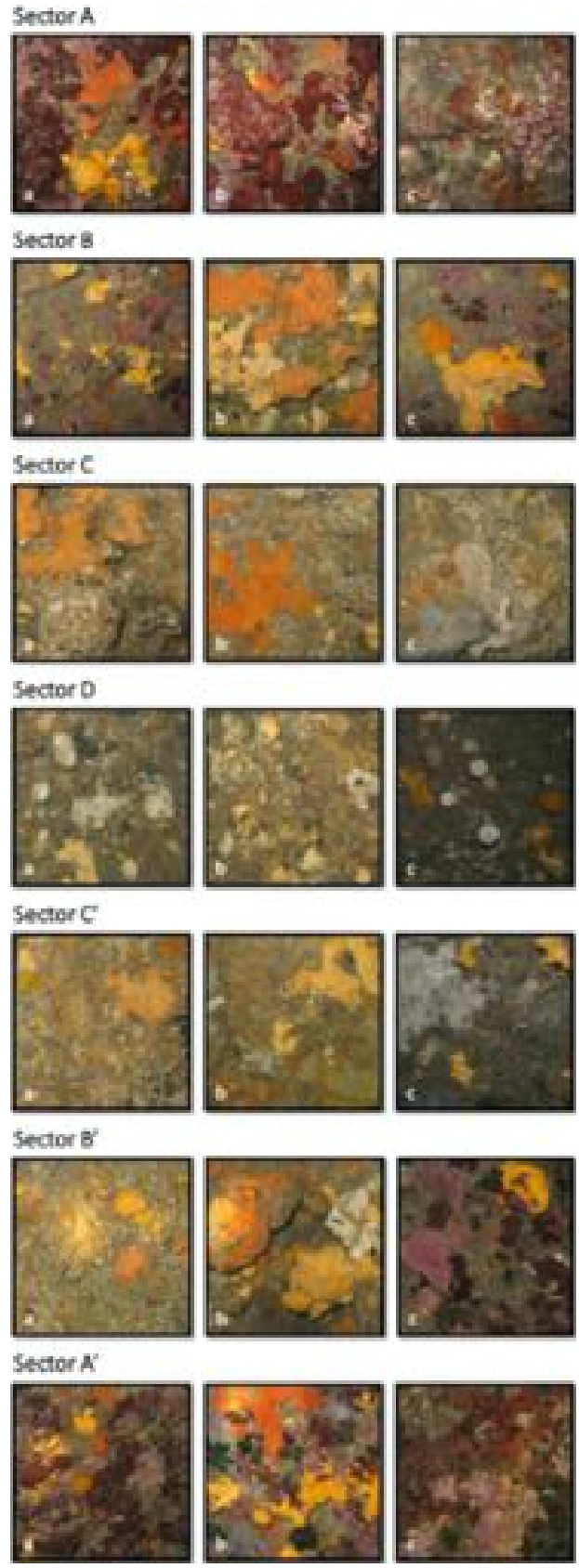

Fig. 3. Views of benthic communities in the studied cave. Indicative photoquadrats from each sector of the cave: a. left wall, b. right wall, c. floor (Photos: V. Gerovasileiou).

Table 1
Higher taxonomic group (HTG), percent biotic coverage (BC), distribution in different cave sectors (A–D, A′–C′) along transects 1 & 2 (Di T1, T2) and position (Po) of the taxa recorded in the surveyed cave. F: cave floor; L: left wall; R: right wall. For depiction of cave sectors see Fig. 2. Species in the list are ordered according to the decreasing value of their percent biotic coverage.

Taxon	HTG	BC (%)	Di T1	Di T2	Po
Peyssonnelia sp.1	Rhodophyta	21.8	A B	A′ B′	F L R
Spirastrella cunctatrix Schmidt, 1868	Porifera	13.8	A B C D	A′ B′ C′ D	F L R
Agelas oroides (Schmidt, 1864)	Porifera	9.4	A B C D	A′ B′ C′ D	F L R
Ircinia paucifilamentosa Vacelet, 1961	Porifera	5.9	B C D	B′ C′ D	F L R
Encrusting Bryozoa	Bryozoa	5.7	A B C D	A′ B′ C′ D	F L R
Phorbas tenacior (Topsent, 1925)	Porifera	4.5	A B C D	A′ B′ C′ D	F L R
Turf-forming algae	Macroalgae	3.4	A B	A′	F L R
Peyssonnelia sp.2	Rhodophyta	3.3	A B	A′ B′	F L R
Petrosia (Petrosia) ficiformis (Poiret, 1789)	Porifera	3.3	A B C D	A′ B′ C′ D	F L R
Lithophyllum sp.	Rhodophyta	3.1	A B	A′ B′	F L R
Ircinia oros (Schmidt, 1864)	Porifera	2.5	B C	B′ C′	F L R
Haliclona sp.	Porifera	2.4	C D	A′ B′ C′ D	F L R
Non-calcified Bryozoa	Bryozoa	2.3	A B C D	A′ B′ C′ D	F L R
Haliclona (Soestella) mucosa (Griessinger, 1971)	Porifera	2.3	A B C D	A′ B′ C′ D	F L R
Dendroxea lenis (Topsent, 1892)	Porifera	2.1	A B C D	B′ C′ D	F L R
Serpulidae	Polychaeta	2.0	A B C D	A′ B′ C′ D	F L R
Terpios gelatinosa (Bowerbank, 1866)	Porifera	1.6	A B C D	A′ B′ C′ D	F L R
Thymosiopsis cuticulatus Vacelet&Perez, 1998	Porifera	1.5	B C D	B′ C′ D	F L R
Madracis pharensis (Heller, 1868)	Anthozoa	1.2	B	B′	L R
Miniacina miniacea (Pallas, 1766)	Foraminifera	1.0	A B C D	A′ B′ C′ D	F L R
Fasciospongia cavernosa (Schmidt, 1862)	Porifera	0.9	A B C	A′ B′	F L R
Hexadella pruvoti Topsent, 1896	Porifera	0.7	A B C D	A′ B′ C′ D	F L R
Merlia normani Kirkpatrick, 1908	Porifera	0.7	B C	B′ C′	F L R
Palmophyllum crassum (Naccari) Rabenhorst	Chlorophyta	0.7	A B	A′ B′	F L R
Myrmekioderma spelaeum (Pulitzer-Finali, 1983)	Porifera	0.4		C′ D	F R
Neophrissospongia sp.	Porifera	0.4	C D	B′ C′ D	F L R
Didemnum sp.	Ascidiacea	0.4	A B C	A′ B′	L R
Leptopsammia pruvoti Lacaze-Duthiers, 1897	Anthozoa	0.4	A B C D	A′ B′ C′ D	F L R
Spongia (Spongia) virgultosa (Schmidt, 1868)	Porifera	0.3	A B	A′	F L R
Spongia (Spongia) officinalis Linnaeus, 1759	Porifera	0.3	A B	B′	L R
Axinella damicornis (Esper, 1794)	Porifera	0.2	A B C D	A′ B′ D	F L R
Acanthella acuta Schmidt, 1862	Porifera	0.2	A B	A′ B′	F L R
Dictyonella incisa (Schmidt, 1880)	Porifera	0.2	A B C	B′ C′	F L R
Sarcotragus foetidus Schmidt, 1862	Porifera	0.2	A B	B′	F L R
Aplysilla rosea (Barrois, 1876)	Porifera	0.1		B′ C′	F L
Caryophyllia (Caryophyllia) inornata (Duncan, 1878)	Anthozoa	0.1	A B C D	A′ B′ C′ D	F L R
Mesophyllum sp.	Rhodophyta	0.1	A		L
Dictyota dichotoma (Hudson) J.V. Lamouroux	Ochrophyta	0.1	A	A′	F L
Zanardinia typus (Nardo) P.C. Silva	Ochrophyta	0.04	A		F
Haliclona (Halichoclona) fulva (Topsent, 1893)	Porifera	0.03	C	B′	L R
Cliona schmidtii (Ridley, 1881)	Porifera	0.03	B C	B′ C′	F L R
Polycyathus muellerae (Abel, 1959)	Anthozoa	0.03	C D	A′ C′ D	L R
Adeonella pallasii (Heller, 1867)	Bryozoa	0.02	A		L R
Valonia macrophysa Kützing	Chlorophyta	0.02	A B	A′	F L R
Hoplangia durotrix Gosse, 1860	Anthozoa	0.01	A		R
Amphiroa sp.	Rhodophyta	0.01		A′	R

3.2. Biotic coverage — spatial patterns

The biotic coverage of the substrate (including Serpulidae tubes) decreased from the entrance sectors A and A′ (∼80%) towards the innermost sector D of the cave (∼40%) (Fig. 4a). The quadrats of the middle part of the cave (sectors, B, B′, C and C′) had a similar percent coverage of about 60%. The rest of the quadrat area was covered with sediment and unidentified non-living biogenic material (BM), which increased towards the inner part reaching 35% coverage in sector C. The highest sediment coverage (31%) was observed in sector D and the lowest (7%) in sector C (Fig. 4a).

The two walls showed a similar pattern of decreasing coverage towards the inner part of the cave (Fig. 4). Mean biotic coverage of the right wall was higher than on the left (Fig. 4b, c). Sediment covered the floors of all sectors, reaching up to 84% of the substrate in sector D (Fig. 4d).

Macroalgae dominated the quadrats of sectors A and A′ covering up to 60% of the substrate, whereas they covered around 10% of sectors B and B′ and were absent from the inner sectors (Fig. 5a). Sponges were found in all cave sectors covering considerable substrate area (from 17% in A and A′ to 52% in C′). Sector D was covered almost exclusively with sponges (94% of the biotic coverage) (Fig. 5a), while the mean coverage of the remaining taxa was less than 9% each.

The coverage pattern of the cave walls was similar (Fig. 5b, c), with some worth-mentioning differences of the left wall: (i) macroalgae were found in sector B, but not on the respective right wall, (ii) many anthozoans were found in sector B′ (13% – mainly *Madracis pharensis* facies) but were absent from the right wall, and (iii) more bryozoans were recorded in sector C′ (11%) compared to the opposite wall (2%).

The floor quadrats at the entrances (A and A′; Fig. 5d) were dominated by macroalgae, whose coverage decreased and was shared with sponges in sectors B and B′. From these sectors inwards, the cave floor was covered almost entirely with sponges, with much lesser coverage of serpulids and bryozoans in sector C (2.5 and 8% respectively).

3.3. Spatial diversity patterns

Mean species richness (Fig. 6a) increased from the two entrances (S=14.3 in both) towards sectors B and B′ (S=16.7 and 15.1 respectively), and then decreased inwards (S=12.2 in sector D).

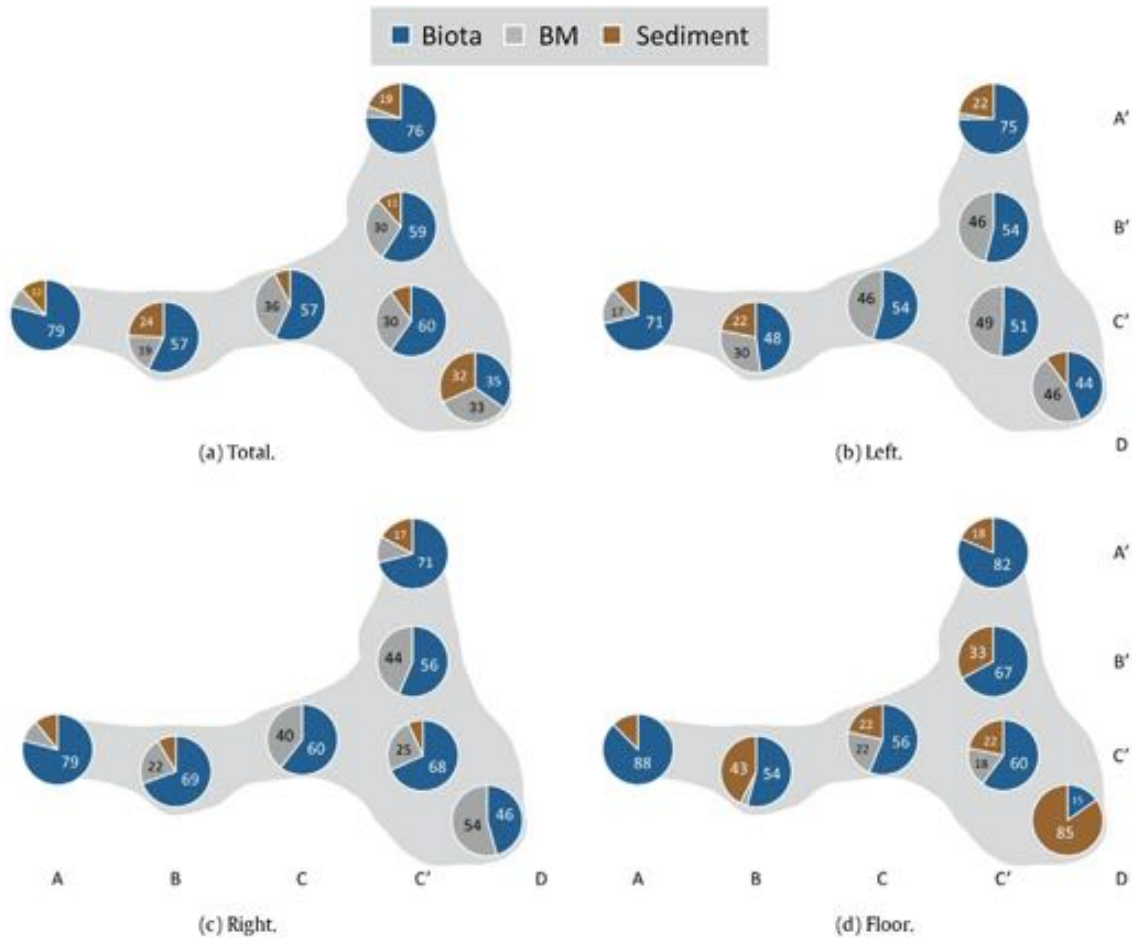

Fig. 4. Mean percent coverage of the substrate by living organisms, unidentified non-living biogenic material (BM) and sediment in each cave sector. a: total; b: left wall; c: right wall; d: floor.

Shannon–Wiener diversity (Fig. 6a) fluctuated along the horizontal cave axis taking a minimum value at the entrances (H′=1.4) and a maximum value in sectors B and B′ (H′=2.04 and 2.1 respectively). Pielou's evenness (Fig. 6a) followed a similar pattern with a minimum value at the entrances (J′=0.5) and a maximum value in the inner part (J′=0.8).

Concerning the biodiversity indices of the cave walls (Fig. 6b, c), the entrance was richer in species number (a maximum number of 20 taxa was recorded on the left wall of sector A). Species richness (S) decreased towards the inner part of the cave to a minimum of 14 taxa in sector D of the left wall and sector C′ of the right wall. Shannon–Wiener diversity (H′) and Pielou's evenness (J′) showed a similar fluctuation pattern across the longitudinal cave axis, overall increasing from the entrances to the inner D sector (Fig. 6b, c).

On the cave floor (Fig. 6d), species richness and Shannon–Wiener diversity increased from sector A and A′ to B and B′ and then decreased towards sector D. The floor was considerably poorer in number of species (mean of 11 taxa) with the fewest (S=7) taxa at the entrances and the most in sectors B and B′ (S=15). Shannon–Wiener diversity was minimum (H′=0.6) in sector A′ and maximum (H′=2) in B′. Pielou's evenness increased from the entrances (0.5 at A and 0.3 at A′) to the inner part (0.73 at D).

In general, the three diversity indices showed similar fluctuation patterns on the opposite walls, but were differentiated on the floor, where lower values were recorded. Species richness, Shannon–Wiener diversity and Pielou's evenness varied significantly in relation to the sector (Se), position within each sector (Po), and combination of these factors with the only exception being Pielou's evenness for the combination of sector and position (Table 2). The results of the pair-wise analyses showed that the source of variability of species richness in relation to sector was the difference between sector B and sectors A, C, D, C′, A′, while that of Shannon–Wiener diversity and Pielou's evenness was the difference between sector A and sectors B, D, B′ as well as between sector A′ and sectors B, D, C′, B′. The source of variability of all three indices in relation to position was the difference between the floor and the two walls.

3.4. Spatial patterns of ecological gradient

Multidimensional scaling (MDS) revealed 5 main groups of photoquadrats (Fig. 7). The entrance floors were grouped together with a similarity of 63.8% (group 1). The entrance walls were placed in a different group, along with the floors of sectors B and B′ as well as the left walls of B, with a similarity of 63.5% (group 2). A third group (similarity 57.8%) was formed by the quadrats of sectors C and C′, the walls of B′ and the right walls of B (group 3). The fourth group (similarity 54.9%) consisted of the D sector quadrats, apart from one that was differentiated (group 5), as well as one quadrat of sector C′ floor (group 4).

SIMPER analysis showed that group 1 was dominated by the macroalgae *Peyssonnelia* sp. 1, *Peyssonnelia* sp. 2 and turf-forming algae that contributed 25% of the dissimilarity, separating this group from the others. Fifty percent of the dissimilarity between groups 2 and 3 was due to ten taxa, among which the macroalgae *Peyssonnelia* sp.1, *Lithophyllum* sp., and *Peyssonnelia* sp. 2, and the sponges *Phorbas tenacior* and *Agelas oroides* had higher coverage in group 2 whereas the sponges *I. paucifilamentosa*, *Dendroxea lenis*,

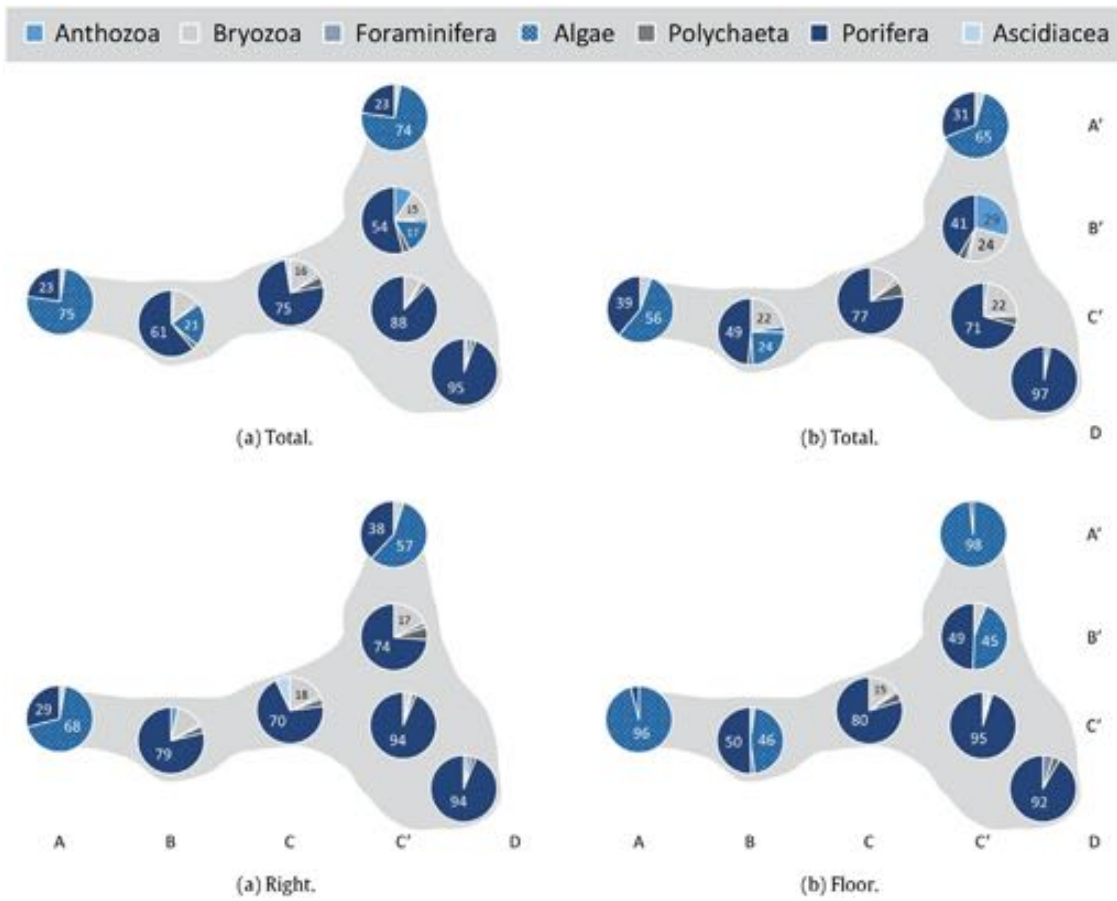

Fig. 5. Mean percent biotic coverage of the substrate by different taxa in each cave sector. a: total; b: left wall; c: right wall; d: floor.

Table 2
Results of PERANOVA analysis for two factors, sector (Se) and position (Po), concerning the diversity indices in the studied cave (d.f.: degrees of freedom, F: pseudo-F value of the F distribution, p: significance level, S: species richness, H′: Shannon-Wiener diversity, J′: Pielou's evenness, ** significance level 0.01, * significance level 0.05).

Source of variability	d.f.	S		H′		J′	
		F	p	F	p	F	p
Se	6	3.8683	0.006**	10.228	0.001**	10.184	0.001**
Po	2	38.072	0.001**	19.732	0.001**	5.2732	0.009**
Se x Po	12	5.0607	0.001**	2.8468	0.013*	1.5663	0.152
Residual	42						
Total	62						

S. cunctatrix, *I. oros*, and the serpulid polychaetes in group 3. Fifty percent of the dissimilarity between groups 3 and 4 was due to nine taxa, among which the sponges *S. cunctatrix*, *I. paucifilamentosa*, and *A. oroides*, and the encrusting and non-calcified bryozoans had higher coverage in group 3, whereas the sponges *Haliclona* sp., *Myrmekioderma spelaeum*, *H. mucosa*, and *D. lenis* in group 4.

Results of the PERMANOVA test showed that sector and position, as well as the combination of the two, had a significant effect on the structure of the communities in the cave (Table 3). Sectors A and A′ were an exception as they had similar species composition. Also, the communities on the cave floor were significantly different from those of the walls, whereas the latter were not different in their structure.

Table 3
Results of PERMANOVA analysis for two factors, sector (Se) and position (Po), concerning the community differentiation in the studied cave (d.f.: degrees of freedom, SS: sum of squares, MS: mean squares, F: pseudo-F, P: significance level, ** significance level 0.01).

Source of variability	*d.f.*	*SS*	*MS*	*F*	*P*
Se	6	57 968	9661.4	17.22	0.001**
Po	2	11 235	5617.6	10.012	0.001**
Se x Po	12	23 605	1967.1	3.5061	0.001**
Residual	42	23 565	561.06		
Total	62	3			

4. Discussion

Diverse morphological types of marine caves, such as semi-submerged, submerged, blind caves and tunnels, are characterized by different hydrodynamic regimes and light penetration levels that result in distinct spatial biodiversity patterns (Riedl, 1966; Bianchi and Morri, 1994). The specific topographic changes in each cave cause a gradient of the environmental parameters, which contributes to the spatial heterogeneity of benthic communities (Sgorbini et al., 1988; Benedetti-Cecchi et al., 1996). Specifically,

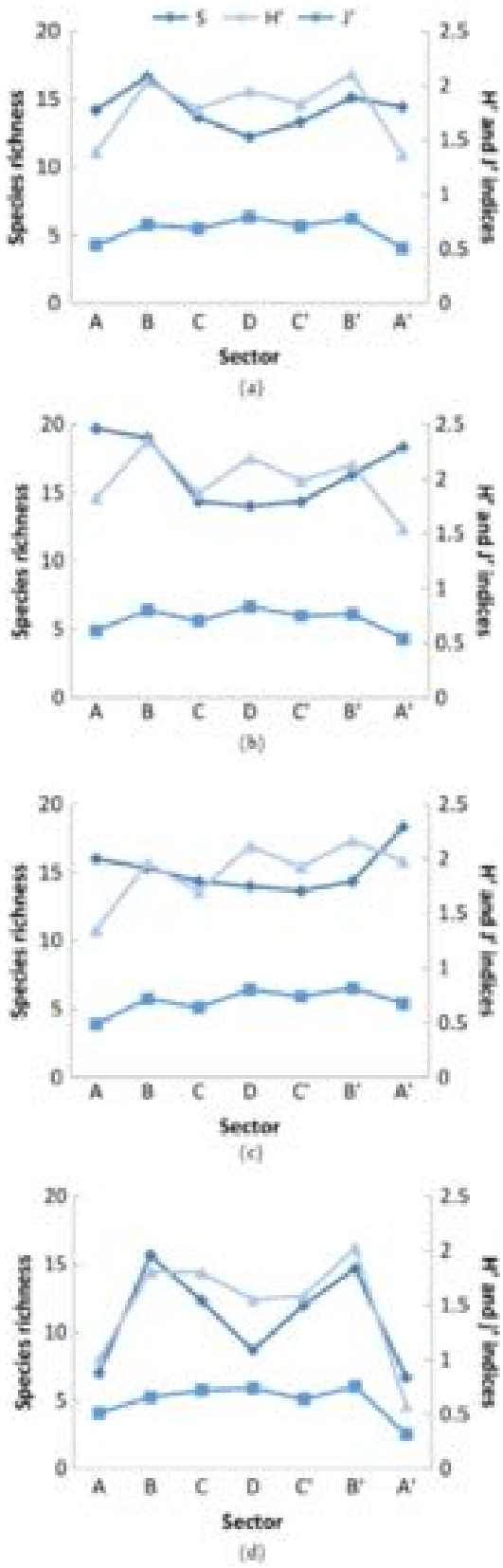

Fig. 6. Diversity measured by Species richness (S), Shannon–Wiener diversity (H′) and Pielou's evenness (J′) indices in each cave sector. a: total; b: left wall; c: right wall; d: floor.

in semi-submerged multi-entranced tunnels, zonation patterns of benthos are less apparent than in entirely submerged blind caves due to the increased light penetration and water renewal further in the inner part of the cave (Ugolini et al., 2003), as well as the higher rate of recruitment (Denitto et al., 2007). Here, a semi-submerged cave was studied for the first time in the eastern Mediterranean Sea extending the previous knowledge of this particular cave type from the western Mediterranean and the Adriatic Sea (e.g. Sarà, 1958, 1961, 1964; Rützler, 1965; Corriero et al., 2000).

The studied cave was found to host considerable biodiversity with 46 taxa of sessile flora and fauna, of which 54% were sponges (Table 1). Porifera dominated the cave habitat both in terms of number of species (but it must be underlined that not all members of other major groups were identified to species level) and substrate coverage; indeed, almost half of the Mediterranean sponge fauna has been recorded in caves (Gerovasileiou and Voultsiadou, 2012). Therefore, most studies regarding benthic communities in semi-submerged caves focus on sponges (e.g. Sarà, 1958, 1961, 1964, 1968; Labate, 1965; Rützler, 1965; Corriero et al., 2000; Bell, 2002; Radolović et al., 2015). With 25 species, the sponge assemblage of the studied cave is comparable – in terms of both species number and composition – with that of most Mediterranean shallow semi-submerged caves (see Table S4 in Gerovasileiou and Voultsiadou, 2012). Sixteen percent of the Mediterranean cave-dwelling keratose sponges (Manconi et al., 2013) were recorded in the examined photoquadrats, including the commercial bath sponge *Spongia officinalis* as well as *Ircinia paucifilamentosa*, a cave-dwelling species which is endemic to the Aegean Sea (Voultsiadou et al., 2016). In addition, the lithistid sponge species *Neophrissospongia* sp. had considerable coverage (0.4%) in the internal cave sectors C and D (Table 1), also representing the first record of this genus in the Aegean Sea (Voultsiadou et al., 2016). Gerovasileiou and Voultsiadou (2012), in their overview of the sponge fauna in Mediterranean marine caves, suggest that this habitat seems to be favourable for particular taxa such as keratose and lithistid sponges, with some species of the latter group described as cave-exclusive. Such species are limited to a small number of caves in insular areas of the western Mediterranean (e.g. Manconi and Serusi, 2008) and Levantine Sea (Pérez et al., 2004). Further taxonomic study of the collected material and additional sampling, given the limitations in visual identification of certain taxa, would raise the known diversity from this cave, especially for rare taxa with cryptic habits. However, the microscopic identification of qualitative samples yielded, besides the abovementioned rare sponges, the bryozoan *Adeonella pallasii* which is endemic to the eastern Mediterranean Sea (Gerovasileiou and Rosso, 2016).

Concerning the dominant taxa and their coverage pattern in the studied cave, sponges and macroalgae prevailed; as expected, macroalgae were only found in the well-lit entrance zones. In particular, encrusting calcified red algae, which are the main building components of the coralligenous community (Ballesteros, 2006), grew within only a few metres from the entrance, in dim light conditions (Alvisi et al., 1994), and were no longer recorded beyond 10–15 m, where they were replaced by sponges. Despite this variability in space, sponges covered considerable part of the substrate throughout the cave (Cinelli et al., 1977; Corriero et al., 2000).

Multivariate resemblance analysis revealed major groups that in terms of species composition correspond to a shallow-water enclave of the pre-coralligenous community (C) in the two entrance zones, where red algae (e.g. *Peyssonnelia* and *Lithophyllum* spp.) dominated, and to the semi-dark cave community (GSO), which occupied the largest part of the cave, where sponges (e.g. *Spirastrella cunctatrix* and *Agelas oroides*) dominated (Pérès and Picard, 1949). Furthermore, a transitional zone with turf-forming algae and sciaphilic rhodophytes was observed on the floors of

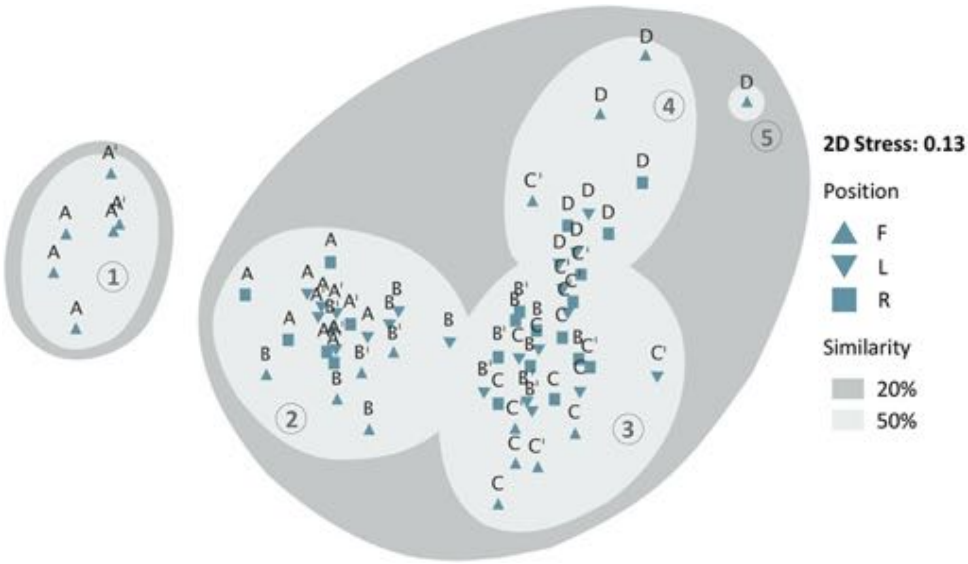

Fig. 7. MDS analysis depicting the photoquadrat similarity based on the percent coverage in each position (F: floor; L: left wall; R: right wall) of the cave sectors (A–D; A′–C′). The distinct photoquadrat groups are numbered 1–5 (see main text for details).

the well-lit cave entrances, corresponding to zone I of the scheme proposed by Bianchi and Morri (1994) for marine caves. The inner sector of the cave (D), although not entirely dark, and the darker blind tunnels further inside corresponded to zones IV-V of the same bionomic scheme, with a mean biotic coverage of 35% and only a few sessile taxa present (e.g. discoloured sponges *Petrosia ficiformis*, few foraminifers, scleractinians and encrusting bryozoans).

The biotic coverage decreased from the entrances to the inner part of the cave, though with no sharp decline, as observed in most semi- and entirely submerged blind caves, in which light intensity and water movement sharply decrease inwards (Harmelin et al., 1985; Balduzzi et al., 1989; Corriero et al., 2000; Martí et al., 2004; Gerovasileiou and Voultsiadou, 2016). This is most probably related to the geomorphological characteristics of the cave (shallow, semi-submerged, with two wide entrances) that contribute to greater light penetration and water renewal. The studied cave is located in an area exposed to intense wind often resulting in strong currents between the two entrances as indicated by the presence of planktonic organisms in the water column (e.g. jellyfish, ctenophores) observed during fieldwork.

The observed diversity gradient is generally in line with previous observations in both semi- and totally submerged caves (Sarà, 1962; Corriero et al., 1997, 2000; Bell, 2002) although different patterns have also been described (Balduzzi et al., 1989; Martí et al., 2004; Gerovasileiou and Voultsiadou, 2016). Nevertheless, despite the observed differentiations, the aforementioned researchers agree that the increase in diversity at the middle cave zones is related to the replacement of macroalgae by sessile invertebrates as a result of reduced light penetration. On the other hand, the decrease of diversity in the inner cave zones is related to the increasing water confinement (Bianchi and Morri, 1994), which results in less food for the filter feeders (Bianchi et al., 2003) and lower larval dispersal (Palau et al., 1991). Species evenness was higher in the inner part of the cave denoting that several species had a considerable surface cover (Balduzzi et al., 1989; Bell, 2002; Gerovasileiou and Voultsiadou, 2016), contrary to the entrances where only few rhodophytes dominated.

Apart from the spatial differentiation in diversity and community structure in relation to distance from the entrances, differences were also observed between the examined positions within each cave sector possibly due to varying sedimentation and illumination regimes caused by different inclination. Heterogeneity of sessile communities between opposite and adjacent cave walls or between walls and ceilings has been reported from other caves as well (Dellow and Cassie, 1955; Bell, 2002; Bussotti et al., 2006; Gerovasileiou et al., 2017).

Local small-scale heterogeneity in the structure of marine cave benthos is mostly related to variability in geomorphology forming microhabitats such as inclined surfaces, bulges or recesses (Bussotti et al., 2006; Gerovasileiou et al., 2013). In this respect, 3D cave topography imaging could enable a better understanding of spatial variability of cave communities (Gerovasileiou et al., 2013). In the studied cave, this was evident in the grouping of right-wall photoquadrats of sector B, representing a shadowy cavity on the wall.

Although Trypia Spilia is located within a NATURA 2000 site of special interest, it had not been studied before for its diversity and benthic community patterns. This first assessment revealed a heterogeneous habitat with considerable species richness including rare taxa. Nevertheless, the internal beach in one of the cave blind tunnels, functioning as a resting place for the endangered Mediterranean monk seal, was almost entirely covered with marine litter and debris (authors' personal observations).

The quantitative study of this semi-submerged cave not only contributes to the poorly known cave biodiversity of the eastern Mediterranean Sea but also offers the baseline for comparative studies in different morphological cave types throughout the Mediterranean basin as well as for future monitoring and management program design and implementation.

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Conflict of interest

The authors have declared that no competing interests exist.

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