



## Marine caves of the Southern Tyrrhenian Sea: a First Census of Benthic Biodiversity

Gianfranco Scotti<sup>1</sup>, Pierpaolo Consoli<sup>1</sup>, Valentina Esposito<sup>1,2\*</sup>, Renato Chemello<sup>3</sup>, Teresa Romeo<sup>1</sup> and Franco Andaloro<sup>1</sup>

<sup>1</sup>ISPRA (Institute for Environmental Protection and Research), Department of environmental monitoring and protection and biodiversity conservation, Italy

<sup>2</sup>Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS), Sezione Oceanografia, Italy

<sup>3</sup>Department of Earth and Marine Sciences, University of Palermo, Italy

\*Corresponding author: Valentina Esposito, ISPRA, Department of environmental monitoring and protection and biodiversity conservation, Via dei Mille 46, 98057 Milazzo, ME, Italy, Tel: +39 0650074063; Fax: +39 0909241832; E-mail: [valentina.esposito@isprambiente.it](mailto:valentina.esposito@isprambiente.it)

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### Abstract

This is the first paper documenting research on a selection of marine caves located along the coast of Capo Milazzo in the southern Tyrrhenian Sea. Three submarine and one semi-submerged caves were surveyed and sampled using underwater photo sampling. Surveys have only taken into account the sessile species belonging to the main taxa: Porifera, Anthozoa, Bryozoa and Polychaeta. Diversity indices and abundances were calculated for three sections within each explored cave: the Entrance Zone, Intermediate Zone and Bottom Zone. The richest group was Porifera with 21 taxa, followed by cnidarians, (Anthozoa), with 8 taxa, Polychaeta (5 taxa), and Bryozoa (5 taxa). Among Porifera, the presence of *Petrobiona massiliana*, a protected species according to SPA/BIO Protocol and the Bern Convention, must be highlighted. The encrusting forms were dominant in the Bottom Zone, the massive forms in the Intermediate Zone and the arborescent forms in the Entrance Zone. Generally, the percentage coverage of each morphological group showed a decline in the Intermediate Zone and a general increase in the Dark Zone within each cave. The S, H' and J values showed different trends in the five caves. These differences, also evidenced by Permanova analysis, depend on the topographic specificity of each cave which, in turn, affects the gradients of the biotic and abiotic parameters. Finally, no horizontal gradient of rarefaction of the benthic sessile fauna has been detected. This study represents an important step for the management and conservation practices of these fragile ecosystems, especially in view of the forthcoming establishment of the Marine Protected Area.

**Keywords** Marine caves; Benthic biodiversity; Photographic census; Mediterranean Sea; Conservation

### Introduction

According to Annex 1 of the 'Habitats' EC Directive 92/43, the submerged and semi-submerged caves, (code 8330), are natural habitats of community interest. Habitat and associated species, (semi-dark cave habitats), have been included in the Action Plan, (AP), regarding the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea [1]. Management procedures involve enacting laws aimed at regulating human activities likely to affect dark populations and permit their long-term conservation.

The AP asserts (B.2-Setting up MPAs) that "the institution of a Marine Protected Area intended to permit more efficacious conservation of these dark assemblages must be based on the identification of emblem sites on the basis of the criteria: uniqueness or rarity, particular importance for species biological stages, importance for threatened, endangered or declining habitats or species, vulnerability and reduced recuperative capacity after disturbance, biological productivity, biological diversity and naturality" [1].

Mediterranean marine caves play a valuable role as 'reservoirs' for the restocking of valuable species [2-5] and show a significant ecological [6] value in relation to their function of habitat/refuge

and/or ecological islands. Moreover, the marine caves [1] are considered a link between closed habitats, (hard and soft substrata, seagrass bed, coralligenous assemblages) [6,7]. The caves also play an important role economically for a local diving centre due to the high frequency of requests from divers to explore them (pers. comm.). Management action will need to be evaluated in order to reduce and prevent the impact of recreational divers on the benthic community [8,9].

Over the past 15 years, several studies focused on the biodiversity of marine caves [4,10-15], their conservation [9,16-19] and the occurrence of non indigenous species [20] and references therein] have been carried out worldwide. In Sicily, several studies were carried out on Bryozoa, Brachiopoda, Serpuloidea and Floristic macroalgal diversity of some submerged caves [21-25].

The presence of submerged and semi-submerged caves has also helped to strengthen the decision making criteria in support of the establishment of marine protected areas in the Mediterranean Sea [26]; about 66% of these protected areas include submerged caves [27].

About 4000 caves have been surveyed in the Mediterranean in 14 European countries [28]. The biodiversity, also linked to the presence of alien species [20], and the community structure of these particular habitats have been investigated. But there is also a large gap between the studies on biodiversity and animal community structure between the western and eastern Mediterranean caves [13], with 3262 western

Mediterranean caves surveyed versus only 738 in the eastern Mediterranean.

In Italy, several studies have been conducted on the benthic populations or single species of the underwater caves [6,11,16,29-33] and have often been limited to Marine Protected Areas [8,9,13,19,21,22,34-47].

There are still many caves to survey and further intensive studies on the biology and ecology of these areas are needed.

The description of the caves considered in this paper represents the first contribution to the knowledge of these environments along the north-eastern coast of Sicily.

Although the seabeds along the promontory of Capo Milazzo have been designated as Marine Protected Areas of forthcoming establishment and the terrestrial part of the promontory represents a Site of Community Interest (SCI), no scientific information is available on the faunal composition of local submerged and semi-submerged caves.

The purpose of this work is therefore: a) to provide the first data on the 2-D morphology of three submerged and one semi-submerged caves at Capo Milazzo; b) to provide further data on the biodiversity of these environments, paying special attention to the presence of protected species included in the lists of international conventions.

## Material and Methods

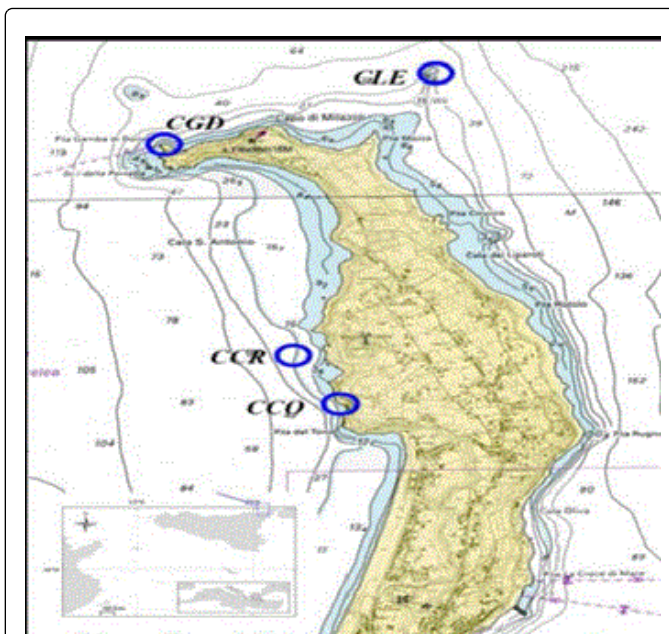
### Study Site

The promontory of Capo Milazzo is a small peninsula that stretches northwards for about 6 km from the northern coast of Sicily, (Figure 1), with a maximum width of about 1.3 km. The coastal profile appears steep and rugged.

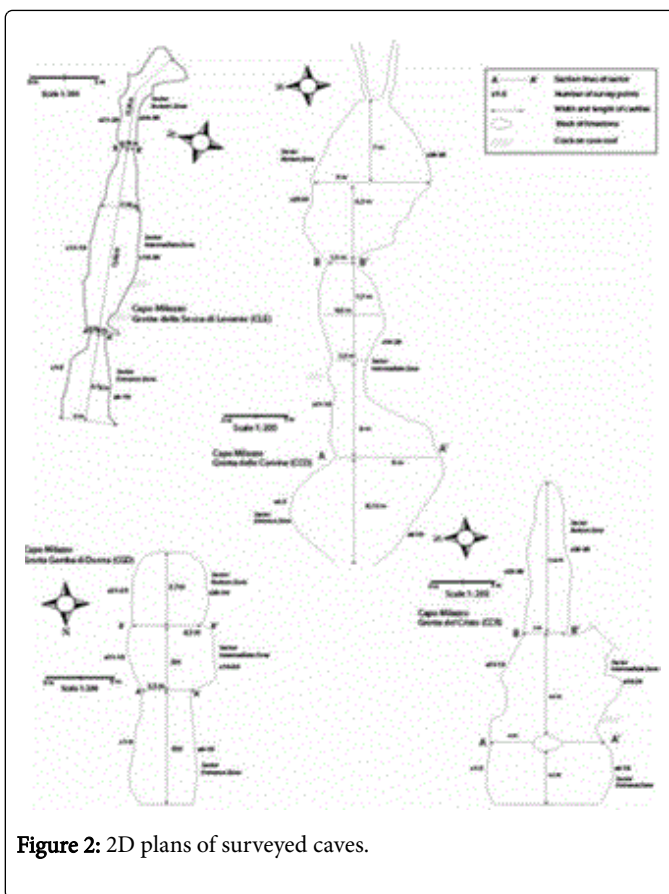
The exposed area is classified as a Site of Community Importance, (code ITA030032 'Capo Milazzo'), according to the EC Habitats Directive 92/43, (ordinary supplement n. 167 to the Official Gazette no. 170 of 24 July 2007). In addition, since January 2014, the submerged part of the promontory of Capo Milazzo was included in the list of the Marine Protected Areas of Gathering, (Law 27 December 2013, n. 147 ordinary supplement n. 87 to the Official Gazette n. 302 of 12.27.2013), and then, from August 2014, following the economic, social and environmental investigations commissioned by the Italian Ministry of the Environment and Protection of Land and Sea to ISPRA, Capo Milazzo was proposed as an MPA.

The bedrock of the peninsula is formed by metamorphic rocks covered by Upper Miocene reef limestones and Upper Pliocene- Lower Pleistocene marls and marly limestone [48].

The studied caves are located at different depths, (from 0 to -30 m u.s.l), and distances from the coast and show different morphogenesis. Three submerged caves ("Delle Corvine" (CCO) "Secca di Levante" (CLE) and "Del Cristo" (CCR) and one semi-submerged, "Gamba di Donna" (CGD)), have been described (Figure 2).



**Figure 1:** Study area with caves location.



**Figure 2:** 2D plans of surveyed caves.

## Sampling

Sampling was conducted during summer 2010. Photographic samples were taken on vertical rocky walls using a Canon G12 PowerShot with an underwater case and two electronic strobes. Within each site five 20 × 20 cm square frames were positioned, randomly, on both vertical walls of the cave and at three levels of distance, (sectors), from the entrance: Entrance Sector, Intermediate Sector and Bottom Sector. Each sampling level was chosen relying on cave morphology: total length, presence of chambers and/or niches.

A metal pole was attached to the camera to indicate the height the camera needed to be held above the benthos to ensure that the width of the photograph was 400 cm<sup>2</sup>. Each photograph was high quality and between 6 and 9 MB in size.

Then, the sampling led to the collection of 10 spatial replicates per sector, (S1-5-S6-10 Figure 2), for a total of 30 samples for each cave. The images were transferred to a PC for the taxonomic recognition at the lowest taxonomic level, (whenever possible), while the coverage percentages for each species or colony were calculated using photoQuad software [49]. Additionally, during the survey, some specimens of each taxon were collected in order to identify at specific level the benthic fauna present in the photoquadrats. Surveys have only taken into account the sessile fauna in the caves belonging to the following taxa: Porifera, Anthozoa, Bryozoa and Polychaeta. Photographic sampling was chosen as it provides a good compromise between optimal resolution and conservation of the habitat, although it could probably lead to the underestimation of small and cryptic species. Furthermore, the non-destructive methodological approach obtaining biodiversity data through the analysis of images is suitable for studying marine benthic communities in Marine Protected Areas and vulnerable habitats.

Four morphological categories have been identified [50]: Encrusting (EN), Massive (MA), Arborescent (AR), and Tubular (TU). For each category and for each sector, coverage in cm<sup>2</sup> and percentage were calculated and the differences in morphological categories were observed through the horizontal development of the caves, (Entrance-Bottom Cave).

## Data analysis

The macrobenthic assemblages were characterized by calculating the following diversity indices for each cave and cave sectors, (Entrance Zone - Intermediate Zone - Bottom Zone): species richness, (S), expressed as the total number of species observed in the images collected in each sampling sector; the Shannon-Wiener diversity index, (H') [51], calculated as where pi is the proportion of specimens in the ith species, and the Pielou's evenness index, (J), expressed as  $J=H'/\ln S$ ; where H' is the Shannon-Wiener diversity index value and S is the species richness.

Statistical analyses were performed using the PRIMER6 & PERMANOVA+ software packages [52,53]. To evaluate potential variations in the values of each of the three diversity indices in relation to the factors "cave" and "sector", a two-way crossed non-parametric univariate analysis of variance, (PERMANOVA), was performed. Data was analyzed on the basis of Euclidean distance, (4999 permutations). Pairwise comparisons were computed when significant differences, (p<0.05), among factor levels were detected.

## Results

### Description and plans of surveyed caves

**Secca di Levante cave (CLE):** The cave, formed by marine erosion on the calcareous rock, is located about 400 m off the coast of Capo Milazzo. The entrance is at -30 m depth and the cave extends horizontally for about 40 m in a NW-SE direction, having an average width of 2.60 meters, and consisting of 3 connected rooms. The final part is completely dark, while the intermediate part has cracks in the roof that allow in light filtering from outside and that connect it with the top of the shoal at a depth of 18 m. Coarse sand and organic detritus originate from the disintegration of organisms that colonise the walls and the vault covering the bottom up to 15 m from the entrance. This cave is subject to strong currents. Muddy sand covers the back part of the cave bottom. This cave, as it is easy to explore, and for the presence of numerous encrusting taxa, (Porifera, Bryozoa), and numerous cavities inhabited with Plesionika sp., Conger conger and Phycis phycis, has been visited by the diving centre as well as independent divers.

**Gamba di donna cave (CGD):** This is a small semi-submerged cave located on the westernmost tip of Capo Milazzo. It develops horizontally within a carbonatic rock layer running N-S. The entrance is located at a depth of -1.5 m. The cave extends for about 14 m inside the promontory without further links with the outside.

It is only accessible from the sea. The entrance, about 1.5 meters wide and 2 meters high, 1 m of which is outside the water, is visible from the surface, but develops largely below the surface level.

**Del Cristo cave (CCR):** This cave is located inside a small rocky outcrop about 50 m off the west coast of the cape. It is part of a small system of cavities and caves from -5 to -20 m depth. The cave is 27 m long; its entrance is located at a depth of -18 m. The Intermediate Zone is linked to the outside through a cracking of the roof. It has a modestly sized side tunnel of about 2 meters. The floor of the cave is covered by a thin, sandy sediment and is littered with rocks and boulders. This cave is often visited by divers, also due to the presence of a statue of Christ placed there years ago.

**Delle Corvine cave (CCO):** The cave is located on the south-western side of the promontory. It has a predominantly horizontal development within the metamorphic rock. The entrance is at a depth of -12 m; the cave extends for about 50 m and consists of 4 main cavities connected to each other by narrow passages. At about 16 m from the entrance there is a small secondary branch that connects the cave with the external environment at a depth of -5 m. The last room ends with two small tunnels that are not accessible to divers. The semi-dark area is limited to the first 10 m from the entrance, while the remaining part is dark. The floor of the cave is covered by fine sediments. Inside there are numerous individuals of *Sciaena umbra*. It is the only cave, among those documented in this work, where there is the presence of individuals of *Grammonus ater* (Osteichthyes-Bythitidae).

### Taxonomic composition

Overall, 37 taxa belonging to 4 phyla were observed (Table 1). The most numerous group was Porifera, with 21 taxa, followed by Cnidaria (Anthozoa) with 8 taxa, Annelida, (5 taxa of Polychaeta), and Bryozoa (3 taxa).

Porifera were represented by species frequently found in cave environments such as *Petrosia ficiformis*, *Spirastrella cunctatrix*,

*Clathrina clathrus* and *Agelas oroides* [10,13,16,41]. Of remarkable importance is the presence of protected species *Petrobiona massiliana*, (Annex II of the SPA/BIO Protocol of the Barcelona Convention and Annex II of the Bern Convention), inside the Gamba di Donna (CGD) cave.

| Morphological groups | Classes          | Species                               | CCO   | CLE  | CCR   | CGD   | Cave sector |
|----------------------|------------------|---------------------------------------|-------|------|-------|-------|-------------|
| MA                   | Calcarea         | <i>Clathrina clathrus</i>             |       | 4.4  |       |       | EZ,IZ,BZ    |
| EN                   | Calcarea         | <i>Petrobiona massiliana</i>          |       |      |       | 16.79 | EZ,IZ,BZ    |
| MA                   | Homoscleromorpha | <i>Corticium sp.</i>                  |       | 1.5  |       |       | IZ,BZ       |
| MA                   | Demospongiae     | <i>Aaptos sp.</i>                     | 2.5   | 5.7  |       |       | EZ,IZ,BZ    |
| MA                   | Demospongiae     | <i>Agelas oroides</i>                 | 1.6   | 5.8  | 18.44 |       | EZ,IZ,BZ    |
| MA                   | Demospongiae     | <i>Axinella cf. verrucosa</i>         | 2.8   |      |       |       | EZ          |
| MA                   | Demospongiae     | <i>Axinella cf. damicornis</i>        | 3.2   |      |       |       | EZ          |
| MA                   | Demospongiae     | <i>Axinella sp.</i>                   | 2.5   | 1.8  | 7.75  |       | EZ,IZ,BZ    |
| MA                   | Demospongiae     | <i>Chondrosia reniformis</i>          |       | 18.6 | 50    |       | EZ,IZ,BZ    |
| EN                   | Demospongiae     | <i>Crambe sp.</i>                     |       |      | 30.65 |       | EZ,IZ,BZ    |
| EN                   | Demospongiae     | <i>Dendroxea sp.</i>                  |       |      |       | 11.42 | EZ,IZ,BZ    |
| EN                   | Demospongiae     | <i>Dysidea fragilis</i>               |       |      | 40.83 |       | EZ, BZ      |
| EN                   | Demospongiae     | <i>Dysidea sp.</i>                    |       |      | 21.31 |       | EZ,IZ,BZ    |
| MA                   | Demospongiae     | <i>Haliclona sp.</i>                  | 3.5   |      | 25.25 |       | IZ,BZ       |
| EN                   | Demospongiae     | <i>Haliclona (Halichoclona) fulva</i> |       | 8.4  |       |       | IZ          |
| MA                   | Demospongiae     | <i>Ircinia cf. oros</i>               | 10.2  |      | 70    |       | EZ,IZ,      |
| MA                   | Demospongiae     | <i>Ircinia sp.</i>                    | 2.4   | 9.7  | 16    |       | IZ          |
| MA                   | Demospongiae     | <i>Ircinia variabilis</i>             | 4.8   |      | 63    |       | IZ,BZ       |
| MA                   | Demospongiae     | <i>Petrosia ficiformis</i>            | 17.7  | 5.9  |       |       | EZ,IZ,BZ    |
| EN                   | Demospongiae     | <i>Phorbas sp.</i>                    |       |      | 19.88 |       | EZ,IZ       |
| EN                   | Demospongiae     | <i>Spirastrella cunctatrix</i>        |       |      | 28.86 | 7.6   | EZ,IZ,BZ    |
| MA                   | Anthozoa         | <i>Astroides calycularis</i>          |       | 15.6 | 7.62  | 21.04 | EZ,IZ,      |
| AR                   | Anthozoa         | <i>Cornularia cornucopiae</i>         |       |      |       | 62.5  | EZ          |
| TU                   | Anthozoa         | <i>Lepsosammia pruvoti</i>            | 15.43 | 4.8  | 3.4   | 5.3   | EZ,IZ,BZ    |
| AR                   | Anthozoa         | <i>Pachyceriantus solitarius</i>      | 1     |      |       |       | IZ          |
| TU                   | Anthozoa         | <i>Paracyathus pulchellus</i>         | 31.25 |      |       |       | EZ          |
| AR                   | Anthozoa         | <i>Parazoanthus axinellae</i>         |       |      |       | 25    | EZ          |
| MA                   | Anthozoa         | <i>Phyllangia americana mouchezii</i> | 13    |      |       |       | IZ          |
| EN                   | Anthozoa         | <i>Polycyathus muelleriae</i>         | 25    |      |       | 45    | EZ,IZ,BZ    |
| EN                   | Polychaeta       | <i>Filograna implexa</i>              |       | 16   |       |       | BZ          |
| EN                   | Polychaeta       | <i>Josephella marenzelleri</i>        | 39.25 |      | 41    |       | IZ,BZ       |
| EN                   | Polychaeta       | <i>Protula tubularia</i>              | 12.5  | 2.6  | 1.9   |       | EZ,IZ,BZ    |
| EN                   | Polychaeta       | <i>Serpula vermicularis</i>           | 12.5  |      | 1.3   |       | EZ,BZ       |



|    |              |                                  |       |      |  |            |
|----|--------------|----------------------------------|-------|------|--|------------|
| EN | Polychaeta   | <i>Vermiliopsis infundibulum</i> | 18.56 |      |  | EZ, BZ     |
| AR | Gymnolaemata | <i>Margaretta cereoides</i>      |       | 27.5 |  | EZ         |
| AR | Gymnolaemata | <i>Myriapora truncata</i>        | 26.75 | 14.3 |  | EZ, IZ     |
| MA | Gymnolaemata | <i>Reteporella grimaldii</i>     |       | 4.6  |  | EZ, IZ, BZ |

**Table 1:** Taxonomic list of species with relative morphological groups, and % coverage assessed in each of the studied cave. MA: massive; EN: encrusting; AR: arborescent; TU: tubular. EZ: entrance zone; IZ: Intermediate zone; BZ: bottom zone; CCO: Corvine cave; CLE: Secca di Levante cave; CCR: Cristo cave; CGD: Gamba di donna cave.

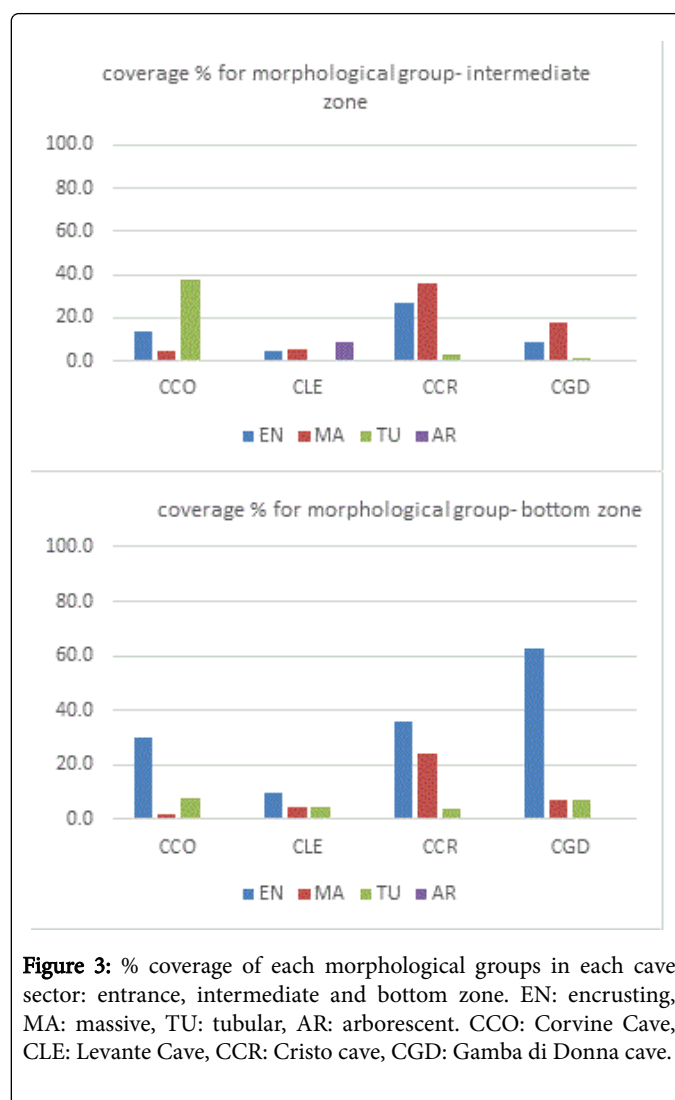
Anthozoans, mostly belonging to the coralligenous assemblages, such as *Parazoanthus axinellae* and *Leptopsammia pruvoti*, are also represented by taxa belonging to the community of dark and semi-dark caves, such as *Cornularia cornucopiae*, *Paracyathus pulchellus* and *Polycyathus muelleriae* [54-56].

Among the bryozoans, shade-tolerant species and coralligenous taxa, (*Reteporella grimaldii*), and representatives of photophilic communities under strong currents, (*Margaretta cereoides*), were found only in the first sectors, (Entrance Zone), of the CLE caves.

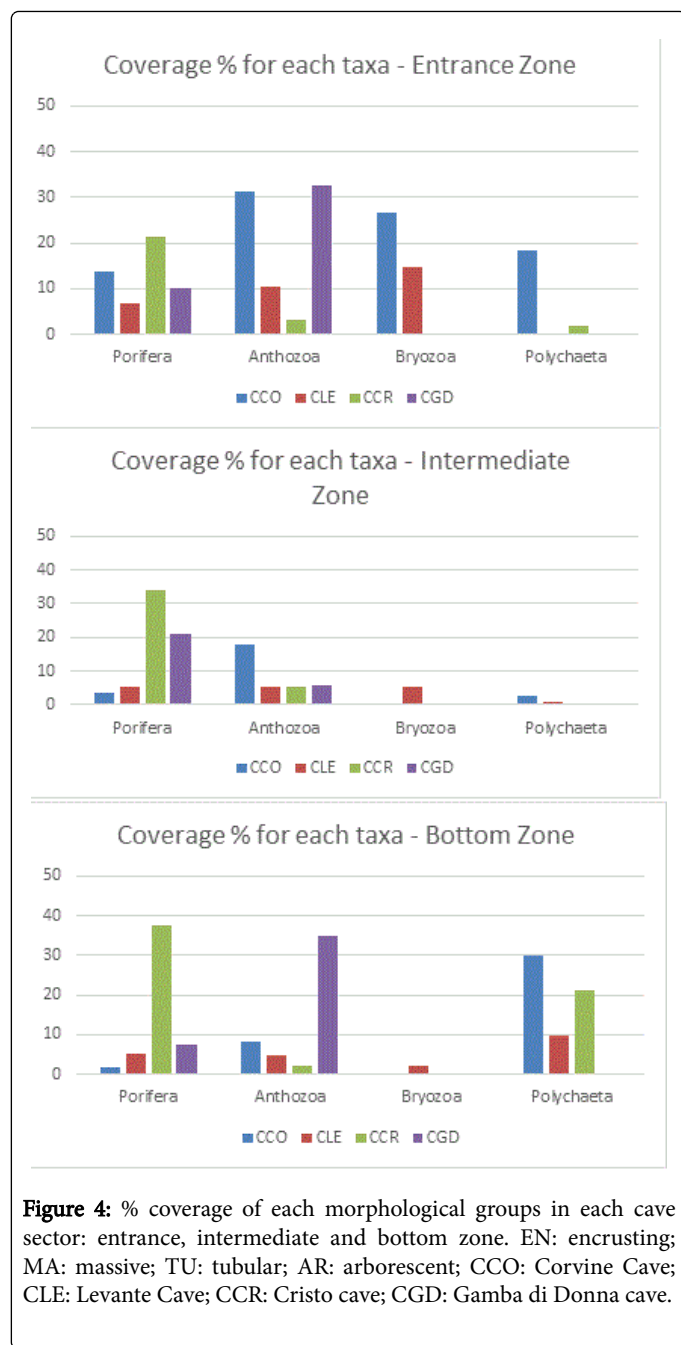
With regards to polychaetes, species found inside the caves of Capo Milazzo are exclusively serpulids typical of shady and coralligenous environments, (*Vermiliopsis infundibulum*, *Filograna implexa*, *Josephella marenzelleri*, *Protula tubularia*), and are widely distributed in the Italian submarine caves [57].

Considering the three sectors of the 4 investigated caves and the 4 morphological categories, (Encrusting - EN, Massive - MA, Arborescent - AR, Tubular - TU), the encrusting forms showed a percentage of coverage higher in the Bottom Zone in all explored caves, whereas the massive and tubular forms were more abundant in the Intermediate Zone. The Entrance zone presents all morphological categories with a dominance of arborescent and massive forms (Figure 3).

Percentage of coverage of each taxa show highest values at the Entrance Zone, (71.44%), a decrease in the Intermediate Zone, (42.15%) and an increase in the Bottom Zone, (49.96%) within each cave. Anthozoans, (*Cornularia cornucopiae*, *Parazoanthus axinellae*), and encrusting forms of some species of sponges, (*Dendroxea sp.*, *S. cunctatrix*, *Ircinia variabilis*), covered the walls at the entrance of the caves. In the Intermediate Zone of each cave, sponges grow significantly, reaching considerable sizes (*A. oroides*, *P. ficiformis*), while the percent coverage of Anthozoa and Polychaeta decreases. *Reteporella grimaldii* and *Myriapora truncata* represent the only Bryozoa species located in the Intermediate Zone. In the Bottom Zone, percent coverage of encrusting forms of serpulids, (*Vermiliopsis infundibulum*, *Serpula vermicularis*, *Josephella marenzelleri*) and porifera (*Dysidea fragilis* and *I. variabilis*) (Figure 4), increases and showed the highest coverage values.



**Figure 3:** % coverage of each morphological groups in each cave sector: entrance, intermediate and bottom zone. EN: encrusting, MA: massive, TU: tubular, AR: arborescent. CCO: Corvine Cave, CLE: Levante Cave, CCR: Cristo cave, CGD: Gamba di Donna cave.



### Biodiversity patterns of the benthic assemblages

The average values of the diversity indices for each sector of the caves are reported in Table 2.

On the whole, the richest and most diverse macrobenthic community resulted in CLE, ( $S=5.3 \pm 2.1$ ;  $H'=1.2 \pm 0.5$ ), while the lowest average values of the diversity indices were recorded in CGD, ( $S=2.3 \pm 1.2$ ;  $H'=0.5 \pm 0.4$ ). Regarding the sectors, the highest average values of species richness, ( $S=7.0 \pm 1.9$ ), and the Shannon diversity index, ( $H'=1.6 \pm 0.2$ ), were recorded in the Intermediate Zone of CLE.

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$S$ ,  $H'$  and  $J'$  values showed different trends in each of the five caves.

| Cave              | Sector       | S   | H'  | J   |
|-------------------|--------------|-----|-----|-----|
| Corvine (CCO)     | entrance     | 2   | 0.2 | 0.5 |
|                   | intermediate | 3.6 | 1   | 0.8 |
|                   | bottom       | 2.8 | 0.6 | 0.6 |
|                   | total        | 2.8 | 0.6 | 0.6 |
| Cristo (CCR)      | entrance     | 5.2 | 0.4 | 0.8 |
|                   | intermediate | 3.6 | 1   | 0.9 |
|                   | bottom       | 3.4 | 0.7 | 0.6 |
|                   | total        | 4.1 | 0.7 | 0.8 |
| Gamba donna (CGD) | entrance     | 3   | 0.7 | 0.7 |
|                   | intermediate | 1.8 | 0.3 | 0.7 |
|                   | bottom       | 2   | 0.7 | 1   |
|                   | total        | 2.3 | 0.5 | 0.8 |
| Levante (CLE)     | entrance     | 4.2 | 0.7 | 0.8 |
|                   | intermediate | 7   | 1.6 | 0.9 |
|                   | bottom       | 4.8 | 1.4 | 0.9 |
|                   | total        | 5.3 | 1.2 | 0.9 |

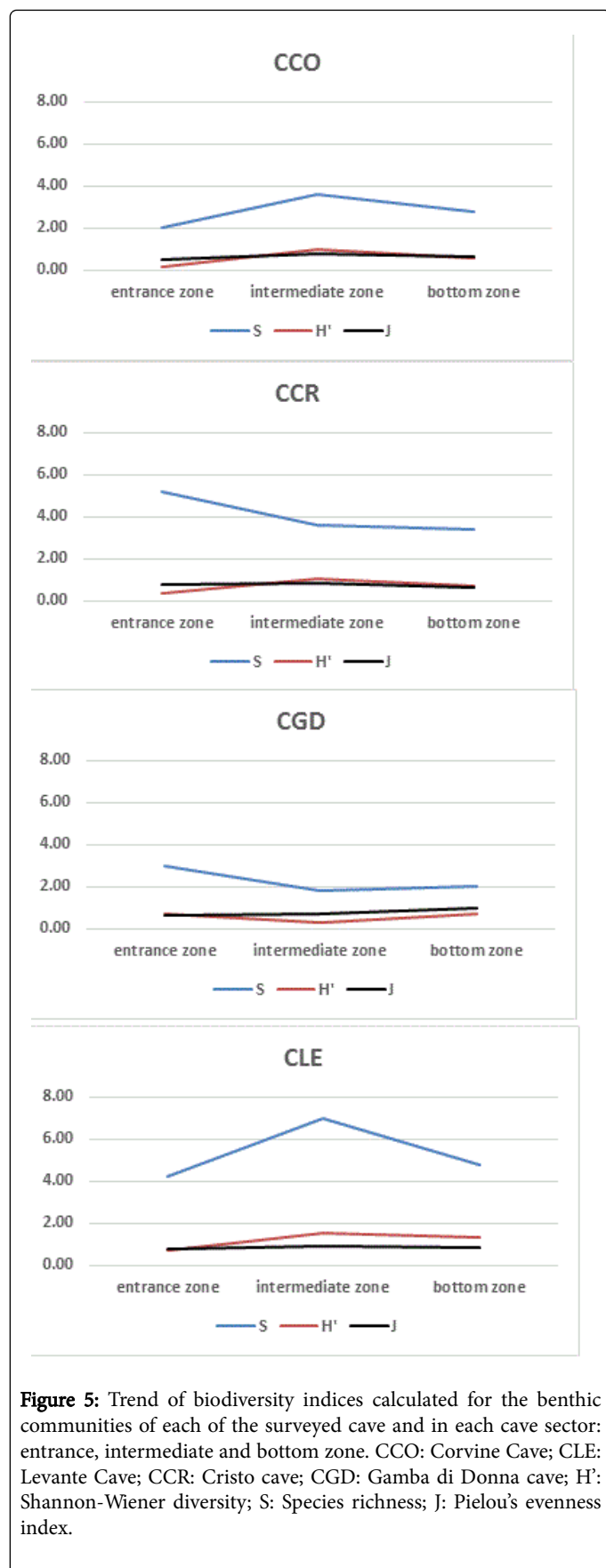
**Table 2:** Average values of the diversity indices calculated for each sector of the explored caves

The Intermediate Zones showed the highest  $H'$  values except in Gamba di Donna, (CGD), (Figure 5). Diversity values decrease in the Bottom Zones of the 4 examined caves, (Table 2). This aspect has already been found in other Mediterranean [12-14] and Black Sea [57] caves.

Species richness ( $S$ ), follows the trend of diversity ( $H'$ ), in CCO, CLE and CGD. Reverse trends of  $S$  are found in CCR.

Results of the two-way crossed PERMANOVA performed on the Shannon diversity index, ( $H'$ ), indicated significant differences, ( $p<0.01$ ), for all the considered factors. The two-way crossed PERMANOVA performed on the species richness index, ( $S$ ), revealed significant differences between caves, ( $F=13.393$ ;  $p<0.01$ ), and between sectors across all caves, ( $F=3.2587$ ;  $p<0.05$ ), but no significant differences between cave sectors. Pairwise comparisons performed on the factor "Cave" showed significant differences among all caves except between CCO and CGD (Table 3).

The pairwise test conducted between levels of the "Cave" factor pointed out Levante, (CLE), as significantly different from all the other caves, ( $p<0.01$ ), (Table 3).



The same test conducted on the “Cave sector” factor indicated significant differences among all the levels considered, ( $p < 0.01$ ), except for the comparison between the Bottom Zone and Intermediate Zone.

Examining the Pielou Evenness Index, (J), the PERMANOVA analysis revealed significant differences for the Cave factor, ( $F = 3.0864$ ;  $p < 0.05$ ), and the interaction factor Cave x Sector ( $F = 2.3545$ ;  $p < 0.05$ ). Pairwise comparisons performed on the Cave factor, showed significant differences between CCO Vs CCR and CCO Vs CLE, (Table 3).

| Species Richness |       |     | Shannon diversity |       |     | Pielou Evenness |       |     |
|------------------|-------|-----|-------------------|-------|-----|-----------------|-------|-----|
| Groups           | T     | p   | Groups            | T     | p   | Groups          | T     | p   |
| CCO vs CCR       | 3.042 | **  | CCO vs CCR        | 1.172 | n.s | CCO vs CCR      | 2.277 | *   |
| CCO vs CGD       | 1.254 | n.s | CCO vs CGD        | 0.029 | n.s | CCO vs CGD      | 1.028 | n.s |
| CCO vs CLE       | 4.642 | *** | CCO vs CLE        | 4.765 | *** | CCO vs CLE      | 2.659 | *   |
| CCR vs CGD       | 3.689 | *** | CCR vs CGD        | 1.182 | n.s | CCR vs CGD      | 1.038 | n.s |
| CCR vs CLE       | 2.179 | *   | CCR vs CLE        | 4.462 | *** | CCR vs CLE      | 0.455 | n.s |
| CGD vs CLE       | 4.755 | *** | CGD vs CLE        | 4.517 | *** | CGD vs CLE      | 1.417 | n.s |

**Table 3:** Results of the pairwise comparisons among caves performed for each index. CCO: Corvine Cave, CLE: Levante Cave, CCR: Cristo cave, CGD: Gamba di Donna cave. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; n.s. = not significant.

## Discussion

This study aims to improve the knowledge of the macrobenthic diversity of some submerged and semi-submerged caves around Capo Milazzo, an area selected as an MPA. The sampling method, not invasive and ethically acceptable for a forthcoming MPA, surely contributed to the knowledge of these particular environments.

According to our results, the diversity index showed a clear pattern, generally increasing from the entrance to the intermediate zone in most of the explored caves, (CCO, CLE and CCR).

The high values of H' and S observed in the Intermediate Zones may be due to openings and cracks in the cave roofs. A small number of species were found in the Intermediate Zone of Gamba di Donna, (CGD), cave, which doesn't have external links. In addition, being a semi-submersible cave exposed to strong northwestern winds, it is subject to strong hydrodynamism generated by N-NW winds and this condition may be responsible for the lower values of S, H' and J observed in its Intermediate Zone.

The Intermediate Zone, in all the examined caves, appears to represent a discontinuity area in terms of total coverage percentages, distribution of animal populations and presence of rare species, (*Haliclona fulva*, *Ircinia sp.*, *Phyllangia americana mouchezii*). In particular, the different morphology of the four examined caves could be responsible for the benthic fauna variability. In fact, differences in the assemblage of the caves probably depend on the topographic

specificity of each cave which, in turn, affects the gradients of biotic and abiotic parameters [14,16,55,58-60]. Strong currents in the CLE cave, for example, may be responsible for the presence of *Margaretta cereoides* in the Entrance Zone.

In conclusion, it is possible to recognize a pool of species, distributed along a horizontal axis, Entrance-Bottom Zone, whose specific composition includes: coral species located mostly in the Entrance Zone, (*Parazoanthus axinellae*, *Paracyathus pulchellus*, *Cornularia cornucopiae*), species of the "semi-obscure" biocenosis, (shaded areas), and "dark", (sensu Pérès & Picard), [61] located in the darkest parts of the caves, (Bottom Zone), (*Petrobionta massiliana*, *Spiraserpula massiliensis*, *Haliclona fulva*).

In some cases these species aggregate together generating different patterns of abundance and coverage which probably depend on abiotic factors such as light, (that may filter through cracks even in the intermedian areas of the caves; i.e. CCR, CLE), hydrodynamism, (strong currents), and cave morphology, (presence of branches and niches).

Sponges and the cnidarians anthozoans represent the most representative taxa at the entrance and middle areas of each cave. The sea worms serpulids, with encrusting sponges, have a significant role in the internal sector, (Bottom Zone).

Improving the available knowledge on these particular marine habitats and their species assemblages is extremely important considering that recreational activities such as diving may negatively affect them. Monitoring studies of these caves and their biodiversity are required for the management and conservation practices of these fragile ecosystems, especially in view of the new MPA establishment.

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