

## Soft-bottom diversity patterns in marine caves; Lessons from crustacean community



Carlos Navarro-Barranco <sup>a,\*</sup>, José M. Guerra-García <sup>a</sup>, Luis Sánchez-Tocino <sup>b</sup>, Pablo Jiménez-Prada <sup>a</sup>, Sara Cea <sup>a</sup>, José Carlos García-Gómez <sup>a</sup>

<sup>a</sup> Laboratorio de Biología Marina, Dpto. Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, Avda Reina Mercedes 6, 41012 Sevilla, Spain

<sup>b</sup> Departamento de Biología Animal, Facultad de Ciencias, Universidad de Granada, Campus Universitario de Fuentenueva, s/n., 18071 Granada, Spain

### ARTICLE INFO

#### Article history:

Received 6 June 2012

Received in revised form 8 April 2013

Accepted 10 April 2013

Available online xxxx

#### Keywords:

Crustacea

Diversity patterns

Marine caves

Mediterranean Sea

Soft-bottom communities

### ABSTRACT

Previous marine cave studies have been generally qualitative, focusing on hard benthic communities of single caves. The ecological patterns of marine cave assemblages, particularly those with soft-sediment bottoms, are poorly known. The aim of this study was to investigate ecological patterns of macroinfauna inhabiting marine caves. Using a multifactorial design, the soft-bottom crustacean fauna of six previously unsampled Mediterranean marine caves was studied. To investigate the influence of the marine cave habitat on local crustacean assemblage two stations were compared in each cave; one internal site from within the marine cave cavity and one external site, in sediments near the opening of the cave. The caves selected had a wide range of profundity in order to explore the influence of depth on the community. External sediments had higher species richness, and a significant decline in diversity values inside the caves was found at all marine cave locations studied. The abundance of organisms was strongly influenced by depth, with deeper internal cave sites having lower abundance than the external stations, while more shallow internal cave sites provide a more stable environment, which allows higher density of individuals. The results described here for soft-sediment marine cave bottoms are in contrast to previous observations in hard benthic marine cave communities, where small-scale variability was similar for interior and exterior cave habitats. The results of this study show that even in the absence of endemic cave taxa, the species assemblage at each cave was clearly different from that present in the exterior habitat and also from that present in other cave sediments. This high variability and strong individuality observed in soft-bottom marine caves suggest that there are many and complex factors controlling these communities.

© 2013 Published by Elsevier B.V.

## 1. Introduction

Marine cave habitats are characterized by distinct biocene, fauna and ecological features (Cicogna et al., 2003; Ott and Svoboda, 1976; Pérès, 1967; Riedl, 1966; Sarà, 1974). Although marine cave assemblages were of great interest over the last decades, knowledge on marine cave environments is still fragmentary. Most studies have focused on a small number of taxa and/or small geographic areas. The studies are mainly qualitative, providing inventories of species and descriptions of spatial distribution patterns.

Within marine caves considerable effort has been devoted to hard substrate benthic communities, however the number of investigations dealing with the macroinfauna living in soft sediment is scarce (Akoumianaki and Hughes, 2004; Bamber et al., 2008; Monteiro-Marques, 1981; Navarro-Barranco et al., 2012). Many studies that focus on single marine caves are difficult to extrapolate to

other cave environments or geographic areas. To-date quantitative studies which have been conducted simultaneously across different caves, have focused on hard-substrate communities only (Bibiloni et al., 1989; Bussotti et al., 2006; Martí et al., 2004a). Only a few quantitative and experimental studies have sort of determine the broad ecological factors governing the marine cave habitats (e.g. Benedetti-Cecchi et al., 1996, 1998; Denitto et al., 2007; Gili et al., 1986; Zabala et al., 1989), being the soft-bottom cave communities in particular poorly studied.

Using a multifactorial sampling design, the spatial variations (internal vs. external habitats) of the soft-bottom crustacean assemblages are explored in six marine caves along the coast of Granada (southern Spain). The study focused on crustacea taxa specifically as one of the most diverse and abundant taxa within the macrobenthic fauna inhabiting marine sediments (Dauvin et al., 1994; Lourido et al., 2008; Prato and Biantolino, 2005). Moreover, crustaceans play an important role in structuring benthic assemblages, and they are also sensitive to many environmental conditions, including depth, sediment composition and also pollution (Carvalho et al., 2012; De Grave, 1999; de-la-Ossa-Carretero et al., 2012; Duffy and Hay, 2000; Gómez-Gesteira and Dauvin, 2000; Guerra-García and García-Gómez, 2004).

\* Corresponding author. Tel.: +34 954556229.

E-mail address: [carlosnavarro@us.es](mailto:carlosnavarro@us.es) (C. Navarro-Barranco).

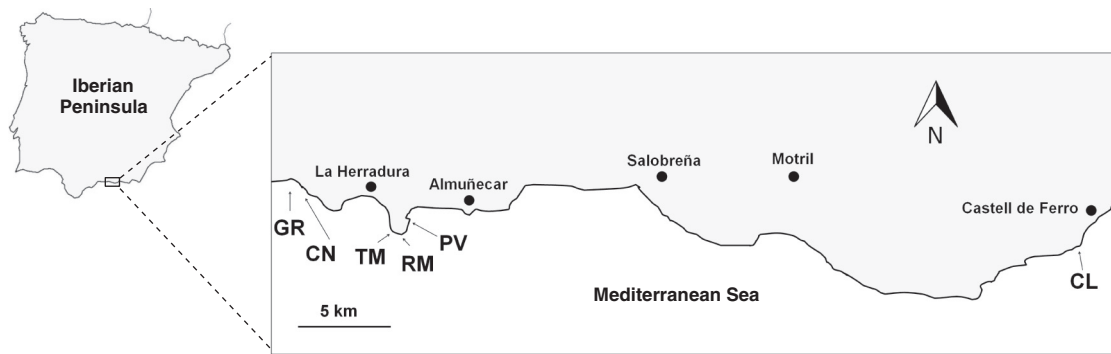


Fig. 1. Study area and position of marine caves studied. GR = Gorgonias; CN = Cantarriján; TM = Treinta Metros; RM = Raja Mona; PV = Punta del Vapor; CL = Calahonda.

The study area is a karstic region with a relatively low human impact, which allows us to have many submarine caves with little anthropogenic influence and, a priori, similar characteristics in a short stretch of coastline (about 30 km). The caves represent a wide range of depths from 5 to 30 m. Riedl (1966) indicates that a displacement associated with the depth occurs in cave communities. When depth increases, cave species move towards the entrance due to the attenuation of light, hydrodinamism etc. outside the cave. To our knowledge, not many efforts have been devoted to test the importance of depth in marine cave communities.

Consequently, the main objective of this study is to investigate diversity patterns of crustacean community according to the depth and the location for soft-sediment habitats of marine caves.

## 2. Materials and methods

### 2.1. Study site

Six karstic marine caves of Granada's coast were selected to carry out this study (Fig. 1): Cueva de las Gorgonias (GR) ( $36^{\circ}44'17''\text{N}$ ,  $46^{\circ}46'42''\text{W}$ , 6 m deep), Cueva de Cantarriján (CN) ( $36^{\circ}44'16''\text{N}$ ,  $3^{\circ}46'41''\text{W}$ , 8 m deep), Cueva de los Treinta Metros (TM) ( $36^{\circ}43'12''\text{N}$ ,  $3^{\circ}44'9''\text{W}$ , 30 m deep), Raja de la Mona (RM) ( $36^{\circ}43'10''\text{N}$ ,  $3^{\circ}44'6''\text{W}$ , 30 m deep), Cueva de la Punta del Vapor (PV) ( $36^{\circ}43'22''\text{N}$ ,  $3^{\circ}42'35''\text{W}$ , 12 m deep) and Cueva de Calahonda (CL) ( $36^{\circ}42'46''\text{N}$ ,  $3^{\circ}22'18''\text{W}$ , 19 m deep). All of them presented similar length (10–25 m) and morphology, with a single submerged entrance followed by a rectangular blind-ending tunnel without air chambers.

### 2.2. Sample collection

The samples were taken during July and August 2011. Two sampling stations were selected in each cave: one in the exterior area and another inside the cave (each one approximately 10 m from the cave mouth). Four replicate samples were taken for the crustacean study at each station using a hand-held rectangular core of  $0.025\text{ m}^2$  to a depth of 10 cm by SCUBA diving. Samples were washed using a 0.5 mm mesh sieve with seawater, preserved in 70% ethanol and stained with Rose Bengal. Each sample was examined in the laboratory using binocular microscopes. All crustacean specimens were counted and identified to species level where possible.

In addition to the macrofaunal samples, three additional sediment cores were collected at each station for physicochemical analyses of the sediments. Samples were immediately stored frozen until the laboratory analyses. Granulometric parameters were determined following the method proposed by Guitián and Carballas (1976). Organic matter and nitrogen percentage in the sediment, and the composition of phosphorus, lead and copper, were determined following the same methodology of Navarro-Barranco et al. (2012).

### 2.3. Data analysis

Mean and standard deviation of crustacean abundances, number of species and Shannon–Wiener diversity index (Shannon and Weaver, 1963) were calculated for each sampling station. Spatial patterns of these parameters were examined using an Analysis of Variance (ANOVA) with the following factors: position (Po), depth (De) and site (Si). Po was a fixed factor with two levels: internal and external marine cave sediments. De, a fixed factor orthogonal with Po, presented three levels: shallow (for caves between 0 and 10 m deep), medium (10–20 m) and deep (20–30 m). Si was a random factor nested with De and with 2 levels (two marine caves at each depth level). Four

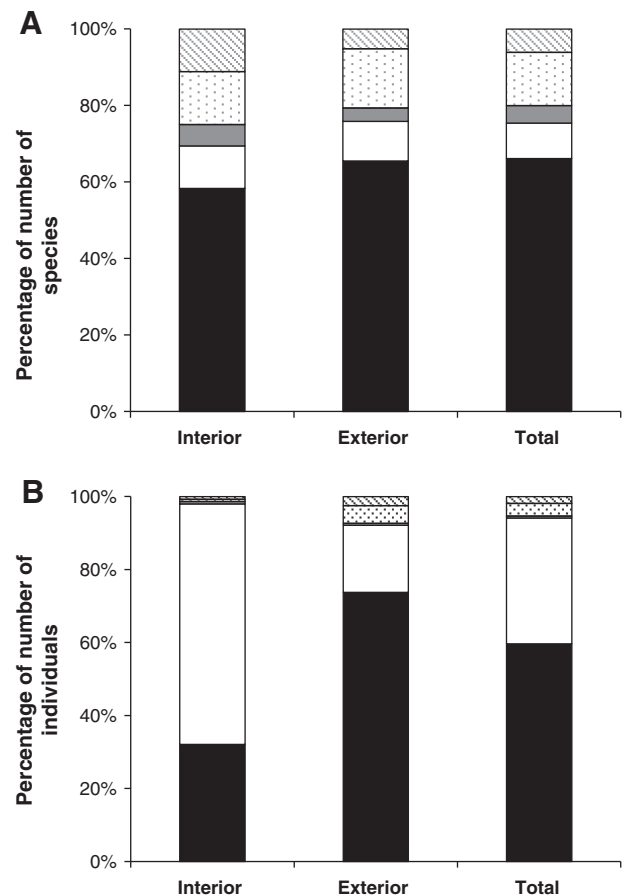


Fig. 2. Percentage (%) of crustaceans groups inside caves, outside caves and the whole study calculated in function of number of species (A) and number of individuals (B). Striped sections represent the percentage of decapods, dotted sections represent cumaceans, gray sections tanaids and black sections represent the percentage of amphipods.

**Table 1**

Results of the multivariate analysis PERMANOVA for crustacean assemblages, based on Bray–Curtis dissimilarities of fourth root transformed data. Df = degrees of freedom; MS = mean square; P = level of significance; \* =  $p < 0.05$ .

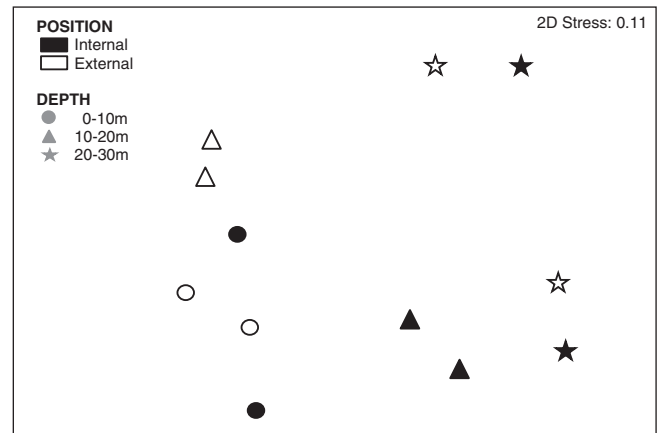
Source of variation	df	MS	F	P
Position = Po	1	17342	3.8116	0.0392*
Depth = De	2	21828	1.7228	0.1569
Site(De) = Si(De)	3	12670	19.476	0.0196*
Po × De	2	9131.7	2.0071	0.1373
Po × Si(De)	3	4549.7	6.994	0.0196*
Residual	36	650.52		

samples of sediment were considered from each cave ( $n = 4$ ). Prior to ANOVA analyses, the homogeneity of variances was tested using Cochran's test and appropriate transformations were applied to the data when necessary (Underwood, 1997). When ANOVA indicated a significant difference for a given factor, the source of difference was identified using the Student–Newman–Keuls (SNK) tests.

Following the same three-factor design, a distance-based Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001) was carried out to test differences in the crustacean species composition. Sixty-five taxa were included in the multivariate data set, and analysis was based on fourth root transformed data. The similarity matrix was generated using the Bray–Curtis similarity index. Terms found to be significant in the analysis were examined individually using appropriate pair-wise comparisons. Non-Parametric Multidimensional Scaling (nMDS) was carried out to examine differences in the crustacean assemblage composition among positions and depths (Clarke and Warwick, 1994). The similarity percentages procedure (SIMPER) was used 'a posteriori' to calculate the contribution of each species to the observed dissimilarity between positions. A cut-off criterion was applied to allow identification of a subset of species whose cumulative percentage contribution reached 90% of the similarity or dissimilarity values and was considered as 'important' in characterizing or separating positions. The same three-factor design applied before was used to explore differences in the abundance of the most important species, as identified by the SIMPER, as characterizing or separating positions.

The similarity values for each station (obtained from the comparison among replicates in PERMANOVA) can be used to estimate the small-scale variability in the crustacean assemblage composition. One-way ANOVA was used to test whether the homogeneity in species composition within stations changes between positions.

A Principal Component Analysis (PCA) was carried out to observe the relationships between environmental measures and sampling stations. Data analyses of ANOVAs were performed using the GMAV5



**Fig. 3.** Two-dimensional nMDS plot for species composition. Data were fourth root transformed.

program (Underwood et al., 2002), and multivariate analyses were carried out using the PRIMER v.6 + PERMANOVA package (Clarke and Gorley, 2001).

### 3. Results

A total of 65 species were recorded during this study. Thirty-six species were found inside the caves, while 58 were recorded at the exterior sites. In both stations, amphipods had the highest species richness, with 44 species in total, followed by cumaceans (9 species), tanaids (6), decapods (4) and isopods (2) (Fig. 2A). Amphipods were also the most abundant group in the study, with approximately 60% of individuals. Inside the cave, tanaids was the group with higher abundance of organisms (Fig. 2B). However, their abundance was highly variable (while in Cantarriján cave, 95% of the species belong to tanaids; this value was only 5% in the other shallow cave).

PERMANOVA analysis showed significant differences in the composition of species between external and internal stations (Table 1) and SIMPER showed a dissimilarity of 83.1% in the species composition between both habitats. Within positions, cave stations only presented a similarity of 4.9%, whereas the stations situated outside the caves had 47.7% of similarity. Table 2 showed the most important species in characterizing and separating positions. Only the abundance of *Siphonocetes sabatieri*, *Diogenes pugilator*, *Megaluropus monasteriensis* and *Pariambus tipicus* was significantly different between positions, with higher abundances in the external stations. Although some taxa, such as all

**Table 2**

Taxa identified as important in characterizing and separating positions, according to the similarity and dissimilarity values obtained by SIMPER analysis. Mean values  $\pm$  SD in cave and open stations were also showed for each species. P = level of significance for the null hypothesis of non differences in abundance between stations. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .

Species	Internal stations		External stations		Internal vs. external stations	
	Abundance	% Similarity	Abundance	% Similarity	% Dissimilarity	P
<i>Siphonocetes sabatieri</i>	–	–	4656 $\pm$ 1267	32.97	22.03	*
<i>Apeudopsis latreillii</i>	2848 $\pm$ 724	30.75	1365 $\pm$ 238	53.78	13.62	n.s.
<i>Harpinia pectinata</i>	990 $\pm$ 373	13.78	106.67 $\pm$ 40	<0.5	13.62	n.s.
<i>Leptocheirus hirsutimanus</i>	–	–	208 $\pm$ 85	<0.5	4.64	n.s.
<i>Apeudes</i> sp.	88 $\pm$ 36	<0.5	140 $\pm$ 57	<0.5	4.13	n.s.
<i>Photis longipes</i>	8 $\pm$ 2	1.6	150 $\pm$ 59	1.02	3.81	n.s.
<i>Harpinia crenulata</i>	148 $\pm$ 38	17.64	58 $\pm$ 24	<0.5	3.57	n.s.
<i>Periculodes longimanus</i>	98 $\pm$ 29	9.71	163 $\pm$ 45	1.43	1.84	n.s.
<i>Diogenes pugilator</i>	26 $\pm$ 5	<0.5	180 $\pm$ 28	1.44	1.78	*
<i>Metaphoxus fultoni</i>	5 $\pm$ 2	<0.5	335 $\pm$ 119	0.98	1.63	n.s.
<i>Harpinia antennaria</i>	38 $\pm$ 15	<0.5	7 $\pm$ 3	<0.5	1.29	n.s.
<i>Pariambus tipicus</i>	–	–	190 $\pm$ 49	1.66	0.89	**
<i>Ampelisca</i> sp.	–	–	40 $\pm$ 16	<0.5	0.89	n.s.
<i>Deflexilodes griseus</i>	28 $\pm$ 6	4.81	13 $\pm$ 4	<0.5	0.52	n.s.
<i>Kupellonura mediterranea</i>	27 $\pm$ 5	8.02	23 $\pm$ 7	<0.5	<0.5	n.s.
<i>Megaluropus monasteriensis</i>	–	–	31.67 $\pm$ 7	2.42	<0.5	*

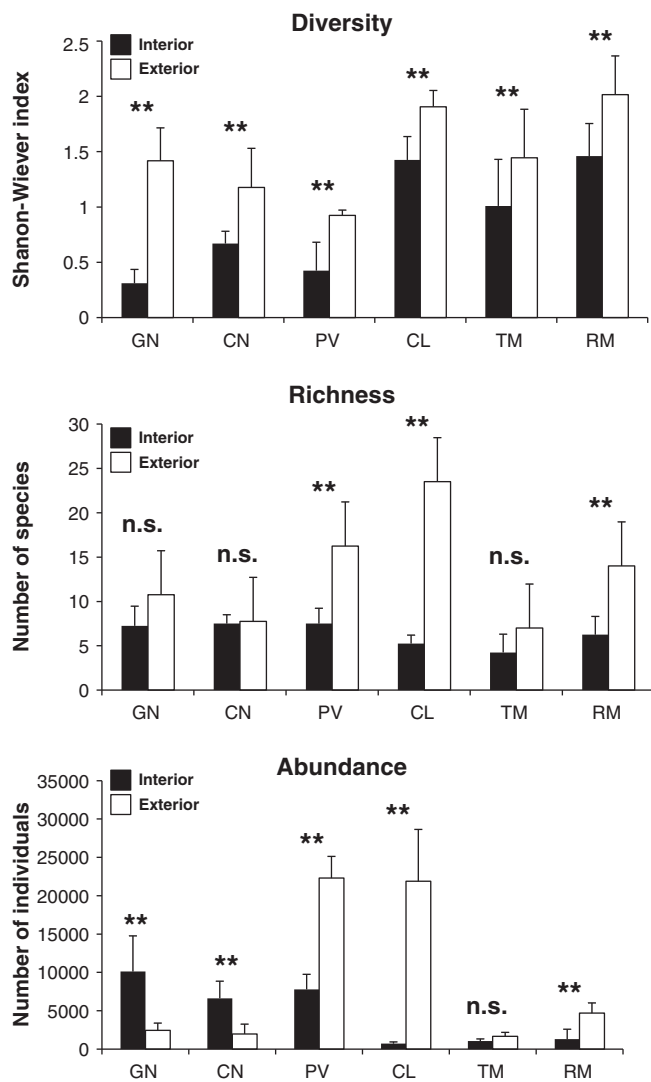
**Table 3**

Results of the three-factor ANOVA for Shannon–Wiener diversity, species richness and abundance of individuals for the crustacean community. No transformation was necessary for diversity and richness values. Abundance data were  $\text{Sqrt}(x + 1)$  transformed. df = degrees of freedom; MS = mean square; P = level of significance; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .

Source of variation	df	Shannon diversity			Species richness			Abundance		
		MS	F	P	MS	F	P	MS	F	P
Position = Po	1	4.28	34.35	0.009***	567.18	13.52	0.035*	152.44	4.38	0.128
Depth = De	2	1.38	0.83	0.515	135.77	3.59	0.16	349.15	7.71	0.065
Site(De) = Si(De)	3	1.66	20.88	0.000***	37.85	3.53	0.003***	45.27	10.26	0.000***
Po × De	2	0.13	1.06	0.448	143.06	3.41	0.168	450.48	12.93	0.033*
Po × Si	3	0.12	1.57	0.214	41.93	6.13	0.002**	34.83	7.9	0.000***
Residual	36	0.08			6.84			4.41		

*Harpinia* species, showed higher abundance inside the caves, no species presented a significant preference to cave stations. The nMDS analysis (Fig. 3) did not reflect a clear separation in species composition between internal and external stations. The single exception being the medium depth stations, for which external and cave stations were clearly separated.

Results for the three-way ANOVA comparing diversity, species richness and abundance showed different patterns for each case (Table 3).



**Fig. 4.** Mean values  $\pm$  SD of diversity, number of species and number of individuals  $m^{-2}$  for each sampling station. Significance of differences between positions was also represented. \*\* =  $p < 0.01$ ; n.s. = non significant. GR = Gorgonias; CN = Cantarriján; TM = Treinta Metros; RM = Raja Mona; PV = Punta del Vapor; CL = Calahonda.

Although all caves showed significant differences between internal and external stations for abundance values, with the exception of Treinta metros cave (Fig. 4), no consistent pattern was found, with Po × De and Po × Si(De) interactions. Species richness was always higher in the external stations than in the internal stations. However, according to SNK tests, these differences were only significant for PV, CL and RM. Diversity values presented the clearest pattern, with significant higher values outside the caves, consistent for all depths and site levels.

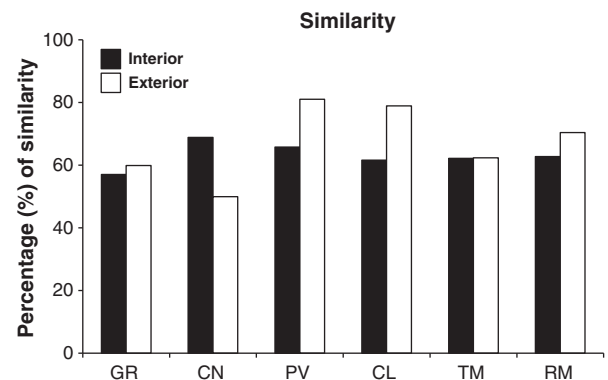
The similarity values for species composition between replicates within stations are represented in Fig. 5. One-way ANOVA did not find significant differences among positions, so it can be assumed that there was similar small-scale variability in both habitats.

Table 4 shows the physicochemical characteristics of the sediment for each station. In the PCA analysis (Fig. 6), axis 1 explains a 49% of variation and correlated positively with the percentage of medium sands and negatively with the amount of silt and clay, organic matter, nitrogen, phosphorous and lead, while axis 2, which explains a 32% of variation, was strongly and positively correlated with the gravel content. Although there are some stations with a particular behavior, cave stations often present higher values of nitrogen, phosphorous, organic matter, lead and percentage of silt and clay. On the other hand, the external stations were dominated by thicker fraction of sediments. Sediment properties (e.g. grain size) often vary with depth but our granulometric and chemical data did not reflect any clear relationship with depth. Therefore, the changes in the community descriptors were easier to relate with depth alone.

## 4. Discussion

### 4.1. Species composition

This study reveals that soft-bottom cave communities are clearly different from soft-bottom communities in open habitats. Each cave has a characteristic crustacean assemblage, which differed from adjacent



**Fig. 5.** Similarity values (Bray–Curtis index) between replicates within each station. GR = Gorgonias; CN = Cantarriján; TM = Treinta Metros; RM = Raja Mona; PV = Punta del Vapor; CL = Calahonda.

**Table 4**  
Mean values  $\pm$  SD of the granulometric data and chemical variables in the sediment per station; O.M. = organic matter.

Cave	Station	Chemical data					Granulometric data		
		O.M. (%)	N (%)	P (mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	% Gravels	% Medium sands	% Silt and clay
Gorgonias	Interior	0.84 $\pm$ 0.15	0.042 $\pm$ 0.000	448.49 $\pm$ 5.4	9.27 $\pm$ 0.2	12.15 $\pm$ 0.07	1.25 $\pm$ 0.33	1.65 $\pm$ 0.5	26.83 $\pm$ 2.4
	Exterior	0.23 $\pm$ 0.00	0.009 $\pm$ 0.000	281.04 $\pm$ 4.68	8.39 $\pm$ 0.32	9.73 $\pm$ 0.1	0.07 $\pm$ 0.01	25.64 $\pm$ 0.22	2.3 $\pm$ 0.17
Cantarriján	Interior	0.74 $\pm$ 0.04	0.024 $\pm$ 0.000	363.08 $\pm$ 9.04	8.77 $\pm$ 0.31	12.31 $\pm$ 0.09	1.15 $\pm$ 0.41	16.2 $\pm$ 0.37	10.83 $\pm$ 1.73
	Exterior	0.18 $\pm$ 0.03	0.009 $\pm$ 0.000	283.52 $\pm$ 4.92	9.63 $\pm$ 0.36	11.37 $\pm$ 0.19	9.79 $\pm$ 0.01	19.3 $\pm$ 0.00	3.34 $\pm$ 0.02
Pta. Vapor	Interior	0.76 $\pm$ 0.02	0.050 $\pm$ 0.000	639.36 $\pm$ 12.9	11.94 $\pm$ 0.2	15.39 $\pm$ 0.13	3.89 $\pm$ 1.64	4.57 $\pm$ 0.39	31.19 $\pm$ 6.11
	Exterior	0.44 $\pm$ 0.10	0.023 $\pm$ 0.000	408.11 $\pm$ 10.2	7.81 $\pm$ 1.14	11.68 $\pm$ 1.17	0.93 $\pm$ 0.27	16.73 $\pm$ 2.1	3.56 $\pm$ 0.56
Calahonda	Interior	0.98 $\pm$ 0.02	0.068 $\pm$ 0.000	778.70 $\pm$ 21.79	16.36 $\pm$ 0.49	17.42 $\pm$ 3.63	3.1 $\pm$ 1.35	3.27 $\pm$ 0.35	65.5 $\pm$ 3.42
	Exterior	0.40 $\pm$ 0.03	0.029 $\pm$ 0.000	506.33 $\pm$ 17.75	9.05 $\pm$ 0.62	7.86 $\pm$ 0.14	0.76 $\pm$ 0.31	2.36 $\pm$ 0.35	13.16 $\pm$ 0.45
Treinta Metros	Interior	1.86 $\pm$ 0.02	0.117 $\pm$ 0.000	786.90 $\pm$ 2.63	18.56 $\pm$ 0.34	19.02 $\pm$ 0.67	3.11 $\pm$ 1.05	3.71 $\pm$ 0.1	58.51 $\pm$ 4.05
	Exterior	1.95 $\pm$ 0.05	0.174 $\pm$ 0.000	579.36 $\pm$ 3.01	15.38 $\pm$ 0.14	10.10 $\pm$ 0.32	4.15 $\pm$ 1.29	6.55 $\pm$ 1.15	46.11 $\pm$ 4.76
Raja Mona	Interior	0.79 $\pm$ 0.02	0.077 $\pm$ 0.000	545.62 $\pm$ 17.48	7.97 $\pm$ 0.72	9.81 $\pm$ 0.48	24.5 $\pm$ 3.38	10.68 $\pm$ 1.88	8.19 $\pm$ 4.34
	Exterior	0.56 $\pm$ 0.01	0.043 $\pm$ 0.000	570.48 $\pm$ 12.39	8.39 $\pm$ 0.57	2.75 $\pm$ 0.1	10.99 $\pm$ 1.16	26.09 $\pm$ 1.39	3.55 $\pm$ 0.9

exterior sediments, and also between marine caves. This high degree of individuality in marine caves has been well reported in the literature (Ros et al., 1989; Sarà, 1974; Vacelet et al., 1994). Despite having unique assemblage patterns, marine caves did not have endemic fauna in this study. There were no exclusive cave taxa among the most abundant species, with all the Crustacea taxa recorded at both internal and external habitat sites. The species richness was also similar in both habitats, with amphipods as the dominant Crustacea group, which is a common feature in soft-bottom communities (Lourido et al., 2008; Sanchez-Moyano et al., 2005).

#### 4.2. Small-scale spatial variability

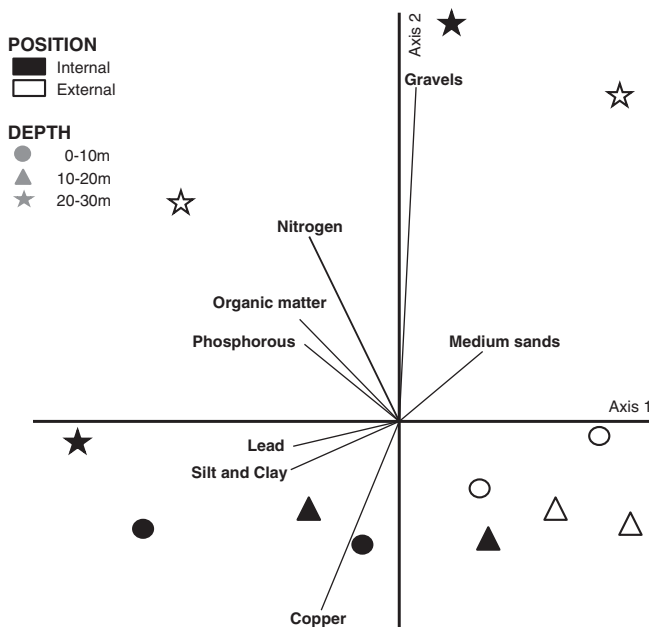
Marine caves had often been considered as simple, stable and homogenous environments (Harmelin et al., 1985; Ott and Svoboda, 1976). However, spatial studies in hard benthic communities revealed that the distribution patterns of sessile fauna inside caves could be very complex, with high variability on a scale of few meters (Benedetti-Cecchi et al., 1996, 1998). The innermost parts of marine caves usually show a prevalence of species with clumped distribution (Martí et al., 2004a). The spatial heterogeneity at small scales in soft-bottom communities was poorly studied and it had never been investigated in marine caves (Kendall and Widdicombe, 1999). Marine

soft sediments can also present high spatial heterogeneity at small scales, with significant variations at scale of meters or even less (Morrissey et al., 1992). An apparently homogeneous habitat as the deep sea, which often has been considered analogous to cave habitats (Akoumianaki and Hughes, 2004; Harmelin, 1997; Vacelet et al., 1994), can present an elevated variability in the sediment due to small-scale biotic and abiotic disturbances (Levin and Thomas, 1988; Snelgrove, 1998; Snelgrove et al., 1992).

In contrast to observations for hard benthic communities, our results suggest that there are no differences in the small-scale variability of soft benthic communities between cave and external habitats. This difference between soft and hard benthic macrofauna is difficult to explain because there are many factors influencing the heterogeneity in the spatial patterns of both habitats. The scale at which the heterogeneity is detected in soft sediments depends on many poorly-known factors: taxa considered and their life history (dispersal capability, site selection by larvae or juvenile, etc.), sediment characteristics (e.g. grain size) or biotic interactions (competition, facilitation, etc.) (Kendall and Widdicombe, 1999; Norén and Lindergarth, 2005; Thrush, 1991). In hard benthic communities, no small-scale differences in the settlement patterns have been found into the caves, so that the high patchiness in the cave walls might derive from other post-settlement factors (Denitto et al., 2007). According to many studies, interactions between species can play an important role determining the spatial distribution of cave organisms (Benedetti-Cecchi et al., 1996; Martí et al., 2004b, 2005; Turon et al., 2009). Thus, further studies are necessary to understand the relevance of such factors in the distribution patterns of cave fauna.

#### 4.3. Diversity, species richness and abundance of organisms

Although a decrease in diversity, richness and abundance values from entrance to the inward end of marine caves is a common feature for hard benthic communities (e.g. Balduzzi et al., 1989; Cicogna et al., 2003; Gili et al., 1986; Martí et al., 2004b; True, 1970; Zabala et al., 1989), our results reveal that some of these parameters do not act in the same way in soft-bottom communities. A decrease in diversity values into the cave was the only constant pattern found. The usual explanation for this trend is that the trophic supply decreases (Fichez, 1990a,b; Zabala et al., 1989), but in some cases, as our caves, that decrease in the organic matter concentrations inside the caves is not detected. In such cases, other factors had been proposed to explain the loss of biodiversity of cave communities: physical gradients inside the cave (light, oxygen, salinity, etc.), the limited capacity of the larvae for dispersion and settlement or the persistence of microlayer gradients along the walls (Gili et al., 1986; Harmelin, 1997; Harmelin et al., 1985; Zabala et al., 1989). All these explanations were proposed for hard benthic communities but may also apply to the soft-bottom communities. Moreover, there are other environmental gradients related to sediment characteristics (grain size, heterogeneity, heavy metals concentration,



**Fig. 6.** Results of PCA for physicochemical variables of the sediment.

etc.) which can explain the biodiversity decrease into the caves too (Navarro-Barranco et al., 2012).

The species richness values showed a similar pattern: all caves present higher number of species in the external stations, and the analysis reveals the existence of significant differences among positions. However, this general pattern was not consistent across caves (Po  $\times$  Si(De) interaction).

Although depth is one of the main factors affecting the structure and composition of marine macrobenthic communities (Hoey et al., 2004; Martínez and Adarraga, 2001; Snelgrove, 1998), our ANOVA analyses did not reflect any depth influence in species richness or diversity. In fact, the number of species into the caves remains almost constant between depths. Some studies suggest the existence of a parabolic relationship between depth and both parameters (Gray, 1997; Rex, 1993), but these patterns were not found in all habitats and for small depth ranges (Bergen et al., 2001). Thus, these generalizations have been questioned, and the depth relationship with diversity and species richness in soft sediments still remains unclear (Gray, 2002; Poore and Wilson, 1993; Snelgrove, 1998). ANOVA analyses only showed a significant depth influence for abundance values. A decrease in abundance values inside the caves was expected, but only deeper stations showed this pattern. At shallow sites, there were fewer numbers of individuals outside the caves. The reason could be related to the bottom stability, a factor highly correlated with the depth (Hernández-Arana et al., 2003; Snelgrove, 1998). At shallow sites, marine caves provide a more stable environment, protected from the influence of waves, wind and storm events, which allows the establishment of higher number of individuals (Bamber et al., 2008). This explanation cannot be applied to semi-submerged caves, which present a different behavior, generally with higher water turbulence (Bell, 2002; Corriero et al., 2000). Our abundance data for open habitats were very similar to those obtained by Carvalho et al. (2012) in the southern Portugal coast, where they reported a decrease of amphipod density in shallow sediments (<10 m) related with an environment harshness in terms of wave impact. Below this depth (between 10 and 25 m), they found a density peak, due to the abatement of the hydrodinamism.

As Bussotti et al. (2006) pointed out for hard benthic cave fauna, it can be concluded that, although some general and consistent patterns can be extracted for soft-bottom communities, the high degree of variability among caves makes it very complicated to understand the main processes that govern the marine cave communities. Numerous factors influence the sediment community, and each cave has different characteristics. However, this high degree of individuality also converts each cave into a unique habitat, making the study of such habitats more interesting.

## Acknowledgment

Financial support of this work was provided by the *Ministerio de Educación y Ciencia* (Project CGL 2011-22474/BOS). Thanks to the 'La Bernarda' dive group, especially to Carlos Gonzalez, for collaboration in the field sampling. Thanks to Dr. Lauren Hughes, who conducted the English revision of the manuscript. We would also thank biologists E. Baeza, P. Cabezas and M. Ros for help with sample identifications and Pilar Burgos (IRNA, CSIC) for her contribution to chemical analyses of sediments. This work forms part of C.N-B's Ph.D. Thesis, supported by the *University of Sevilla* (PIF Grant). [SS]

## References

- Akoumianaki, I., Hughes, J.A., 2004. The distribution of macroinfauna along a Mediterranean submarine cave with sulphur springs. *Cah. Biol. Mar.* 45, 355–364.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.* 26, 32–46.
- Balduzzi, A., Bianchi, C.N., Boero, F., Cattaneo-Vietti, R., Pansini, M., Sará, M., 1989. The suspension-feeder communities of a Mediterranean sea cave. *Sci. Mar.* 53, 387–395.
- Bamber, R.N., Evans, N.J., Robbins, R.S., 2008. The marine soft-sediment benthic communities of Hong Kong: a comparison of submarine cave and open habitats. *J. Nat. Hist.* 42, 953–965.
- Bell, J.J., 2002. The sponge community in a semi-submerged temperate sea cave: density, diversity and richness. *Mar. Ecol.* 23, 297–311.
- Benedetti-Cecchi, L., Airoldi, L., Abbiati, M., Cinelli, F., 1996. Exploring the causes of variation in assemblage of benthic invertebrates from a submarine cave with sulphur springs. *J. Exp. Mar. Biol. Ecol.* 208, 153–168.
- Benedetti-Cecchi, L., Airoldi, L., Abbiati, M., Cinelli, F., 1998. Spatial variability in the distribution of sponges and cnidarians in a sublittoral marine cave with sulphur-water springs. *J. Mar. Biol. Assoc. UK* 78, 43–58.
- Bergen, M., Weisberg, S.B., Smith, R.W., Cadien, D.B., Dalkey, A., Montagne, D.E., Stull, J.K., Velarde, R.G., Ranasinghe, J.A., 2001. Relationship between depth, sediment, latitude, and the structure of benthic infaunal assemblages on the mainland shelf of southern California. *Mar. Biol.* 138, 637–647.
- Bibiloni, M.A., Uriz, M.J., Gili, J.M., 1989. Sponge communities in three submarine caves of the Balearic Islands (Western Mediterranean): adaptations and faunistic composition. *Mar. Ecol.* 10 (4), 317–334.
- Bussotti, S., Terlizzi, A., Fraschetti, S., Belmonte, G., Boero, F., 2006. Spatial and temporal variability of sessile benthos in shallow Mediterranean marine caves. *Mar. Ecol. Prog. Ser.* 325, 109–119.
- Carvalho, S., Cunha, M.R., Pereira, F., Pousão-Ferreira, P., Santos, M.N., Gaspar, M.B., 2012. The effect of depth and sediment type on the spatial distribution of shallow soft-bottom amphipods along the southern Portuguese coast. *Helgol. Mar. Res.* <http://dx.doi.org/10.1007/S10152-011-0285-9>.
- Cicogna, F., Bianchi, C.N., Ferrari, G., Forti, P., 2003. Grotte marine: cinquant'anni di ricerca in Italia. CLEM, Ministero dell' Ambiente e della tutela del Territorio, Rome.
- Clarke, K.R., Gorley, R.N., 2001. PRIMER (Plymouth Routines in Multivariate Ecological Research) v5: User Manual/Tutorial. PRIMER-E Ltd., Plymouth.
- Clarke, K.R., Warwick, R.M., 1994. Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation. Natural Environment Research Council, UK.
- Corriero, G., Liaci, L.D., Ruggiero, D., Pansini, M., 2000. The sponge community of a semi-submerged Mediterranean cave. *Mar. Ecol.* 21, 85–96.
- Dauvin, J.C., Iglesias, A., Lorgeré, J.C., 1994. Circalittoral suprabenthic coarse sand community from the Western English Channel. *J. Mar. Biol. Assoc. UK* 74, 543–562.
- De Grave, S., 1999. The influence of sedimentary heterogeneity on within maerl bed differences in infaunal crustacean community. *Estuar. Coast. Shelf Sci.* 49, 153–163.
- de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lisazo, J.L., Dauvin, J.C., 2012. Sensitivity of amphipods to sewage pollution. *Estuar. Coast. Shelf Sci.* 96, 129–138.
- Denitto, F., Terlizzi, A., Belmonte, G., 2007. Settlement and primary succession in a shallow submarine cave: spatial and temporal benthic assemblage distinctness. *Mar. Ecol.* 28, 35–46.
- Duffy, J.E., Hay, M.E., 2000. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecol. Monogr.* 70, 237–263.
- Fichez, R., 1990a. Decrease in allochthonous organic inputs in dark submarine caves, connection with lowering in benthic community richness. *Hydrobiologia* 207, 61–69.
- Fichez, R., 1990b. Absence of redox potential discontinuity in dark submarine cave sediments as evidence of oligotrophic conditions. *Estuar. Coast. Shelf Sci.* 31, 875–881.
- Gili, J.M., Riera, T., Zabala, M., 1986. Physical and biological gradients in a submarine cave on the western Mediterranean coast (NE Spain). *Mar. Biol.* 90, 291–297.
- Gómez-Gesteira, J.L., Dauvin, J.C., 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. *Mar. Pollut. Bull.* 40, 1017–1027.
- Gray, J.S., 1997. Gradients in marine biodiversity. In: Ormond, R., Gage, J., Grassle, J.F. (Eds.), *Marine Biodiversity: Patterns and Processes*. Cambridge University Press, Cambridge, pp. 18–34.
- Gray, J.S., 2002. Species richness of marine soft sediments. *Mar. Ecol. Prog. Ser.* 244, 285–297.
- Guerra-García, J.M., García-Gómez, J.C., 2004. Crustacean assemblages and sediment pollution in an exceptional case study: a harbour with two opposing entrances. *Crustaceana* 77, 353–370.
- Gutián, F., Carballas, T., 1976. *Técnicas de análisis de suelos*, 2nd ed. Pico Sacrp, Santiago de Compostela.
- Harmelin, J.G., 1997. Diversity of bryozoans in a Mediterranean sublittoral cave with bathyal-like conditions: role of dispersal processes and local factors. *Mar. Ecol. Prog. Ser.* 153, 139–152.
- Harmelin, J.G., Vacelet, J., Vasseur, P., 1985. Les grottes sous-marines obscures: un milieu extrême et un remarquable biotope refuge. *Tethys* 11, 214–229.
- Hernández-Arana, H.A., Rowden, A.A., Attrill, M.J., Warwick, R.M., Gold-Bouchot, G., 2003. Large-scale environmental influences on the benthic macroinfauna of the southern Gulf of Mexico. *Estuar. Coast. Shelf Sci.* 58, 825–841.
- Hoey, G.V., Degraer, S., Vincx, M., 2004. Macrobenthic community structure of soft-bottom sediments at the Belgian continental shelf. *Estuar. Coast. Shelf Sci.* 59, 599–613.
- Kendall, M.A., Widdicombe, S., 1999. Small scale patterns in the structure of macrofaunal assemblages of shallow soft sediments. *J. Exp. Mar. Biol. Ecol.* 237, 127–140.
- Levin, L., Thomas, C.L., 1988. The ecology of xenophophores (Protista) on eastern Pacific seamounts. *Deep-Sea Res.* 35, 2003–2027.
- Lourido, A., Moreira, J., Troncoso, J.S., 2008. Assemblages of peracarid crustaceans in subtidal sediments from the Ría de Aldán (Galicia, NW Spain). *Helgol. Mar. Res.* 62, 289–301.
- Martí, R., Uriz, J.M., Ballesteros, E., Turon, X., 2004a. Temporal variation of several structure descriptors in animal-dominated benthic communities in two Mediterranean caves. *J. Mar. Biol. Assoc. UK* 84, 573–580.
- Martí, R., Uriz, J.M., Ballesteros, E., Turon, X., 2004b. Benthic assemblages in two Mediterranean caves: species diversity and coverage as a function of abiotic parameters and geographic distance. *J. Mar. Biol. Assoc. UK* 84, 557–572.

- Martí, R., Uriz, J.M., Turon, X., 2005. Spatial and temporal variation of natural toxicity in cnidarians, bryozoans and tunicates in Mediterranean caves. *Sci. Mar.* 69, 485–492.
- Martínez, J., Adarraga, I., 2001. Distribución batimétrica de comunidades macrobentónicas de sustrato blando en la plataforma continental de Guipúzcoa (Golfo de Vizcaya). *Bol. Inst. Esp. Oceanogr.* 17, 33–48.
- Monteiro-Marques, V., 1981. Peuplements des planchers envasés de trois grottes sous-marines de la région de Marseille. *Etude préliminaire*. *Tethys* 10, 89–96.
- Morrisey, D.J., Howitt, L., Underwood, A.J., Stark, J.S., 1992. Spatial variation in soft-sediments benthos. *Mar. Ecol. Prog. Ser.* 81, 197–204.
- Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., García-Gómez, J.C., 2012. Soft-bottom crustacean assemblages in Mediterranean marine caves: the cave of Cerro-Gordo (Granada, Spain) as case study. *Helgol. Mar. Res.* 6 (4), 567–576.
- Norén, K., Lindergarth, M., 2005. Spatial, temporal and interactive variability of infauna in Swedish coastal sediments. *J. Exp. Mar. Biol. Ecol.* 317, 53–68.
- Ott, J.A., Svoboda, A., 1976. Sea caves as model systems for energy flow studies in primary hard bottom communities. *Pubbl. Stn. Zool. Napoli* 40, 477–485.
- Péres, J.M., 1967. The Mediterranean benthos. *Oceanogr. Mar. Biol. Annu. Rev.* 5, 449–533.
- Poore, G., Wilson, G.D.F., 1993. Marine species richness. *Nature* 361, 597–598.
- Prato, E., Biandolino, F., 2005. Amphipod biodiversity of shallow water in the Taranto seas (north-western Ionian Sea). *J. Mar. Biol. Assoc. UK* 85, 333–338.
- Rex, M.A., 1993. Global-scale patterns of species diversity in the deep-sea benthos. *Nature* 365, 636–639.
- Riedl, R., 1966. *Biologie der Meereshöhlen*. Verlag Paul Parey, Hamburg/Berlin.
- Ros, J., Romero, J., Ballesteros, E., Gili, J.M., 1989. Buceando en las aguas azules. El bentos. In: Margalef, R. (Ed.), *El Mediterraneo Occidental*. Omega, Barcelona, pp. 235–297.
- Sanchez-Moyano, E., García-Asencio, I., García-Adiego, E., García-Gómez, J.C., Leal-Gallardo, A., Ollero, C., Fraidiás-Amarillo, J., 2005. Vigilancia ecológica del litoral andaluz. *Consejería de Medio Ambiente, Junta de Andalucía, Sevilla*.
- Sarà, M., 1974. Il popolamento delle grotte marine: interesse di una salvaguardia. *Pubbl. Stn. Zool. Napoli* 40, 50–505.
- Shannon, C.E., Weaver, N., 1963. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.
- Snelgrove, P.V.R., 1998. The biodiversity of macrofaunal organisms in marine sediments. *Biodivers. Conserv.* 7, 1123–1132.
- Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F., 1992. Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. *Limnol. Oceanogr.* 41, 605–614.
- Thrush, S.F., 1991. Spatial patterns in soft-bottom communities. *TREE* 6 (3), 75–79.
- True, M.A., 1970. Etude quantitative de quatre peuplements sciaphiles sur substrat rocheux dans la région marseillaise. *Bull. Inst. Océanogr. Monaco* 69 (1401), 1–48.
- Turon, X., Martí, R., Uriz, J.M., 2009. Chemical bioactivity of sponges along an environmental gradient in a Mediterranean cave. *Sci. Mar.* 73, 387–397.
- Underwood, A.J., 1997. *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.
- Underwood, A.J., Chapman, M.G., Richards, S.A., 2002. *GMAV-5 for Windows*. An Analysis of Variance Programme. University of Sydney, Australia.
- Vacelet, J., Boury-Esnault, N., Harmelin, J.G., 1994. Hexactinellid cave, a unique deep-sea habitat in the scuba zone. *Deep-Sea Res.* 41 (7), 965–973.
- Zabala, M., Riera, T., Gili, J.M., Barange, M., Lobo, A., Peñuelas, J., 1989. Water flow, trophic depletion, and benthic macrofauna impoverishment in a submarine cave from the western Mediterranean. *Mar. Ecol.* 10, 271–287.