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Meiofaunal diversity and nematode assemblages in two submarine caves of a Mediterranean marine protected area

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Abstract

Submarine caves are environments of great ecological interest because of the occurrence of peculiar conditions, such as the attenuation of light and reduced water turnover, which can determine oligotrophic conditions from the entrance to the interior part of the cave. These environmental gradients may influence the distribution of the communities inhabiting submarine caves. In this study we investigated the meiofaunal community and nematode assemblages from the sediments inside and outside two submarine caves in Ustica Island Marine Protected Area (southwest Italy): Grotta Falconiera and Grotta dei Gamberi. Consistently with a general pattern of distribution reported by several studies on benthic organisms, our results showed a decrease in the abundance and changes in the taxa composition of the meiofaunal community along the exterior-interior axis of the caves, also highlighting the dissimilarity between the dark and semi-dark communities. We found a significant influence of the availability of organic matter (i.e. phytopigment concentrations) on the distribution and composition of both the meiofauna and the nematode community inside the caves. Different nematode assemblages characterized the inside and the outside of the two caves, with species occurring exclusively in the sediment of both caves, particularly in the dark portions, and completely absent in the external sediments. Environmental features of submarine caves may affect food resources inside the caves and consequently trophic nematode assemblages. Our results showed a difference in feeding strategies between nematodes inhabiting the caves and those living outside, suggesting that in the two caves investigated, bacteria might represent the most important food source for nematodes.

Keywords: Marine caves, meiobenthos, community structure, nematode communities, functional biodiversity, Mediterranean sea.

Introduction

Submarine caves represent a habitat enclosing a great and sharp variability of environmental parameters (light, physicochemical water properties, hydrodynamism, etc.), and supporting a high biodiversity (Benedetti Cecchi *et al*., 1996; Villora-Moreno, 1996; Bussotti *et al*., 2006, 2015; Todaro *et al*., 2006a; Gerovasileiou *et al*., 2015). For these reasons, the biota inhabiting the sea caves has generated great interest for many marine biologists, and studies conducted in this environment revealed the existence of unique communities characterized by high endemism, several protected and alien species (Chevaldonne & Lejeusne, 2003; Martí *et al*., 2004a, Gerovasileiou *et al*., 2015) or by more generalist taxa which take refuge there (Vacelet *et al*., 1994; Bussotti *et al*., 2015; Gerovasileiou *et al*., 2015). Due to their ecological importance and vulnerability to human pressures (e.g. recreational diving, Di Franco *et al*., 2010; Guarnieri *et al*., 2012), marine caves have been recognised as priority habitats for conservation purposes (Bussotti *et al*., 2015; Gerovasileiou *et al*., 2015), and are protected by the European community in the Habitats Directive (European Union, Council Directive 92/43/EEC). The variations in light intensity, hydrodynamics and trophic availability, can influence the distribution of the fauna in the submarine caves (Benedetti Cecchi *et al*., 1996; Martí *et al*., 2004b; Bussotti *et al*., 2006). Most of the studies reported a general decline in species richness, biomass and abundance of the benthic organisms from the outermost to the innermost portions

of the caves (Martí *et al*., 2004a, Navarro-Barranco *et al*., 2013). This gradient is generally explained by attenuation of light and reduced water turnover, which can determine oligotrophic conditions towards the inner part of the caves (Fichez, 1990; Airoldi & Cinelli, 1997). Most of the information on submarine caves concerned hard-substrate benthic communities (Martí *et al*., 2004b, Bussotti *et al*., 2006; Micael *et al*., 2006; Denitto *et al*., 2007), while the soft-substrate cave communities (Akoumianaki & Hughes, 2004; Bamber *et al*., 2008; Navarro-Barranco *et al*., 2012) are still poorly studied. Due to their peculiar environmental and biotic characteristics, submarine caves may constitute a sheltered and favourable environment for meiofaunal communities, thus representing a very interesting habitat for researchers dealing with meiofauna biodiversity and ecology (Todaro *et al*., 2006b). Studies on the meiofauna communities in marine caves are generally scarce and have been published only over the last two decades (Villora-Moreno, 1996; Gallo-D'Addabbo *et al*., 2001; Boesgaard & Kristensen, 2001). Most of these studies described new species of some meiofauna taxa, such as Tardigrades (Villora-Moreno, 1996; Gallo-D'Addabbo *et al*., 2001), Kinorhynchs (Sørensen *et al*., 2000), Priapulids (Todaro & Shirley, 2003) and Gastrotrichs (Todaro *et al*., 2006b). Until now the study of the entire meiofaunal community in Mediterranean submarine caves has been neglected and detailed information regarding the taxa of Nematoda (in terms of taxonomic and trophic group composition) is not available. Nematodes, representing generally the dominant taxon of meiofauna in marine sediments, are considered a model to describe spatial pattern of biodiversity in marine benthic systems (Lambshead, 2004), and, due to their high trophic diversity, they provide detailed information on the trophic status of the entire meiobenthic communities (Danovaro *et al*., 2009). Only Zhou & Zhang (2008) have described the nematode assemblages occurring in two submarine caves in Hong Kong, showing the lack of an endemic nematode species association inside these caves, but the presence of differences in nematode trophic composition between the outside and the inside of the caves. The lower hydrodynamism and reduced currents can result in a different trophic structure of the communities associated with the sediment inside the submarine caves compared with those outside. Moreover, the limitation of light, by inhibiting the growth of benthic microalgae, may cause a change in food composition and availability for cave meiofaunal community (Todaro *et al*., 2006b; Zhou & Zhang, 2008). All the issues mentioned above suggest the relevance of meiofauna as a driver of marine caves biodiversity. From this perspective, information on meiofaunal diversity can be pivotal to proper management and conservation measures for marine caves, with this issue being particularly crucial in the context of marine protected areas (MPAs).

The aims of the present study were to investigate a) meiofaunal abundance and community structure related to trophic conditions (in terms of quantity and quality of sedimentary organic matter); b) nematode abundance, species richness, community structure, body biomass, maturity index and functional (trophic) diversity, in two submarine Mediterranean caves.

Materials and Methods

Study sites and sampling strategy

The study was performed in two marine caves (i.e. sites) in Ustica Island MPA (southwest Italy): Grotta dei Gamberi and Grotta Falconiera (Fig. 1). Grotta dei

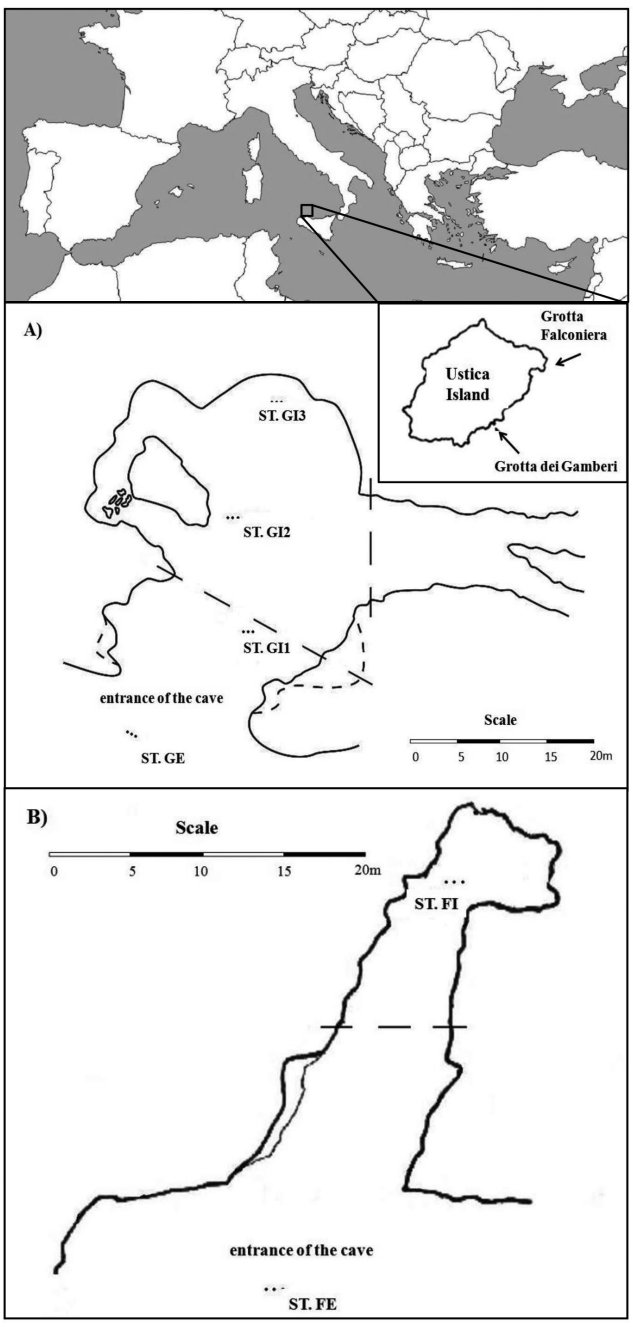

Fig. 1: The investigated Caves: A) Grotta dei Gamberi and B) Grotta Falconiera with the position of the sampling stations outside (GE and FE) and inside the caves (GI1, GI2 and GI3 in Grotta dei Gamberi and FI in Grotta Falconiera) (modified from Colantoni *et al.,* 1991).

Gamberi (38°41'34'' N; 13°11'01'' E) is one of the main diving locations in Ustica Island, especially in the summer (Di Franco *et al*., 2010). The cave opens into a basaltic cliff and is completely submerged. It presents two openings, one is very large (17.3 m width, 6 m height, at about 42 m depth) which the divers use as the entrance, and the other a narrower one (1 m width, 1.5 m height, at about 26 m depth) normally used as the exit. The cave has a large main hall irregularly shaped with the N / E axis about 43 m long and the E/W axis about 40 m wide. On the eastern side of the main hall a side branch opens up E / W ending in two tunnels, providing a passage to the outside (Fig. 1A).

Grotta Falconiera (38°42'37'' N; 13°12'09'' E) is about 28 m long with a large opening (20 m wide at the entrance) located between 27 and 34 m depth, continuing into a tunnel (9 m wide) straight for about 28 m. The cave is mostly illuminated, except for its end portion (Fig. 1B).

The sediment at the bottom of both caves is sandy and muddy with gross bioclastic fragments (Colantoni *et al*., 1991; Di Franco *et al*., 2010), showing a non-homogeneous grain size distribution ranging from 1 µm to 1.5 cm and the coarsest sediment close to the two openings (Di Franco *et al*., 2009).

Sampling was carried out in April 2009 and the sampling stations within each cave were chosen, after a preliminary survey, to compare the different sections of the caves. The samples were collected at the Grotta Falconiera at one control station 10 m outside the cave entrance (FE) and at one station 25 m inside the cave under dark conditions (FI). At Grotta dei Gamberi samples were collected at one control station 10 m outside the cave entrance (GE), one station with semi-dark conditions inside the cave located at 12 m from the entrance of the cave (GI1), one station at 20 m from the entrance (GI2) and one station in the innermost part of the cave with dark conditions (GI3), 33 m from the cave entrance.

Sediment samples were manually collected in 3 replicate (n = 3) cores operated by SCUBA divers (diameter 3.7 cm, 10.7 cm² surface area) down to a depth of 10 cm, for the analysis of phytopigments, biochemical components of organic matter and for meiofauna and nematode diversity.

Immediately after sampling, the sediment cores were sectioned into different layers (0-1, 1-5 and 5-10 cm) and stored at -20°C for organic matter and fixed with 4% buffered formaldehyde in filtered (0.4 µm) seawater solution for the meiofauna until analysis was performed in laboratory.

Phytopigments and biochemical variables

Chlorophyll-a and phaeopigments analyses were carried out according to Lorenzen & Jeffrey (1980). For all the sites, pigments were extracted (12 h at 4°C in the dark) from triplicate superficial (0-1 cm) sediment samples (n = 3), using 5 ml of 90% acetone as the extractant. The extracts were analysed fluorometrically to estimate chlorophyll-a, and, after acidification with 200 µl of 0.1N HCl, to estimate phaeopigments concentrations. Concentrations were normalised to sediment dry weight and reported as µg g^{-1}. Total phytopigments were defined as the sum of chlorophyll-a and phaeopigments (Pusceddu *et al*., 2010). Proteins, carbohydrates and lipids sediment contents were analysed spectrophotometrically according to Danovaro (2010) and concentrations expressed as bovine serum albumin, glucose and tripalmitine equivalents, respectively. For each biochemical assay, blanks were obtained using pre-combusted sediments (450 °C for 4 h). For all the sites, the analyses were performed on triplicate superficial (0-1 cm) sediment samples (n = 3). Carbohydrate, protein and lipid concentrations were converted into carbon equivalents using the conversion factors 0.40, 0.49, and 0.75 mg C mg^{-1}, respectively, and their sum was reported as biopolymeric organic carbon (Pusceddu *et al*., 2010). The algal carbon contribution was calculated as the percentage of phytopigments to biopolymeric C concentrations, after converting phytopigments concentration into carbon equivalents using the mean value of 40 µg C $µg^{-1}$ (Pusceddu *et al*., 2010). We chose the percentage contributions of phytopigment and protein to biopolymeric C concentrations and the values of the protein to carbohydrate ratio as descriptors of the aging and nutritional quality of sediment organic matter (Pusceddu *et al*., 2010).

Meiofauna

For the meiofauna extraction, the sediment samples (in 3 replicates, n = 3) were sieved through a 1000 µm mesh, and a 37 µm mesh was then used to retain the smallest organisms. The fraction remaining in the 37 µm sieve was resuspended and centrifuged thrice with Ludox HS40 (diluted with water to the final density of 1.18 g cm^{-3}), as described by Heip *et al*., (1985). The material collected with the 37 µm mesh sieve was preserved in a 50 ml tube with 4% buffered formalin and stained with Rose Bengal (0.5 g l^{-1}). All the meiobenthic organisms were counted and classified according to taxon, under a stereomicroscope. Here we report only the values integrated down to 10 cm depth.

Nematode biodiversity

All the nematodes from 0-1 cm layer of sediment from each independent replicate (3 replicates, n = 3) were mounted on slides, following the formalin-ethanol-glycerol technique to prevent dehydration (Seinhorst, 1959). All the nematodes were then identified to species level according to the most recent literature (NeMys database, Deprez *et al*., 2005). The species richness (SR) was calculated as the total number of species cumulatively

collected from three replicates ($n = 3$) at each station. The number of species expected for a theoretical sample of 50 specimens, ES (50), was chosen to facilitate comparisons among the stations (Hurlbert, 1971). Margalef Index (D), Shannon-Wiener diversity index (H') and Pielou's evenness (J) were calculated from the sum of the individuals of the three replicates collected in each of the sampling stations, using the DIVERSE routine included in the PRIMER v6.0+ software (Clarke & Gorley, 2006).

We also measured the β-diversity (i.e., turnover diversity, Gray, 2000) between stations as the percentage of the dissimilarity of nematode community species composition, calculated from the resemblance matrices based on Bray-Curtis similarity (SIMPER, included in the PRIMER v6.0+ software). The trophic composition of nematode assemblages was determined according to classification of Wieser (1953): (1A) buccal cavity absent or fine and tubular-selective deposit (bacteria) feeders; (1B) large but unarmed buccal cavity-non-selective deposit feeders; (2A) buccal cavity with scraping tooth or teeth-epistrate (microalgae) feeders; (2B) buccal cavity with large jaws-predators/omnivores. The index of trophic diversity (ITD) was calculated based on the trophic composition of the nematode assemblages as ITD $= g_1^2 + g_2^2 + g_3^2 \ldots + g_n^2$, where g is the relative contribution of each trophic group to the total number of individuals and n is the number of trophic groups. For n = 4 (as in the present study), ITD ranges from 0.25 to 1.00 (Heip *et al*., 1985; Gambi *et al*., 2003). The Maturity Index (MI) was determined for the nematode assemblage as the weighted average of the individual colonizer-persister (c-p) species (Bongers *et al*., 1991). In particular, Bongers distinguished r-strategist species (colonizers or c-p 1), which are more tolerant of environmental variations, and k-strategist species (persisters or c-p 5), which are more sensitive. Nematode biomass was calculated from the biovolume estimates using the Andrassy (1956) formula ($V = L \times W^2 \times 0.063 \times 10^{-5}$, in which body length, L, and width, W, are expressed in μm). Each body volume was multiplied by an average density (1.13 g cm^{-3}) to obtain the body mass (μg DW: μg WW = 0.25) and the carbon content was considered to be 40% of the dry weight (Feller & Warwick, 1988) and expressed as μg C. Total nematode biomass was expressed as μg C 10 cm^{-2}.

Statistical analysis

Univariate permutational analysis of variance (PERMANOVA; Anderson, 2001; McArdle & Anderson, 2001) was performed to test for differences in the concentration of phytopigments and biochemical components, meiofaunal abundance, richness of taxa, nematode diversity indexes (SR, ES50, D, H', J', ITD and MI) and nematode biomass considering two factors: site (fixed factor) and station (random factor nested in site). The same statistical design was also adopted in the multivariate context (permutational multivariate analysis of variance; PERMANOVA), performed on biochemical composition of organic matter, meiofaunal community structure and nematode species and trophic composition. The PERMANOVA tests were based on Euclidean distances of previously normalized data (for organic matter variables) or Bray–Curtis similarity matrixes after square root transformation of the data (for meiofaunal and nematodes variables), using 9999 random permutations of the appropriate units (Anderson, 2001; McArdle & Anderson, 2001). When significant differences among stations were observed, post-hoc pairwise tests were performed to ascertain in which stations (inside and outside the caves) the investigated parameters were significantly different. Due to the restricted number of unique permutations in the pair-wise tests, p values were obtained from Monte Carlo samplings (Anderson & Robinson, 2003). SIMPER analyses were performed to assess the percentage of dissimilarity in the composition of the meiofaunal and nematode communities between the two sites (Grotta Falconiera and Grotta dei Gamberi), between the stations inside and outside the caves and between the internal stations of the two caves. SIMPER analyses were also performed to identify which among the investigated taxa/species was mostly responsible for the dissimilarities observed. Ranked matrices of Bray–Curtis similarities, constructed on previously square-root transformed data, were used as input for this test. These similarity matrices were also applied to produce a non-metric, multidimensional scaling 2-dimensional plot (MDS). To identify the potential trophic drivers of differences in meiofaunal abundance and community composition, nematode species assemblages and trophic structure between the inside and outside of the caves, non-parametric multivariate multiple-regression analyses based on Bray-Curtis dissimilarity distances were carried out using the routine DISTLM step-wise (McArdle & Anderson, 2001). Statistical analyses were performed using the PRIMER6+ program (Plymouth Marine Laboratory, Clarke, 1993).

Results

Phytopigments and biochemical variables

Total phytopigments were significantly lower inside than outside the Grotta Falconiera (PERMANOVA, $p < 0.01$) and decreased significantly from the outer to the innermost part of Grotta dei Gamberi, as revealed by pair-wise test (PERMANOVA, $p < 0.01$, Table S1 and S2). At Grotta dei Gamberi, the values ranged from 1.4 ± 0.1 to 4.5 ± 0.7 μg g^{-1} (at the GI3 and GE stations, respectively), at Grotta Falconiera from 3.8 ± 0.2 to 6.4 ± 0.9 μg g^{-1} (inside and outside the cave, respectively) (Table 1). Biopolymeric C concentarions were higher in the sediment inside the caves than outside (ranging from 0.8 ± 0.1 to 1.6 ± 0.1 mg g^{-1} at the GE and FI stations,

respectively, Table 1), and the pair-wise test revealed significant differences at Grotta dei Gamberi between the external station (GE) and the semi-dark stations (GI1 and GI2) (PERMANOVA, $p < 0.05$). The carbohydrate carbon represented the major fraction of biopolymeric carbon, ranging from 43% to 59% (at the FI and FE stations, respectively), followed by protein carbon, ranging from 29% to 44% (at the GI2 and GE stations, respectively) and lipid carbon ranging from 9% to 18% (at the GI3 and GI2 stations, respectively). The quality of the organic matter, in terms of the algal carbon contribution to biopolymeric carbon, was significantly higher outside than inside the two caves (PERMANOVA, pair-wise $p < 0.01$), ranging from 5% and 22% (at the GI2 and GE stations, respectively) (Table 1).

Meiofauna

The results of pair-wise test (Tables S3 and S4) revealed that, in both the sites investigated, the values of meiofaunal abundance were significantly lower (PERMANOVA, $p < 0.001$) at the stations located inside the caves than those outside (Fig. 2). At Grotta dei Gamberi meiofaunal abundance ranged from 868.7 ± 223.2 to 993.0 ± 53.6 ind. 10 cm^{-2} (at the GI2 and GI1 stations, respectively).

Inside the cave nematodes were the dominant taxon (75% of the total meiofaunal community) in the innermost stations (GI3), while in the other two stations (GI1 and GI2) copepods represented the dominant group (53-57%), followed by nematodes (34-29%), polychaetes (5-6%), ostracods (2-4%), turbellarians (0.2-2%), gastrotrichs (0.1-1%) and tardigrades (0.03-0.7%). Inside Grotta Falconiera meiofaunal abundance was 951.7 ± 111.7 ind. 10 cm^{-2}, with nematodes as the dominant taxon (58% of total meiofaunal community), followed by the copepods (33%), polychaetes (5%), ostracods (3%), turbellarians (0.4%) and tardigrades (0.1%). The multivariate multiple regression analysis (DistLM), performed to identify the potential trophic drivers of the differences in the total meiofaunal community, revealed that, in both the caves investigated, the concentration of phytopigments explained 58% of the variance in total abundance and phytopigments together with biopolymeric C concentrations explained 50% of the variance in community composition (Table 2).

Outside of the caves meiofaunal abundance was 2504.0 ± 414.6 and 2524.3 ± 309.9 ind. 10 cm^2, at the FE and GE stations, respectively. Here nematodes were dominant taxon (48-51%), followed by copepods (34-39%), polychaetes (4-14%), ostracods and gastrotrichs (1-2%), tardigrades (0.7-0.9%) and turbellarians (0.3-1%).

The percentage contribution of the other remaining taxa (isopods, sarcomastigofora, halacaroides, bivalves, tanaids, cnidarians, kinorhynchs) was lower than 0.5%, and were pooled and categorised as 'others'. The structure of meiofaunal communities in both the sites investigated is shown in Fig. 3.

Table 1. Concentrations of total phytopigments, biochemical components (proteins, carbohydrates, lipids and biopolymeric C) and quality (protein to carbohydrate ratio, protein and phytopigment percentage contribution to biopolymeric C) of organic matter in the sediments of the sampling stations inside Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside the caves (FE and GE respectively).

Sites	Stations	Phytopigment		Protein		Carbohydrate		Lipid		Biopolymeric C		Protein/ Carbohydrate	Protein/ Biopolymeric C	Phytopigment/ Biopolymeric C
		µg g^{-1}	±SD	mg g^{-1}	±SD	mg g^{-1}	±SD	mg g^{-1}	±SD	mg g^{-1}	±SD		(%)	(%)
Grotta Falconiera	FE	6.4	0.9	0.9	0.1	2.2	0.3	0.2	0.0	1.5	0.1	0.4	30.2	17.2
	FI	3.8	0.2	1.4	0.2	1.8	0.1	0.3	0.0	1.6	0.1	0.8	42.9	9.2
Grotta dei Gamberi	GE	4.5	0.7	0.7	0.1	0.9	0.2	0.1	0.0	0.8	0.1	0.8	43.6	22.4
	GI1	3.1	0.2	1.0	0.2	1.7	0.3	0.2	0.0	1.3	0.0	0.6	39.0	9.7
	GI2	1.6	0.0	0.7	0.1	1.7	0.3	0.2	0.1	1.2	0.1	0.4	29.1	5.3
	GI3	1.4	0.1	0.7	0.1	1.4	0.3	0.1	0.0	1.0	0.1	0.5	35.0	5.9

SD = standard deviation

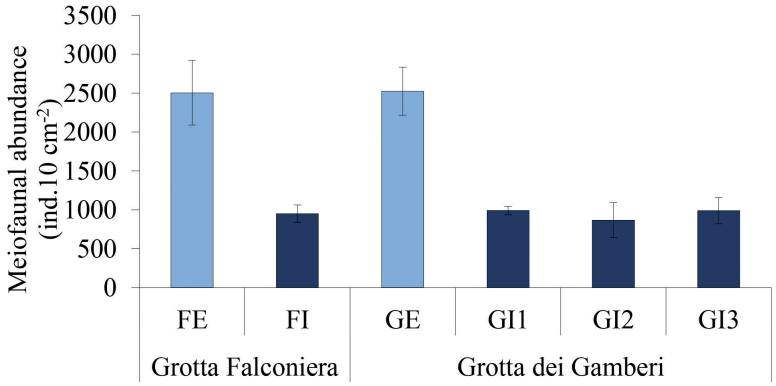

Fig. 2: Meiofaunal abundance (average ± standard deviation) in the sediment at the sampling stations inside Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside the caves (FE and GE respectively).

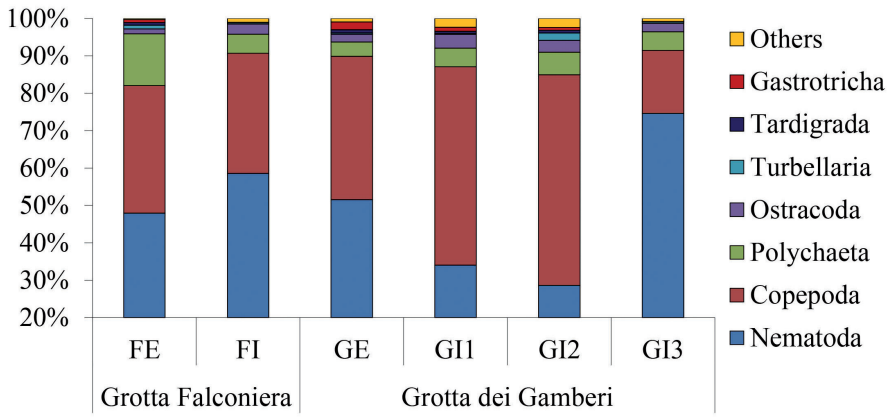

Fig. 3: Community structure of the entire meiofaunal assemblage in the sediment at the sampling stations inside Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside the caves (FE and GE respectively).

Table 2. Results of the multivariate multiple regression analysis carried out to ascertain the role of environmental variables on the a) meiofaunal abundance, b) meiofaunal community composition, c) nematode abundance, d) nematode species composition, e) nematode trophic structure and f) the abundance of each trophic group in the investigated sites.

Variable	SS (trace)	Pseudo-F	P	Prop. (%)	Cumul. (%)
a) Meiofaunal abundance					
Phytopigment	5117.5	22.3	***	0.58	0.58
Biopolymeric C	2234.3	23.3	***	0.25	0.84
b) Meiofaunal community composition					
Phytopigment	3847.9	7.3	**	0.31	0.31
Biopolymeric C	2251.9	5.5	**	0.18	0.50
c) Nematode abundance					
Phytopigment	4623.9	7.8	**	0.33	0.33
Lipid	3138.1	7.4	**	0.22	0.55
d) Nematode species composition					
Phytopigment	11489.0	3.6	***	0.19	0.19
Protein	5369.6	1.8	*	0.09	0.27
e) Nematode trophyc composition					
Phytopigment	1918.7	5.9	**	0.27	0.27
f) *Abundance of each trophyc group*					
1A Phytopigment	6070.2	16.4	***	0.51	0.51
Biopolymeric C	1900.5	7.1	**	0.16	0.66
1B All variable tested			ns		
2A All variable tested			ns		
2B Phytopigment	7207.5	24.7	***	0.61	0.61
Biopolymeric C	1249.5	5.5	*	0.11	0.71

SS = sum of squares; Pseudo-F = F statistic; P = probability level; Var (%) = percentage of variance explained by that variable; Cum (%) = cumulative percentage of variance explained, *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, n.s = not significant.

We did not find significant differences in meiofaunal community composition between the two sites (Table S3); however, the results of pair-wise test revealed significant differences between the stations inside and outside the caves (PEMANOVA, $p < 0.01$, Table S4) and, inside Grotta dei Gamberi, significant differences were also observed between the semi-dark stations (GI1 and GI2) and the dark station (GI3) of the submarine cave (PEMANOVA, $p < 0.01$, Table S4).

SIMPER analyses (Table 3) showed the highest dissimilarity for the entire meiofaunal communities (31%) between the outer station (GE) and the dark station (GI3) of Grotta dei Gamberi. We observed the lowest dissimilarities between the two external stations GE and FE (15%), between the semi-dark stations GI1 and GI2 (13%) and between the innermost station of Grotta dei Gamberi (GI3) and the interior station of Grotta Falconiera (FI) (15%). The SIMPER analyses showed also that the taxa most responsible for the dissimilarities observed were the nematodes and copepods, followed by the polychaetes and gastrotrichs.

MDS showed clear differences between meiofaunal communities inside and outside the caves, also highlighting the dissimilarity observed between the dark (stations

Table 3. Output of the SIMPER analyses carried out on a) meiofaunal community composition and b) nematode species assemblages. The meiofaunal taxa included in this table were responsible of 70% cumulative dissimilarity, the nematode species included were responsible of 15% cumulative dissimilarity, between the two sites, the stations inside Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside the caves (FE and GE respectively) and between the stations inside the two caves.

		SIMPER % Dissimilarity	
a)	Grotta Falconiera vs Grotta dei Gamberi	24.09	Nematoda, Copepoda, Polychaeta, Gastrotricha
	FE vs FI	30.70	Copepoda, Polychaeta, Nematoda, Gastrotricha
	GE vs GI1	25.53	Nematoda, Copepoda, Gastrotricha, Polichaeta
	GE vs GI2	28.61	Nematoda, Copepoda, Gastrotricha, Polichaeta
	GE vs GI3	31.32	Copepoda, Nematoda, Gastrotricha, Tardigrada
	GI1 vs GI2	12.96	Nematoda, Copepoda, Turbellaria, Ostracoda, Isopoda, Cnidaria
	GI1 vs GI3	25.71	Copepoda, Nematoda, Gastrotricha, Tardigrada, Halacaroidea
	GI2 vs GI3	28.30	Nematoda, Copepoda, Turbellaria, Tardigrada, Gastrotrica
	FE vs GE	15.39	Polychaeta, Copepoda, Gastrotricha, Nematoda, Halacaroidea, Turbellaria
	FI vs GI1	20.09	Copepoda, Nematoda, Gastrotricha, Halacaroidea, Isopoda, Tardigrada
	FI vs GI2	21.70	Nematoda, Copepoda, Gastrotricha, Turbellaria, Halacaroidea, Tardigrada
	FI vs GI3	14.50	Copepoda, Nematoda, Anphipoda, Gastrotricha, Tardigrada, Cnidaria, Turbellaria
b)	Grotta Falconiera vs Grotta dei Gamberi	85.24	*Chromaspirina parapontica, Catanema sp., Anticoma acuminata, Desmodora pontica, Tricoma brevirostris, Prochromadorella ditlevseni, Desmodora pilosa, Actinonema pachydermatum*
	FE vs FI	86.20	*Chromaspirina parapontica, Anticoma acuminata, Desmodora sinuata, Meyersia meridionalis, Calomicrolaimus honestus*
	GE vs GI1	87.90	*Catanema sp., Chromaspirina parapontica,Monoposthia costata*
	GE vs GI2	88.28	*Chromaspirina parapontica, Monoposthia costata, Monoposthia mirabilis, Spirinia parasitifera*
	GE vs GI3	98.07	*Chromaspirina parapontica, Anticoma acuminata, Desmodorella tenuispiculum, Tricoma brevirostris*
	GI1 vs GI2	69.73	*Catanema sp., Actinonema pachydermatum, Pselionema simplex, Desmodora sanguinea*
	GI1 vs GI3	89.26	*Catanema sp., Anticoma acuminata, Desmodora pilosa*
	GI2 vs GI3	89.08	*Anticoma acuminata, Desmodorella tenuispiculum, Actinonema pachydermatum, Desmodora pilosa*
	FE vs GE	68.97	*Spirinia parasitifera, Meyersia meridionalis, Calomicrolaimus honestus, Daptonema sp., Monoposthia costata*
	FI vs GI1	82.67	*Catanema sp., Anticoma acuminata, Pselionema simplex, Prochromadorella ditlevseni*
	FI vs GI2	83.68	*Actinonema pachydermatum, Anticoma acuminata, Paradesmodora sp., Catanema sp.,Desmodora sinuata*
	FI vs GI3	87.75	*Desmodorella tenuispiculum, Tricoma brevirostris, Desmodora pontica, Desmodora sinuata, Parasphaerolaimus paradoxus*

FI and GI3) and semi-dark communities (stations GI1 and GI2) (Fig. 4a), in accordance with the results from the SIMPER analysis (Table 3).

Nematode species richness

Nematode species richness inside the caves ranged from 36 species at station GI3 (ES(50) = 24.4) to 57 species at station FI (ES(50) = 33.2). Outside the caves we found 43 species at GE (ES(50) = 26.7) and 51 species at FE (ES(50) = 30). The value of Pielou index was 0.89 outside and 0.92 inside the Grotta Falconiera and ranged from 0.84 at GI1 to 0.91 at GI2 in the Grotta dei Gamberi.

Nematode species richness and the values of Shannon-Wiener, Margalef, Pielou and ES(50) indexes are reported in Table 4. Total nematode biomass showed lower values inside the caves than outside, the pairwise test revealed significant differences in Grotta dei Gamberi (PERMANOVA, $p < 0.01$, Table S4), with values ranging from 80.0 ± 37.5 to 1210.5 ± 204.7 µC 10cm^{-2} (at the stations GI2 and GE, respectively) (Table 4). The dominant nematodes family was Desmodoridae in all the stations (ranging from 11% at GI3 to 54% at GI1) except at the station GI3, where the dominant family was Anticomidae (15% of total nematodes). The second dominant family outside the caves was Monoposthiidae (9% at FE and 14% at GE) completely absent inside the two caves. The pair-wise test revealed significant differences, in terms of species composition, between the outer and the inner nematode assemblages (PERMANOVA, $p < 0.05$, Table S4) of Grotta Falconiera and Grotta dei Gamberi, however inside Grotta dei Gamberi we found a significant difference between the semi-dark part (stations GI1 and GI2) and the dark part (GI3) of the cave (PERMANOVA, $p < 0.05$) as well.

DistLM analysis showed that the variance in nematode species composition, as for total meiofaunal community, was significantly explained by total phytopigment concentrations and proteins content in the sediments (Table 2).

Among the 180 species identified, only 27 species were found both inside and outside the caves, 98 were present exclusively inside the caves and among these 14 species were in common between the two caves. *Chromaspirina parapontica* was the dominant species in the sediments outside the caves (16% at FE and 14% at GE) and was absent inside the caves, as well as *Monoposthia costata* and *Monoposthia mirabilis*. Inside the Grotta Falconiera (FI) and in the innermost part of Grotta dei Gamberi (GI3) the most abundant species was *Anticoma acuminata*, representing 8% and 15% of the total abundance respectively, and was exclusive of these stations. The list of all nematode family and species, and their relative abundance in each station, are reported in Table S5.

SIMPER analyses (Table 3) showed that *Chromaspirina parapontica* and *Anticoma acuminata* were the species that further explained the dissimilarity between the outside (FE) and the inside (FI) of Grotta Falconiera (86%) and between the external (GE) and the innermost parts (GI3) of Grotta dei Gamberi (98%). Inside the Grotta dei Gamberi we found the lowest dissimilarity between GI1 and GI2 (70%) and the highest between GI1 and GI3 (89%). This dissimilarity was explained mostly by *Catanema sp.*, present only in GI1 and GI2 (25% and 6%, respectively), *Anticoma acuminata,* absent in these two stations, and *Desmodora pilosa.*

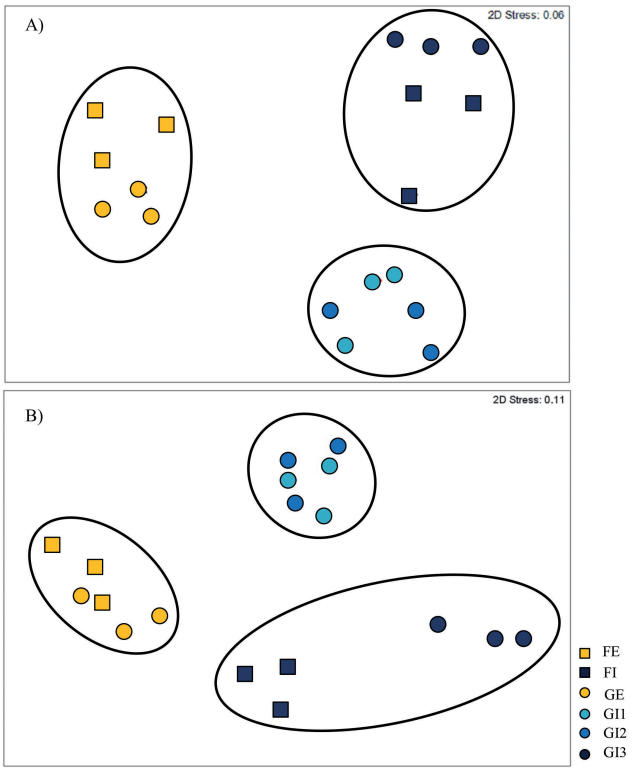

Fig. 4: Multi-dimensional scaling (MDS) analysis performed using A) taxonomic composition of meiofaunal community and B) nematode species assemblage inside (blue symbols) Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside (yellow symbols) the caves (FE and GE respectively). The stations were represented by squares at Grotta Falconiera and by circles at Grotta dei Gamberi. Total number of meiofaunal taxa and nematode species were square-root transformed.

MDS, applied to nematode species composition (Fig. 4b), evidenced clear differences between the communities inside and outside the caves and between the dark (stations FI and GI3) and semi-dark communities (stations GI1 and GI2).

Nematode trophic composition

The index of trophic diversity (ITD) was significantly different between the stations (PERMANOVA, $p < 0.001$, Table S3), ranging from 0.28 at station GI3 to 0.48 at station GI1. The values of the maturity index (MI) ranged from 2.69 to 2.85 (at the FI and FE stations, respectively) (Table 4). Pairwise test (Table S4) revealed

Table 4. Total nematode abundance, biomass and nematode diversity indexes (SR species richness; ES(50) expected species number for 50 individuals; D Margalef index; H' Shannon's index; J' species evenness; MI maturity index; ITD index of trophic diversity) in the sediments at the sampling stations inside Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside the caves (FE and GE respectively).

Sites	Stations	Nematode abundance		Total Nematode biomass		SR	ES(50)	D	J'	H'	MI	ITD
		Ind.10cm^{-2}	±SD	µgC 10cm^{-2}	±SD							
Grotta Falconiera	FE	1196.6	171.7	494.6	263.6	51	30.0	10.6	0.89	3.52	2.85	0.32
	FI	555.3	113.0	435.2	174.8	57	33.2	11.9	0.92	3.74	2.69	0.41
Grotta dei Gamberi	GE	1296.0	161.9	1210.5	354.6	43	26.7	8.9	0.89	3.34	2.85	0.44
	GI1	334.7	76.5	121.6	57.7	43	25.5	8.7	0.84	3.15	2.85	0.48
	GI2	249.3	83.2	80.0	65.0	48	30.8	10.3	0.91	3.53	2.84	0.45
	GI3	738.7	125.4	392.9	210.0	36	24.4	7.5	0.88	3.14	2.80	0.28

significant differences in nematode trophic composition between the stations located inside and outside the two caves (PERMANOVA, $p < 0.05$). In cave sediments selective deposit feeders showed higher abundance than in sediments outside the caves, whilst the contribution of predator/omnivores (2B) were lower. In all the stations the nematodes assemblage was dominated by epistrate or epigrowth feeders (2A) (from 32% to 66% of total nematode abundance at the stations GI3 and GI1, respectively) except at station GI3, where the selective (bacterial) deposit feeders (1A) were the dominant group (34%). However, the inner and the outer parts of the two caves showed a different trophic structure. In the stations outside the caves the second dominant feeding group was represented by predators/omnivores (2B) (29% and 35% of all nematodes at the GE and FE stations, respectively). Inside the caves the predators/omnivores ranged from 11% at station GI3 to 15% at station FI, instead the selective deposit feeders (bacterivores, 1A) were the second dominant group, ranging from 16% at station GI1 to 19% at station FI. This feeding group represented only 6% of all nematodes outside the caves. The relative abundance of the four nematode feeding groups is illustrated in Fig. 5. The multivariate multiple regression analyses revealed that, in both the caves investigated, the phytopigment concentrations explain 51% and 61% of the variance in the abundance of the bacterivores and predators/omnivores, respectively (Table 2).

Discussion

Meiofauna and biochemical variables

A common feature of submarine caves is the environmental spatial heterogeneity over small scales, associated mainly with changes in light intensity, hydrodynamic regime and other factors such as concentration and quality of the suspended particulate matter (Fichez, 1990). This heterogeneity is presumed to have strong effects on distribution of species and their interactions, causing differences in the species colonization at the scale of few meters along the exterior-interior axis of the caves (Fichez, 1990; Benedetti Cecchi *et al*., 1996). Prior studies on the benthic communities from Mediterranean marine caves showed changes in assemblages composition and, generally, a decrease in species richness, density and biomass of organisms both in hard-substrata (Martí *et al*., 2004a, Bussotti *et al*., 2006; Denitto *et al*., 2007) and in soft-substrata communities when compared to those outside the caves (Todaro *et al*., 2006b; Navarro-Barranco *et al*., 2013).

Consistently with these studies, our results showed lower meiofaunal abundance in the sediments inside the two submarine caves in the Ustica Island Marine Protected Area (Grotta Falconiera and Grotta dei Gamberi), than outside, revealing the influence of typical cave environmental gradients on communities

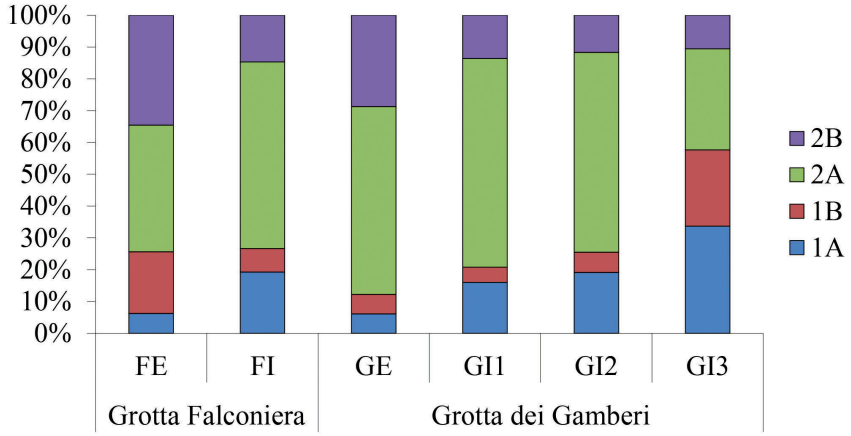

Fig. 5: Nematodes trophic structure. Reported are 1A (deposit feeders), 1B (non-selective deposit feeders), 2A (epistrate feeders) and 2B (predators/omnivores) at the sampling stations inside Grotta Falconiera (FI) and Grotta dei Gamberi (GI1, GI2 and GI3) and outside the caves (FE and GE respectively).

distribution (Bussotti *et al*., 2006; Todaro *et al*., 2006b). Several authors suggested that this common pattern of the fauna in the submarine caves can be caused mostly by the oligotrophic conditions in the inner sections, determined by the decrease in light and hydrodynamism (Fichez, 1990, 1991; Airoldi & Cinelli, 1997). In the marine sediments the meiofaunal distribution depends strongly on the quantity and quality of the organic matter (Soltwedel, 2000; Gambi & Danovaro, 2006); thus the limited food supply in the submarine caves could negatively affect the abundance of meiobenthic organisms in the sediments. Navarro-Barranco *et al*. (2012) in a study on six caves in the western Mediterranean found significant higher concentrations of organic matter inside than outside the caves. Also our results showed higher concentrations of biopolymeric C in the sediments inside than outside the caves, significantly in Grotta dei Gamberi; however, we observed a decrease in the meiofaunal abundance towards the inner parts of the two caves.

Fichez (1990, 1991) suggested that the quality of organic matter might decrease from the outermost to the innermost part of the submarine caves. Consistently with this, we found lower phytopigment concentrations and decreased algal contribution to biopolymeric carbon (which reflect the bio-available fraction of sedimentary organic matter for benthic fauna) in the sediments inside than outside the caves. Our results showed that the phytopigments were the sedimentary trophic component that explained most of the variance in total meiofaunal abundance. This finding is consistent with several studies that showed a positive relationship between meiofaunal abundance and distribution, and pigment concentrations in marine sediments (Danovaro *et al*., 2008; Lampadariou *et al*., 2009). In the inner parts of the two caves meiofaunal community composition was significantly different from that found in the external sediments. Specifically, nematodes and copepods, representing cumulatively the highest fraction of the meiofaunal assemblages, were responsible for most of the dissimilarity between stations inside and outside the caves, followed by polychaetes and gastrotrichs. Nematodes dominated the sediments in the external stations (50% of total meiofaunal abundance), however the nematode contribution to total meiofaunal abundance was higher inside (FI station, 59%) Grotta Falconiera and in the innermost part (GI3 station, 75%) of Grotta dei Gamberi.

The darkness inside submarine caves may induces microalgae disappearance (Cinelli *et al*., 1977), disadvantaging several meiofaunal taxa, such as copepods, which feed mainly on microphytobenthos, unlike nematodes that can feed on different food sources (e.g. bacteria) (Moens & Vincx, 1997; Todaro *et al*., 2006b). Consistently with this, we observed (as highlighted by DistLM analysis) that phytopigments were an important driver of differences in meiofaunal community composition together with biopolymeric C concentrations. Inside Grotta dei Gamberi we found significant differences in meiofaunal community composition, suggesting a horizontal zonation pattern for meiofauna, such as described by Pérès & Picard (1964) for macrobenthos. They identified 'semi-dark' (in the part of the cave that receives light) and 'dark' biocoenosis (in the completely dark parts of the cave, with reduced seawater circulation).

In the semi-dark area of Grotta dei Gamberi (stations GI1 and GI2) the dominant taxa were Copepoda (55% of total community) followed by Nematoda (30%), however Polychaeta, Ostracoda, Turbellaria and rare taxa ("others" category) showed higher abundance in the external sediments than in the innermost part of the cave.

The dominance of copepods on meiofaunal community and the higher abundance of rare taxa in the semi-dark area of Grotta dei Gamberi could be related to the

availability of more light and efficient water exchange, compared with that of the dark area (Fichez, 1990, 1991).

Nematoda diversity

Nematode abundance decreased significantly from the outside to the inside of the caves, as well as the values of total nematode biomass, which were lower inside than outside both caves, significantly at Grotta dei Gamberi. Our results did not show any significant differences between the interior and exterior sediments of the caves investigated in nematode species richness and other biodiversity indexes. The Maturity Index (MI) did not show significant differences between the stations investigated, although we found the lowest values inside the Grotta Falconiera (station FI) and in the innermost station of Grotta dei Gamberi (GI3), due to the increasing importance of the opportunistic nematodes and the decreasing importance of the persistent species.

Nematodes of Dasmodoridae, a family with wide distribution (Ingels *et al*., 2006), dominated in all the stations (outside and inside both caves), except in the innermost sediment of Grotta dei Gamberi (GI3 station), where nematodes of Anticomidae family showed higher abundance. Nematodes from Monoposthiidae were abundant in the external sediments, but absent in the stations inside the two caves (Table S5).

SIMPER analysis revealed high dissimilarity (85%) in nematodes species community between the two caves, due to the different cave topography and position, which could determine the differences in the water circulation and irradiance, making each cave a unique system (Martí *et al*., 2004a; Denitto *et al*., 2007). We found 180 nematodes species, among which 98 were found exclusively in the sediments inside the caves, in contrast to the results from Zhou & Zhang (2008), who did not find nematode species typical of cave sediments. SIMPER analysis on nematode species showed high dissimilarity between assemblages inside and outside the caves, mainly explained by *Chromaspirina parapontica,* a k-strategist specie (Danovaro *et al*., 1995; Semprucci *et al*., 2013), dominant outside the two caves and absent inside them (Table S5). We also observed high dissimilarity inside the Grotta dei Gamberi between the semi-dark and dark parts of the cave (89% dissimilarity between stations GI1 and GI3), suggesting differences in nematode assemblages along the exterior-interior axis, as reported in previous studies for benthic species (Bussotti *et al*., 2006; Denitto *et al*., 2007). Despite the high dissimilarity between the two dark stations (GI3 and FI), we observed that *Anticoma acuminata*, a relatively opportunistic species (c-p = 2; Bongers *et al*., 1991), dominated in the sediments of these stations of both caves and was absent in all other stations, explaining part of the dissimilarity observed among the stations inside and outside the caves.

Nematode trophic composition

For their environmental conditions, submarine caves appear to be very interesting environments for trophic pathway studies (Navarro-Barranco *et al*., 2012). Light availability, energy and organic matter flows may significantly affect food resources inside the caves and consequently trophic nematode assemblages. Our results showed differences in the composition of the trophic groups between the stations inside and outside the caves. We observed significant higher ITD values in the station inside compared to those outside Grotta Falconiera. At Grotta dei Gamberi ITD index was higher in semi-dark stations (GI1 and GI2) than in external sediments, instead in dark station (GI3) the values were significantly lower than outside. In fact, in all the stations, both inside and outside the caves, the trophic structure of nematodes was dominated by epistrate feeders (2A), which represented ~60% of total nematodes inside the two caves. By contrast in the innermost part of Grotta dei Gamberi (station GI3), selective deposit feeders nematodes (bacterivores, 1A) were dominant with non-selective deposit feeders (1B), together representing ~60% of total abundance. A common feature of the nematode assemblages inside the two caves was the higher contribution of selective deposit feeders (bacterivores, 1A) and lower abundance of predators/omnivores (2B), compared to external sediments.

Hart *et al*. (1985) compared the environmental features of the submarine caves with those of the deep-sea habitat (lack of light, limited hydrodynamism and food resources) and hypothesized a close relationship between the organisms inhabiting these two environments. In fact, some deep sea organisms are known to have successfully colonized the submarine caves, supporting this theory (Vacelet *et al*., 1994; Villora-Moreno, 1996). Worldwide, in the deep sea sediments, the dominant nematode trophic groups are deposit- (1A + 1B) and epistrate-feeders (2A), and regarded as potential bacterivores; however, the predators/omnivores are less abundant (Gambi *et al*., 2003; Danovaro *et al*., 2008; Pape *et al*., 2013).

In support of the theory of the similarity between submarine caves and deep sea habitats, our results indicate that nematodes trophic structure inside the caves is characterized by a higher abundance of bacterivores and a lower percentage of predators/omnivores, compared to nematodes trophic structure outside the caves.

The dominance of epistrate feeders inside the two caves, despite the lack of the algal component, such as in the deep sea, can be explained as the ability of the organisms of this trophic group to feed on bacteria, scraping off the microbial covering from the sediment particles (Moens & Vincx, 1997), unlike the nematodes of the same group which live outside the caves, and probably feed mainly on the algal component available. Also Todaro *et al*. (2006b) in a Mediterranean cave showed that the gastrotricha species inside the cave

depended only slightly on the microalgal component for their survival, whereas they fed mainly on bacteria and fungi. Consistently with these results, we assumed that in the two caves investigated, the bacteria might represent the most important food source for nematodes.

Conclusions

In this study we observed that meiofauna community followed the general pattern of distribution of organisms in the cave systems, already reported in the literature for benthic taxa by several authors, showing a significant decrease in abundance from the outer to the inner part of the investigated caves. The inside and the outside of the caves showed significant differences in meiofaunal community structure and in nematode taxonomic and trophic diversity. Consistently with previous studies, our results showed significant influence of environmental gradients, represented by the availability of organic matter (i.e phytopigment concentrations), on the distribution and composition of meiofauna and nematodes community in submarine caves.

Despite the different characteristics of the two caves investigated, we found similar nematode assemblages, in terms of dominant nematode species and trophic group composition in the sediments inside the caves. In particular, in the dark parts of both caves, we identified a nematode assemblage endemic to the cave habitat, characterized by opportunistic nematode species completely absent in the external sediments.

All these elements emphasise the ecological relevance of marine caves in coastal areas and therefore support their inclusion in MPAs in order to maximise the diversity representativeness and ensure their protection.

Acknowledgements

The manuscript is dedicated to the memory of Gaetano Ferruzza, our friend and colleague, who devoted the last years of his career to research on submarine caves, especially at Ustica island, and who tragically passed away on October 6th 2009, along with Dario Romano in a scuba-diving accident while working in a marine cave.

References

Airoldi, L., Cinelli, F., 1997. Sources and biochemical composition of suspended particulate material in a submarine cave with sulphur water springs. *Marine Biology*, 128, 537-545.

Akoumianaki, I., Hughes, J.A., 2004. The distribution of macroinfauna along a Mediterranean submarine cave with sulphur springs. *Cahiers de Biologie Marine*, 45, 355-364.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26, 32-46.

Anderson, M.J., Robinson, J., 2003. Generalised discriminant analysis based on distances. *Australian & New Zealand Journal of Statistics*, 45 (3), 301-318.

Andrassy, I., 1956. The determination of volume and weight of nematodes. *Acta Zoologica*, 2, 1-15.

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. *Journal of Natural History*, 42, 953-965.

Benedetti Cecchi, L., Airoldi, L., Abbiati, M., Cinelli, F., 1996. Exploring the causes of spatial variation in an assemblage of benthic invertebrates from a submarine cave with sulphur springs. *Journal of Experimental Marine Biology and Ecology*, 208, 153-168.

Boesgaard, T.M., Kristensen, R.M., 2001. Tardigrades from Australian marine caves. With a redescription of Actinarctus neretinus (Arthrotardigrada). *Zoologischer Anzeiger*, 240, 253-264.

Bongers, T., Alkemade, R., Yeates, G.W., 1991. Interpretation of disturbance induced maturity decrease in marine nematode assemblages by means of the Maturity Index. *Marine Ecology Progress Series*, 76, 135-142.

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

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

Cinelli, F., Frest, E., Mazzella, L., Pansini, M., Pronzato, R. *et al*., 1977. Distribution of benthic phyto- and zoocoenoses along a hight gradient in a superficial marine cave. p. 173-183. In: *Biology of benthic organisms*. Keegan, B.F., Cèidigh, P.O., Boaden, P.J.S. (Eds). Pergamon, Oxford.

Clarke, K.R., 1993. Non parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18, 117-143.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: *User Manual/ Tutorial*. PRIMER-E, Plymouth.

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

Colantoni, P., Gamba, R., Alvisi, M., 1991. Le grotte sommerse di capo Falconara, dello Scoglio del Medico e dei Gamberi nell'isola di Ustica. *Accademia internazionale di scienze e tecniche subacquee di Ustica*, 6, 45-56.

Danovaro, R., Fabiano, M., Vincx, M., 1995. Meiofauna response to the Agip Abruzzo oil spill in subtidal sediments of the Ligurian Sea. *Marine Pollution Bullettin*, 30 (2), 133-145.

Danovaro, R., Gambi, C., Lampadariou, N., Tselepides, A., 2008. Deep-sea biodiversity in the Mediterranean Basin: testing for longitudinal, bathymetric and energetic gradients. *Ecography*, 31, 231-244.

Danovaro, R., Gambi, C., Höss, S., Mirto, S., Traunspurger, W. *et al*., 2009. Case studies using nematodes assemblage analysis in aquatic habitats. p. 146-171. In: *Nematodes as environmental indicators*. Wilson, M.J., Kakouli-Duarte, T. (Eds). CABI, Wallingford, UK.

Danovaro, R., 2010. *Methods for the study of deep-sea sediments, their functioning and biodiversity*. CRC Press, Boca Raton, USA, 458pp.

Denitto, F., Terlizzi, A., Belmonte, G., 2007. Settlement and

primary succession in a shallow submarine cave: spatial and temporal benthic assemblage distinctness. *Marine Ecology*, 28, 35-46.

Deprez, T., Steyaert, M., Vanaverbeke, J., Speybroeck, J., 2005. *NeMys database*. www. nemys.ugent.be (Accessed March 2014)

Di Franco, A., Ferruzza, G., Baiata, P., Chemello, R., and Milazzo, M. 2009. Preliminary consideration on depositional mechanism and natural gross sedimentation rate in a deep submarine cave. Presentation to the 27th IAS Meeting of Sedimentology, Alghero, Italy, 20-23 September 2009.

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

Feller, R.J., Warwick, R.M., 1988. Energetics. p. 181-196. In: *Introduction to the Study of Meiofauna*. Higgins, R.P., Thiel, H. (Eds). London, Smithsonian Institute.

Fichez, R., 1990. Decrease in allochthonous organic inputs in dark submarine caves, connection with lowering in benthic community richness. *Hydrobiologia*, 207, 61-69.

Fichez, R., 1991. Suspended particulate organic matter m a Mediterranean submarine cave. *Marine Biology* 108, 167-174.

Gallo-D'Addabbo, M., De Zio Grimaldi, S., Sandulli, R., 2001. Heterotardigrada of two submarine caves in San Domino island (Tremiti islands) in the Mediterranean sea. *Zoologischer Anzeiger*, 240, 361-369.

Gambi, C., Vanreusel, A., Danovaro, R., 2003. Biodiversity of nematode assemblages from deep-sea sediments of the Atacama Slope and Trench (South Pacific Ocean). *Deep-Sea Research I*, 50, 103-117.

Gambi, C., Danovaro, R., 2006. A multiple-scale analysis of metazoan meiofaunal distribution in the deep Mediterranean Sea. *Deep-Sea Research I*, 53, 1117-1134.

Gerovasileiou, V., Chintiroglou, C.C., Vafidis, D., Koutsoubas, D., Sini, M. *et al.*, 2015. Census of biodiversity in marine caves of the eastern Mediterranean Sea. *Mediterranean Marine Science*, 16 (1), 245-265.

Gray, J.S., 2000. The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *Journal of Experimental Marine Biology and Ecology*, 250, 23-49.

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

Hart, C.W., Manning, R.B., Iliffe, T.M., 1985. The fauna of Atlantic marine caves: evidence of dispersal by sea floor spreading while maintaining ties to deep waters. *Proceedings of the Biological Society of Washington*, 98, 288-292.

Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. *Oceanography and Marine Biology*. 23, 399-489.

Hurlbert, S.H., 1971. The non-concept of species diversity: a critique and alternative parameters. *Ecology*, 52, 577-58.

Ingels, J., Vanhove, S., De Mesel, I., Vanreusel, A., 2006. The biodiversity and biogeography of the free-living nematode genera Desmodora and Desmodorella (family Desmodoridae) at both sides of the Scotia Arc. *Polar Biology*, 29, 936-949.

Lambshead, P.J.D., 2004. Marine nematode biodiversity. p. 436-467. In: *Nematology advances perspectives*. Chen, Z.X., Chen, S.Y., Dickson, D.V. (Eds). CABI, Wallingford.

Lampadariou, N., Tselepides, A., Hatziyanni, E., 2009. Deep-sea meiofaunal and foraminiferal communities along a gradient of primary productivity in the eastern Mediterranean Sea. *Scientia Marina*, 73, 337-345.

Lorenzen, C., Jeffrey, J., 1980. Determination of chlorophyll in seawater. *Technical Papers in Marine Sciences* (UNESCO), 35, 1-20.

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

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

Micael, J., Azevedo, J.M.N., Costa, A.C., 2006. Biological characterization of a subtidal tunnel in São Miguel island (Azores). *Biodiversity and Conservation*, 15, 3675-3684.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, 82, 290-297.

Moens, T., Vincx, M., 1997. Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the UK*, 77, 211-227.

Navarro-Barranco, C., Guerra-García, J.M., Sánchez-Tocino, L., García-Gómez, J.C., 2012. Soft-bottom crustacean assemblages in Mediterranean marine caves: the cave of Cerro-Gordo (Granada, Spain) as case study. *Helgoland Marine Research*, 6 (4), 567-576.

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

Pape, E., Van Oevelen, D., Moodley, L., Soetaert, K., Vanreusel, A., 2013. Nematode feeding strategies and the fate of dissolved organic matter carbon indifferent deep-sea sedimentary environments. *Deep-Sea Research I*, 80, 94-110.

Pérès, J.M., Picard, J., 1964. Nouveau manuel de bionomie benthique de la Mer Méditérraneè. *Recueil de Travaux Station Marine d'Endoume*, 31 (47), 5-137.

Pusceddu, A., Bianchelli, S., Canals, M., Durrieu De Madron, X. *et al.*, 2010. Organic matter in sediments of canyons and open slopes along European continental margins. *Deep-Sea Research I*, 57, 441-457.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to unhydrous glycerine. *Nematologica*, 4, 67-69.

Semprucci, F., Moreno, M., Sbrocca, S., Rocchi, M., Albertelli, G. *et al.*, 2013. The nematode assemblage as a tool for the assessment of marine ecological quality status: a case-study in the Central Adriatic Sea. *Mediterranean Marine Science*, 14(1), 48-57.

Soltwedel, T., 2000. Metazoan meiobenthos along continental margins: a review. *Progress in Oceanography*, 46, 59-84.

Sørensen, M.V., Jørgensen, A., Boesgaard, T.M., 2000. A

new echinoderes (Kinorhyncha, Cyclorhagida) from a submarine cave in new south Wales, Australia. *Cahiers de Biologie Marine*, 41, 167-179.

Todaro, M.A., Shirley, T.C., 2003. A new meiobenthic priapulid (Priapulida, Tubiluchidae) from a Mediterranean submarine cave. *Italian Journal of Zoology*, 70, 79-87.

Todaro, M.A., Guidi, L., Leasi, F., Tongiorgi, P., 2006a. Morphology of Xenodasys (Gastrotricha): the first species from the Mediterranean Sea and the establishment of *Chordodasiopsis* gen. nov and Xenodasyidae fam. nov. *Journal of the Marine Biological Association of the United Kingdom*, 86, 1005-1015.

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

Vacelet, J., Boury-Esnault, N., Harmelin, J.G., 1994. Hexactinellid cave, a unique deep-sea habitat in the scuba zone. *Deep-Sea Research I*, 41 (7), 965-973.

Villora-Moreno, S., 1996. A new genus and species of deep-sea family Coronarctidae (Tardigrada) from a submarine cave with a deep-sea like condition. *Sarsia*, 81, 275-283.

Wieser, W., 1953. Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. *Arkiv for Zoologi* 2 (4), 439-484.

Zhou, H., Zhang, Z.N., 2008. Nematode assemblages from submarine caves in Hong Kong. *Journal of Natural History*, 42, 9-12.