Meiobenthic copepod fauna of a marine cave (NW Mediterranean) closely resembles that of deep-sea communities

Annika Janssen[1,*], Pierre Chevaldonné[2], Pedro Martínez Arbizu[1]

[1]Senckenberg am Meer, Abt. Deutsches Zentrum für Marine Biodiversitätsforschung, Südstrand 44, 26382 Wilhelmshaven, Germany

[2]Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale (IMBE), UMR CNRS 7263, Aix-Marseille Université, Station Marine d Endoume, Rue de la Batterie des Lions, 13007 Marseille, France

ABSTRACT: The 3PP marine cave near Marseille (NW Mediterranean) is at a depth of only 15 m. Nevertheless, the cave is a unique deep-sea-like habitat zone due to total darkness, lack of water circulation, oligotrophy, and cold homothermy, a thermal regime similar to that of the deep Mediterranean. We studied the meiofaunal community composition and diversity at 3 sampling stations in the cave (entrance, middle, and blind end). Major taxon composition, based on presence/ absence data, did not vary over the transect; however, significant differences in community were found. Tardigrades were the only major taxon restricted to the inner parts of the cave. Copepod diversity decreased towards the inner parts of the cave. The 3PP Cave was characterized by very low abundances of meiofaunal organisms similar to abyssal sites elsewhere. A total of 405 individual copepods were assigned to 27 families and 90 species (75% of them new to science). Significant differences in copepod communities between stations were found at family, genus, and species level. Some harpacticoid taxa generally known from the deep sea, such as *Marsteinia*, *Ancorabolina*, *Paranannopus*, *Nematovorax*, and Argestidae, were represented in the 3PP Cave. A naive Bayes model was used for the first time to classify the communities as 'shallow' or 'abyssal'. The meiobenthic communities at the entrance and the end of the cave were unequivocally classified as 'shallow' and 'abyssal' respectively, while the middle part of the cave presented a mixed community. Our study further highlights the strong faunal and community affinities between marine caves and the deep sea, indicating the existence of dispersal mechanisms from deep waters into the euphotic zone for benthic organisms.

KEY WORDS: 3PP Cave · Marine caves · Meiofauna · Mediterranean Sea · Deep sea · Naive Bayes model

INTRODUCTION

During the last few decades, cave divers have discovered numerous different cave systems, some of them entirely submerged. Among them were marine caves, here considered caves or crevices opening under the sea level and entirely filled with seawater (excluding caves with a strong terrestrial influence and anchialine systems) (Stock et al. 1986). Marine caves share many different ecological features with the deep sea such as darkness, the resulting lack of photosynthetic production, a low input of organic matter, and thus low food supply and reduced water circulation (Riedl 1966). Cave communities as well as deep-sea communities rely on input of organic matter from surface layers.

These limiting conditions lead to a habitat lacking most coastal species. The similarities between caves and the deep sea sometimes allow connections between populations of these 2 systems (Harmelin et al. 1985, Hart et al. 1985, Calado et al. 2004, Bakran-Petricioli et al. 2007). Some particular caves, such as the 3PP Cave in the south of France, display another characteristic in common with the deep sea: cold homothermy. In Winter, littoral coldwater lenses of ca. 13°C are trapped by density at the bottom of descending caves, i.e. the temperature of Mediterranean deep waters (Vacelet et al.1994, Bakran-Petricioli et al. 2007). Several deep-sea organisms, for instance the hexactinellid *Oopsacas minuta* or the bryozoan *Puellina setiformis*, have been found in the darkest parts of marine caves, particularly 3PP Cave (Vacelet et al.1994, Harmelin 1997, Bakran-Petricioli et al. 2007, Glover et al. 2010). Most marine caves along the French Mediterranean coast have their main opening to the open sea at 10 to 20 m depth (Riedl 1966, Harmelin et al. 1985).

To date, knowledge on meiobenthic marine cave communities is scarce. The earliest studies were conducted by Wieser (1954), Pesta (1959), and Riedl (1966). More recently, additional studies were conducted, but essentially with taxonomic emphasis on tardigrades, kinorhynchs, and priapulids, by Palacin & Masalles (1986), Villora-Moreno (1996), Sandulli et al. (2002), Sørensen et al. (2000), Boesgaard & Kristensen (2001), Gallo D'Addabbo et al. (2001), Sandulli et al. (2002), and Todaro & Shirley (2003). Todaro et al. (2006) investigated the nature and abundance of meiofaunal taxa living in a Mediterranean marine cave in southern Italy, in relation to distance from the cave entrance, and over 2 different seasons. Particular attention was paid to the gastrotrichs. Meiobenthic densities were compared along a gradient from the cave entrance to the cave end. However, this was not a cave with cold homothermy, and therefore deep-sea like conditions are not as striking as in 3PP.

The aim of our study is to identify the abundance and diversity of metazoan meiofauna at 3PP Cave in order to assess relatedness between cave and deep-sea communities. Understanding the level of connectivity between these 2 components of the Mediterranean aphotic system is of great importance for the environmental management of coastal zones, and caves such as 3PP may serve as a shallow-water analogue to study deep-sea processes (Glover et al. 2010) in an ever-changing Mediterranean Sea (Lejeusne et al. 2010).

MATERIALS AND METHODS

Study area

The study site is located 30 km east of Marseille, near La Ciotat on the French Mediterranean coast. The area is characterized by a narrow continental shelf and a steep slope intersected by deep canyons (Harmelin 1997). When the 'Mistral' wind blows a very strong wind-induced upwelling, brings up deep cold water masses to the surface (Millot 1979). The rocky shore is interspersed with numerous shallow underwater caves, mainly of karstic origin (Harmelin 1997). Located below the Bec-de-l'Aigle Cape, near La Ciotat (Fig. 1), 3PP Cave (43° 09.47′ N, 05° 36.01′ E) is not karstic but rather runs through an Upper Cretaceous conglomerate. It has a tubular shape with a blind end at a distance of 120 m from the entrance (Fig. 2). In contrast to most karstic caves, which are widely studied in the Mediterranean, the floor of this cave presents an inwardly descending profile. This topography is responsible for coldwater trapping by density, a rare pattern of hydrological stratification, and therefore responsible for the deep-sea character of the cave (Vacelet et al. 1994, Harmelin 1997, Bakran-Petricioli et al. 2007). The entrance of the cave is located 15 m under sea level. The inner water body measures roughly 3500 m³. Throughout the cave, the floor is covered by >1 m muddy deposits (Harmelin 1997).

Sampling and sample treatment

Sampling took place between 7 and 11 May 2007. Within the cave, 15 samples (5 per station) were taken at 3 stations. The first station (from inside to
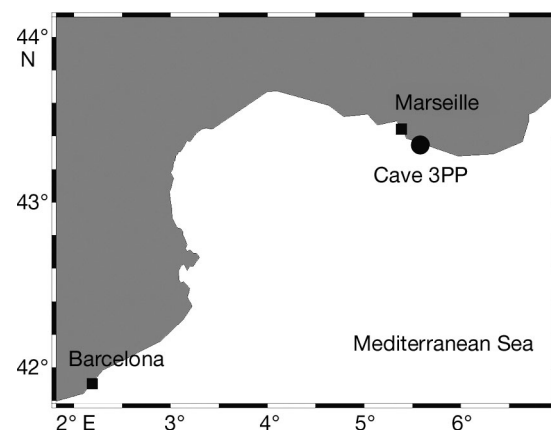

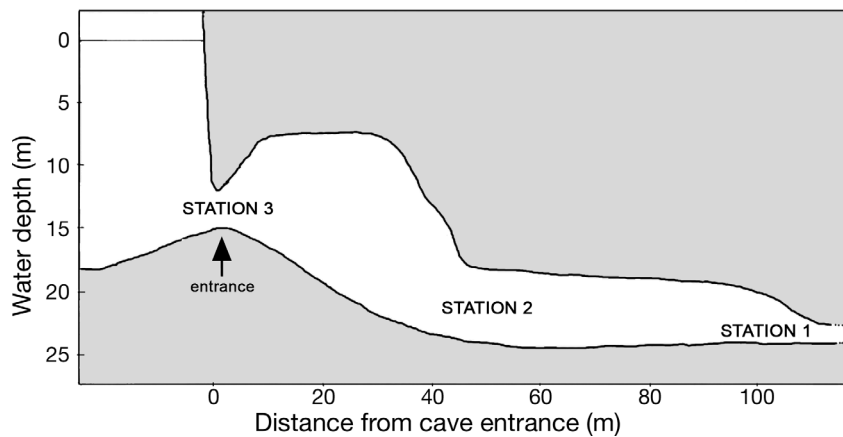Fig. 1. 3PP Cave in the NW Mediterranean Sea

Fig. 2. Schematic cross-section of 3PP Cave and location of the 3 meiofauna sampling stations within the cave (modified after Harmelin 1997)

outside) was located 100 m from the entrance near the cave end, the second station 50 m from the entrance at mid-cave, and the third station was situated at the entrance (Fig. 2). The samples were taken by SCUBA divers using a hand-held corer (5.6 cm in diameter). Two cores from Stn 1 (cave end) lost a small amount of sediment in sample processing. Therefore, they had to be discarded in subsequent quantitative analyses. All samples were fixed with formaldehyde at a final concentration of ~4%. Meiofauna was extracted by means of centrifugation with a colloidal silica polymer (Levasil) as flotation medium and kaolin to cover the heavier particles (McIntyre & Warwick 1984). Centrifugation was repeated 3 times at 1650 × g for 5 min. All animals retained on a 40 µm sieve were counted and sorted under a dissecting microscope to major taxa in the laboratory at Senckenberg am Meer Wilhelmshaven, Abt. Deutsches Zentrum für Marine Biodiversitätsforschung (DZMB), Wilhelmshaven (Germany). For determination of copepod species, most of the individuals were mounted on single slides using glycerol as embedding medium. All adult copepods were identified to species level using Lang (1948), Wells (1976), Huys et al. (1996), Boxshall & Halsey (2004), and original species descriptions.

Statistical analysis

Non-metric multidimensional scaling (nMDS) was used to represent similarities between the stations at community level. In the process, Bray-Curtis similarity (as 1 – Bray-Curtis) for assemblages of major taxa of meiofauna (Bray & Curtis 1957) and cosine similarity for copepod assemblages (Salton & McGill 1983)

were computed on untransformed data (PAST software v. 1.34; Hammer et al. 2001). The qualitative cosine similarity index does not downgrade the importance of low-abundance species and considers only shared attributes. This is appropriate in our study, because we target the detection of changes in species composition between stations, rather than changes in abundance. The Bray-Curtis Index computes the average sum of differences in taxon abundance between samples and is appropriate for comparing meiofauna assemblages at high taxonomic level, where differences are expected in relative abundance rather than in higher taxa presence/absence. Analysis of similarities (ANOSIM) (Clarke & Green 1988), diversity values, and rarefaction curves (developed by Sanders 1968, improved by Hurlbert 1971 and Simberloff 1979) were also computed with the same software. The graphical illustration from rarefaction values was produced with Excel (Microsoft Office Package 2007). The location map was designed with Ocean Data View software (Schlitzer 2010).

In order to test if the communities at the 3 different stations resemble deep-sea rather than shallow-water communities, we trained a naive Bayes model (Langley et al. 1992) using discrete presence-absence of 31 harpacticoid genera in 853 samples (prior class probability of 0.855 for 'shallow', 0.144 for 'abyssal'). Training data were taken from 2 sources. A comprehensive analysis of European shallow-water harpacticoid communities was provided in Veit-Köhler et al. (2010). Harpacticoid genera from the Angola Basin (South Atlantic, DIVA-1 cruise, 5000 m depth) and from the Clarion-Clipperton Fracture Zone (NE Pacific, Nodinaut cruise, 5000 m depth) were kindly provided by K. H. George and R. Mahatma (pers. comm.). Only genera present in the cave samples and in the training dataset were used for modeling. The trained model was then applied to the 15 cave samples, to be classified into 'shallow' and 'abyssal'. Average posterior probability of the 'abyssal' class (5 samples per level) decided the final classification. The underlying statistical property of the naive Bayes model is the application of Bayes' rule. Because we are only interested in relative posterior probabilities (we want to know which posterior probability is higher: 'abyssal' or 'shallow'?), Bayes' rule can be simplified to the following expression:

$$p(A|B) = p(B|A)p(A) \qquad (1)$$

where $p(A|B)$ = probability of A given B (the posterior probability); $p(B|A)$ = probability of B given A (conditional probability or likelihood); and $p(A)$ = probability of A (prior probability). The expression therefore reads as 'the probability that A occurs given B equals the likelihood that B occurs given A, conditioned to the probability that A occurs at all'.

The application of Bayes' rule to the specific problem of classifying the cave samples into communities is discussed later. This can be applied, however, in the same way to any other kind of communities. Calculations were performed using the open-source Konstanz Information Miner (Berthold et al. 2007, www.knime.org) and R using package e1071 (R Development Core Team 2010, Dimitriadou et al. 2011).

RESULTS

The metazoan meiofauna was assigned to 14 major high-rank taxa (Table 1). Nematodes were the most abundant taxon, with a relative abundance (RAD) of 82 to 85%, followed by copepods (+ nauplii, RAD: 7 to 10%), then priapulids and annelids with 2–4% and 2% RAD, respectively. Kinorhynchs, ostracods, tardigrades, and rotifers were observed frequently but in lower numbers. Other meiobenthic taxa, i.e. Acari, Amphipoda, Bivalvia, Gastrotricha, Isopoda, and Loricifera, were represented by single specimens (grouped under 'Others' in Table 1). The presence and absence of major metazoan meiofaunal groups were not found to significantly vary among the 3 sampling stations (entrance, mid-cave, cave end). However, due to the decreasing individual densities from the cave entrance to the cave end, significant differences between the stations were evidenced

with ANOSIM (Fig. 3, Table 2). The only exception was found with tardigrades, whose density increased from the cave entrance to the cave end.

Particular attention was given to the copepods. A total of 405 adult copepods were assigned to 27 families (Table 3). Cletodidae and Miraciidae appeared most frequently at the entrance of the cave (Stn 3) and defined half of all copepods. Often represented were also Ameiridae, Zosimeidae, Pseudotachidiidae, and Ectinosomatidae. In the middle of the cave (Stn 2), the most abundant taxa were Zosimeidae, Pseudotachidiidae, and Ectinosomatidae. Several were represented by single specimens. At the end of
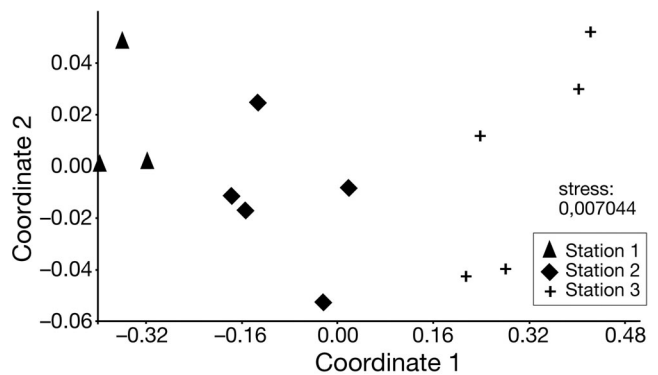

Fig. 3. Non-metric multidimensional plot for Bray-Curtis similarity of samples of meiofaunal assemblages analyzed at higher taxonomic levels. No transformation

Table 2. Pairwise 1-way analysis of similarities (ANOSIM) test for the differences in the composition of meiobenthic assemblages at different stations of 3PP Cave

	Stn 1		Stn 2	
	R	p	R	p
Stn 2	0.98	0.008	–	–
Stn 3	1	0.016	0.918	0.021

Table 1. Mean individual densities and relative abundances (RAD) of meiofaunal taxa sampled from 3PP Cave sediment

Taxon	Stn 3: Cave entrance Density (ind. 10 cm^{-2})	RAD (%)	Stn 2: Mid-cave Density (ind. 10 cm^{-2})	RAD (%)	Stn 1: Cave end Density (ind. 10 cm^{-2})	RAD (%)
Nematoda	967 ± 270.7	85	309 ± 79.3	85	143 ± 52.3	82
Copepoda (+ nauplii)	102 ± 49.8	9	26 ± 5	7	16 ± 7.9	10
Priapulida (larvae)	27 ± 26.5	2	13 ± 5.3	4	3 ± 2.7	2
Annelida	27 ± 7.7	2	6 ± 1.5	1	3 ± 2.5	2
Kinorhyncha	13 ± 4.1	1	2 ± 0.8	<1	1 ± 0.9	<1
Ostracoda	5 ± 3	<1	1 ± 1.2	<1	1 ± 0.6	<1
Rotifera	3 ± 3.6	<1	3 ± 1.6	1	1 ± 1.1	<1
Tardigrada	0 ± 0	0	3 ± 2	1	5 ± 3.5	3
Others	10 ± 2.6	1	5 ± 1.5	1	2 ± 0.6	1

Table 3. Relative abundance (RAD) and total number of individual copepods (Σ) sorted by copepod families, at the different 3PP Cave sampling stations

	Stn 3 RAD (%)	Stn 3 Σ	Stn 2 RAD (%)	Stn 2 Σ	Stn 1 RAD (%)	Stn 1 Σ
Harpacticoida						
Ameiridae	8	25	6	4	–	–
Ancorabolidae	–	–	<1	1	9	3
Argestidae	–	–	<1	1	3	1
Canthocamptidae	2	6	–	–	–	–
Cletodidae	37	112	–	–	–	–
Cletopsyllidae	<1	1	–	–	–	–
Cyclopinidae	<1	1	<1	1	–	–
Ectinosomatidae	4	12	7	5	12	4
Harpacticidae	–	–	<1	1	–	–
Huntemannidae	<1	1	–	–	–	–
Idyanthidae	3	8	–	–	–	–
Laophontidae	2	4	<1	1	–	–
Longipediidae	<1	1	–	–	–	–
Miraciidae	28	84	6	4	–	–
Neobradyidae	<1	1	–	–	–	–
Normannelidae	1	6	–	–	–	–
Orthopsyllidae	1	2	–	–	–	–
Parastenhelidae	<1	1	<1	1	–	–
Peltidiidae	1	3	–	–	–	–
Pseudocyclopidae	–	–	<1	1	–	–
Pseudotachiidae	4	13	14	10	9	3
Tegastidae	<1	1	<1	1	–	–
Thalestridae	–	–	<1	1	–	–
Zosimeidae	6	18	46	32	35	12
Harpacticoida sp. 1	–	–	6	4	6	2
Calanoida						
Stephiidae	<1	1	<1	1	–	–
Cyclopoida						
Oithonidae	–	–	<1	1	26	9
Total copepods	–	301	–	70	–	34

the cave (Stn 1), the most abundant taxa were Zosimeidae, Oithonidae, and Ectinosomatidae. Ancorabolidae and Pseudotachidiidae were also represented by several specimens.

Quantitative and qualitative analysis at species level displayed divergent copepod communities between the entrance and the stations well inside the cave (Stns 1 and 2). The significant differences in the cosine index were also verified by ANOSIM (Table 4, Fig. 4). A total of 90 copepod working species were

Table 4. Pairwise 1-way analysis of similarities (ANOSIM) test for the differences in the composition of benthic copepod species assemblages at 3 stations of 3PP Cave

	Stn 1 R	Stn 1 p	Stn 2 R	Stn 2 p
Stn 2	0.38	0.048	–	–
Stn 3	0.78	0.008	0.98	0.008

identified, and almost 3/4 of them were new to science. The dominant species at the cave entrance were *Cletodes longicaudatus* and Miraciidae sp. 2 followed by *Typhlamphiascus bouligandi* and *Stylicletodes reductus* (Table 5). The dominant genus in the middle of the cave was *Zosime*. Three unknown, but obviously different species were most frequently represented: *Zosime* sp.1, sp. 2, and sp. 3. Stn 1 was characterized by a very low individual density of copepods. The most abundant taxon was an unknown species of *Oithona*, followed by the 3 unknown species of *Zosime* that we also found in the middle of the cave.

Diversity analysis (Table 6) using several diversity indices (Margalef index, Pielou's evenness, Shannon index, Simpson index) and the rarefaction method showed a higher diversity at the cave entrance in comparison with the end of the cave (Fig. 5).

Table 7 shows the conditional probability of belonging to the class 'shallow' and 'abyssal' of the 31 genera as reported from the literature. The posterior probability of assignment to the class 'abyssal' or 'shallow' is presented for the 15 cave samples in Table 8. Taking the average posterior probability of the 5 replicates per station, the innermost Stn 1 can be classified as 'abyssal' ($p = 0.84$), whereas the mid-cave Stn 2 and the entrance Stn 3 were classified as
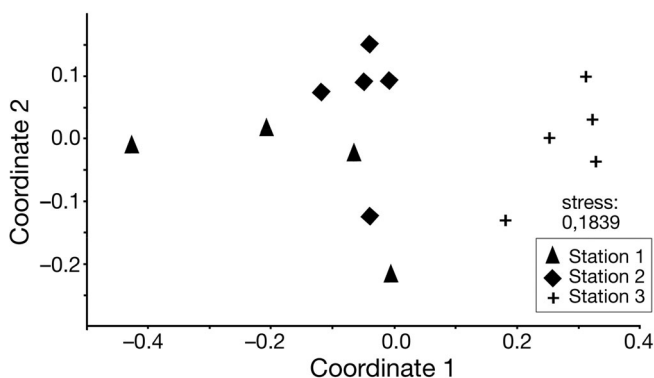

Fig. 4. Non-metric multidimensional plot for cosine similarity of samples of copepod assemblages analyzed at species level. No transformation

Table 5. Relative abundance (RAD) and total number (Σ) of individual copepods sorted by species from the 3 cave sampling stations. Only species with >1 % RAD at ≥1 station are listed

	Stn 3 RAD (%)	Σ	Stn 2 RAD (%)	Σ	Stn 1 RAD (%)	Σ
HARPACTICOIDA						
Harpacticoida sp. 1	–	–	6	4	6	2
Ameiridae						
Ameiridae sp. 1	–	–	3	2	–	–
Ameiridae sp. 5	2	5	–	–	–	–
Ameira parvula Claus, 1866	2	5	<1	1	–	–
Leptomesochra sp. 3	1	4	–	–	–	–
Ancorabolidae						
Ancorabolina cavernicola George & Tiltack, 2009	–	–	<1	1	9	3
Cletodidae						
Cletodidae sp. 3	3	8	–	–	–	–
Cletodes sp. 1	1	3	–	–	–	–
Cletodes sp. 2	1	3	–	–	–	–
Cletodes longicaudatus Boeck, 1872	18	55	–	–	–	–
Stylicletodes reductus	9	28	–	–	–	–
Enhydrosoma sp. 1	2	6	–	–	–	–
Enhydrosoma curvirostre T. Scott, 1894	2	8	–	–	–	–
Ectinosomatidae						
Ectinosomatidae sp. 3	–	–	–	–	6	2
Ectinosomatidae sp. 4	–	–	–	–	6	2
Ectinosoma sp. 2	<1	2	3	2	–	–
Idyanthidae						
Nematovorax gebkelinae Brohldick, 2005	3	8	–	–	–	–
Miraciidae						
Miraciidae sp. 2	15	44	–	–	–	–
Schizopera grimalschii Jakubisiak, 1938	<1	1	4	3	–	–
Delavalia sp. 3	2	5	–	–	–	–
Typhlamphiascus bouligandi Soyer, 1972	10	30	–	–	–	–
Normannelidae						
Normanella minuta Boeck, 1873	2	6	–	–	–	–
Pseudotachidiidae						
Pseudomesochra similis Lang, 1936	<1	2	4	3	6	2
Pseudomesochra sp. 4	1	3	<1	1	–	–
Pseudotachidius coronatus Scott, 1898	2	5	–	–	–	–
Carolinicola sp. 1	<1	1	4	3	–	–
Carolinicola sp. 2	–	–	3	2	–	–
Zosimeidae						
Zosime sp. 1	1	3	23	16	12	4
Zosime sp. 2	2	6	17	12	9	3
Zosime sp. 3	3	9	6	4	15	5
CYCLOPOIDA						
Oithonidae						
Oithona sp. 1	–	–	<1	1	25	9
Others	17	51	21	15	6	2

'shallow' ($p = 0.6$ and $p = 0.86$, respectively). However, by assigning the mid-cave station to 'shallow', 2 out of 3 replicates were classified as 'abyssal' by the model, so we need to consider that this station displays an intermediate or mixed assemblage. The copepod community living at the entrance of the cave is a typical shallow-water one (Fig. 6).

The genera *Pseudomesochra*, *Marsteinia*, *Paranannopus*, *Metahuntemannia*, *Zosime*, *Sarsmeira*, and *Nematovorax* were classified by naive Bayes as abyssal taxa, due to relatively high conditional probabilities (0.33 to 0.73) of being present in abyssal samples and quite low conditional probabilities (0.0 to 0.02) of being present in shallow-water samples. In contrast, *Enhydrosoma*, *Longipedia*, *Typhlamphiascus*, *Haloschizopera*, and *Cletodes* were classified as shallow-water taxa, with conditional probabilities ranging from 0.18 to 0.45 of being present in shallow-water samples and very low conditional probabilities (0.0 to 0.02) of being present in abyssal samples (Fig. 7).

DISCUSSION

Main meiofaunal groups

Significant differences between meiobenthic assemblages at the 3 stations within the 3PP Cave were ascertained with the help of a similarity analysis (Fig. 3, Table 2). The same higher taxa of meiobenthos were represented at each station. Therefore, the significant differences are essentially due to differences in abundance of the dominant taxonomic groups. Abundances decreased considerably from the cave's entrance to the cave's end. Biological communities in marine caves of the Mediterranean Sea are always characterized by a general decrease in the benthic biomass and abundance from the open sea to the

Table 6. Diversity indices comparing the copepods from the 3 cave sampling stations

Diversity index	Stn 3	Stn 2	Stn 1
S, species richness	70	29	11
n, number of individuals	301	70	34
d, Margalef index	12.09	6.59	2.84
J, Pielou's evenness	0.77	0.84	0.91
H ($\log_e$), Shannon index	3.23	2.83	2.19
$1 - \lambda$, Simpson index	0.92	0.91	0.89
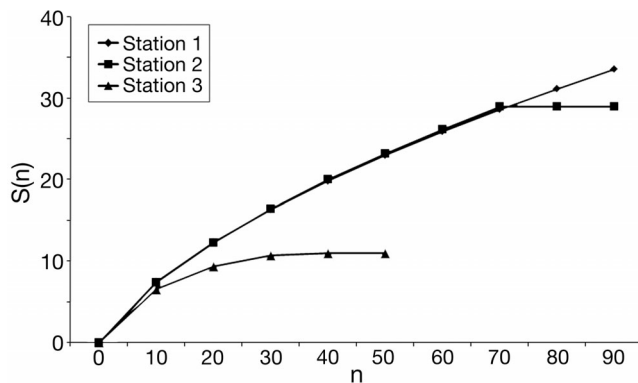
Fig. 5. Rarefaction curves for the species-level copepod assemblages sampled at the 3 stations within 3PP Cave. n: number of individuals, S(n): expected number of species

dark inner parts (Riedl 1966, Cinelli et al. 1977, Harmelin et al. 1985, Gili et al. 1986, Fichez 1991).

The main factor responsible for the impoverishment of organisms inside marine caves is the gradual qualitative and quantitative reduction of food. The trophic depletion of cave ecosystems mainly derives from the negative gradient of 2 physical factors: light and water circulation (Cinelli et al. 1977). At the entrance of the cave, light decreases abruptly until total darkness at the end of Cave 3PP, which causes the extirpation of photosynthetic organisms (Cinelli et al. 1977), therefore preventing autochthonous primary production. In turn, reduced water circulation, or confinement, decreases the supply of nutritional material from the outside, as well as the amount of colonists (Harmelin 1997). The gradual decrease in the mean densities of the total meiofauna seems to indicate that the meiobenthic community in 3PP Cave is structured in accordance with the trophic depletion hypothesis. The study of Grotta Piccola Del Ciolo, a marine cave without coldwater homothermy, located in the south of Italy, has revealed similar results (Todaro et al. 2006).

In Table 9, the individual densities (ind. per 10 cm²) of the total meiofauna and the 2 dominant taxa (nematodes and copepods) of 3PP Cave are compared to individual densities for the marine sublittoral Gulf of Lions at 30 m (McIntyre 1969) and Grotta Piccola del Ciolo (Todaro et al. 2006). Due to the close proximity (cave opens at 15 m) of Stn 3 to the outside sublittoral environment, individual densities there fall within the range of values obtained by McIntyre (1969) for the same geographical area.

Well inside the cave (Stns 1 and 2), individual densities are considerably lower than the values from the sublittoral Gulf of Lions. Generally, meiofaunal densities of marine caves are very low. This is also supported by Todaro et al. (2006). 3PP Cave, perhaps because of its peculiar descending profile and coldwater homothermy, displays even lower individual densities than at a more common cave such as Grotta Piccola del Ciolo. This latter Italian cave is also much shallower (3 to 4 m water depth) and is only partially submerged, which warrants rather significant water movement inside the cave. Water renewal and water movements in 3PP Cave (which is entirely submerged) are much more reduced than in other marine caves, due to the presence of a thermocline at cave entrance (Harmelin 1997). All this further reduces food transfer and colonization success from the outside, and hence the finding of very low abundances of meiobenthic assemblages.

Copepod assemblage at family level

Until now, there has been no investigation of marine cave meiofauna focusing on the copepod communities, particularly at genus or species levels. Early studies of Mediterranean marine caves displayed rather different nematode communities inhabiting marine caves compared to the littoral zone (Wieser 1954).

The analyses of copepod assemblages within 3PP Cave also reveal distinct communities at the entrance of cave (littoral zone) and the back areas of the cave. Several copepod families are restricted to different stations within the cave. Therefore, they seem to be strongly dependent on local environmental conditions prevailing at these specific areas. For instance, the Cletodidae, Ameiridae, and Miraciidae are almost exclusively represented at the entrance of the cave. These taxa are typical representatives of the Mediterranean sublittoral meiofauna (Bodiou & Chardy 1973, Chertoprud et al. 2007). The copepod families Argestidae, Ancorabolidae, Huntemannidae, Neobradyidae, and Idyanthidae are consid-

Table 7. Conditional probability assignments for 'present' and 'absent' to classes 'shallow' and 'abyssal' of the 31 genera in the training dataset (values rounded to 3 decimals)

	Present		Absent	
	Abyssal	Shallow	Abyssal	Shallow
Ameira	0.268	0.171	0.731	0.829
Ancorabolina	0.024	0.000	0.976	1.000
Arenosetella	0.016	0.101	0.984	0.899
Cletodes	0.000	0.184	1.000	0.816
Dactylopodella	0.000	0.004	1.000	0.996
Delavalia	0.138	0.000	0.862	1.000
Ectinosoma	0.106	0.111	0.894	0.889
Enhydrosoma	0.000	0.448	1.000	0.552
Esola	0.000	0.001	1.000	0.999
Haloschizopera	0.024	0.232	0.976	0.768
Harpacticus	0.000	0.099	1.000	0.901
Hemimesochra	0.000	0.025	1.000	0.975
Laophonte	0.000	0.093	1.000	0.907
Leptomesochra	0.016	0.009	0.984	0.990
Longipedia	0.000	0.368	1.000	0.631
Marsteinia	0.691	0.000	0.309	1.000
Metahuntemannia	0.398	0.000	0.602	1.000
Nematovorax	0.341	0.000	0.659	1.000
Nitocra	0.000	0.004	1.000	0.996
Normanella	0.000	0.081	1.000	0.919
Paralaophonte	0.000	0.027	1.000	0.973
Paranannopus	0.398	0.000	0.602	1.000
Pseudameira	0.000	0.042	1.000	0.958
Pseudomesochra	0.732	0.001	0.268	0.999
Pseudotachidius	0.122	0.001	0.878	0.999
Psyllocamptus	0.000	0.004	1.000	0.996
Sarsameira	0.333	0.019	0.667	0.981
Schizopera	0.000	0.003	1.000	0.997
Stylicletodes	0.008	0.016	0.992	0.984
Typhlamphiascus	0.000	0.281	1.000	0.719
Zosime	0.398	0.022	0.602	0.978

Table 8. Posterior probability of assignment to classes 'abyssal' or 'shallow' for the 15 cave samples (values rounded to 3 decimals)

Sample	Stn	Naive Bayes winner	Posterior probability	
			Abyssal	Shallow
1	1	Abyssal	1.000	0.000
2	1	Shallow	0.215	0.785
3	1	Abyssal	1.000	0.000
4	1	Abyssal	1.000	0.000
5	1	Abyssal	1.000	0.000
6	2	Abyssal	1.000	0.000
7	2	Shallow	0.000	1.000
8	2	Abyssal	0.998	0.002
9	2	Shallow	0.000	1.000
10	2	Shallow	0.000	1.000
11	3	Shallow	0.144	0.856
12	3	Shallow	0.144	0.856
13	3	Shallow	0.144	0.856
14	3	Shallow	0.144	0.856
15	3	Shallow	0.144	0.856

ered typical deep-sea groups (Seifried 2004), and were represented at the innermost area of 3PP Cave in general. However, there are also generalist families which seem not to be affected by the environmental gradient within the cave, i.e. Zosimeidae, Pseudotachidiidae, and Ectinosomatidae. From a quantitative point of view, these differences become even more obvious. These families, common to all sampling stations, were, however, always more abundant at Stn 3 (Table 3).

Copepod assemblages at species level

Nevertheless, a more detailed picture of copepod assemblages can be obtained by looking at species composition (George 2005). The similarity analysis at species level supports a clear separation of copepod assemblages between Stns 3 and 1 and also between Stns 3 and 2. There was no clear separation of copepod assemblages between Stns 2 and 1 (Fig. 4, Table 4), even though more dissimilarities than similarities exist between these stations. Most differences are caused by single specimens, but Stns 2 and 1 otherwise share numerous species. The significant differences between stations observed in copepod assemblages are caused by characteristic species which are exclusively present at single stations. At Stns 3, 2, and 1, the number of characteristic species was 58, 16, and 4, respectively.

From a quantitative point of view, differences between stations were even more obvious due to the fact that the generalist taxa were almost always represented in higher abundances at Stn 3. Furthermore, none of the dominant species of Stn 3, i.e. *Cletodes longicaudatus*, *Stylicletodes reductus*, Miraciidae sp. 2, and *Typhlamphiascus bouligandi*, was represented at another station. The presence of these prevalent, characteristic species is likely related to the environmental conditions controlling the cave entrance. The characteristic species of Stn 1, i.e. *Oithona* sp. 1, *Ancorabolina cavernicola*, as well as Harpacticoida sp. 1, seem to be restricted to the conditions of greater confinement and food scarcity prevailing towards cave end, conditions which are similar to that of the deep Mediterranean.

The diversity analysis, whichever index was used (Margalef's and Shannon's), shows a higher diversity at the entrance of the cave. Both indices give a great weight to species richness. The results from rarefaction curves also point to a higher diversity at Stn 3 compared to Stns 2 and 1, caused by the higher species richness.
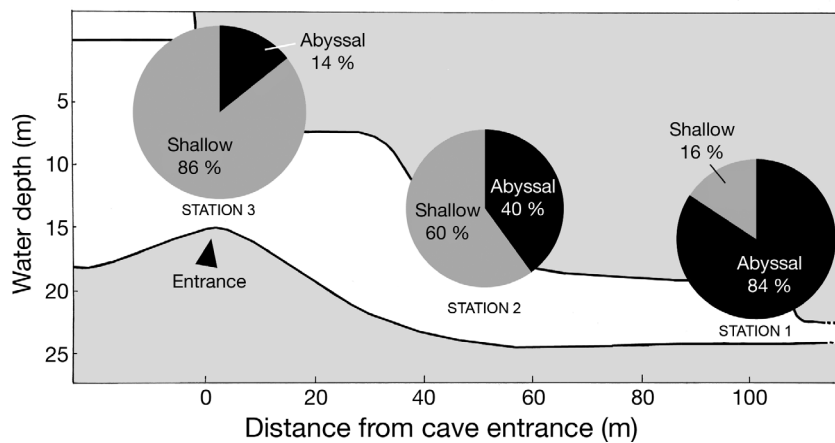
Fig. 6. Average posterior probability (expressed as %) of classes 'shallow' or 'abyssal' over 5 replicates per station at increasing distance from entrance
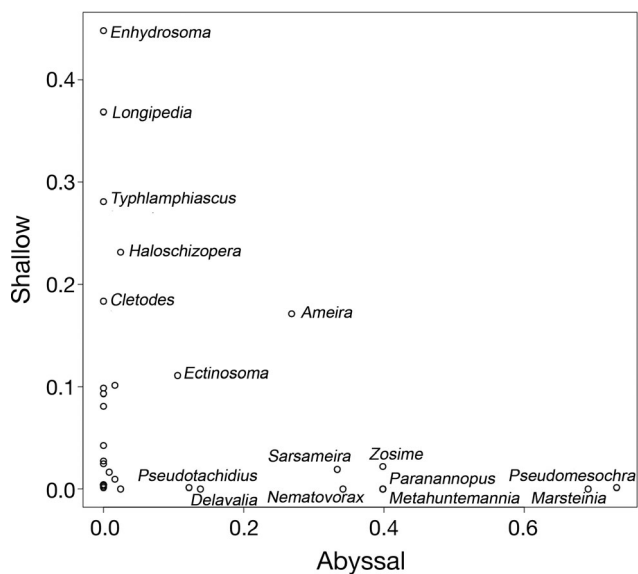

Fig. 7. Conditional probability of the 31 genera reported from literature for class 'shallow' and 'abyssal' if genus is present in the sample. For clarity, only the 16 most significant genera out of 31 are labeled

Meiofaunal assemblages from 3PP Cave versus the deep Mediterranean

The deep-sea fauna of the Mediterranean is characterized by a rather low degree of endemism and a low diversity compared to the deep-sea fauna of the NE Atlantic. The Gibraltar sill (280 m deep) has historically been regarded as a physical barrier to the potential colonization of the Mediterranean from the richer deep Atlantic fauna (Bouchet & Taviani 1992). The faunal composition of the deep Mediterranean displays a number of characteristics separating them

from most other deep-sea faunas in the world. The meiofaunal assemblage mainly consists of eurybathic species. Below 500 m, the fauna has 6 times more species in common with the upper layers than strictly deep-water species (Bouchet & Taviani 1992).

Meiofaunal densities at the entrance of 3PP Cave are very similar to those known for the Mediterranean shallow waters. The cave entrance is located within the sublittoral zone of 15 m depth. Therefore, the meiofaunal densities fall within the expected range. In contrast, the individual densities at cave end are comparable with densities known from the deep Mediterranean. The relative abundances of copepods and nematodes are also consistent with the relative abundances known for the Mediterranean deep sea at 600 m (Table 9). Further, the abundance of the tardigrades within the cave must be highlighted. They occur most frequently towards cave end. The tardigrades of 3PP Cave belong to the genus *Trogloarctus*. This genus, belonging to the deep-sea family Coronarctidae, was described by Villora-Moreno (1996) in the darkest parts of 3PP Cave. With the notable exception of *Trogloarctus*, species of Coronarctidae are almost exclusively known from sediments deeper than 1600 m. General features of the family may be considered as adaptations to deep-sea habitat, and therefore Coronarctidae seem to be a bathyal–abyssal taxon (Villora-Moreno 1996).

Knowledge of copepod systematics, diversity, and species composition in the deep sea is scarce, including descriptions of only ~500 species to date. Of the harpacticoids from benthic deep-sea samples, >95% are new to science (Seifried 2004). Many copepod families, like ameirids, canthocamptids, cletodids, miraciids, and ectinosomatids for instance, are cosmopolitan and present at any depth. Therefore, specimens of the 3PP Cave belonging to these families could originate from the shallow zones as well as from deeper areas of the Mediterranean. Previous studies on the meiofaunal assemblages of the deep Mediterranean are mostly restricted to higher-level meiofaunal taxa or only comprise planktonic copepods (Scotto di Carlo et al. 1984, 1991, de Bovée et al. 1990, Danovaro & Fabiano 1995, Tselepides et al. 2004). However, there are also many copepod families which are typical and mostly represented in the deep sea. The families Argestidae, Pseudotachidiidae, Ancorabolidae, Huntemannidae, Zosi-

Table 9. Comparison of individual densities (means ± SD) and relative mean abundances (RAD) of metazoan meiobenthos at 3PP Cave and at Grotta Piccola del Ciolo, a marine cave without coldwater homothermy. Values for the marine sublittoral Gulf of Lions and for the deep Mediterranean are also provided

Reference	Nematoda		Copepoda		Meiofauna
	Density (ind. 10 cm^{-2})	RAD (%)	Density (ind. 10 cm^{-2})	RAD (%)	Density (ind. 10 cm^{-2})
3PP Cave (this study)					
Entrance (Stn 3)	967 ± 270.7	85	45 ± 19.1	4	1146 ± 311.3
Mid (Stn 2)	309 ± 79.3	84	12 ± 2.7	3	364 ± 85.1
End (Stn 1)	143 ± 52.3	82	9 ± 3.7	5	173 ± 78.1
Grotta Piccola del Ciolo (Todaro et al. 2006)					
Entrance	1742.5	90	38.7	2	1932.8
Mid	502.6	89	24	4	564.8
End	612	86	6.7	1	709.6
Marine sublittoral Gulf of Lions, 30 m					
(McIntyre 1969)	362–1142	70–81	100–198	7–13	513–1416
Mediterranean deep sea, 600 m					
(Tselepides et al. 2004)	~250	70–82	~20	2–11	~300

meidae, Neobradyidae, and Idyanthidae are considered typical deep-sea groups (Seifried 2004). Many specimens of 3PP Cave copepods from the inner cave stations belong to these typical deep-sea taxa. A new copepod species, *Ancorabolina cavernicola* (George & Tiltack 2009), was also recently described from the same set of 3PP Cave samples. Up to that time, species of *Ancorabolina* were exclusively known from the deep sea, so they were considered to be at least bathyal. Therefore, the occurrence of *A. cavernicola* in the 3PP Cave may then probably be due to the particular and specific deep-sea-like conditions prevailing in this cave (George & Tiltack 2009). This is yet another indication that environmental conditions in this peculiar cave make it a natural mesocosm for studies of the deep Mediterranean.

Naive Bayes classification

The naive Bayes model also indicates that the copepod community living at the end of the cave resembles those of abyssal habitats (0.84 posterior probability). The mid-cave community could not be unequivocally identified as shallow or abyssal. Some of the samples were predicted to be shallow and some abyssal (3:2 respectively). This may be an indicator for a mixed community with shallow and abyssal components. At the entrance of the cave, all investigated samples were predicted to be shallow.

Naive Bayes classifiers have been extensively used in document classification and email spam filtering (Yang et al. 2002), as well as for instance classifying bacteria according to their rRNA sequence (Wang et

al. 2007). They require a small amount of training data to produce accurate models (Zhang 2004). However, the use of naive Bayes classifiers to analyze the resemblance of benthic communities between sampled stations is a novel approach of our study. We have been unable to find any previous work using this kind of statistical analysis for marine benthic communities, although it has recently been used indirectly to classify benthic communities by image analysis or LIDAR multi-signal machine learning classification (Lytle et al. 2010, Collin et al. 2011).

Bayes' rule is applied 'to get from the probability of the data, given the model, to the probability of the model given the data' (Kruschke 2011: p. 53). In our case, we use the probability of presence or absence of every copepod genus (the data), given that the sample comes from an abyssal or a shallow station (the model), to calculate the probability that a cave sample belongs to the 'abyssal' or 'shallow' class (the model), given the presence or absence of copepod genera (the data). We 'naively' assume that the presence or absence of a genus in a class ('abyssal' or 'shallow') is unrelated to the presence or absence of any other genus in that class. By doing so, every genus contributes with its own conditional probability independently of the likelihood of class membership of the sample. In the Bayesian analysis, the final classification is produced by combining both sources of information, the prior class probability and the likelihood of class membership given the attributes present, to form a posterior probability using Bayes' rule. Our training dataset consists of 123 abyssal samples and 730 shallow-water samples. So the probability that a random sample from this collection

belongs to class 'abyssal' is 123/853 = 0.144, and accordingly, the probability of belonging to class 'shallow' is 730/853 = 0.855. These are called prior probabilities in Bayesian terminology. The posterior probability of class membership ('abyssal' or 'shallow') of a cave sample is obtained by multiplying the prior probability of that class by the likelihood that the sample belongs to that class given the presence or absence of the genera. The likelihood of class membership itself is obtained by multiplying by each other the conditional probabilities of class membership of each genus given its presence or absence in the training dataset. How do we obtain the conditional probability of class membership for each genus? Say the genus *Ameira* is present in 33 out of 123 abyssal samples and in 125 out of 730 shallow-water samples in the training dataset. So if this genus is present in a cave sample, it will contribute with a conditional probability of 33/123 = 0.268 to the membership of this sample to the class 'abyssal' and 125/730 = 0.171 to the class 'shallow'. However, this genus will contribute with 0.731 conditional probability to the class 'abyssal' and 0.828 to the class 'shallow' if it is absent in the cave sample (Table 7). Remarkably (either case is true), presence or absence will contribute with a different conditional probability to the final likelihood of the sample to belong to either class.

Note that the posterior probability of class membership of a cave sample is computed separately for the classes 'abyssal' and 'shallow', and is later normalized so that the sum of both posterior probabilities equals 1. The sample is then assigned to the class with highest posterior probability. Also notice that a genus absent from a given class in the training dataset (e.g. no abyssal sample contains the genus *Cletodes*) will produce a conditional probability of 0 for that class (Table 7), which will result in the posterior probability of that class being also 0 (by multiplication by 0), canceling the contribution of any other genus to the posterior probability of that class. This is avoided by Laplacian correction, adding a minimal number (Laplacian factor) when computing conditional probabilities, so that all of them are positive and non-zero. Zero values contained in Table 7 are due to decimal rounding.

Finally, note that the sample space (the set of all possible events) for computing the prior probability and the likelihood of class assignment is inferred from a set of open-sea benthic shallow-water and deep-sea samples, but we are predicting into a different sample space, the cave, with unknown probability properties. So the correct reading of our results (e.g. for the class 'abyssal') is: if this sample were taken from the open seas, then it would most likely come from the abyss according to the genera present and to our current knowledge.

The advantage of naive Bayes classifiers lies in the assumption of conditional independence of attributes (Zhang 2004), which makes the algorithms computationally inexpensive. The attributes in the present study correspond to 'genera'. This is important in the present context, because the genera present in the cave represent only a subset of the potential abyssal and shallow-water genera present outside the cave, namely only those represented by species that could survive in the cave out of those species that eventually colonized the cave. In addition, in every sample, we only collected a subset of the pool of cave species. The naive Bayes approach is robust to this fuzziness. Once the model is trained, the presence of each genus in the sample contributes with its independent probability to the general class assignment of the sample. The final result mimics human reasoning: In our mind, the absence of some deep-sea genera in a cave sample does not prevent us from recognizing an assemblage of genera as a 'deep-sea-like' community, if most of the genera found in the sample are indeed characteristic of deep-sea habitats. But our belief in an 'abyssal' class will decrease drastically if some genera expected to be present, because they are only and always present in abyssal samples, are missing, or if genera that are only and always present in shallow-water samples are present in the cave sample.

The cave–deep sea connection

Evidence of significant ties between marine cave fauna and deep-sea fauna has been discussed earlier by Riedl (1966) and Hart et al. (1985), and is supported by certain taxa within the Crustacea, e.g. *Munidopsis polymorpha* and Mictacea (Bowman & Iliffe 1985, Just & Poore 1988), as well as the deep-sea hexactinellid *Oopsacas minuta*, the bryozoan *Puellina setiformis*, the Bresiliid shrimp *Bresilia saldanhai*, and the deep-sea tardigrade genus *Trogloarctus*, which have been found in the darkest parts of marine caves, particularly 3PP Cave (Vacelet et al. 1994, Harmelin 1997, Calado et al. 2004, Bakran-Petricioli et al. 2007, Glover et al. 2010). *Ancorabolina cavernicola*, the harpacticoid recently discovered from the present dataset from 3PP Cave (George & Tiltack 2009), also belongs to this group of taxa.

An explanation for the colonization of deep-sea species in 3PP Cave is provided by the powerful upwellings within the Gulf of Lions. With the common upwelling of deep-water masses, which occurs when the Mistral wind blows, meiofaunal assemblages from the deep are likely lifted to shallower waters. Only when they colonize favorable places like the 3PP Cave can they succeed in establishing viable communities. These colonists find in 3PP Cave some of the conditions they are best adapted to: low temperature, low food supply, low disturbance levels, absence of light, and probably a sediment granulometry and organic matter content that are very similar to that of deeper sediments. It may also be that they just survive where others cannot, i.e. where they find a sustainable level of competition. Only very few studies have provided records of environmental data in marine caves. Riedl (1966) was the first to propose a model of light attenuation as a function of depth, cave configuration, and length, but it remained mostly theoretical until Passelaigue (1989) provided detailed measurements and comparisons with the deep sea: light levels are equivalent 55 to 65 m (approx. 3PP Stn 2) inside a shallow cave in Marseille and at 350 to 400 m of depth in a nearby canyon. Longer caves, or caves with sharp turns, therefore present even smaller amounts of light, or no light at all, resembling even deeper oceanic depths. Actual *in situ* water circulation data have rarely been collected. Velocity levels are usually low, and deployment of oceanographic instruments is not always possible. Attempts at measuring water flow have been made using fluorescent dyes, or the dissolution of plaster balls (e.g. Harmelin 1969, Zabala et al. 1989). Results are often ambiguous and direct flow measurements still lacking. Fichez (1991) estimated a residence time of 8 d for the water mass in the darkest part of one cave. A rare current velocity measurement of 1 cm s^{-1} has been obtained, in a place where current flow was visible to divers (Vacelet 1996). The hydrodynamic conditions are such that the allochthonous organic inputs have been measured as equivalent to a depth of 1000 m (Fichez 1990a). The amount of suspended particulate organic matter decreases inside the caves, but also its organic content (Fichez 1991, Rastorgueff et al. 2011). Chlorophyll *a* values further demonstrate the marked oligotrophic character of the inside parts of dark caves (Fichez 1990b). As a global rule, the deep waters are significantly colder than shallow waters. Therefore, shallow cave waters are unlikely to realize the temperature conditions of the deep sea, except at high latitudes. However, a significant exception lies in the Mediterran-

ean, where the deep-water homothermy at ca. 13°C meets the surface winter conditions of at least the northernmost part of the basin. Thermal conditions have been described in common Mediterranean caves by e.g. Harmelin (1969) and Passelaigue (1989), where temperatures inside the caves generally follow that of adjacent shallow waters, although caves are buffered from outside variations. There was a paradigm shift when the 3PP Cave was discovered, due to the cold water (ca. 13 to 15°C) trapping year-round (Vacelet et al.1994, Vacelet 1996, Harmelin 1997). Later, more cold water trapping caves with a descending profile were discovered (Bakran-Petricioli et al. 2007), all presenting faunistic peculiarities, demonstrating even stronger ecological relatedness to the deep sea.

Current knowledge of the benthic communities suggests a unimodal diversity–productivity relationship, with a peak of diversity at intermediate levels (Rex & Etter 2010). Using ocean depth and distance from cave entrance as surrogates for productivity (i.e. particulate organic carbon [POC] flux or food availability), we would expect a peak in diversity in the mid-slope and in the mid-cave station. The unimodal distribution, however, seems to fail at extreme oligotrophic seas like the Eastern Mediterranean (Tselepides et al. 2000) or the Arctic Laptev Sea (Vanaverbeke et al. 1997). Remarkably, the Laptev Sea study showed a steady decrease in number of nematode genera with depth and also mentioned the same pattern for copepods. This indicates that at extreme oligotrophic conditions, the range of food availability only accounts for the descending side of the general unimodal curve, so that a steady decrease is observed. The same pattern is probably also true for marine caves and possibly other extremely oligotrophic habitats. However, our 3 stations are insufficient to characterize the true relationship between diversity and distance from the entrance (i.e. food source) of the cave. We may have ignored a peak of diversity between the entrance and the 50 m station or between the 50 m and 100 m stations.

Nevertheless, marine caves will never compete in terms of number of species with the deep sea. On the one hand, we have the largest open benthic ecosystem on earth, with few important barriers (the abyss), and on the other hand, it is a very small and almost enclosed system, with limited immigration pathways (the cave). Because marine caves cannot compare in size and connectivity with the deep seabed, they cannot harbor the entire diversity of small-sized taxa observed in the deep sea, but rather a very small subset of it.

The limiting environmental factors impede the survival of littoral species far within the cave and allow the establishment of deep-sea species at least within remote parts of the cave. In the present study, we have further explored the strong connections prevailing between marine caves and the deep sea. Additional work is in progress to to focus on the analogy between 3PP Cave meiofauna and that of the nearby deep-sea canyons. Temporal, trophic, or evolutionary processes are very difficult and costly to address in the deep sea. Shallow-water analogues such as 3PP could be used as accessible mesocosms to study such processes (Glover et al. 2010), particularly when the Mediterranean, including its deepest parts, experiences dramatic changes (Lejeusne et al. 2010).

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