Research Article

Meiobenthos and nematode assemblages from different deep-sea habitats of the Strait of Sicily (Central Mediterranean Sea)

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Abstract

Much attention is currently devoted at upgrading our knowledge on biodiversity and functioning of deep water ecosystems. Information is constantly enriched by researchers, even from basins as the long-studied Mediterranean Sea. In such a perspective, we studied meiobenthic and nematode communities inhabiting muddy sediments from three different habitats at bathyal depths in the Strait of Sicily: a cold-water coral site (CS) in the Maltese Coral Province, a muddy bottom in the same area (MS), and a hydrocarbon imprinted pockmark site (PS) in the Gela Basin. The average meiofauna density at CS (1343 ind/10 cm^2) and MS (1804 ind/10 cm^2) is much higher than that reported in literature for similar habitats; it is also markedly more elevated than that recorded at PS (224 ind/10 cm^2). Although nematodes of the three sites show different abundances, they share similar assemblage structure. Nematodes (avg. 86%) and copepods (avg. 9.3%) were the most abundant meiofaunal taxa at all sites followed by annelids, kinorhynchs and turbellarians. Nematodes were composed by 21 families and 46 genera, with *Terschellingia* as most abundant genus (12.4%), followed by *Microlaimus* (11%), *Daptonema* (11%), *Thalassomonhystera* (10.8%), *Acantholaimus* (9.5%) and *Sabatieria* (8.7%). The genera *Thalassomonhystera*, *Terschellingia, Microlaimus*, *Daptonema*, *Chromadorita*, *Sabatieria*, and *Anticoma* display a dominance in at least one station. The taxonomic structure of meiofaunal communities of the studied sites is rather similar but differences in relative abundance are evident.

Keywords: Community structure, meiobenthos, nematoda, cold-water corals, pockmark, Strait of Sicily, Mediterranean.

Introduction

The Mediterranean Sea is considered one of the most explored areas since antiquity, and boasts a remarkable biodiversity from the coastal zones down to bathyal settings (Taviani, 2014). Much is known about its resident macrobenthos, and new taxonomic and ecological information is steadily increased by the concurrent advancement of underwater exploratory technologies and the implementation of molecular approaches. Also the distribution patterns of the deep-sea meiobenthos are relatively well known (Danovaro & Fabiano, 1995; Gambi *et al.,* 2010; Sevastou *et al.,* 2013, and references therein). In general, meiobenthos density in the deep-sea Mediterranean is significantly lower as compared to the adjacent open Atlantic Ocean, and it is decreasing eastwards (Danovaro & Fabiano, 1995; Gambi *et al.,* 2010; Sevastou *et al.,* 2013). Such traits are likely governed by differences in trophic regimes, with a marked oligotrophy in the Mediterranean when compared with the Atlantic, and by a subdued influence of the organic-rich Atlantic water masses moving towards East Mediterranean (Gambi

et al., 2010, for review). This is, however, mostly true for the large-scale oceanic deep-sea habitats (open continental slope, canyons, and basins), whereas such widely spread but local mid- and small-scale deep-sea habitats as cold seeps, pockmarks, mud volcanos, hot vents, deep-water coral reefs, etc., are still scarcely studied (Zeppilli *et al.,* 2011; Vanreusel *et al.,* 2010a).

In the present study, meiobenthic and nematode communities from a cold-water coral site (CS), a closely located muddy site (MS) and a hydrocarbon seepage pockmark in the Gela Basin (PS) from the bathyal zone of the Strait of Sicily are compared. Cold-water coral habitats are bioconstructions composed primarily by slow-growing frame-building coral species which may result in biodiversity hotspots, highly vulnerable to human and natural impacts. Pockmarks are sedimentary depressions caused by the seepage of hydrocarbon-enriched fluids through the seabed. Although at times characterized by toxic compounds in sediments that negatively affect non-chemosynthetic benthic life, these habitats generate extensive spatial heterogeneity for microbes and macrofau-

402

Medit. Mar. Sci., 16/2, 2015, 402-412

na promoting high levels of biodiversity (Levin, 2005).

Cold-water coral grounds and cold seeps are often characterized by own, unique benthic communities reflecting high microhabitat heterogeneity and specific food sources (for meiobenthos, see e.g. Shirayama and Ohta, 1990; Raes and Vanreusel, 2005; Vanreusel et al., 2009; Bongiorni et al., 2010; Pape et al., 2011; Lampadariou et al., 2013). Previous studies on nematode assemblages from cold seeps revealed high variability in their density, taxonomic composition, and assemblage structures, as a function of the geographic region, depth, macrobenthic communities, remoteness from the centre of seepages (Shirayama and Ohta, 1990; Olu et al., 1997; Van Gaever et al., 2006, 2010; Portnova et al., 2010a, b; Vanreusel et al., 2010a, b; Zeppilli et al., 2011). They can exhibit high densities and, at the same time, a very low diversity (Van Gaever et al., 2006; Portnova et al., 2010b).

It was shown that nematode assemblages inhabiting sediments surrounding the living corals and related coral rubble were characterised by a higher species richness than nematode assemblages from the slope sediments and by own specific nematode taxa, which were not found in the adjacent slope sediments (Bongiorni et al., 2010). Deep-water corals form a number of microhabitats, which are occupied by different nematode assemblages (Raes and Vanreusel, 2006). The aim of the present study was to investigate the community structure patterns of meiobenthic and nematode assemblages in three different Central Mediterranean deep-sea habitats. Furthermore, it represents the first attempt to study the meiofauna of this particular area in the Strait of Sicily.

Material and Methods

Study area

Our study area includes three different sites in the Strait of Sicily: the Gela Basin (south of Sicily), a small escarpment (southwest of Malta), and a bathyal muddy bottom located next to the edge of the Malta trough (Fig. 1). The Gela Basin represents the Plio-Quaternary foredeep of the Maghrebian fold-and-thrust belt and is a site of widespread and repeated mass failure deposits. Several mass-transport deposits are recognizable in the Gela Basin as fluid-rich and poorly consolidated squeezed sediments, containing pockmarks fields colonized, since the early Holocene at least (<11 kyrs), by chemosymbiotic macrofauna such as lucinids (*Lucinoma kazani, Myrtea amorpha*), and vesycomids (*Isorropodon perplexum*) bivalves and vestimentiferans (*Lamellibrachia*) (Taviani et al., 2013; Taviani, 2014). No living chemosymbiotic macrofauna has been observed at present at this site, with *Myrtea amorpha* only providing a subrecent age (Taviani et al., 2013). The only living macrofaunal element that seems consistently associated with seepage sites is the ghost callianassid shrimp *Calliax*. Active hydrocarbon seepage at the Gela site seems at present absent or strongly reduced (Taviani et al., 2013).

The Malta Cold-Water Coral Province is a spectacular site where lush colonies of cnidarians and sponges contribute to making an important deep-sea habitat (Taviani et al., 2011). This area, first described by Schembri et al. (2007), is characterized by a sub-vertical escarpment which is connected to a large plateau between Malta and Linosa Trough. This vertical wall is an important site of coral growth being settled by communities dominated by scleractinians (i.e., *Madrepora, Lophelia, Dendrophyllia* and the solitary *Desmophyllum*), antipatharians (i.e., *Leiopathes* and *Antipathes*), soft and calcified gorgonaceans, including red coral (*Corallium rubrum*), and associated macroinvertebrates (Schembri et al., 2007; Freiwald et al., 2009; Taviani et al., 2011; Angeletti and Taviani, 2011).

Sediment sampling

The study area was surveyed during the MEDCOR cruise (December 2009) by RV "Urania". A large volume (ca. 60 l) Van Veen grab was used for sediment sampling at seven stations (Table 1). Two stations were sampled from the Maltese Coral Province, south of Malta (see references in Schembri et al., 2007; Taviani et al., 2011); this sampling site will be called CS (coral site) in the rest of the article. One station was selected from a muddy bottom slope (muddy site, MS). Four stations were chosen in the Gela Basin from a Pockmark field area described by Taviani et al. (2013); this site was called PS (pockmark site) in the study (Fig. 1).

Each sediment sample was treated as follow: a brief visual description of the macroscopic elements (geobiological) on the surface was first provided; then the redox potential profile was obtained at the sediment surface and at 1 cm sediment layer interval within the top 7 cm, in order to identify the Redox Potential Discontinuity (RPD) layer (Fig. 2). Three subsamples for meiobenthic studies were collected using a perspex tube of 2.5 cm in diameter (4.90 cm²). These latter were fixed with buffered (pH 6.9) 5% formalin-seawater solution. Meiofauna and nematodes assemblage analyses were performed in the uppermost 5 cm sediment layer.

Meiofauna was extracted from muddy sediment by centrifugation with LUDOX AM solution, retained on a 43 μm sieve and stained with Rose Bengal; finally the organisms were sorted and counted under a stereomicroscope (Higgins and Thiel, 1988). One hundred nematodes were randomly withdrawn from each core and mounted on permanent slides (Seinhorst, 1959). The nematode specimens were identified to the genus level under a 100x oil immersion objective using a Leica DM 2500 microscope and the NeMys online identification key (Steyaert et al., 2005). The genus-level analyses provide suitable taxonomic sufficiency for elucidating major macro-ecological patterns and for large-scale between-habitats comparisons of communities, and overtake the difficult and time-consuming taxonomic identification of nematodes to the species level (Vanreusel et al., 2000, 2010b).
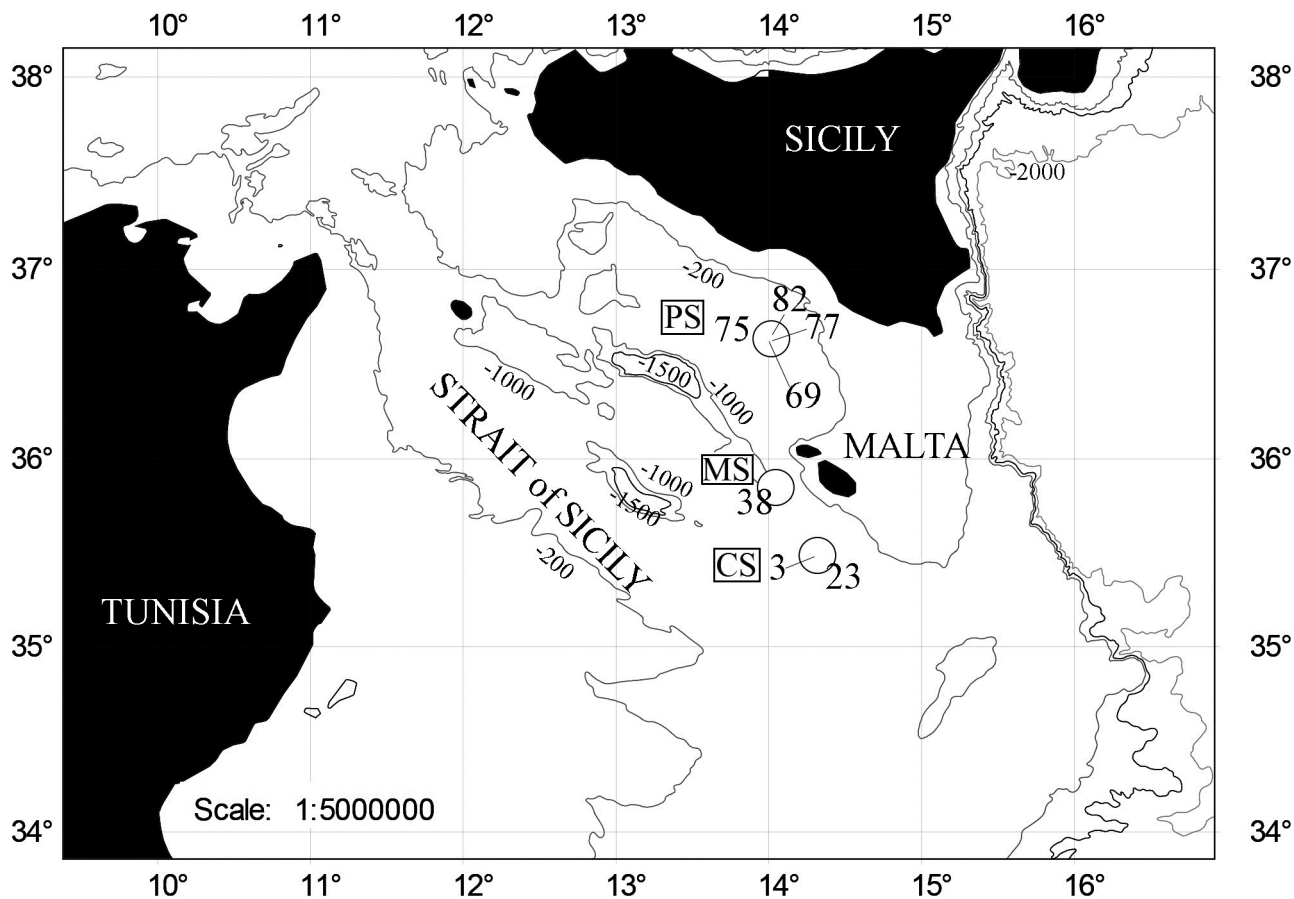
Medit. Mar. Sci., 16/2, 2015, 402-412

403

Fig. 1: Sampling area. Coral Site (CS): St. 3, 23. Muddy Site (MS): St. 38. Pockmark Site (PS): St. 69, 75, 77, 82.

Table 1. Location, coordinates, depth, date of sampling stations and number of examined nematode individuals. CS = coral site; MS = muddy site; PS = pockmark site.

Area	Station #	Date (UTC)	Latitude (N)	Longitude (E)	Depth (m)	Number of nematode individuals examined
South Malta	CS 3	11/12/2009	35°31.011'	14°05.997'	476	100
South Malta	CS 23	13/12/2009	35°30.672'	14°11.460'	726	100
West Malta	MS 38	14/12/2009	35°48.798'	14°04.901'	1033	100
Gela	PS 69	19/12/2009	36°45.631'	14°00.147'	824	100
Gela	PS 75	21/12/2009	36°45.807'	14°01.255'	833	100
Gela	PS 77	21/12/2009	36°45.743'	13°58.880'	832	*67
Gela	PS 82	21/12/2009	36°45.451'	13°59.137'	834	*98

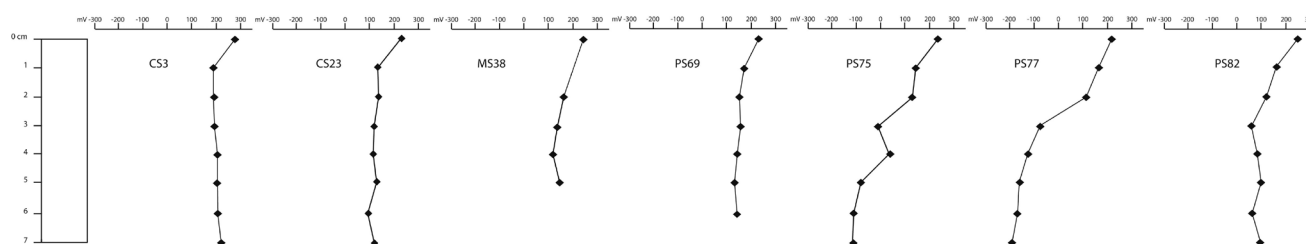*Total number of nematode individuals present in the sample.

Fig. 2: Eh profiles measured in the seven grab samples. On the left side is represented the sediment depth (cm). Redox potential in the samples PS75, PS77 and PS82 changes from positive to negative values below 4 cm sediment depth (see text for explanation).

404

Medit. Mar. Sci., 16/2, 2015, 402-412

Data analysis

The software packages PAST (Hammer *et al.*, 2001) and PRIMERv6 (Clarke & Gorley 2006) were used for statistical analysis. Analysis of similarity (ANOSIM), based on Bray-Curtis similarity distances (square root transformed), were used to analyze the multivariate data (percentage composition of nematode assemblages in samples). The SIM-PROF test was used to evaluate the degree of distinctions between samples. Similarity percentage (SIMPER) analysis, based on Bray-Curtis similarity distance, was used to assess which taxa were primarily responsible for any observed differences between groups of samples. Differences between the sets were visualized using non-metric multidimensional scaling (nMDS) plots.

Nematode diversity at genus level was measured using Margalef's richness (*d*), $\log_e$- and $\log_2$-based Shannon-Wiener's (*H'*), Pielou's evenness (*J'*) indices, and the estimated genus richness for the sample of 51 and 100 specimens (*EG(51)* and *EG(100)*, respectively) were used.

The genera found in the study sites were compared with known nematofauna described from the Mediterranean continental slope and canyons (as inhabiting the same depth range) with aid of the test of taxonomical distinction analysis (TAXDTEST) performed in the PRIMERv6 software. The Δ+ test (average taxonomic distinctness) used in this procedure shows the probability that the taxonomic distinctness of the given list of taxa represents a random selection from a specified faunistic complex. For creating a dataset, the following references were used: *Soetaert & Vincx, 1987; Decraemer & Soetaert, 1989; Soetaert, 1989; Soetaert & Heip, 1995; Soetaert et al.. 1995; Lampadariou & Tselepides, 2006; De Leonardis et al., 2008*. The taxonomic levels of 'genus-subfamily-family-superfamily-suborder-order-subclass-class' were used.

Results

Abiotic conditions

All sediments (top 5 cm layer) were composed by mud (avg. silt 87% and clay 13%). Relatively high values of the redox potential were found at all stations from the coral site (CS) and the muddy site (MS), and station 69 from the pockmark site (PS) in all sediment layers with positive values in the range of 200-350 mV (Fig. 2, Table 2); this indicates good oxidized conditions at the above mentioned stations. The remaining PS stations showed lower (55 mV) or negative (as low as –295 mV) redox potentials in the sediment layers deeper than 2 cm, indicating the presence of reduced compounds and a RPD (Redox Potential Discontinuity) layer at this sediment depth.

Total meiobenthic density

The average total meiobenthic density at CS and MS was much higher than at PS (1342±87, 1803±96, and 224±34 ind/10 cm², respectively).

The highest linear correlation (0.751) was found between the total meiobenthic density (in the upper 5 cm sediment layer) and the mean redox potential for the upper 5 cm of sediment; however this correlation was not statistically significant (p=0.051). A very low correlation was found between the meiobenthic density and the redox potential from surface and 0-1 cm sediment layers (r=0.342, p=0.425 for the average redox potential value for these two uppermost sediment layers), whereas the correlation was higher for the lower sediment layers (r=0.726, p=0.064).

Composition of meiobenthic communities

Six major meiobenthic groups (*nauplii* and copepod adults are considered a single group) were recognized in the samples (Table 3). In total, the most abundant taxon in the study area was Nematoda (86±2%) followed by Copepoda and *nauplii* (9.3±0.9%). Other groups were much less abundant: Annelida 2.3±1.4%; Kinorhyncha 1.6±1.1%; Turbellaria 0.5±0.2%; and Ostracoda 0.3±0.1%. Turbellaria and Ostracoda were virtually absent in the PS site.

The relative abundance of nematodes was higher in CS (average 86,8%) and MS (average 88,4%) than in PS (79.6%) Copepoda, Annelida and Kinorhyncha were more abundant in PS (Table 3). However, the one-way ANOSIM test showed no significant distinctions between CS, MS and PS in composition (relative abundance) of major meiofaunal groups (R=0.537, p=0.086). Scanty groups such as Turbellaria and Ostracoda were not or rarely found in PS, probably due to the much lower meiofaunal densities at this sampling site.

Nematode assemblages and diversity

Nematodes were totally represented by 21 families and 46 genera at all sites (Table 4). In total, the genus *Terschellingia* was the most abundant genus (12.4% of all examined nematodes) followed by *Microlaimus* (11%), *Daptonema* (11%), *Thalassomonhystera* (10.8%), *Acantholaimus* (9.5%) and *Sabatieria* (8.7%) (Table 4). The genera *Thalassomonhystera*, *Terschellingia, Microlaimus*, *Daptonema*, *Chromadorita*, *Sabatieria*, and *Anticoma* dominated at least on one station (Table 5).

The genus composition (relative abundance) of nematofauna inhabiting CS was much more homogeneous in comparison with PS (similarities within groups were 59% and 41%, respectively). The SIMPROF test indicated that nematode stations can be split in three groups (A, B and C) based on the relative composition of nematode assemblages, and the difference in relative abundance of nematode genera between these groups is significant (p=0.048). Group A includes all CS and MS stations and two PS stations (#69 and #75) with a high similarity within the group at genus level (66%); PS station #77 and #82 showed specific assemblages (groups B

Medit. Mar. Sci., 16/2, 2015, 402-412

405

Table 2. Redox potential (mV) at sediment surface, separate sediment layers, and for the whole upper 5 cm sediment layer (mean ± standard error).

Station #	Surface	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	Mean 0-5 cm sediment layer
CS 3	278	189	192	193	205	203	210±14
CS 23	311	198	202	181	177	194	211±20
MS 38	242	190	160	133	115	142	164±19
PS 69	230	170	150	155	141	130	163±15
PS 75	235	145	132	-10	40	-80	77±50
PS 77	268	216	162	-26	-76	-111	72±66
PS 82	248	160	120	55	79	95	126±28

Table 3. Density (ind./10 cm^2) of meiobenthos and separate meiobenthic groups (and their average percentage) for samples from coral, muddy and pockmark sites.

Meiobenthic taxa	Coral Site CS 3	CS 23	Average %	Muddy Site MS 38	%	Pockmark Site PS 69	PS 75	PS 77	PS 82	Average %
Nematoda	1231	1102	86.8	1595	88.4	196	250	108	158	79.6
Copepoda + *nauplii*	157	120	10.3	126	7.0	22	31	15	26	10.5
Annelida	23	16	1.5	39	2.2	5	11	15	16	5.3
Kinorhyncha	18	10	1.0	15	0.8	23	8	3	6	4.4
Turbellaria	2	3	0.2	21	1.2	2	0	0	0	0.2
Ostracoda	0	5	0.2	8	0.4	0	0	0	0	0
Total	1429	1255	−	1803	−	248	300	140	206	−

Table 4. Nematode orders, families, and genera (sorted alphabetically) found in the study area and their relative portion (% of total number of individuals examined) in the nematode assemblage.

Taxon	%	Taxon	%	Taxon	%
o. **Chromadorida**		Desmodoridae	0.9	Thoracomopsidae	2.6
Ceramonematidae	0.4	*Chromaspirina*	0.3	*Mesacanthion*	2.6
Metadasynemoides	0.4	*Desmodora*	0.6	Tripyloidadae	0.5
Chromadoridae	20.8	Epsilonematidae	0.5	*Bathylaimus*	0.5
Acantholaimus	9.5	*Epsilonema*	0.5	o. **Monhysterida**	
Chromadora	0.8	Leptolaimidae	0.5	Diplopeltidae	0.9
				Ceramonematidae	0.4
				Metadasynemoides	0.4
Chromadorella	0.5	*Leptolaimus*	0.5	*Araeolaimus*	0.4
Chromadorita	3.2	Microlaimidae	11.5	*Southerniella*	0.5
Dichromadora	3.0	*Aponema*	0.5	Linhomoeidae	12.7
Euchromadora	0.8	*Microlaimus*	11.0	*Desmolaimus*	0.3
Hypodontolaimus	0.9	Selachinematidae	0.4	*Terschellingia*	12.4
Metachromadora	0.3	*Richtersia*	0.4	Monhysteridae	10.8
Neochromadora	0.6	o. **Enoplida**		*Thalassomonhystera*	10.8
Parachromadorita	0.2	Anticomidae	5.1	Xyalidae	14.7
Prochromadora	0.2	*Anticoma*	5.1	*Ammotheristus*	0.8
Spilophorella	0.8	Dorylaimidae	0.4	*Daptonema*	11.0
Comesomatidae	11.2	*Mesodorylaimus*	0.4	*Elzalia*	0.8
Cervonema	0.7	Ironidae	0.5	*Paramonhystera*	0.5
Comesoma	0.8	*Dolicholaimus*	0.5	*Promonhystera*	1.1
Sabatieria	8.7	Oncholaimidae	4.0	*Rynchonema*	0.5
Cyatholaimidae	1.4	*Oncholaimus*	0.5		
Longicyatholaimus	0.3	*Viscosia*	3.5		
Marylynnia	0.9	Oxystominidae	0.8		
Paracanthoncus	0.2	*Halalaimus*	0.8		

Table 5. Percentage (%) of most abundant genera for separate stations, sampling areas, and groups of samples. Only genera which were dominant (>10%) or subdominant (>5%) at least at one station are listed. The division of samples into three groups (A, B, and C) is shown as well (according to SIMPROF analysis).

Site	Coral Site		Muddy Site	Pockmark Site				Group A (in average)
Station	CS 3	CS 23	MS 38	PS 69	PS 75	PS 77	PS 82	
Group of samples	A	A	A	A	A	B	C	
Terschellingia	11.0	10.0	15.0	22.0	13.0	6.0	6.0	14.2
Thalassomonhystera	10.0	7.0	18.0	20.0	12.0	3.0	4.2	13.4
Microlaimus	8.0	14.0	9.0	6.0	17.0	26.9	1.0	10.8
Sabatieria	6.0	12.0	9.0	8.0	18.0	7.5	0	10.6
Acantholaimus	8.0	5.0	8.0	12.0	10.0	3.0	18.4	8.6
Daptonema	5.0	19.0	9.0	2.0	7.0	22.4	17.3	8.4
Theristus	11.0	7.0	4.0	8.0	4.0	0	2.0	6.8
Anticoma	23.0	2.0	5.0	3.0	1.0	0	0	6.8
Viscosia	2.0	5.0	1.0	1.0	2.0	16.4	1.0	2.2
Mesacanthion	0	3.0	2.0	0	0	4.5	9.2	1.0
Dichromadora	2.0	0	0	1.0	1.0	0	16.3	0.8
Chromadorita	0	1.0	0	1.0	0	0	19.4	0.4

and C, respectively) (Fig. 3). The genus *Terschellingia* dominated in group A (14%), followed by *Thalassomonhystera* (13%), *Microlaimus* (11%), *Sabatieria* (11%), *Acantholaimus, Daptonema* and *Theristus* (average percentage for the group A was 9%, 8%, and 7%, respectively). The dominant genera of group B were *Microlaimus* (27%), *Daptonema* (22%), and *Viscosia* (16%); *Chromadorita* (19%), *Acantholaimus* (18%), *Daptonema* (17%) and *Dichromadora* (16%) prevailed in group C (Table 5).

No significant distinctions in diversity indices were found between CS and MS (Mann-Whitney tests: > 0.05 for all pairs of diversity indices). However, nematode diversity was noticeably higher in group A as compared with the groups of samples B and C (Table 6). No significant linear correlations between diversity indices and redox potential values were found.

In the TAXDTEST analysis, CS and MS samples were pooled in one group because of high resemblance

of their taxonomic composition. According to this analysis, the list of genera from the CS+MS is within 95% confidence limits of the taxonomic distinctness Δ+ (i.e. complexity of taxonomic structure) of the nematofauna inhabiting the Mediterranean continental slope (Fig. 4). However it shows a reduced taxonomic distinction in comparison to the nematofauna known from Mediterranean canyons. The list of nematode genera found in the PS is far outside of the 95% confidential limits of Δ+ for both slope and canyon nematofauna.

Discussion

Meiobenthic density and community structure

The study sites are situated on the upper (PS: 476-726m depth) and the mid-continental slope (CS and MS: 824-1033m depth). The meiobenthic densities for this depth range are normally 100-1000 (ca. 750 in average) and 4-400 (300 in average) ind/10 cm^2 for the Western and Central parts of the Mediterranean, respectively (Danovaro and Fabiano, 1995; Gambi *et al.,* 2010). The meiobenthos densities observed in CS and MS (average 1342 ind/10 cm^2 and 1803 ind/10 cm^2) are much higher than the above mentioned densities typical for this part of the Mediterranean. Indeed, they also seem to be in contradiction with data by Raes and Vanreusel (2006) from the cold-water coral framework in the Porcupine Seabight (NE Atlantic), where the density of nematodes (the most abundant meiobenthic taxon representing 90-95% of metazoan meiobenthos) was much lower (ca. 300 ind/10 cm^2). However, the latter study refers to nematode density from a degraded coral bottom, whereas, our study focused on sediment occurring beneath living corals, possibly more enriched in food resources.

The total meiobenthic density in PS samples was 140-300 ind/10 cm^2, which could be considered as typical for this area at such a depth range. For the nearest mud
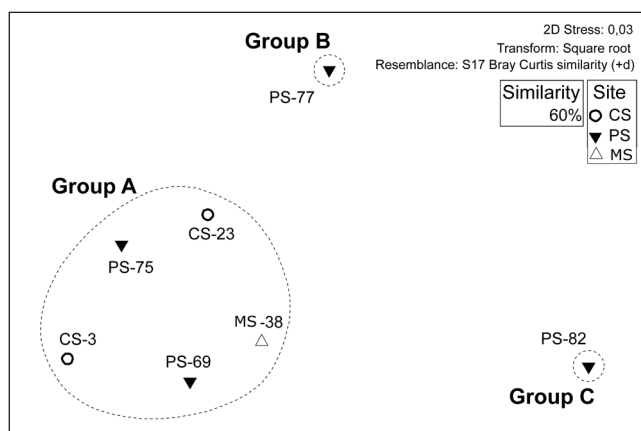

Fig. 3: Non-parametrical MDS ordination of samples from coral site (CS), muddy site (MS) and pockmark site (PS) based on nematode genus percentage abundance using the Bray–Curtis similarity index. Samples are subdivided in three groups according to SIMPROF test (see text and tables).

Medit. Mar. Sci., 16/2, 2015, 402-412

407

Table 6. Nematode diversity indices. See explanation in the text (*2.3 Data Analyses*).

Station #	Group of samples	D	J'	H'($\log_e$)	H'($\log_2$)	EG(51)	EG(100)
CS 3	A	4.13	0.814	2.44	3.52	15.2	20
CS 23	A	5.21	0.818	2.63	3.80	17.4	25
MS 38	A	5.21	0.820	2.64	3.80	18.0	25
PS 69	A	4.78	0.744	2.33	3.37	15.9	23
PS 75	A	4.34	0.788	2.40	3.48	15.2	21
PS 77	B	2.38	0.841	2.02	2.91	10.5	−*
PS 82	C	2.40	0.827	2.06	2.96	9.7	−*

* Less than 100 nematode individuals were present and examined in these samples.
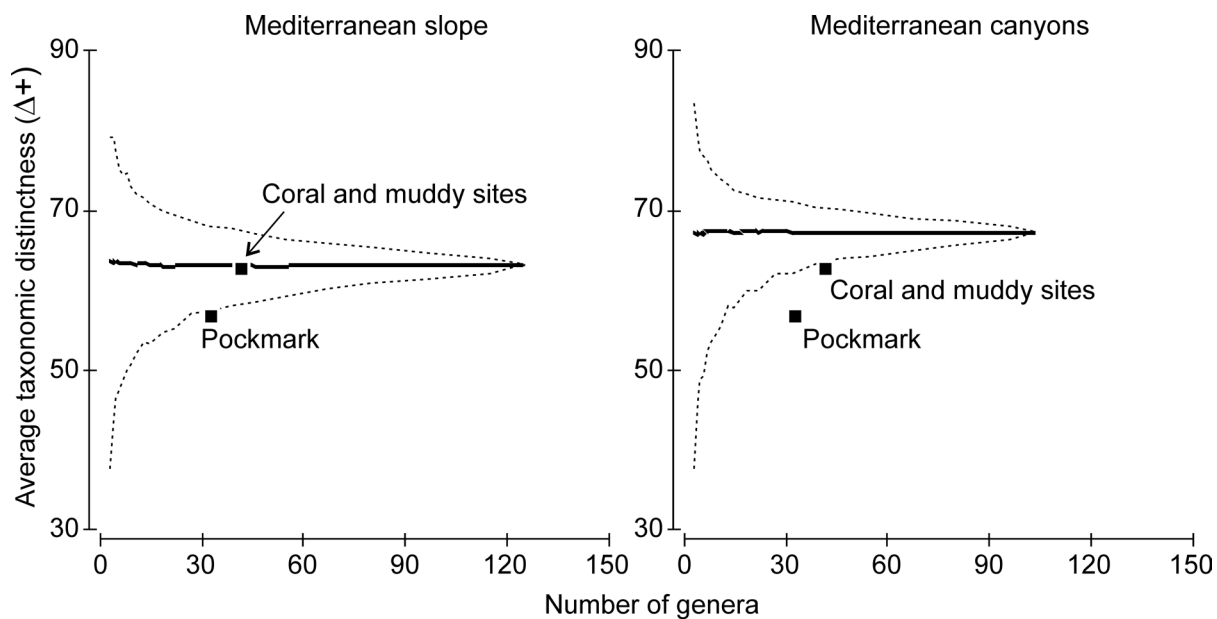
Fig. 4: Taxonomic distinctness (D+) of the nematode fauna (genus presence/absence) from the coral, muddy and pockmark sites in the Sicily Strait compared to the slope (left) and canyon (right) nematode faunas of the Mediterranean. Mean values (bold lines) are shown with upper and lower 95% confidential limits (dashed lines). The positions of the nematofauna from the coral site, muddy site and the pockmark site are marked with black squares.

volcano located in the Sicily Strait (ca. 50 km apart from the PS, depth 140-165 m), the total meiobenthic density varying from 227 to 538 ind/10 cm^2 was previously recorded (Zeppilli *et al.,* 2011), this value is higher than in the present data. The pockmark meiobenthic density was also reported as being much lower than in adjacent sediments in the western Mediterranean Sea (Zeppilli *et al.,* 2012). In fact, the meiofauna from reduced sediments colonized by chemosymbiotic macrofauna often shows significantly higher standing stocks when compared with the adjacent non-seep sediment (Shirayama and Ohta, 1990; Olu *et al.,* 1997; Pape *et al.,* 2011; Lampadariou *et al.,* 2013); sometimes its density does not significantly exceed the density of meiofauna from surrounding sediments (Olu *et al.,* 1997; Pape *et al.,* 2011). Unfortunately, it was not possible to compare the total meiobenthic density in PS with the density from the adjacent sediment. One of the possible explanations for such differences in density may rely upon the occurrence in most pockmarks of more reduced sediment conditions starting at layers

deeper than 2 cm. Indeed, three out of four samples from PS (#75, #77 and #82) are characterized by low redox potential values indicating subsurface reduced conditions. On the contrary, samples from CS and MS showed higher redox potential through the whole 5-cm sediment layer, documenting more oxidised sediments.

The studied sites were inhabited by meiobenthic communities with similar structure showing no significant differences in percentages of the most abundant taxa (nematodes, copepods, annelids). Nematodes were the most abundant taxon (average of 83% for all samples), followed by copepods with *nauplii* (9%) and annelids (2.3%). The composition on metazoan meiobenthic community at higher taxon level follows the general pattern characteristic for the continental slope of the temperate zone (Mokievskii *et al.,* 2007). The relative percentage values of the most abundant meiobenthic groups fall within the limit reported from the Mediterranean slope (Soetaert *et al.,* 1991; Danovaro *et al.,* 1995, 2000, 2013; Tselepides *et al.,* 2004; Lampadariou and Tselepides,

408

Medit. Mar. Sci., 16/2, 2015, 402-412

2006; De Leonardis *et al.,* 2008; Bianchelli *et al.,* 2010).

The ubiquitous presence of kinorhynchs is noticeable, confirming that this phylum is mainly composed by mud-dwelling species, well represented also in deep-sea sediments (e.g. Gutzmann *et al.,* 2004).

Nematode diversity

At genus level, the diversity indices in nematode assemblages in all studied sites were comparable or even higher than in other bathyal Mediterranean localities. For instance, De Leonardis *et al.* (2008) reported that the H´($\log_e$) index was 1.5-2.0, and J´ was 0.6-1.0 at genus level in the shelf and slope nematode assemblages from the South Adriatic Sea and the Gulf of Taranto (in comparison with 2.06-2.64 and 0.79-0.84, respectively, in the present study). According to the Δ+ test, it may be concluded that nematode assemblages under study are closely related to counterparts from the Mediterranean continental slope with respect to the complexity of their taxonomic structure. The taxonomical distinction of nematofauna from CS, MS and PS is significantly lower than in the Mediterranean canyons (the latter ones show higher D+ as compared with the Mediterranean continental slope). PS nematofauna has remarkably lower Δ+ than nematofauna from the CS and MS.

The nematode diversity is coherent with the composition of assemblages: two samples from PS (#77 and #82) had a significantly lower nematode diversity than all other samples (Group A samples). Low Δ+ measurement in PS and low diversity indices in two pockmark samples are in accordance with the established notion that nematode assemblages from extreme ecosystems imprinted by reduced conditions, like cold seeps and hot vents, often show a low diversity (Shirayama and Ohta, 1990; Dando *et al.,* 1991; Van Gaever *et al.,* 2010; Copley *et al.,* 2007; Portnova *et al.,* 2010b; De Groote and Vanreusel, 2011; Hauquier *et al.,* 2011). Thus, low diversity indices might indicate that samples PS77 and PS82 are influenced by more stressing environmental conditions, such as those closer to seepage (De Groote and Vanreusel, 2011; Van Gaever *et al.,* 2010).

In our study, in PS samples, J´, H´($\log_e$), H´($\log_2$), and EG(51) indices of 0.74-0.84, 2.0-2.4, 2.9-3.5, and 9.7-15.9, were respectively measured. Hauquier *et al.* (2011), reported H´($\log_e$) and EG(51) indices of 0.76 and 4.5 respectively, in the cold seep located in the western Weddel Sea (off Antarctic Peninsula). Average J´ and H´($\log_e$) for three investigated cold seeps were given as 0.49±0.28 and 1.41±1.05 (mean ± standard error), respectively in the review by Vanreusel *et al.* (2010a). Therefore, the nematode generic diversity in the PS was sensibly higher than in the above mentioned studies. As to the cold-water coral habitat, EG (100) was 33-40 in the samples from the cold-water coral framework in the Porcupine Seabight (NE Atlantic) at a depth 970-1005 m; this value is rather higher than in the CS samples (20-25).

Composition of nematode assemblages

Nematode assemblages of Group A (which included all CS and MS samples, and half of PS samples) displayed a high similarity within the group, in spite of the fact that the nematode density at CS and MS was much higher than at PS. The other two samples (PS77 and PS82) had their own assemblage structures with special complex of dominating genera.

All studied sites could be considered as having the same assemblage structure, with some differences in two samples. Possibly, these differences in the assemblage structure might be explained by environmental factors (different distance from seep fluid emission points, microhabitats with special environments, etc.) other than redox potential of sediments. As previously pointed out, cold water coral grounds and pockmarks are known to enhance the spatial heterogeneity giving rise to a number of microhabitats which can be occupied by diverse assemblages, thus increasing meiofaunal diversity (e.g. Raes and Vanreusel, 2006; Bongiorni *et al.,* 2010; Semprucci *et al.,* 2013; Van Gaever *et al.,* 2006, 2010). The density and structure of benthic communities may vary considerably depending upon sampling locations that could account for differences between samples. Nematode assemblages from two PS samples (stations #77 and #82) had significantly lower diversity indices, possibly as a consequence of their proximity to the seep fluid emission (cf. Van Gaever *et al.,* 2006; Portnova, 2010b). On the other hand, the fact that most samples (Group A) share similar assemblage structure may indicate a resemblance of habitats at these two sites. Deep-water coral and cold seeps habitats are characterized by very different environments, and it is not reasonable to assume that the observed resemblance of nematode assemblages from these two sites reflects identical feeding conditions, as the total meiobenthos density at CS and MS was much higher than at PS. A possible explanation for this resemblance could be that PS samples were collected rather far from the seep fluid emission points, as also shown by the quite high redox potential in 0-2 cm sediment layer; moreover, the diversity of nematode assemblages within the samples of Group A was also high, in comparison to nematode assemblages living close to the seep fluid emission (Van Gaever *et al.,* 2006; Portnova, 2010b).

The dominating genera differ from known nematode assemblages of the Mediterranean slope and canyons, where the strong predominance of the genus *Sabatieria* is marked in most papers (e.g. Soetaert *et al.,* 1995; Soetaert & Heip, 1995). De Leonardis *et al.* (2008) found that *Astomonema* (a genus displaying maximum abundances in reduced sulphide or methane seep sediments), *Hopperia* and *Paramonhystera* were the most abundant at a depth below 200 m in the south Adriatic Sea and in the Gulf of Taranto.

Nematode assemblages inhabiting cold water coral habitats are still scarcely studied. To date, only the coral reef from the Porcupine Seabight (NE Atlantic) was in-

Medit. Mar. Sci., 16/2, 2015, 402-412

409

vestigated (Raes and Vanreusel, 2006; Raes *et al.,* 2008). According to this study, *Desmoscolex* was the dominating genus in the sediments underlying the coral reef, followed by *Pselionema, Sabatieria, Acantholaimus* and *Desmodora.* Again, the dominating genera in our present study were different, and only few subdominating genera were present in our samples (*Sabatieria, Acantholaimus*).

The nematofauna of cold seep sites studied so far at comparable conditions elsewhere in the ocean, also differs considerably from what described at the Gela site. For example, in the neighbouring mud volcano (ca. 50 km apart), *Sabatieria, Desmodora,* and *Richtersia* were the most abundant genera (Zeppilli *et al.,* 2011). The families Comesomatidae and Xyalidae dominated in other cold seeps in the Western Mediterranean (Zeppilli *et al.,* 2012), whereas Linhomoeidae, Monhysteridae, Microlaimidae, and Xyalidae prevailed in the PS. The genus *Astomonema* was predominating in the North Sea pockmark followed by *Leptolaimus* (Dando *et al.,* 1991). The genus *Sabatieria* strongly dominated in the reduced sediments of several deep-sea cold seeps in the Mediterranean (De Groote and Vanreusel, 2011; Pape *et al.,* 2011; Lampadariou *et al.,* 2013). *Halomonhystera* were noted as a strong dominant in several studies on cold seeps from polar regions; other dominants from these regions included *Terschellingia, Sabatieria, Tricoma* and *Laimella* (Van Gaever *et al.,* 2006; Portnova *et al.,* 2010b; Hauquier *et al.,* 2011; for review see Vanreusel *et al.,* 2010a). *Daptonema* prevailed in the cold-seep nematode assemblage off Hatsushima, Central Japan followed in order of abundance by *Chromadorita* (Shirayama and Ohta, 1990). In summary, the predominance of the genera *Terschellingia* and *Sabatieria* recorded at PS well corresponds with what observed in many other cold seeps inside and outside the Mediterranean Sea.

Conclusions

The meiobenthic and nematode communities inhabiting muddy sediments from three contrasting habitats at bathyal depths in the Strait of Sicily (cold-water coral, muddy bottoms, and pockmarks) have been examined at taxonomic level to unveil similiarities and differences. In all situations, the general taxonomic structure of meiofaunal communities is rather similar with noticeable differences in relative abundances. The meiofauna is dominated by nematodes (avg. 86%, with 46 genera into 21 families) and copepods (avg. 9.3%), with annelids, kinorhinchs and turbellarians as minor taxa.

Our study revealed that in the cold water coral and muddy bottoms of this sector of the Mediterranean Sea the meiobenthic community is considerably more abundant than that reported in literature for comparable habitats, while meiofaunal density at pockmarks (PS) is comparatively lower than CS and MS, possibly limited by Eh subsurface conditions. Nematodes of the three sites show different abundances, although they share similar assemblage structure.

References

Angeletti, L., Taviani, M., 2011. Entrapment, reservation and incipient fossilization of benthic predatory molluscs within deep-water coral frames in the Mediterranean Sea. *Geobios*, 44, 543-548.

Bianchelli, S., Gambi, C., Zeppilli, D., Danovaro, R., 2010. Metazoan meiofauna in deep-sea canyons and adjacent open slopes: a large-scale comparison with focus on the rare taxa. *Deep-Sea Research I*, 57, 420-433.

Bongiorni, L., Mea, M., Gambi, C., Pusceddu, A., Taviani, M. *et al.,* 2010. Deep-water scleractinian corals promote higher biodiversity in deep-sea meiofaunal assemblages along continental margins. *Biological Conservation*, 143 (7), 1687-1700.

Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth.

Copley, J.T.R., Flint, H.C., Ferrero, T.J., Van Dover, C.L., 2007. Diversity of meiofauna and free-living nematodes in hydrothermal vent mussel beds on the northern and southern East Pacific Rise. *Journal of Marine Biological Association U.K.*, 87, 1141-1152.

Dando, P.R., Austen, M., Burke, Jr. R.A., Kendall, M.A., Kennicutt, III M.C. *et al.,* 1991. The ecology of a North Sea pockmark with an active methane seep. *Marine Ecology Progress Series*, 70, 49-63.

Danovaro, R., Fabiano, M., 1995. Meiofaunal abundance and distribution in bathyal sediments of the Mediterranean Sea: an overview. *Biologia Marina Mediterranea*, 1, 217-225.

Danovaro, R., Della Croce, N., Eleftheriou, A., Fabiano, M., Papadopoulou, N., *et al.* 1995. Meiofauna of the deep Eastern Mediterranean Sea: distribution and abundance in relation to bacterial biomass, organic matter composition and other environmental factors. *Progress in Oceanography*, 36, 329-341.

Danovaro, R., Tselepides, A., Otegui, A., Della Croce, N., 2000. Dynamics of meiofaunal assemblages on the continental shelf and deep-sea sediments of the Cretan Sea (NE Mediterranean): relationships with seasonal changes in food supply. *Progress in Oceanography*, 46, 367-400.

Danovaro, R., Carugati, L., Corinaldesi, C., Gambi, C., Guilini, K., *et al.* 2013. Multiple spatial scale analyses provide new clues on patterns and drivers of deep-sea nematode diversity. *Deep-Sea Research II*, 92, 97-106.

Decraemer, W., Soetaert, K., 1989. Peculiar new deep-sea desmoscolecid species of the genus *Protricoma* (Nematoda)

410

Medit. Mar. Sci., 16/2, 2015, 402-412

from Corsica. *Zoologica Scripta*, 18 (3), 223-229.

De Groote, A., Vanreusel, A., 2011. Meiofauna of deep-sea cold seeps in the Eastern Mediterranean area, with special emphasis on nematode biodiversity and connectivity. p. 24. In: *VLIZ Young Scientists Day,* Book of abstracts. Mees J, Seys J (Eds.). Brugge, Belgium, 25 February 2011. VLIZ Special Publication, 48.

De Leonardis, C., Sandulli, R., Vanaverbeke, J., Vincx, M., De Zio, S., 2008. Meiofauna and nematode diversity in some Mediterranean subtidal areas of the Adriatic and Ionian Sea. *Scientia Marina*, 72 (1), 5-13.

Freiwald, A., Beuck, L., Rüggeberg, A., Taviani, M., Hebbeln, D., et al. 2009. The white coral community in the central Mediterranean Sea revealed by ROV surveys. *Oceanography*, 22, 58-74.

Gambi, C., Lampadariou, N., Danovaro, R. 2010. Latitudinal, longitudinal and bathymetric patterns of abundance, biomass of metazoan meiofauna: importance of the rare taxa and anomalies in the deep Mediterranean Sea. *Advances in Oceanography and Limnology*, 1, 167-197.

Gutzmann, E., Martínez Arbizu,P., Rose, A., Veit-Kohler, G., 2004. Meiofauna communities along an abyssal depth gradient in the Drake Passage. *Deep-Sea Research II*, 51, 1617-1628.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4 (1), 4, 9pp.

Hauquier, F., Ingels, J., Gutt, J., Raes, M., Vanreusel, A., 2011. Characterisation of the Nematode Community of a Low-Activity Cold Seep in the Recently Ice-Shelf Free Larsen B Area, Eastern Antarctic Peninsula. *PLoS ONE*, 6 (7), e22240.

Higgins, R.P., Thiel, H., 1988. *Introduction to the study of meiofauna*. Smithsonian Institution Press, Washington D.C., London, 488 pp.

Lampadariou, N., Tselepides, A., 2006. Spatial variability of meiofaunal communities at areas of contrasting depth and productivity in the Aegean Sea (NE Mediterranean). *Progress in Oceanography*, 69, 19-36.

Lampadariou, N., Kalogeropoulou, V., Sevastou, K., Keklikoglou, K., Sarrazin, J., 2013. Influence of chemosynthetic ecosystems on nematode community structure and biomass in the deep eastern Mediterranean Sea. *Biogeosciences*, 10, 5381-5398.

Levin, L.A., 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. *Oceanography and Marine Biology Annual Review*, 43, 1-46.

Mokievskii, V.O., Udalov, A.A., Azovskii, A.I., 2007. Quantitative distribution of meiobenthos in deep-water zones of the World Ocean. *Oceanology*, 47, 797-813.

Olu, K., Lance, S., Sibuet, M., Henry, P., Fiala-Medioni, A. *et al.,* 1997. Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados accretionary prism. *Deep-sea Research II*, 44, 811–841.

Pape, E., Bezerra, T.N., Vanneste H., Heeschen K., Moodley M., *et al.,* 2011. Community structure and feeding preference of nematodes associated with methane seepage at the Darwin mud volcano (Gulf of Cádiz). *Marine Ecology Progress Series*, 438, 71-83.

Portnova, D., Haflidason, H., Todt C., 2010a. Nematode species distribution pattern at the Nyegga Pockmarks. P. 174. In: *Fourteenth International Meiofauna Conference, Ghent, 11-17 July 2010.* Hendrik, G., Nara Bezerra, T., Cnudde, C. *et al.* (Eds.).

Portnova, D., Mokievsky, V., Soltwedel, T., 2010b. Nematode

species distribution patterns at the Håkon Mosby Mud Volcano (Norwegian Sea). *Marine Ecology*, 32, 24-41.

Raes, M., Vanreusel, A., 2005. The metazoan meiofauna associated with a cold-water coral degradation zone in the Porcupine Seabight (NE Atlantic). pp. 821-847. In: *Cold-water corals and ecosystems*. Freiwald A, Roberts JM (Eds.). Springer-Verlag, Berlin Heidelberg.

Raes, M., Vanreusel, A., 2006. Microhabitat type determines the composition of nematode communities associated with sediment-clogged cold-water coral framework in the Porcupine Seabight (NE Atlantic). *Deep-Sea Research I*, 53, 1880−1894.

Raes, M., Decraemer, W., Vanreusel, A., 2008. Walking with worms: coral-associated epifaunal nematodes. *Journal of Biogeography*, 35, 2207-2222.

Schembri, P.J., Dimech, M., Camilleri, M., Page, R., 2007. Living deep-water *Lophelia* and *Madrepora* corals in Maltese waters (Strait of Sicily, Mediterranean Sea). *Cahiers de Biologie Marine*, 48, 77-83.

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

Semprucci, F., Colantoni, P., Baldelli, G., Sbrocca, C., Rocchi, M. *et al.,* 2013. Meiofauna associated with coral sediments in the Maldivian sub-tidal habitats (Indian Ocean). *Marine Biodiversity*, 43, 189-198.

Sevastou, K., Lampadariou, N., Polymenakou, P.N.,Tselepides, A., 2013. Benthic communities in the deep Mediterranean Sea: exploring microbial and meiofaunal patterns in slope and basin ecosystems. *Biogeosciences*, 10, 4861-4878.

Shirayama, Y., Ohta, S., 1990. Meiofauna in a cold-seep community off Hatsushima, central Japan. Journal of the *Oceanography Society of Japan*, 46, 118-124.

Soetaert, K., 1989. The genus *Desmoscolex* (Nematoda, Desmoscolecidae) from a deep-sea transect off Calvi (Corsica, Mediterranean). *Hydrobiologia*, 185 (2),127-143.

Soetaert, K., Vincx, M., 1987. Six *Richtersia* species (Nematoda, Selachinematidae) from the Mediterranean Sea. *Zoologica Scripta*, 16 (2), 125-142.

Soetaert, K., Heip, C., 1995. Nematode assemblages of deep-sea and shelf break sites in the North Atlantic and Mediterranean Sea. *Marine Ecology Progress Series*, 125, 171-183.

Soetaert, K., Heip, C., Vincx, M., 1991. The meiobenthos along a Mediterranean deep-sea transect off Calvi (Corsica) and in an adjacent canyon. *Marine Ecology*, 12 (3), 227-242.

Soetaert, K., Vincx, M., Heip, C., 1995. Nematode community structure along a Mediterranean shelf-slope gradient. *PSZN I Marine Ecology*, 16(3), 189-206.

Steyaert, M., Deprez, T., Raes, M., Bezerra, T., Demesel, I. *et al.,* 2005. *Electronic Key to the free-living marine Nematodes*. World Wide Web electronic publication. www.nemys.ugent.be. (Accessed 14 November 2014).

Taviani, M., 2014. Marine chemosynthesis in the Mediterranean Sea. pp 69-83. In *The Mediterranean Sea: Its history and present challenges*. Goffredo S, Dubinsky Z (eds) Springer Science+Business Media Dordrecht.

Taviani, M., Angeletti, L., Antolini, B., Ceregato, A., Froglia, C. *et al.,* 2011. *Geo-biology of Mediterranean Deep-Water Coral Ecosystems*. Marine Research at CNR, 6, 705-720.

Taviani, M., Angeletti, L., Ceregato A., Foglini F., Froglia C., *et al.,* 2013. The Gela Basin pockmark field in the strait of Sicily (Mediterranean Sea): chemosymbiotic faunal and carbonate signatures of postglacial to modern cold seepage. *Biogeosciences*, 10, 4653-4671.

Medit. Mar. Sci., 16/2, 2015, 402-412

411

Tselepides, A., Lampadariou, N., Hatziyanni, E., 2004. Distribution of meiobenthos at bathyal depths in the Mediterranean Sea. A comparison between sites of contrasting productivity. *Scientia Marina*, 68 (3), 39-51.

Van Gaever, S., Moodley, L., De Beer, D., Vanreusel, A., 2006. Meiobenthos at the Arctic Håkon Mosby Mud Volcano with a parental caring nematode thriving in sulphide-rich sediments. *Marine Ecology Progress Series*, 321, 143-155.

Van Gaever, S., Raes, M., Pasotti, F., Vanreusel, A., 2010. Spatial scale and habitat-dependent diversity patterns in nematode communities in three seepage related sites along the Norwegian Sea margin. *Marine Ecology*, 31, 66-77.

Vanreusel, A., Clough, L., Jacobsen, K., Ambrose, W., Jivaluk, J., *et al.,* 2000. Meiobenthos of the central Arctic Ocean with special emphasis on the nematode community structure. *Deep-Sea Research I*, 47, 1855-1879.

Vanreusel, A., De Groote, A., Gollner, S., Bright, M., 2010a. Ecology and biogeography of free-living nematodes associated with chemosynthetic environments in the deep sea: a review. *PLoS ONE* 5(8), e12449.

Vanreusel, A., Fonseca, G., Danovaro, R., Da Silva, M.C., Esteves, A.M. *et al.,* 2010b. The importance of deep-sea habitat heterogeneity for global nematode diversity. *Marine Ecology*, 31 (1), 6-20.

Zeppilli, D., Mea, M., Corinaldesi, C., Danovaro, R., 2011. Mud Volcanoes in the Mediterranean Sea are hot spots of exclusive meiobenthic species. *Progress in Oceanography*, 91 (3), 260-272.

Zeppilli, D., Canals, M., Danovaro, R., 2012. Pockmarks enhance deep-sea benthic biodiversity: a case study in the western Mediterranean Sea. *Diversity and Distributions* 18, 832-846.

412

Medit. Mar. Sci., 16/2, 2015, 402-412