From microbes to macrofauna: an integrated study of deep benthic communities and their response to environmental variables along the Malta Escarpment (Ionian Sea)

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SUMMARY: A comparative study for abundance, biomass and diversity was carried out for the prokaryote, meiofauna and macrofauna communities at three depth stations (1200, 1800 and 2100 m) along the Malta Escarpment (Mediterranean Sea). Our investigation showed a two-fold increase with depth in prokaryote abundance; the contribution of prokaryote biomass to the total benthic biomass was predominant at all depths. Bacteria were the dominant prokaryote component and Archaea formed a considerable fraction (20%-30%) of the prokaryote assemblages. The meio- and macrofauna abundances and meiofauna biomass did not decrease significantly with depth but macrofauna biomass did. The α diversity did not follow a clear bathymetric trend for both nematode and macrofauna species. Probably because of the large number of eurybathic nematode genera, nor did the turnover diversity in nematode composition change down the depth gradient. Conversely, for the macrofauna there was a perceptible change in community composition between the shallowest station and the two deeper stations. Food availability affected only the macrobenthic component. The increase in the prokaryote organisms with depth and the dominance of nematodes and macrofauna deposit feeders suggest active grazing by the two benthic components on microbes. This would transfer energy to the higher trophic levels through the microbial compartment.

Keywords: prokaryote, meiofauna, macrofauna, abundance, biomass, diversity, Malta Escarpment, Mediterranean Sea.

RESUMEN: DESDE MICROORGANISMOS A LA MACROFAUNA: UN ESTUDIO COMPARATIVO DE LAS COMUNIDADES BENTÓNICAS PROFUNDAS Y SU RESPUESTA A LAS VARIABLES AMBIENTALES A LO LARGO DEL TALUD DE MALTA (MAR JÓNICO). – Se llevó a cabo un estudio comparativo de las comunidades de procariotas, meiofauna y macrofauna sobre la abundancia, biomasa y diversidad. Fue realizado en tres estaciones en varias profundidades (1200, 1800 y 2100 m) a lo largo del talud de Malta (mar Mediterráneo). Nuestra investigación muestra que, aumentando la profundidad, la abundancia de procariotas se duplica. La contribución de la biomasa procariota al total de la biomasa bentónica predominó en todas las profundidades. Las bacterias eran la fracción procariota dominante y junto con los Archaea representaban al 20-30% del total de procariotas. Por otra parte la abundancia de la meiofauna y macrofauna y la biomasa de la meiofauna no disminuían significativamente con la profundidad, mientras que la biomasa de la macrofauna sí lo hacía. La diversidad α no siguió una tendencia batimétrica muy clara para las especies de los nematodos y macrofauna. Probablemente debido a la gran abundancia de géneros de nematodos eurybatiales, la tasa de renovación de la diversidad en la composición de nematodos tampoco cambió por el gradiente de profundidad. En cambio, para la macrofauna hubo un cambio apreciable en la composición de la comunidad entre la estación más superficial y las dos estaciones más profundas. La disponibilidad de alimentos afectó sólo al componente macrobentónico. El aumento con la profundidad de los organismos procariotas, el dominio de los nematodos y la macrofauna que se alimenta de los materiales depositados sugieren un medio activo para los dos componentes bentónicos microbianos. Esto daría lugar a una transferencia de energía a niveles tróficos superiores a través del compartimento microbiano.

Palabras clave: procariotas, meiofauna, macrofauna, abundancia, biomasa, diversidad, talud de Malta, mar Mediterráneo.

INTRODUCTION

The continental slopes are the areas of steep descent from the continental shelf to the ocean floor and constitute less than 20% of the oceans of the world (Levin and Sibuet 2012). Although estimates remain controversial, continental margin environments might account for more than 20% of total marine productivity, and for a significantly greater proportion of organic matter export, with potentially up to 50% of the biological pump transfer of organic carbon to the deep ocean and 15% of the net air-to-sea CO_2 flux (Mackenzie and Lerman 2006). In addition, at least 80% of the mass of terrigenous materials reaching the ocean is deposited in continental margin environments, and more than 90% of the total organic carbon accumulation in the ocean occurs in continental margin sediments (Mackenzie and Lerman 2006). Furthermore, these sediments host a large proportion of the unknown biodiversity and are repositories of deep-sea biomass (Ramirez-Llodra *et al.* 2010). The enhanced levels of biodiversity along slopes are hypothesized to be a source of biodiversity for continental shelves and deeper basins as well (Danovaro *et al.* 2009). Recently it has been demonstrated that higher levels of biodiversity can sustain a higher ecosystem functioning and efficiency (Danovaro *et al.* 2008a). Indeed it is extremely important to clarify the sources of deep-sea biodiversity and to identify the mechanisms that may drive this diversity and its distribution (Levin and Sibuet 2012).

For all these reasons, continental slopes are remarkable sites for investigations of the variability of the standing stock and of the diversity of all of the size-class components of the benthic fauna, as well as of the environmental variables (e.g. hydrostatic pressure and food availability) that are the potential drivers for these communities. Previous investigations have shown that the distribution, biomass and structure of deep benthic communities along depth gradients of continental margins are highly variable from site to site and at different spatial scales (Galil 2004, Guilini *et al.* 2011).

On the whole, there is a considerable decrease in meiofauna and macrofauna abundance and biomass with increasing water depth, which represents a well-known pattern in benthic marine ecology (Rex *et al.* 2006, Ramirez-Llodra *et al.* 2010). However, this general rule does not apply to all size classes of deep-sea communities. For instance, the number of bacterial and archaeal operational taxonomic units does not appear to change significantly with increasing water depth (Rex *et al.* 2006, Danovaro *et al.* 2010). A single general driver explaining the spatial variation of benthic biodiversity is clearly reductive (Coll *et al.* 2010), and various biological and environmental factors have been proposed to explain changes in species diversity with depth. These have included habitat heterogeneity, food resources, oxygen availability, temperature and water currents. However, for each deep-sea benthic group of organisms (from microfauna to megafauna), these fac-

tors can act in different combinations, which can mask other local or regional factors and generate unpredictable biotic responses (Levin *et al.* 2001).

The different benthic size components (microfauna, meiofauna and macrofauna) of the slope ecosystems of the oceans throughout the world have been investigated in different studies (e.g. Kröncke *et al.* 2000, Galéron *et al.* 2000, Rex *et al.* 2006), although there is still little information available for the Mediterranean Sea (Albertelli *et al.* 1999; Danovaro *et al.* 2010). However, an integrated analysis of size-class groups of benthic species that belong to different functional groups (Gage and Tyler 1991, Flach 1999) is seen as the best approach to understand deep-sea ecology and functioning (Flach 1999, Galéron *et al.* 2000). Furthermore in a scenario of developing understanding of the critical role of biodiversity for the functioning of marine ecosystems, it is crucial to clarify how biodiversity varies in space and which mechanisms/factors are responsible for these variations (Narayanaswamy *et al.* 2010).

To accomplish this overall objective, we sampled the microfauna, meiofauna and macrofauna and measured the key environmental variables at three different depths (1200, 1800 and 2100 m) along the continental margin of the Malta Escarpment in the Ionian Sea (Mediterranean Sea) during the *Tyrrmounts09* expedition (May 2009) on the R/V *Urania*.

Although this sampling was across a relatively short depth transect (only three depths, according to limited ship time), the aims of this investigation were the following:

(i) To describe the benthic size-class components in terms of abundance, biomass, community structure and diversity in relation to the different water depths, and to determine whether they show co-variations.

(ii) To investigate potential relationships within the benthic communities between any variations in the three biotic components and the three known potential drivers (depth, grain size and food availability).

MATERIALS AND METHODS

Study site and sampling

The sampling was carried out along the Malta Escarpment, to the southeast of Capo Passero (Fig. 1), from 8 to 19 May, 2009. The Malta Escarpment is an area of continental slope in the Ionian Sea with a drop of more than 3000 m, and it is the dominant morphological offshore feature of eastern Sicily; it separates the Hyblean-Malta plateau from the deep Ionian Basin (Argnani and Bonazzi 2005). The Malta Escarpment has been the subject of many geological investigations (e.g. Catalano *et al.* 2001, Argnani and Bonazzi 2005), although little data and information are currently available relating to its deep benthic communities (e.g. meiofauna, macrofauna).

Sediment samples were collected at three depths (hereinafter also referred to as the depth stations) along
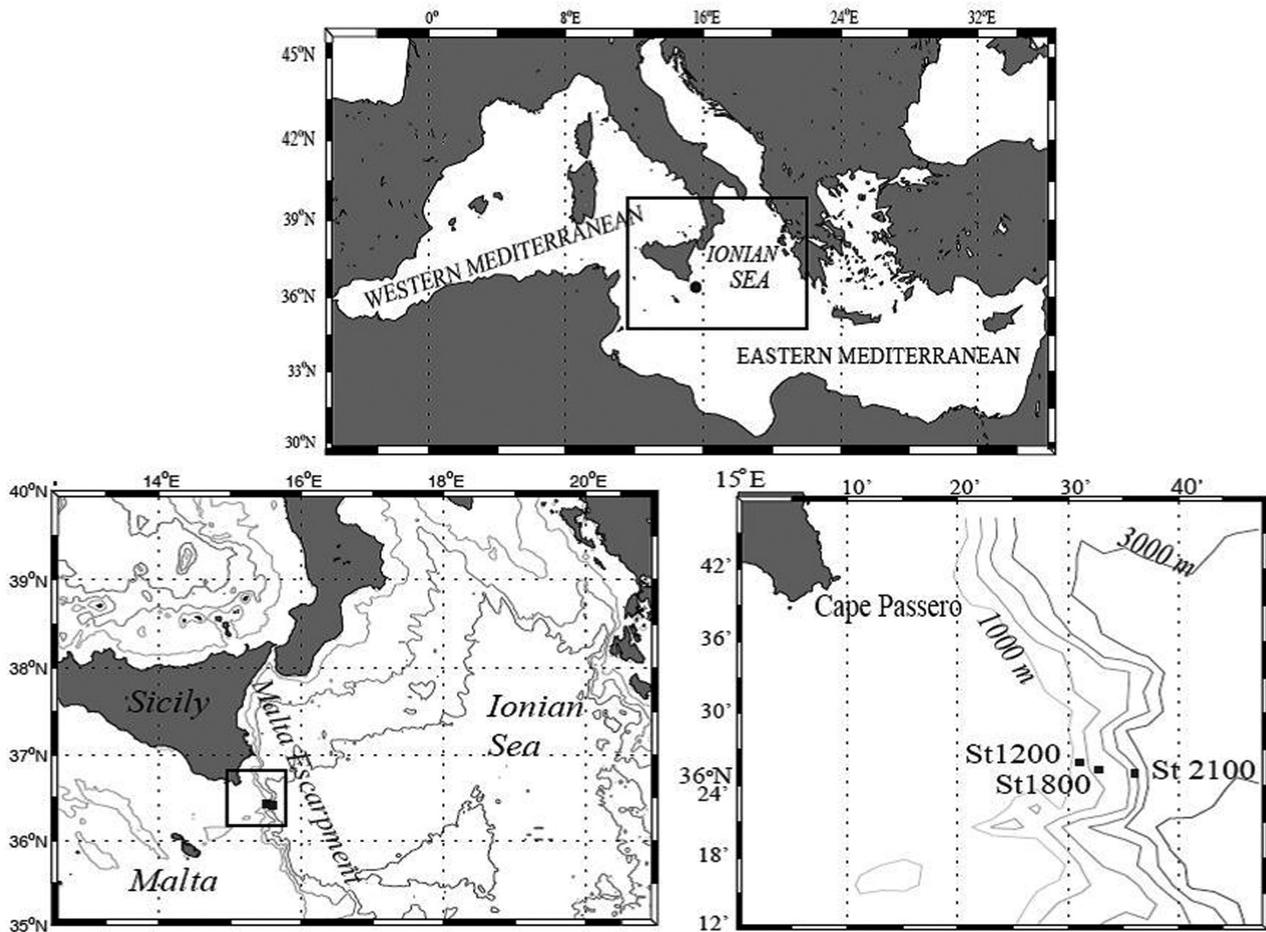
Fig. 1. – Map of the study area along the Malta Escarpment, showing the three water depth stations: St1200, St1800, St2100.

a single transect: 1200 m, 1800 m and 2100 m for a total regional distance between sites of 9 kilometres (~ 3 and ~ 6 km between the 1200 and 1800 m stations and between the 1800 and 2100 m stations, respectively). At each depth station, three independent replicates were collected for the analysis of the macrofauna, using a circular box corer that covered an area of 803.84 cm^2 (inner diameter, 32 cm). Sub-samples of the prokaryotes, meiofauna and sediments (for the measurement of organic matter content and sediment grain size) were taken from three separate box corer hauls from each station.

Measured variables

The temperature, conductivity and dissolved oxygen were measured at each depth station using a SeaBIRD 911 CTD probe. As performed for the macrofauna (see below), the grain-size analyses were done on the top 20 cm of sediment for each replica. These samples were dry-sieved to separate the coarser part (≥63 μm) from the finer part (<63 μm), which was analysed with a Helos/KF Sympatec laser spectrometer. According to many studies conducted in the deep sea (e.g. Pusceddu

et al. 2010, Pape *et al.* 2013) and for an easier comparison with other work, total protein, carbohydrate, lipid, chlorophyll-*a* and phaeopigment contents were determined from superficial (0-1 cm) sediment samples (±1 g) and following the standard techniques (Danovaro *et al.* 1999). The carbohydrate (CHO), protein (PRT) and lipid (LIP) concentrations were converted into carbon equivalents using the conversion factors 0.40, 0.49 and 0.75 μgC μg^{-1}, respectively, and normalized to sediment dry weight after desiccation (60°C, 24 h). The biopolymeric organic carbon (BPC) was calculated as the sum of the carbon equivalents of protein, lipid and carbohydrate (Fabiano *et al.* 1995). Chloroplast pigment equivalents (CPEs) were defined as the sum of the chlorophyll-*a* and phaeopigment concentrations.

As reported in Danovaro (2010), the total prokaryote counts were determined from the top 1 cm layer of the sediment using staining with acridine orange (Luna *et al.* 2002) and expressed as cells m^{-2} and cells g^{-1} (Fig. 1a, supplementary information Table S1). The prokaryote biomass was estimated using an ocular micrometer and is expressed as mgC m^{-2} and μgC g^{-1} (supplementary information Table S1), with the assignment of the prokaryote cells to different size classes

based on their maximum width and length (Fry 1990); these were then converted into biovolumes using the estimate of the average carbon content of 310 fgC μm^{-3} (Fry 1990). The total numbers and biomass were normalized to the sediment dry weight after desiccation (60°C for 24 h).

To investigate the prokaryote community structure, fluorescence in situ hybridization (FISH) was used, with rRNA-targeted oligonucleotide probes and signal amplification (catalysed reporter deposition; CARD), as previously described (Molari and Manini 2012). The oligonucleotide probes used were EUB338-mix (EUB338, 5'-GCTGCCTCCCGTAGGAGT-3', EUB338-II, 5'-GCAGCCACCCGTAGGTGT-3', and EUB338-III, 5'-GCTGCCACCCGTAGGTGT-3') to target total *Bacteria*, ARCH915 (5'- GTGCTC-CCCCGCCAATTCCT-3') to target total *Archaea*, CREN537 (5'-TGACCACTTGAGGTGCTG-3') to target *Crenarchaeota* Marine Group I, EURY806 (5'-CACAGCGTTTACACCTAG-3') to target *Euryarchaeota* Marine Group II, and NON338 (5'-ACTC-CTACGGGAGGCAGC-3') as negative control.

For the meiofauna analysis, sediment was taken from each box corer using a plexiglas tube with a 3.6-cm internal diameter; this sample was immediately fixed with 4% buffered formalin and rose bengal. For our purposes, only the sediment from the top 5 cm was sieved through 300 µm and 20 µm mesh, as most of the meiofauna are usually concentrated in the top 2 cm, or at least in the top 0 cm to 6 cm (Tselepides *et al*. 2004). The fraction remaining on the 20 µm sieve was resuspended and centrifuged with using Ludox HS40 (Danovaro, 2010). The meiofauna specimens were counted (individuals [ind] m^{-2} and ind 10 cm^{-2}; supplementary information Table S1) and identified to their major taxa under a stereomicroscope. One hundred nematodes from each replicate (or all of the nematodes where there were fewer than 100 specimens) were hand-picked and mounted on permanent slides for the analysis of nematode diversity. The nematodes were identified to the species level (due to the presence of many unknown deep-sea species, the species level is indicated, for example, as sp1, sp2, sp3, etc.) according to Platt and Warwick (1983), Warwick *et al*. (1998), and the NeMys database (Deprez *et al*. 2005). The nematode volumes and biomass (mgC m^{-2} and µgC 10 cm^{-2}; Supplementary Information Table S1) were calculated using the Andrassy (1956) formulae, and the nematode wet weights were converted into carbon biomass according to Jensen (1984). Then nematode feeding types (1A, 1B, 2A, 2B) were determined following the classification provided by Wieser (1953). The nematode trophic diversity index (ITD; Heip *et al*. 1985) and maturity index (Bongers 1990) were calculated. The nematode trophic diversity indices ranged from 0.25 (high diversity; all trophic groups present) to 1.00 (low diversity; only one group present). The maturity index is used to estimate the stability of a community, and this index can range from 1 (r-strategy nematodes) to 5 (K-strategy nematodes).

Accordingly with different works on deep-sea macrobenthos, in which the first 15-20 cm were usually considered (e.g. Flach and de Bruin 1999, Witte 2000), the macrofauna was sampled in the first 20 cm of the sediment of all of the box corer samples (three replicates per depth station). The sediments were gently washed over 300 µm mesh size, and the part that remained was fixed in borax-buffered seawater with 10% formalin, and stained with rose bengal before sorting in the laboratory. In the present study, these macrofauna samples included large Nematoda, Copepoda and Ostracoda. All of the organisms were counted and identified under a stereomicroscope to the lowest possible taxonomic level, to provide abundance (ind m^{-2}) values per species. The biomass was measured as mg wet weight m^{-2} and then converted to mgC m^{-2} using the conversion factors of Rowe *et al*. (1991). Four major macrofauna trophic groups were identified, according to the literature (Sars 1882, Norman and Stebbing 1886, Fauchald and Jumars 1979, Gage and Tyler 1991): surface deposit feeders; subsurface deposit feeders; carnivores/scavengers; and filter feeders/suspension feeders.

The nematode and macrofauna species diversities were measured using the H' log-base 2 Shannon-Wiener index (Shannon and Weaver 1949) and the evenness J' index (Pielou 1975). The species richness was calculated as the total number of species collected at each depth station. The species abundance data were converted into rarefaction diversity indices (Sanders 1968, as modified by Hurlbert 1971), and the expected number of species ES(n) for a theoretical sample of n=51 nematodes and n=31 macrobenthic individuals was calculated for each depth station.

For the estimation of the meiofaunal and macrofaunal diversity and feeding mode, we are aware of the weakness of the results due to the problem that many macrofaunal organisms have been recognized at a higher taxonomic level and for the meiofauna only the group of nematodes has been taken in consideration, even if it was always the most abundant and usually considered as representative of the whole community (Danovaro 2010).

Statistical analyses

To test for differences in the biological and environmental variables according to the three water depths, one-way analysis of variance (ANOVA) was applied using the STATISTICA8 software. Prior to the analyses, the homogeneity of variance was tested with the Cochran test, and when necessary, the data were appropriately transformed. When significant differences were encountered, a Tukey post hoc comparison test (at $p<0.05$) was performed.

Statistical differences in the nematode and macrobenthic species compositions and community structure compositions of the major taxa (i.e. turnover diversity) among the investigated depths were

TABLE 1. – Sediment variables for the three increasing water depths along the Malta Escarpment. Data are means ± standard deviation. CPE, chloroplast pigment equivalent; PRT/CHO, protein-to-carbohydrate ratio; BPC, biopolymeric organic carbon.

Depth (m)	%sand	%silt	%clay	CPE (µg g^{-1})	Proteins (mg g^{-1})	Carbohydrates (mg g^{-1})	Lipids (mg g^{-1})	PRT/CHO	BPC (mgC g^{-1})
1200	2.3	28.7	63.2	4.15±0.59	1.67±0.28	0.45±0.08	0.86±0.23	3.68±0.14	1.64±0.33
1800	0.3	30.9	62.4	8.35±0.46	1.58±0.20	0.54±0.03	0.56±0.02	2.92±0.37	1.41±0.09
2100	1.6	34.5	56.5	1.63±0.25	1.27±0.07	0.60±0.18	0.32±0.08	2.20±0.55	1.11±0.12

detected using ANOSIM analysis. All absolute data were presence/absence transformed prior to analyses, and a similarity matrix based on Bray-Curtis similarity was produced. One-way similarity percentage (SIMPER), non-parametric, statistical routines were conducted on the presence/absence transformed data using Bray-Curtis similarity matrices to determine which meiofauna or macrofauna taxa contributed most to the dissimilarities between stations at different depths. Non-metric multi-dimensional scaling analysis was performed (Plymouth Routines In Multivariate Ecological Research; PRIMER 6; Plymouth Marine Laboratory, UK) to obtain graphic visualization of the similarities (Bray-Curtis) among the macrofauna and nematode assemblages of the depth stations.

Spearman rank correlation analysis was initially carried out to identify the relationships between the prokaryote, meiofauna and macrofauna abundances, biomass, trophic structures, and diversity indices (for nematodes and macrobenthic organisms) and the investigated environmental parameters (depth, grain size and organic matter content). Non-parametric multiple regression analysis (McArdle and Anderson 2001) was performed to detect possible correlations between biological descriptors (abundance, biomass, community trophic structure and diversity indices) and environmental variables (depth, grain size and organic matter content), using distance-based multivariate analysis for a linear model (DISTLM), with forward selection of predictor variables (i.e. environmental variables) carried out with tests by permutation (Anderson, M.J., University of Auckland, New Zealand). The multiple regression tests were based on Bray-Curtis dissimilarities (untransformed data); p values were obtained by 4999 permutations of the original data. ANOSIM and SIMPER analyses were performed using the PRIMER 6 software package.

RESULTS

Environmental parameters

The water mass along the Malta Escarpment from the shelf to 3000 m depth was homogeneous, with temperatures ranging from 13.50°C to 13.75°C, and salinity ranging from 38.74 to 38.76. The dissolved oxygen content ranged from 4.13 ml l^{-1} to 4.30 ml l^{-1}. The velocity of the currents was 0.05 to 0.10 m s^{-1}. Most of the sediments were clay (on average, 60.7%), followed by silt (31.4%) and small amounts of sand (<2.5%), at all three depths (Table 1).

TABLE 2. – Output of one-way ANOVA and Tukey post hoc tests carried out across the three increasing water depths, to determine the significances of the differences in the sediment organic matter concentrations and benthic components. F, ANOVA F statistic; p, probability level, as ***=p<0.001, **=p<0.01, *=p<0.05, and ns, not significant. Tukey post hoc tests specified as significant differences between stations. CPE, chloroplast pigment equivalent; PRT/CHO, protein-to-carbohydrate ratio; BPC, biopolymeric organic carbon; ES(51), expected species number; H'(log$_2$), Shannon diversity index log$_2$.

Variables	F	P	Tukey post-hoc
Total phytopigments	164.5	***	1200<1800>2100
Proteins	3.36	ns	ns
Carbohydrates	1.3	ns	ns
Lipids	10.63	**	1200>2100
Prot:Carb ratio	10.84	**	1200>2100
C biopolymeric	4.83	ns	ns
Prokaryotes density	8.46	*	1200<1800<2100
Prokaryotes biomass	25.78	**	1200<1800<2100
%EUB	0.62	ns	ns
%ARCH	3.79	ns	ns
%EURY	4.12	ns	ns
%CREN	6.69	*	1800>2100
Meiofauna density	0.61	ns	ns
Meiofauna biomass	0.35	ns	ns
n° TAXA	8.45	*	1800>1200
IDT	0.19	ns	ns
MI	0.18	ns	ns
J'	0.13	ns	ns
ES(51)	1.72	ns	ns
H'(log$_2$)	3.62	ns	ns
SR	0.57	ns	ns
1A %	6.84	*	1800>1200
1B %	2.8	ns	ns
2A %	28.95	***	1200<1800>2100
2B %	6.61	*	1200>1800
Macrofauna density	0.67	ns	ns
Macrofauna biomass	7.74	*	1200>2100
n° TAXA	1	ns	ns
SR	0.64	ns	ns
J'	0.15	ns	ns
ES(31)	0.37	ns	ns
H'(log$_2$)	0.5	ns	ns
SDF %	0.73	ns	ns
SSDF %	2.77	ns	ns
CNV/SCV%	0.67	ns	ns
FF/SF %	2.8	ns	ns

The total phytopigments expressed as the CPEs are reported in Table 1. The CPEs differed significantly between all of the depths (Table 2), with the highest values at 1800 m depth.

The distributions of the biochemical components of the sedimentary organic matter showed different patterns for all of the variables investigated (Table 1). Proteins formed the dominant class of organic compounds in the top 1 cm of the sediments, with *ca*. three-fold higher concentrations than the carbohydrates and

lipids. While the total protein and total carbohydrate concentrations did not differ significantly between the depth stations (Table 2), the total lipid concentrations showed a significantly lower concentration at the deepest station (2100 m; Tables 1, 2). The BPC defines the sum of the protein, carbohydrate and lipid components, and its level represents an indicator of the food availability in the sediment. The BPC distribution mirrored that of the dominant class, protein (Table 1). The protein-to-carbohydrate ratio (Table 1, PRT/CHO) is indicative for both the aging and the quality of the organic matter content. Here values of this ratio greater than 1 indicate relative high quality and high food availability for organisms (Pusceddu *et al.* 2010). This protein-to-carbohydrate ratio in the present study significantly decreased with increasing depth (Table 2, PRT/CHO), although the mean values remained greater than 1 at all depths (Table 1, PRT/CHO), indicating high food quality for all of these depth stations.

Benthic standing stocks

The total benthic prokaryote abundance and biomass (Fig. 2a, supplementary information Table S1) almost doubled with depth, from $1.83\pm0.27\times10^{12}$ cells m^{-2} to $3.34\pm0.71\times10^{12}$ cells m^{-2}, and from 59.23 ± 4.99 mgC m^{-2} to 117.58 ± 16.37 mgC m^{-2}, respectively. Prokaryotes were the only biotic component to show any significant increase with depth (Table 2).

The meiofauna abundance showed no clear bathymetric trend (Fig. 2b, supplementary information, Table S1), although it showed a pronounced inter-replica variability, especially for the shallowest depth station (1200 m). The mean values of the meiobenthic abundance ranged from $1.8\pm1.8\times10^{5}$ ind m^{-2} (at 1200 m) to $3.0\pm1.2\times10^{5}$ ind m^{-2} (at 1800 m), with no significant differences between the three depths (Fig. 2b, Table 2). There was a general, although not significant, decrease in biomass with depth (Fig. 2b, Table 2). The total mean meiobenthic biomass varied from a minimum of 9.6 ± 5.5 mgC m^{-2} (at 2100 m) to a maximum of 19.2 ± 13.7 mgC m^{-2} at the shallowest station (at 1200 m; Fig. 2b). The total macrofauna abundance showed means ranging from 557 ± 312 ind m^{-2} (at 1200 m) to 365 ± 144 ind m^{-2} at the deepest station (at 2100 m), without any significant differences between the three depths (Fig. 2c). Unlike the abundance pattern, there was a clear (>10-fold) and significant decrease in biomass detected with increasing water depth (Fig. 2c, Table 2), with means ranging from 28.2 ± 14.6 mgC m^{-2} (at 1200 m) to 1.8 ± 1.0 mgC m^{-2} (at 2100 m; Fig. 2c).

Figure 3 shows the changes in the biomass contributions to the total benthic biomass (sum of total prokaryote, meiofauna and macrofauna biomass) of each of the benthic components with depth, expressed as the ratios between the benthic standing stocks. The contribution of prokaryotes to the total biomass significantly increased with increasing depth (p<0.01), due to the large increase in the prokaryote biomass, in con-
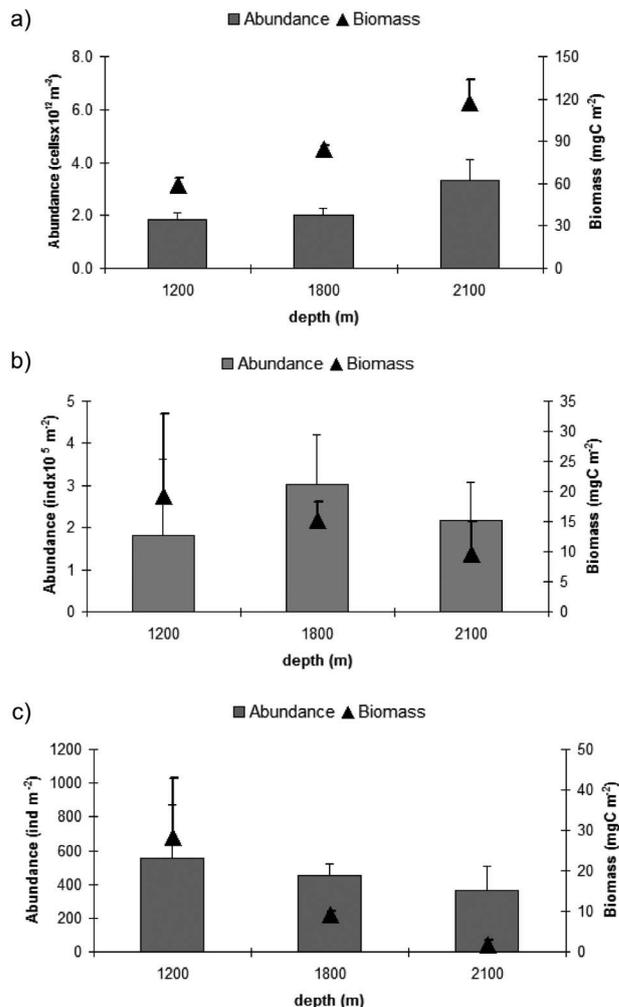

FIG. 2. – Distributions of the different benthic components for the three increasing water depths. (a) Total prokaryote abundance (cells × 10^{12} m^{-2}) and biomass (mgC m^{-2}). (b) Total meiofauna abundance (ind m^{-2}) and biomass (mgC m^{-2}). (c) Macrofauna abundance (ind m^{-2}) and biomass (mgC m^{-2}). Data are means ± standard deviation.

trast to the meio- and especially macrofauna biomass contributions, which decreased with depth (Fig. 3).

Prokaryote, meiofauna and macrofauna community structures

Overall, the number of cells visualized using the EUB338 and ARCH915 probes accounted for 87% to 92% of the total prokaryote abundance (Table 3). The abundance of bacteria and Archaea increased with increasing water depth (Table 3), although without showing significant differences (Table 2). The contribution of bacteria to the total prokaryote abundance was always dominant, with similar percentages (62% to 70%) at all three depths (Table 3). The use of specific probes for the domain of the Archaea revealed two major groups at all three depths: Crenarchaeota and Euryarchaeota, with Crenarchaeota always present in greater numbers than Euryarchaeota (Table 3). The

TABLE 3. – Total and relative abundance of the different components of the prokaryote communities for the three increasing water depths. Data are means and ± standard deviation.

Depth (m)	Abundance (cells $10^8 g^{-1}$)				Relative abundance (%)			
	Bacteria	Archaea	Euryarchaeota	Crenarchaeota	Bacteria	Archaea	Euryarchaeota	Crenarchaeota
1200	1.10±0.26	0.34±0.13	0.18±0.03	0.24±0.07	66.84	20.74	10.93	14.01
1800	1.12±0.01	0.56±0.01	0.14±0.06	0.33±0.05	61.53	30.73	7.82	18.25
2100	2.10±0.04	0.60±0.12	0.17±0.01	0.27±0.06	69.79	19.94	5.75	8.93

relative distribution of Crenarchaeota varied significantly with water depth, with the highest contribution at the intermediate depth (1800 m) (Tables 2, 3).

For the meiofauna community composition, a total of seven major taxa were identified and usually characterized the deep meiobenthic populations: Nematoda, Copepoda with their nauplii, Ostracoda, Kinorhyncha, Turbellaria, Tardigrada and Gastrotricha (Fig. 4a). All of these seven taxa were represented at the intermediate depth station (1800 m), which was the most diversified of the three depth stations. As expected, at all depths, nematodes were the dominant group, providing from 87% (at 1800 m) to 98% (at 1200 m) of the total meiobenthic abundance. The other taxa were, in order of dominance, Gastrotricha (maximum 9% at 1800 m, but absent at 1200 m) and Copepoda with their nauplii (maximum 3% at 2100 m), with the remaining taxa at <1% (Fig. 4a). In terms of biomass the nematodes were confirmed as dominant everywhere, while the contribution to the biomass of the other taxa had the same order of dominance as for abundances (Fig. 4b). The relative contribution of these other taxa to meiofauna biomass, however, was greater than that for abundance. The ANOSIM analysis revealed significant differences in the meiofauna taxa composition between the 1200-m and 1800-m depth stations (R=88.9%, p<0.01), with the major contribution to this difference formed by Gastrotricha and "others" (52.1% and 26.3%, respectively; SIMPER analysis), in terms of both abundance and biomass.

The number of macrofauna taxa decreased with depth, from 10±1 at 1200 m to 8±2 at 2100 m, although without showing any significant differences. Polychaeta was the most abundant group, as usual for deep-sea sediments, and their relative contribu-
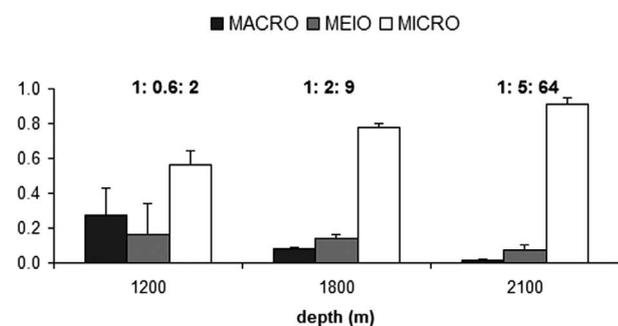
tion to the community structure decreased with depth (from 49.9% at 1200 m to 29.2% at 2100 m; Fig. 5a). Conversely, the macrobenthic nematodes increased in abundance with depth, from 7.5% at 1200 m to 21.1% at 2100 m. The Crustacea, which were mainly represented by Isopoda, Amphipoda and Tanaidacea, were the second most abundant group, with the greatest contribution at 1800 m (30.3%). The Mollusca had the highest contribution at the deepest station (2100 m; 20.6%), mainly because of the high numbers of Bivalvia, and especially *Nucula* sp1. The ANOSIM analysis showed significant differences in the macrofauna taxa composition between the 1200-m and 2100-m depth stations (R=100%; p<0.01), mainly because of Oligochaeta, Sipuncula and Nemertina at the shallowest depth station (1200 m; with their contributions to the dissimilarity of 21.6%, 21.7% and 20.8%, respectively; SIMPER analysis).

The Polychaeta were also dominant in terms of macrofauna biomass (Fig. 5b). At the deepest station (2100 m), they were accompanied by a high contribu-

FIG. 4. – Meiofauna community structures for the major taxa for the three increasing water depths, as indicated. (a) Abundance. (b) Biomass. Others: Ostracoda, Kinorhyncha, Turbellaria, Tardigrada combined.

FIG. 3. – Mean ratios for the prokaryotes (MICRO), meiofauna (MEIO) and macrofauna (MACRO) biomass values on total benthic biomass (mgC m^{-2}) for the three increasing depths. Data are means ± standard deviation. Ratios for macro: meio: micro are also shown.
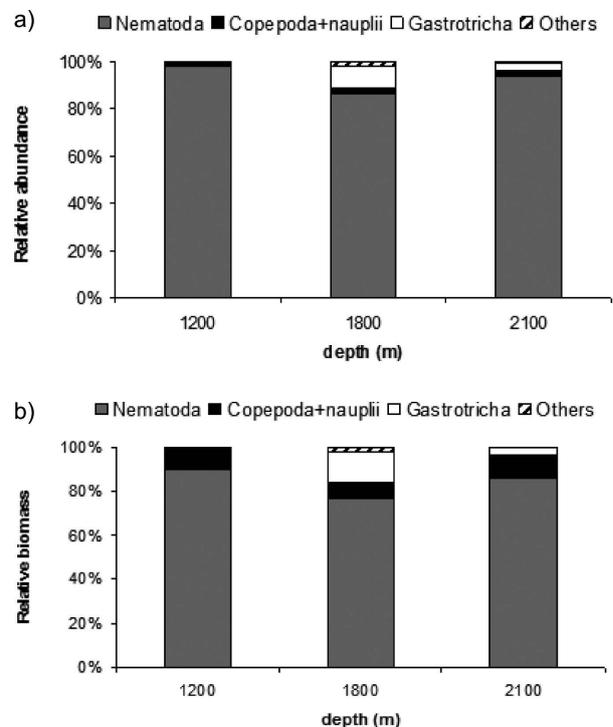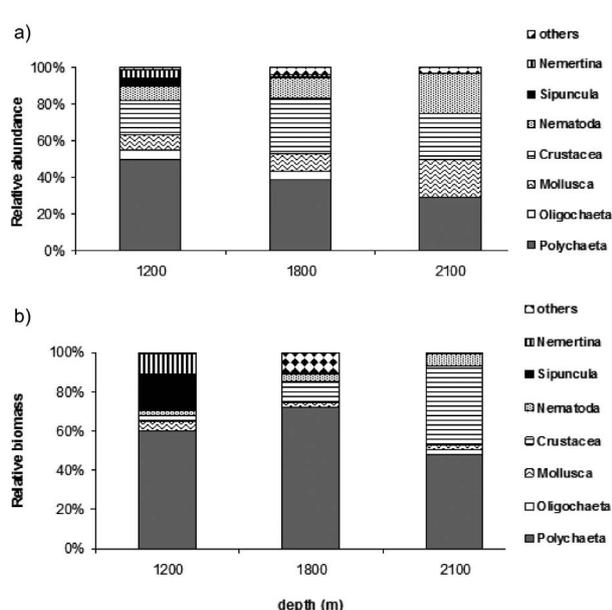
a)

b)

Fig. 5. – Macrofauna community structure of the major taxa (as indicated) for the three increasing water depths. (a) Abundance. (b) Biomass. Others: Hydrozoa, Porifera and Echiura combined.
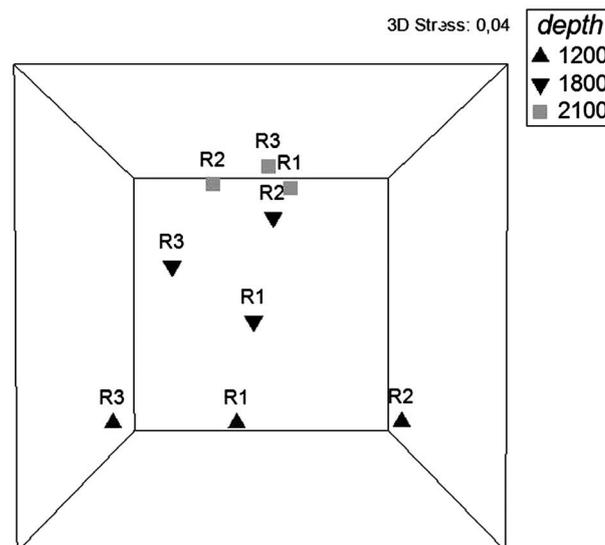

Fig. 6. – Non-metric multi-dimensional scaling ordination plot based on macrofauna species assemblages across the three increasing water depths. All replicates are shown.

tion of Crustacea (33.5%) because of the presence of large Amphipoda belonging to the families of Phoxocephalidae and Lysianassidae. The Crustacea was the only macrofauna group of which the biomass increased with depth. The Mollusca and macrobenthic nematode contributions to the total macrofauna biomass did not exceed 5% at any of the depth stations (Fig. 5b). The ANOSIM analysis revealed no significant differences between the three depths in term of the biomass contributions of the different taxa.

Nematode and macrofauna diversities

Overall, 21 families, 61 genera of Nematoda and a total of 81 species were identified (supplementary information, Table S2). The highest number of families (19) and species (57) was seen at the 1800-m depth station. The species richness or "sample" diversity (i.e. α diversity) of the nematodes is reported in Table 4. This α diversity (SR) did not change significantly with depth (Table 2), although the diversity and equitability (J') indices were greatest at 1800 m depth. The high values of J' at all of the three depths showed that there

were no dominant nematode species (Table 4). The ANOSIM analysis, which was performed to analyse any changes in nematode species composition between the stations at different depths, showed no significant differences in the nematode assemblages. The maturity index ranged from 3.0±0.1 (at 1200 m) to 3.2±0.1 (at 1800 m), indicating a greater abundance of nematodes with K-strategies at all three depths, including the genera *Halalaimus, Desmoscolex, Leptolaimus* and *Sphaerolaimus* (for a full species list, see supplementary information, Table S2).

A total of 68 macrofauna organisms were identified at the lowest possible taxonomic level (supplementary information, Tables S3 and S4). The macrofauna diversity indices are given in Table 4. As in the α diversity of the nematode composition, none of the diversity indices or the Pielou index of equitability showed any significant differences with increasing water depth. The index of equitability (J') showed an absence of any dominant species also in the macrobenthic communities.

Conversely to what has been reported for nematode compositions, the ANOSIM analysis revealed that the composition of the macrofauna assemblages significantly differed between the 1200-m and 1800-m stations (R=35%; p<0.05) and between the 1200-m

TABLE 4. – Diversity indices for the nematodes (meiofauna) and macrofauna for the three increasing water depths. Data are means ± standard deviation. H'($\log_2$), Shannon-Wiener diversity index $\log_2$; ES(n), expected species number, as ES(51) for meiofauna and ES(31) for macrofauna; J', Pielou's index of equitability.

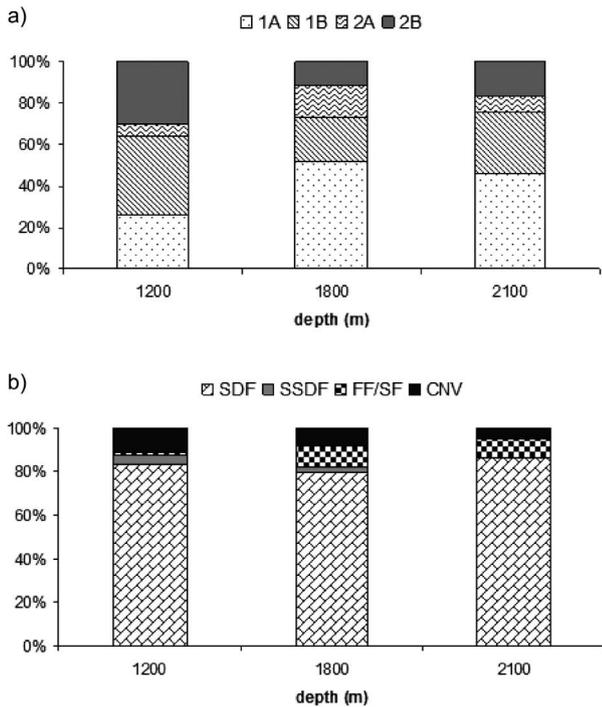|	Depth (m)	Species richness	H'($\log_2$)	ES(n)	J'
Meiofauna	1200	25.3±9.1	4.0±0.3	18.3±5.5	0.89±0.06
	1800	31.3±6.8	4.6±0.2	20.1±2.0	0.93±0.01
	2100	30.0±5.3	4.4±0.2	17.3±3.7	0.90±0.00
Macrofauna	1200	25.3±11.5	4.3±0.8	19.5±4.0	0.94±0.00
	1800	23.7±3.2	4.3±0.2	24.7±3.1	0.93±0.01
	2100	18.7±5.0	3.9±0.4	23.2±3.1	0.93±0.06

FIG. 7. – Relative proportions of the different trophic groups (as indicated) for the three increasing water depths. (a) Nematodes. 1A, selective deposit feeders; 1B, non-selective deposit feeders; 2A, epistrate feeders; 2B, predators. (b) Macrofauna. SDF, surface deposit feeders; SSDF, sub-surface deposit feeders; FF/SF, filter feeders/suspension feeders; CNV, carnivores/scavengers.

and 2100-m stations (R=85%; p<0.01). The high coefficients of dissimilarities from the SIMPER analysis confirmed the ANOSIM findings: 62.6% dissimilarity between the 1200-m and 1800-m stations, and 72.7% dissimilarity between the 1200-m and 2100-m stations. The non-metric multi-dimensional scaling representation in Figure 6 clearly shows the separation between the stations at the different depths.

Nematode and macrofauna feeding guilds

As expected for deep-sea nematode communities, the two most represented feeding groups at the 1800-m and 2100-m stations were the selective deposit feeders (1A; 52% and 46%, respectively). At 1200 m, the most represented were the non-selective deposit feeders (1B; 38%), which ingest small food particles and bacteria (Fig. 7a). However, the contributions of the other trophic groups were not negligible. The predators (2B) were significantly high at the shallowest station (1200 m; 30.7%; Fig. 7a), mainly because of *Sphaerolaimus* spp. The group of epistrate feeders (2A) was significantly more represented at 1800 m (15%), in agreement with the higher CPE. For the Malta Escarpment the nematode ITD index showed mean values ranging from 0.31±0.01 (at 1200 m) to 0.37±0.04 (at 1800 m), with no significant differences between the three depth stations.

Considering the macrobenthic trophic structure, the contributions of each trophic group showed no significant differences with depth, in contrast to the nematodes (Table 2). Surface deposit feeders were the most common trophic macrofauna group at all of the stations (Fig. 7b), with a maximum of 87% at 2100 m and a minimum of 80% at 1800 m. The second group was represented by the carnivores and scavengers (11%) at 1200 m, mainly because of the Polychaeta, such as *Glycera* sp., Arabellidae and Syllidae, and the Nemertina. On the other hand, at 1800 m and 2100 m, the group of filter feeders/suspension feeders (11% and 9%, respectively) was the second most represented, because of Bivalvia (*Kelliella* sp.), Hydrozoa, Porifera, and Polychaeta (Chaetopteridae). There were also sub-surface deposit feeders at 1200 m and 1800 m (5% and 2.3%, respectively). Though surface deposit feeders dominated the community structure, the other trophic groups were not negligible.

DISCUSSION

Variabilities in the deep-sea benthic components along the Malta Escarpment

The tremendous geomorphological, hydrographic, geochemical, and biogenic heterogeneity on the seafloor influences the diversity of the margins at multiple spatial scales (Levin and Sibuet 2012). Until recently, the majority of research on these margins focused on slopes with soft sediments (Rex and Etter 2010), which represent by far the most extensive continental margin habitat and are home to one of the most diverse marine communities known (Grassle and Maciolek 1992). The imperative for finding, cataloguing, and understanding margin diversities derives from the many key functions, goods and services provided by continental margin ecosystems, and by the increasingly deep human footprint on our continental slopes (Ramirez-Llodra *et al.* 2010). Furthermore, a description of multilayer patterns in standing stock and biodiversity that includes the different benthic size components is essential to adopt an integrated approach to the study of deep-sea systems (Boissonas *et al.* 2002).

A review by Rex *et al.* (2006) showed overall that there is a significant exponential decrease in both abundance and biomass of meiofauna, macrofauna and megafauna with increasing water depth. Bacterial abundance and biomass, however, show no decline with depth. Our research on the benthic prokaryote abundance and community structure along the Malta Escarpment has provided data that are consistent both with pooled datasets from different regions (Schmidt *et al.* 1998, Rex *et al.* 2006) and with studies carried out at local scales elsewhere in the Mediterranean (Boetius *et al.* 1996, Deming and Carpenter 2008). Bacteria were the dominant prokaryote component, in agreement with the results from similar hybridization studies carried out in marine sediments (Vetriani *et al.* 1999,

TABLE 5. – Spearman rank order correlations between the biological descriptors and environmental variables across the three increasing water depths. Marked correlations are significant at p<0.05 (italic numbers) and p<0.01 (bold numbers). CPE, chloroplast pigment equivalent; PRT/CHO, protein-to-carbohydrate ratio; BPC, biopolymeric organic carbon; ES(51) and ES(31), expected species number; H'(log$_2$), Shannon diversity index log$_2$.

	depth	% sand	% silt	% clay	CPE	PRT	CHO	LIP	PRT/CHO	BPC	cell m^{-2}	mgC m^{-2}
prokaryote (cell m^{-2})	*0.843*	-0.264	**0.843**	**-0.843**	-0.500	-0.600	0.150	*-0.900*	**-0.683**	**-0.750**	1.000	0.900
prokaryote (mgC m^{-2})	*0.949*	-0.474	**0.949**	**-0.949**	-0.433	*-0.767*	0.250	*-0.950*	**-0.883**	**-0.900**	0.900	1.000
%EUB	0.316	0.316	0.316	-0.316	*-0.667*	-0.550	-0.183	-0.317	-0.383	-0.600	0.500	0.433
%ARCH	-0.053	*-0.685*	-0.053	0.053	*0.733*	0.550	0.350	0.150	0.150	0.400	-0.183	-0.100
%EURY	*-0.685*	0.422	*-0.685*	0.685	0.383	*0.667*	-0.400	0.600	**0.867**	**0.750**	-0.450	-0.750
%CREN	-0.422	-0.422	-0.422	0.422	*0.800*	0.600	0.067	0.517	0.350	0.533	-0.567	-0.383
meiofauna (Ind m^{-2})	0.158	-0.316	0.158	-0.158	0.117	-0.133	0.017	-0.383	-0.400	-0.317	0.367	0.367
meiofauna (mgC m^{-2})	-0.158	-0.316	-0.158	0.158	0.417	0.233	0.000	-0.033	0.000	0.017	0.117	0.017
n° TAXA	0.485	**-0.888**	0.485	-0.485	0.358	-0.009	0.562	-0.562	-0.536	-0.264	0.426	0.485
ITD	0.369	-0.580	0.369	-0.369	0.167	-0.067	0.350	-0.367	-0.400	-0.217	0.333	0.483
MI	0.369	-0.580	0.369	-0.369	0.183	-0.117	0.367	-0.567	-0.517	-0.267	0.383	0.433
J'	0.000	-0.474	0.000	0.000	0.583	0.033	-0.017	0.033	0.050	0.117	-0.367	-0.117
ES(51)	0.422	-0.527	0.422	-0.422	0.050	-0.067	0.450	-0.300	-0.367	-0.250	0.283	0.300
H'(log2)	0.474	**-0.791**	0.474	-0.474	0.217	-0.100	0.617	-0.383	-0.533	-0.300	0.217	0.367
SR	0.239	-0.239	0.239	-0.239	-0.109	0.067	0.462	-0.160	-0.235	-0.101	0.227	0.118
1A %	*0.685*	-0.738	*0.685*	*-0.685*	0.067	-0.483	0.350	*-0.767*	*-0.750*	-0.600	0.517	**0.733**
1B %	-0.399	**0.718**	-0.399	0.399	-0.277	0.202	-0.395	0.513	0.605	0.319	-0.202	-0.420
2A %	0.318	**-0.873**	0.318	-0.318	0.510	0.251	0.536	-0.234	-0.293	0.000	0.176	0.268
2B %	-0.662	**0.767**	-0.662	0.662	-0.109	0.510	-0.351	0.628	0.644	0.611	-0.393	-0.653
macrofauna (Ind m^{-2})	-0.369	0.264	-0.369	0.369	0.050	0.367	0.067	0.600	0.383	0.517	-0.617	-0.500
macrofauna (mgC m^{-2})	*-0.949*	0.474	*-0.949*	0.949	0.417	0.750	-0.217	0.917	0.850	0.883	**-0.883**	**-0.983**
n° TAXA	-0.514	0.216	-0.514	0.514	0.197	0.607	0.316	0.573	0.462	**0.735**	*-0.693*	*-0.735*
SR	-0.423	0.053	-0.423	0.423	0.268	0.577	0.351	0.594	0.410	**0.695**	*-0.703*	-0.611
J'	0.105	0.264	0.105	-0.105	-0.450	-0.150	0.333	-0.200	-0.150	-0.050	0.100	0.000
ES(31)	-0.264	-0.105	-0.264	0.264	0.300	0.617	0.500	0.383	0.350	**0.667**	-0.483	-0.517
H'(log2)	-0.422	0.053	-0.422	0.422	0.283	0.600	0.367	0.533	0.433	**0.717**	-0.650	-0.617
SDF %	0.079	0.318	0.079	-0.079	-0.418	-0.201	-0.234	-0.176	-0.142	-0.276	0.452	0.326
SSDF %	*-0.693*	0.346	**-0.693**	**0.693**	0.201	0.237	-0.237	**0.749**	0.402	0.347	*-0.785*	-0.621
CNV/SCV%	-0.265	0.291	-0.265	0.265	-0.033	0.134	0.050	0.142	0.285	0.268	-0.243	-0.385
FF/SF %	0.485	-0.646	0.485	-0.485	0.281	-0.043	0.221	-0.383	-0.264	-0.162	0.298	0.358

Ishii *et al.* 2004). As shown by other authors (Vetriani *et al.* 1999, Molari and Manini 2012), Archaea abundance formed a considerable fraction (20%-30%) of the deep benthic prokaryote assemblages. Their increasing abundance with water depth is in agreement with other studies (Schippers *et al.* 2005, Molari and Manini 2012). Looking more in detail at the Archaea composition, the counts for Crenarchaea were higher than those for Euryarchaea at all of the depth stations. A Crenarchaea dominance has been reported in other marine benthic archaeal assemblages (Teske and Sorensen 2008). This finding also agrees with studies in the mesopelagic and bathypelagic domains (Herndl *et al.* 2005). Furthermore, we also found other archaeal benthic clusters in the deep-sea surficial sediments that were not hybridized by the CREN537 and EURY806 probes. This finding is consistent with previous diversity studies that have reported a complex archaeal population structure of the deep-sea seafloor, and high archaeal diversity in marine sediments (Vetriani *et al.* 1999, Auguet *et al.* 2009). The total meiofauna abundance and biomass did not decrease significantly with depth, in contrast with results from other slope areas, where significant decreases in meiofauna stock with depth have been reported (Cartes *et al.* 2002, Tselepides and Lampadariou 2004); however, the present data are similar to reports by Tselepides *et al.* (2004) and Lampadariou and Tselepides (2006) from eastern Mediterranean slopes. In the present study, the short bathymetric transect considered may well make it difficult to detect a clear decreasing trend in meiofauna standing stock. Nevertheless, the pronounced inter-replicate variability was consistent with previous studies on small-scale distributions of meiofauna, which have shown that meiobenthic communities can be highly variable even within a few tens of centimetres (De Bovée *et al.* 1990, Agnes *et al.* 2011). The mean values of the meiofauna abundance and biomass along the Malta Escarpment correspond to the range of values reported for other slope areas and for bathyal depths along the Mediterranean Sea continental margins (Soetaert and Heip 1995, Tselepides *et al.* 2004, Lampadariou and Tselepides 2006, just for stations in the Aegean sea), although these are lower in comparison to other areas at similar depths from other oceans (Soltwedel 2000).

Nematodes usually have higher diversity with increasing depth (Coll *et al.* 2010, Danovaro *et al.* 2010), although a clear trend of increasing or decreasing nematode diversity and species richness with depth is not always found (Lampadariou and Tselepides 2006). Along the Malta Escarpment, neither the α diversity nor the turnover diversity of the nematode species composition showed significant and clear changes between the depth stations, probably becaue of the many eurybathic nematode genera found at almost all of these depth stations. Some of the most represented genera, such as *Halalaimus, Leptolaimus, Syringolai-*

mus, Sabatiera, Sphaerolaimus, Metasphaerolaimus, Linhystera and *Desmoscolex*, are widespread in the deep sea, and they inhabit a wide bathymetric range and a variety of habitats (e.g., Soetaert and Heip 1995, Lampadariou and Tselepides 2006, Vanreausel *et al.* 2010). Furthermore, the high values of the maturity indices, which were here always *ca.* 3, showed how all of these depths were characterized by more K-selected genera and stable nematode communities (Hasemann and Soltwedel 2011).

Similar to the meiofauna, the macrofauna abundance along the Malta Escarpment did not decrease with depth, in contrast to some other studies conducted in the central-eastern and western Mediterranean Sea (Kröncke *et al.* 2003, Mamouridis *et al.* 2011). On the other hand, there was a lower biomass at the deepest station, following the generally recognized trend of decreased biomass with increased water depth (Rex *et al.* 2006). The biomass appears to be generally more affected by the depth rather than the density. Indeed, for the macrofauna, Rex *et al.* (2006) reported a steeper slope of decreasing biomass rather than abundance at increasing water depths. Tselepides *et al.* (2000) showed a similar trend in the south Aegean sea: a sharper decrease with depth in biomass rather than in the number of macrofauna organisms. Our macrofauna abundances seen here along the Malta Escarpment are greater than those reported for the eastern Mediterranean basin, although the biomass values are similar (Tselepides *et al.* 2000, Kröcke *et al.* 2003), and they are higher than those reported in a study conducted in the western Mediterranean basin (Tahey *et al.* 1994). Comparable macrofauna abundances were found by Kröncke *et al.* (2003) in the Ionian Sea, though they reported a higher total biomass. Our biomass values are consistent with those reported from stations below 4000 m in depth by Kröncke *et al.* (2003). However, comparisons with all of these studies must be made with care, as the mesh sizes used have often differed widely between studies (Soltwedel 2000).

One of the most evident changes in the community structure with increased depth along the Malta Escarpment was a turnover of the abundances of Polychaeta *versus* the macrobenthic nematodes. Similar changes were seen by Flach and de Bruin (1999) for the Goban Spur slope and by Sharma *et al.* (2011) in the Gulf of Mexico, where they showed a positive relationship between the dry biomass of the macrobenthic nematodes and the water depth, as in the present study. All of the macrobenthic nematodes identified along the Malta Escarpment belong to the groups of non-selective deposit feeders (1B) and carnivores (2B), as reported by Sharma *et al.* (2011) and Sharma and Bluhm (2011), and these are functionally different from the meiobenthic nematodes from this area (which mostly belong to groups 1A and 1B).

As along the Malta Escarpment, nematodes of different size classes have been shown to have different functional roles and to partition food sources

(Sharma and Bluhm 2011). The high proportion of deposit-feeding nematodes in the deep sea reflects the important role of bacteria in their nutrition (Danovaro *et al.* 2008b). Furthermore, the dominance of large detritivores and deposit-feeding nematodes indicates that they play important roles in the carbon recycling in the benthic food web. Predators and omnivores comprised the second most dominant feeding type to characterize these depth stations in the present study. Most predators and omnivores are also facultative scavengers (Heip *et al.* 1985), and this combination of feeding types is a successful feeding strategy in food-limited habitats, such as the deep sea (Sharma *et al.* 2011). Also, the gain in importance with depth of molluscs, such as *Nucula* sp1, and crustaceans, such as Ischnomesidae (Isopoda) and *Leptognathia* sp1 (Tanaidacea), can be expected, as these are widespread organisms that inhabit the deep sea and that show high plasticity in their feeding strategies (Gage and Tyler 1991). Nevertheless, polychaetes remained one of the most represented groups, with some abundant families, such as Spionidae, Paraonidae, Cirratulidae and Heterospionidae. These have been commonly reported in other studies (e.g. Karakassis and Eleftheriou 1997) as successful inhabitants of the deep sea (Fauchald 1977). Although the values of the α diversity did not change between these depth stations, as was seen for the nematode communities, diversified macrofauna populations emerged at the different depths. The separation between the 1200-m and 1800-2100-m depth stations provided by the non-metric multi-dimensional scaling and confirmed by the ANOSIM analysis indicate a change in the macrofauna composition along the bathymetric gradient that was more evident than was seen for the nematode communities. Additionally, the low coefficient of similarity (36%) between the replicates from the shallowest depth station indicates a particularly patchy distribution of organisms at 1200 m depth. Conversely, the deeper communities showed more inter-replicate and among-station homogeneity, in agreement with the idea of a higher homogeneity in benthic populations that characterize the lower slope areas, as suggested by Carney (2005). The differences between the depths was affected by the presence of "rare" species that occurred as singletons or in very low numbers in each sample within the replicates at the same depth (particularly at 1200 m), as well as between the depths. This phenomenon has been widely reported in deep-sea macrobenthic communities (Grassle and Macioleck 1992, Rex and Etter 2010).

Contributions of different benthic size classes to total biomass and energy transfer

A comparison between the biomass estimates of the different benthic components can provide information on the energy transfer pathways in benthic food webs (Albertelli *et al.* 1999). A more rapid decrease in the macrobenthos standing stock, and generally in the big-

TABLE 6. – DISTLM-forward analysis: results of the forward selection procedure. Variable, significant explanatory variable(s); SS(Trace), portion of sum of squares relative to the analysed predictor variable; pseudo-F, statistic; *p*, significance level (in italics the significant *p* values); prop, proportion of variation explained by the explanatory variable.

	Variable	SS(Trace)	pseudo-F	*p*	prop
Prokaryotic abundance	Depth	4.2371	6.376	0.0232	0.48
Prokaryotic biomass	% Clay	48.2857	59.150	0.0006	0.89
	Depth	2.9729	6.2974	0.048	0.06
Meiofauna N° taxa	% Sand	2936.6803	7.8967	0.0074	0.53
Nematoda trophic group (2A%)	% Sand	121.5000	38.5617	0.003	0.85
Nematoda trophic group (2B%)	% Sand	661.5000	13.9263	0.0166	0.67
Macrofaunal biomass	BPC	1128.0079	17.2322	0.0006	0.71

ger organisms, is a universal phenomenon that involves complex changes in the relative importance of each of the different size groups (Rex and Etter 1998, Rex *et al.* 2006). Usually this restriction in size arises from low amounts of food (Wei *et al.* 2010). In the present study, the significant increase in prokaryote biomass of the total biomass suggests two things: (i) a greater ability of prokaryotes to exploit the organic carbon in the sediments, as compared to the other two benthic classes; and consequently, (ii) a partial channelling of the carbon to the higher trophic levels through the prokaryote component (Danovaro *et al.* 2000). As deposit feeding is one of the most widely adopted feeding strategies with both meiobenthic and macrobenthic organisms along the investigated area, this suggests grazing on microbes by organisms belonging to the higher trophic levels and could be indicative of a direct food chain, as already described for benthic communities inhabiting food-limited areas (Boetius *et al.* 1996, Kröncke *et al.* 2000, Kröncke and Turkey 2003).

Different benthic size classes in relation to the environmental variables

In energetically stressed environments, such as the deep sea, it is often difficult to identify processes that affect the distribution and abundance of different biotic assemblages. This is because both biological and physical factors are involved, they are not independent, and their relationships with the taxa of interest are frequently non-linear. The amount of CPEs in deep-sea sediments has been shown to be a function of the superficial primary production, and of the depth and efficiency of removal along the water column as the particles of organic matter sink to the seafloor (Pusceddu *et al.* 2010). The overall refractory nature of the autotrophic fractions in the sediments along the Malta Escarpment, which show the very low chlorophyll-a levels of between 0.2% and 2.6% of the total CPEs, has also been observed in other deep-sea sediments (Garcia *et al.* 2007). Despite this, the potential quality of the food that characterizes the Malta Escarpment sediments is closer to the richer western Mediterranean deep sediments than to those of the more oligotrophic east basin or south Adriatic Sea (Pusceddu *et al.* 2010). This sediment composition also supports a macrofauna community that is dominated by surface deposit feeders at all of these depths, instead of sub-surface deposit

feeders and carnivores; indeed, a high percentage of surface deposit feeders is usually associated with medium-to-high quality of organic matter (Wieking 2002, Mamouridis *et al.* 2011), which confirms that food is one of the most important variables that influence benthic community structures (Mamouridis *et al.* 2011).

Significant correlations were seen between the macrofauna biomass and the quantity and quality of the organic matter, as well as between the diversity indices and the food availability. The DISTLM analysis (Table 6) confirmed that 71% of the macrobenthic biomass was explained by the BPC content. Again, the lipid content was positively correlated with the macrofauna biomass, as also previously reported by Cartés *et al.* (2002). Several studies have recognized that together with food availability and depth, grain size is one of the factors that can shape the benthic communities in the deep sea (Karakassis and Eleftheriou 1997, Stora *et al.* 1999). This is the case for the macrofauna species distribution along the Malta Escarpment, which is apparently significantly related to the sediment grain size.

If a particular influence of food on the macrobenthic population appears to be clear, for the meiofauna only the group of deposit feeders produced a significant correlation with the prokaryote biomass. The relationships between meiofauna abundance and food quality/availability are controversial. Several studies have reported a strong influence of food availability not only on the meiofauna standing stock, but also on the diversity (e.g. Garcia *et al.* 2007, Ingels *et al.* 2011). Conversely, Hasemann and Soltwedel (2011) showed the same apparent lack of relationship between meiofauna and organic matter content as we see along the Malta Escarpment. Hasemann and Soltwedel (2011) inferred that factors that influence the nematode community have different effects in different areas. Vanhove *et al.* (1999) underlined that the spatial variability of the meiofauna and the different aggregations are related to different groups of factors (i.e. physical, chemical and biological) that can act together on meiobenthic communities. Soetaert and Heip (1995) and Johnson *et al.* (2007) indicated that the amount of sedimentary food is not the best proxy for food availability for the benthos, and that there are often no correlations between meiofauna and food. Along the Malta Escarpment, the meiofauna appeared to be more influenced by the sand content, which could explain most of the variability in the diversity (i.e. the number of taxa) and

feeding groups. The relationship between the benthic communities and the grain size is not unusual in deep sediments (Etter and Grassle 1992), and it has been reported as one of the environmental variables that influences meiofauna distribution, and especially nematodes (Heip *et al.* 1985).

Along the Malta Escarpment, it here emerges that the differences in prokaryote community abundances are not the result of any single controlling factor, but are the result of the interactions between the different environmental and ecological settings (i.e. grain size, CPE, quality and quantity of organic matter). The abundance of total prokaryotes, Bacteria and Archaea did not show a significant positive correlation with the quantity and availability of the sedimentary organic matter. Along the Mediterranean basin, many other studies have shown that the total prokaryote numbers and biomass are relatively insensitive to variables that are indicative of food availability (Danovaro *et al.* 1993, Boetius *et al.* 1996, Tholosan and Bianchi 1998, Bianchi *et al.* 2003). However, the importance of organic compound resources as a controlling factor of prokaryote assemblages has been confirmed by many other studies (Turley and Dixon 2002, Deming and Carpenter 2008). The discrepancy in these data can be attributed to the presence of different numbers of dead/dormant cells, as in all of these studies, the relationship was analysed among the total prokaryote parameters and the quantity/availability of organic matter.

It is possible to conclude that different environmental variables can differentially influence each one of these benthic size components along the Malta Escarpment. Other factors not considered in the present study that are fundamental in the shaping of slope benthic communities (e.g. environmental data related to the pelagic realm, such as vertical and later food inputs) must now be taken into further consideration to better explain the community distributions and variabilities.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

The following material is available through the web page
http://www.icm.csic.es/scimar/supplm/sm03811SMA.pdf

TABLE S1. – Replicates, depth, prokaryote abundance (cell g⁻¹) and biomass (μgC g⁻¹); meiofaunal abundance (ind 10 cm⁻²) and biomass (μgC 10 cm⁻²).

TABLE S2. – List of all nematoda species identified; mean abundance values (ind 10 cm⁻²) for each depth are reported.

TABLE S3. – List of macrofauna organisms identified; mean abundance values (ind m⁻²) for each depth are reported. n.i., not identified.

TABLE S4. – List of macrobenthic nematodes identified; mean abundance values (ind m⁻²) for each depth are reported.

From microbes to macrofauna: an integrated study of deep benthic communities and their response to environmental variables along the Malta Escarpment (Ionian Sea)

ELISA BALDRIGHI, STEFANO ALIANI, ALESSANDRA CONVERSI,
MARC LAVALEYE, MIRENO BORGHINI and ELENA MANINI

Supplementary material

TABLE S1. – Replicates, depth, prokaryote abundance (cell g⁻¹) and biomass (μgC g⁻¹); meiofaunal abundance (ind 10 cm⁻²) and biomass (μgC 10 cm⁻²).

Replicate	Depth (m)	Prokaryote abundance (cells g⁻¹)	Prokaryote biomass (μgC g⁻¹)	Meiofaunal abundance (ind 10 cm⁻²)	Meiofaunal biomass (μgC 10 cm⁻²)
R1	1206	1.64E+08	5.33	143.51	9.95
R2	1246	1.89E+08	5.78	379.41	46.17
R3	1264	1.40E+08	4.88	24.6	1.48
R1	1780	1.59E+08	7.61	235.90	11.66
R2	1779	1.97E+08	7.83	438.39	16.38
R3	1768	1.92E+08	7.39	230.01	17.39
R1	2071	3.81E+08	12.09	271.29	15.40
R2	2087	2.62E+08	10.51	110.09	4.58
R3	2120	2.59E+08	9.15	268.34	8.78

TABLE S2. – List of all nematoda species identified; mean abundance values (ind 10 cm⁻²) for each depth are reported.

Species	1200	1800	2100	Species	1200	1800	2100
Aegialoalaimus	1	2	3	*Molgolaimus*	0	0	0
Ammotheristus sp1	0	1	1	*Nannolaimoides*	0	0	0
Ammotheristus sp2	0	0	1	*Nemanema*	1	0	2
Amphimonhystrella sp1	3	3	4	*Oxystomina* sp1	0	1	3
Amphimonhystrella sp2	0	0	0	*Oxystomina* sp2	1	0	1
Aponema	0	0	0	*Paracomesoma*	0	1	0
Calligyrus	1	4	2	*Paracyatholaimus*	0	0	1
Choanolaimus	0	0	0	*Paradesmodora*	0	1	0
Chromadorina	0	0	1	*Paralinhomoeus*	0	0	0
Cylicolaimus	0	0	0	*Paralongicyatholaimus* sp1	1	1	1
Daptonema	0	0	0	*Paralongicyatholaimus* sp2	0	0	0
Desmoscolex sp1	1	6	1	*Pareudesmoscolex*	0	1	0
Desmoscolex sp2	0	3	0	*Paroxystomina*	0	0	0
Desmoscolex sp3	0	2	0	*Pierrickia*	1	2	1
Diplopeltoides	0	0	0	*Polygastrophora* sp1	2	3	2
Disconema sp1	0	1	1	*Polygastrophora* sp2	0	0	0
Disconema sp2	0	0	1	*Polysigma* sp1	0	1	1
Elzalia sp1	0	1	1	*Polysigma* sp2	0	1	0
Elzalia sp2	1	4	1	*Procamacolaimus*	0	0	1
Euchromadora	0	0	0	*Prochromadorella*	1	0	0
Graphonema	0	0	1	*Psammonema*	0	1	0
Halalaimus sp1	2	6	11	*Pselionema*	1	2	0
Halalaimus sp2	1	0	0	*Quadricoma*	1	1	2
Halichoanolaimus	0	0	0	*Rhabditis*	0	0	0
Haliplectus	0	0	0	*Sabatiera* sp1	9	4	9
Hopperia	0	1	0	*Sabatiera* sp2	10	3	2
Laimella	0	0	0	*Sigmaphoranema*	0	1	0
Leptolaimoides	0	0	1	*Sphaerolaimus* sp1	1	1	1
Leptolaimus sp1	1	6	5	*Sphaerolaimus* sp2	12	1	1
Leptolaimus sp2	0	6	4	*Sphaerolaimus* sp3	0	0	2
Linhystera sp1	2	5	8	*Spirinia*	0	0	0
Linhystera sp2	1	0	1	*Syringolaimus* sp1	1	4	1
Linhomoeus	0	0	0	*Syringolaimus* sp2	2	0	3
Longicyatholaimus	0	3	0	*Thalassomonhystera*	0	0	0
Metacomesoma	1	0	0	*Terschellingia* sp1	3	3	3
Metacyatholaimus	0	1	0	*Terschellingia* sp2	0	0	0
Metacylicolaimus	0	0	0	*Terschellingia* sp3	0	0	0
Metasphaerolaimus sp1	4	1	5	*Tricoma*	1	1	1
Metasphaerolaimus sp2	1	0	0	*Trochamus*	0	0	0
Metepsilonema	0	1	0	*Wieseria*	0	0	2
Minolaimus	0	1	0				

TABLE S3. – List of macrofauna organisms identified; mean abundance values (ind m^{-2}) for each depth are reported. n.i., not identified.

Organism	Depth (m)		
	1200	1800	2100
Ampharetidae sp1	4	4	0
Ampharetidae sp2	4	0	4
Lysianassidae sp1	8	0	4
Phoxocephalidae sp1	0	4	4
Amphipoda	0	4	8
Aplacophora _Solenogasters	4	0	4
Arenicolidae sp1	4	0	0
Arabellidae sp1	12	0	0
Capitellidae sp1	4	0	0
Chetopteridae sp1	0	12	8
Cirratulidae sp1	8	0	4
Cirratulidae sp2	4	8	4
Copepoda harpacticoida sp1	12	8	4
Copepoda harpacticoida sp2	8	29	17
Copepoda harpacticoida sp3	0	4	12
Cossuridae sp1	8	12	0
Echiurida sp1	4	0	4
Glycera sp1	8	0	0
Glycera sp2	0	0	0
Heterospionidae sp1	46	12	0
Heterospionidae sp2	0	66	21
Hydrozoa sp1	0	4	0
Hydrozoa sp2	0	17	4
Macrostylidae sp1	21	46	4
Macrostylidae sp2	4	0	0
Leptanthura cf. tenuis	4	0	0
Ilyarachnidae sp1	4	0	0
Anthuroidae sp1	4	0	0
Hyssura cf.producta	4	0	0
Ischnomesidae sp1	0	12	17
Desmosomidae sp1	0	8	0
Calathura sp1	0	4	0
Isopoda	4	0	0
Kellia sp1	8	4	12
Lumbrineris sp1	0	4	0
Magelonidae sp1	4	12	0
Nematoda	41	41	79
Nemertina sp1	12	8	0
Nemertina sp2	8	8	0
Nucula sp1	25	37	54
Oligocaheta sp1	12	8	0
Oligocaheta sp2	12	4	0
Oligochaeta sp3	4	4	0
Oligochaeta sp4	0	4	0
Orbinidae sp1	4	0	0
Ostracoda sp1	12	4	8
Ostracoda sp2	12	0	0
Owenidae sp1	0	0	4
Paraonidae sp1	21	21	17
Paraonidae sp2	4	4	0
Pilargidae sp1	0	8	0
Sipuncula sp1	17	4	0
Sipuncula sp2	8	0	0
Skenea sp1	0	0	4
Spionidae sp1	46	12	17
Spionidae sp2	37	12	4
Spionidae sp3	8	4	0
Spionidae sp4	0	4	0
Spongia sp1	0	8	4
Syllidae sp1	12	4	8
Syllidae sp2	4	0	0
Leptognathia filiformis	12	8	0
Leptognathia unguicillata	4	0	0
Typhlotanais sp1	0	0	8
Terebellidae sp1	4	8	0
Terebellidae sp2	12	0	0
Terebellidae sp3	4	0	0
Yoldia sp1	4	0	0
Xyluphaga sp1	0	0	21

TABLE S4. – List of macrobenthic nematodes identified; mean abundance values (ind m^{-2}) for each depth are reported.

Species	Depth (m)
	1200
Linhystera sp1	12
Monhystera sp1	4
Oxystomina sp1	4
Polygastrophora sp1	0
Pareurystomina sp1	4
Trissonchulus sp1	21
	1800
Anoplostoma sp1	17
Belbolla	4
Linhystera sp1	0
Oncholaimellus sp1	0
Polygastrophora sp1	12
Pareurystomina sp1	0
Promonohystera sp1	8
Trissonchulus sp1	17
Thalassoalaimus sp1	0
Wieseria sp1	4
	2100
Daptonema sp1	8
Monhystera sp1	12
Oxystomina sp1	8
Polygastrophora sp1	17
Pheronus sp2	29
Wieseria sp1	4