

# Drivers of deep Mediterranean megabenthos communities along longitudinal and bathymetric gradients

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**ABSTRACT.** The Mediterranean deep sea, being isothermal and longitudinally trophic-graded, is an optimal natural benchmark to test for macro-ecological patterns of species distribution. The relevance of environment-biota interactions on deep-sea megafauna in the Mediterranean Sea, a matter still largely overlooked, is addressed here along longitudinal and bathymetric axes. Benthic and nektobenthic megafauna were collected in the 3 basins of the Mediterranean: the western (Catalan Sea and southern Balearic), central (western Ionian) and eastern (south of Crete) basins, with an otter-trawl Maireta system and an Agassiz dredge between 1200 and 4000 m depth. Simultaneously, environmental data were collected on surface production, near-bottom physical parameters, sediment grain size and carbon content. Megafaunal biomass was higher in the Catalan Sea, decreasing eastward and with depth. Species diversity and evenness were relatively constant in the western and central Mediterranean at all depths, whereas these indices decreased with depth in the eastern Mediterranean.  $\beta$ -diversity analyses indicated a high species turnover between areas. The 3 basins presented significantly different environmental conditions. Sediment particulate organic carbon, surface fluorescence and sediment grain size were the 3 environmental variables that best explained the distribution of megabenthos along the longitudinal Mediterranean axis. These results show that the food supply, from either the surface or from the adjacent deep-sea floor, is critical in regulating the biodiversity of deep-sea Mediterranean megafauna and that this diversity is pooled region-wide. The heterogeneity of resources may be essential in maintaining these high levels of local and regional diversity.

**KEY WORDS:** deep-sea; Mediterranean; megafauna; environmental factors; POC; biodiversity

## INTRODUCTION

The regulation of benthic environments by physical and geochemical variables is, at present, a pivotal topic in the study of marine ecosystems. Global-scale studies have just begun to explore the complex interactions between environment and biota, yet are admittedly limited by the scant information available from the deep sea (Tittensor et al. 2010, Wei et al. 2010). The vast deep-sea floor remains mostly unexplored, despite being the largest ecosystem on the planet and it has a substantial reserve of undescribed biodiversity (Grassle & Maciolek 1992, Ramírez-Llodra et al. 2010a). Bolstered by worldwide programs such as

the Census of Marine Life, the exploration of deep-sea environments has been subject to a considerable growth in the last decade (Danovaro et al. 2010).

A series of factors have been proposed as potential drivers for the composition and diversity of deep benthic communities (Levin et al. 2001, Carney 2005). Most deep-ocean ecosystems, excluding reducing systems such as hydrothermal vents or cold seeps, are heterotrophic, depending on allochthonous organic matter inputs from the surface strata (Gage & Tyler 1991). This input of food to the deep-sea floor is, in turn, directly linked to the primary production of the sunlit

layers (Billett et al. 1983, Corliss et al. 2009), river runoff, atmospheric events (Company et al. 2008), climate (Smith et al. 2009) and, to an unknown extent, human activities (Smith et al. 2008). Degradation processes in the water column, which affect organic matter availability on the bottom, have also been proposed to be influential (Soltwedel et al. 2009). In this context, it is essential to address the potential relationship between biodiversity and environmental factors to highlight the strengths and weaknesses of these ecosystems, ultimately leading to a better understanding of their functioning and of the possibilities for their management.

The Mediterranean Sea, a latitude-constrained semi-enclosed sea, presents environmental characteristics that make it of particular interest for the study of its deep benthic communities (Bethoux et al. 1999, Sardà et al. 2004a). Compared with the Atlantic ocean, a nearly constant high temperature below 200 m (13 to 14 °C, depending on basin) in the Mediterranean favours enhanced degradation of food particles by pelagic microbes, further reducing the quantity of food available to the deep benthos (Tyler 2003). Throughout the continental slope, a strong zonation of benthic megafauna can be observed, associated with a constant reduction in abundance, biomass and diversity, accentuated under 1500 m (Pérès 1985, Company et al. 2004, D'Onghia et al. 2004). Below the 2600 and 2700 m isobaths, biomasses are extremely low and population densities are reduced to minimum levels (Tecchio et al. 2011).

Variations in physical conditions are known to strongly influence the response of large size classes of benthos such as macro- and megafauna. Conversely, small size classes are more dependent on biotic inter-relationships (Gage & Tyler 1991), although in recent years an effect of the environment has been observed on these biota fractions as well (Danovaro et al. 2008). These physical conditions are known to vary along the west-east axis in the Mediterranean, forming a decreasing gradient of organic matter availability

at the seafloor (Azov 1991, Danovaro et al. 1999). In addition, since the start of deep-sea exploration in the Mediterranean, it has been known that although the western and central basins host varied and abundant benthic megafauna communities, in the eastern basin (Levantine) all size classes of deep benthos are highly scarce in the whole continental margin (Sardà et al. 2004b and articles therein). It could thus be hypothesised that communities at deep basin depths in the western Mediterranean are structured similarly to communities in the mid-slope of the eastern basin. However, the relatedness of these 2 gradients (environmental and biotic) is unclear and leaves open a number of questions regarding the deep-water megafauna in the Mediterranean.

Two factors have prevented studies of this type until now: (1) the extreme difficulty in sampling unknown areas of the deep Mediterranean seafloor, especially in the central and eastern basins, results in scarcity of megabenthos datasets below 1000 m depth; those available are usually obtained by pooling together samples taken with different methods; and (2) the absence of integrated sampling plans to retrieve data on both the megabenthos and the abiotic environment.

During a multidisciplinary trans-Mediterranean cruise conducted in 2009, biological sampling was conducted over a large spatial scale in the Mediterranean deep-sea, extending the exploration eastward to the southern Cretan Sea, and simultaneously collecting a set of environmental variables. The objective of the present study was, for the first time, to integrate the patterns of megabenthos standing stock and diversity at the species level, as well as and the environmental conditions at which these communities live, along 2 axes of the Mediterranean deep-sea: depth and longitudinal.

## **MATERIALS AND METHODS**

**Study areas.** The target ecosystem was the fine-mud benthos Mediterranean Sea on the continental slope and in the deep basin, between 1200 and 4000 m in depth (Fig. 1). Environmental

and biological data were collected during two multidisciplinary oceanographic cruises in 2009, in the framework of the BIOFUN multidisciplinary project. A one-month trans-Mediterranean cruise (BIOFUN I) was conducted in June 2009 aboard the R/V *Sarmiento de Gamboa*, in the Southern Balearic Sea in the Western Mediterranean (WM), the Western Ionian Sea in the Central Mediterranean (CM) and the Southern Cretan Sea in the Eastern Mediterranean (EM). Another cruise (BIOFUN II) was conducted aboard the R/V *García del Cid* on the Catalan Sea continental slope (CS), also in the Western Mediterranean, in October 2009. The sampling plan included three main depths in each zone: 1200, 2000 and 2700-3000 m. In addition, stations at 3300 and 4000 m depth in the Western Ionian were included in the sampling plan. Throughout the text, the acronyms CS, WM, CM and EM will be used to identify the study areas.

**Environment variables.** At each station, physical water characteristics were obtained along the water column using a Seabird 9plus CTD. Data were processed using SBE Data Processing software v.7.18d. The variables considered were benthic temperature (°C), benthic salinity (PSU), benthic turbidity (FTU) and fluorescence (RFU). As the data were collected with the same CTD and methodology throughout the cruise, the mean values of the fluorescence data in the 0-150 m depth range were used to estimate surface primary production. Turbidity near the bottom was obtained by averaging the data of the last 5 m of the water column.

At each station, plankton was collected using two nets: a standard WP2 net with a 200 µm mesh for mesozooplankton and a scaled-down version for microplankton with a 53 µm mesh size. Hauls were vertical from 200 m depth to the surface, at a speed of 1 m s<sup>-1</sup>. The filtered water volume was estimated using General Oceanics 2030R flowmeters. After sieving through 200 µm and 2000 µm sieves for micro and mesozooplankton, respectively, the samples were filtered on board using pre-dried and pre-weighted 25 mm

diameter GF/C glass microfiber filters for microplankton and 47 mm diameter for mesozooplankton. The filters were then stored at -20 °C. In the laboratory, the filters were freeze-dried for 48 h and weighed ( $d = 0.0001$  g) to retrieve plankton biomass, which was then standardised for filtered water volume.

Sediment samples were collected using a multiple corer at the same stations. Surface sediment samples (the top 1-cm) were used to investigate grain size and elemental composition. A non-homogenised fraction of approximately 1 g of sediment sample was treated with 10% H<sub>2</sub>O<sub>2</sub> to oxidise organic matter. A Coulter Counter LS230 Laser Particle Size Analyser was used to determine grain size. The results are expressed in percentages of coarse fraction (> 63µm). Another fraction of sediment was homogenised with an agate mortar for organic carbon analysis. The samples were first decarbonated using repeated additions of 25% HCl with 60 °C drying steps in between until no effervescence was observed, and the particulate organic carbon (POC) was measured on a Carlo Erba Flash 1112 elemental analyser at the Scientific Technical Services of the University of Barcelona.

**Megafauna sampling.** A combination of 2 samplers was used to collect the widest range of benthic megafauna: an otter-trawl Maireta system (OTMS) and an Agassiz dredge. The OTMS is a 1-warp benthic otter-trawl designed to work seamlessly on high depth grounds (Sardà et al. 1998). Its stretch mesh size at the cod-end is 40 mm with an outer cover of 12 mm, to allow retrieval of small-sized fractions of megafauna. The net total length is 25 m. SCANMAR sensors were used down to 1200 m depth to estimate net geometry and times of arrival and departure from the bottom. The net horizontal opening was estimated at  $12.7 \pm 1.3$  m, and the vertical opening at 1.4 m with substantially less variability. For trawls deeper than 1200 m, the same values for the opening geometry were assumed, whereas the bottom times were calculated as the period between the end of cable paying out and the start

of cable retrieval. Trawls were conducted at 2.6 – 2.8 knots. The Agassiz dredge had a 2.5 m horizontal opening and 1.2 m vertical opening, a net mesh size of 12 mm, and was trawled at 2.0 knots. Haul bottom times were estimated using the same method as for the OTMS.

**Data analysis.** Every individual collected with the OTMS or the Agassiz dredge was identified to species level or to the lowest taxonomical level possible. A series of taxonomic literature was applied for the identification of fishes (Mercader et al. 2001, Compagno et al. 2005), crustaceans (Zariquiey 1968) and other invertebrates (Tortonese 1965, Villanueva 1992, Saiz-Salinas 1993). Species names were then cross checked with the World Register of Marine Species, available online (WoRMS, <http://www.marinespecies.org/>). Individuals were then counted and weighted.

Calculation of the diversity indices was applied separately on the abundance data from the OTMS and Agassiz dredge; the expected number of species  $ES(25)$  and Pielou's evenness indexes ( $J'$ ) were calculated for  $\alpha$ -diversity (Magurran 2004). Total abundance and biomass of each species were standardised for trawled area (in  $km^2$ ) using swept area data from the SCANMAR system. The species x samples matrix was also presence/absence transformed to permit treating the OTMS and Agassiz samples as a whole. A CLUSTER analysis using the Sørensen similarity index as resemblance measure was carried out, and a SIMPROF permutational routine (1000 restarts) was applied to test for the significance of genuine clustering. Differences in species composition between zones were tested with a single-factor Similarity Percentages routine (SIMPER) using Bray-Curtis similarity and used as a  $\beta$ -diversity estimator (Gray 2000, Danovaro et al. 2008). A faunal list was also compiled from the presence/absence matrix.

Environmental variables were normalised, after benthic turbidity was log-transformed to reduce an initial left-skewness. A one-way ANOSIM test, using Euclidean distance as the measure and basin

as the factor, was conducted on the environmental variables set. A BEST (BIOENV) routine was run to identify environmental variables correlated with differences between communities in terms of both species identities and abundances. This routine searches for the best subset of environmental variables, which optimises the match with the biotic patterns (Clarke & Gorley 2006). To avoid the introduction of errors from possible seasonal patterns, only quantitative OTMS data from the June 2009 trans-Mediterranean cruise (BIOFUN 1) were considered as response variables. BEST was run with Spearman rank as the correlation method and Euclidean distance as the resemblance measure for environmental variables. A global match test was performed, with 1000 permutations, to test for the significance of selections. Ecological analyses were performed with the PRIMER-E 6 software.

## RESULTS

A total of 17 OTMS trawls and 16 Agassiz hauls were conducted successfully (Table 1). Ninety-nine species belonging to 10 different phyla were collected, and are listed in Appendix 1. Of these, one was a new species of decapod crustacean belonging to family Galatheididae, *Munidopsis ariadne*, described by Macpherson (2011) and retrieved at 2000 m depth in the Southern Cretan Sea. Samples at 2000 m in the Central basin and at 2800 m in the Eastern basin were not retrievable with OTMS even after repeated deployments.

The overall taxonomic composition showed that the most speciose groups were bony fishes (Actinopterygii, 40 species), decapod crustaceans (27 species) and molluscs (19 species, mainly Cephalopoda and Gastropoda). The abundance of the different taxonomic groups collected with the OTMS in each study area at 1200, 2000 and 2800 m is presented in Fig. 2. Bony fishes and decapod crustaceans were also the only ubiquitous groups in all areas. The areas with the least number of groups (3) were the 2800 m station in the CM and the 2000 m station in the EM, followed by 4 groups at 2800 m depth in the WM. The maximum

number of groups (10) was found in the CM at 1200 m depth. The other areas hosted between 6 and 8 taxonomic groups.

The total biomass (Fig. 3) measured at 1200 m depth presented significant differences between areas (global Kruskal-Wallis test,  $p = 0.008$ ). The CS biomass was the highest ( $1258.8 \pm 92.2 \text{ kg km}^{-2}$ ), followed by the WM ( $226.0 \pm 20.2 \text{ kg km}^{-2}$ ) and the CM ( $235.6 \pm 26.0 \text{ kg km}^{-2}$ ). The biomass of the catches in the EM was the lowest ( $15.7 \pm 9.1 \text{ kg km}^{-2}$ ), significantly differing from the CS biomass (M-W test,  $p < 0.0001$ ). At 2000 m depth a slight, non-significant (global K-W test,  $p = 0.301$ ) eastward decrease in biomass was evident. Biomass levels at 2800 m were low in all studied areas, ranging from  $41.1 \pm 9.6 \text{ kg km}^{-2}$  in the CS to  $13.0 \text{ kg km}^{-2}$  in the CM.

Diversity estimated by ES(25) on the OTMS samples showed a stable pattern with depth on the CS, ranging from 8.5 to 10.5 (Fig. 4). The same pattern was present in the WM, with values ranging between 10.3 and 11.7. The CM showed a similar diversity between the 1200 m and the deep 2800 m stations, although no data are available for the 2000 m depth. In the EM, diversity abruptly decreased between 1200 m and 2000 m. Pielou's index of evenness ranged between 0.36 in the EM at 2000 m depth and 0.95 at 1200 m also in the EM. In all of the other zones, Pielou's evenness was relatively constant and values ranged between 0.54 and 0.86.

Considering the Agassiz samples, ES(25) showed decreasing patterns in the four areas (Fig. 4). On the CS, diversity decreased from 12.2 at 1200 m depth to 9.1 at 2000 m, although no replicates were available. In the WM, ES(25) decreased between 1200 m and 2000 m, but it remained constant between 2000 m and 2800 m depth. In the CM, no samples were available at 1200 m depth, but diversity decreased slightly between 2000 m and 3300 m and then remained constant down to 4000 m depth. In the EM, diversity decreased steeply with depth. Evenness followed comparable patterns, except in the CM, where the samples from 3300 m and 4000 m presented

higher evenness than that from the 2000 m station. In all comparisons, the sample sizes were too small to perform statistical tests to verify the significance of differences.

Ordination by CLUSTER with SIMPROF analysis showed a significant grouping by depth and zone (Fig. 5). All of the 1200 m samples grouped together, also showing a grouping by zone, whereas at 2000-4000 m the influence of geographic area was less marked.  $\beta$ -diversity estimation by the SIMPER routine showed a relatively low similarity within zones (ranging from 35.9 to 46.2 %) and a high turnover diversity between zones ranging between 61.3 and 74.5 %. The zone with the lowest internal similarity was the CM (27.1 %) which also included the widest depth range between samples (2800 m of depth gradient between the shallowest and deepest stations).

Table 1 shows the environmental variables considered in this study. Significant environmental differences between basins were found by the ANOSIM test (Global  $R = 0.711$ ,  $p = 0.001$ ). Pairwise comparisons of WM-CM and WM-EM were also significant ( $p = 0.0008$  in both cases), whereas the CM-EM contrast was borderline ( $p = 0.029$ ) but still significant. Results from the BEST routine identified a group of three variables that together best explained the community dissimilarity in the longitudinal axis of the Mediterranean: sediment grain size, sediment POC, and surface fluorescence. The results of the global match test ( $p = 0.555$ ,  $p = 0.004$ ) corroborated the significance of that selection. Considering these three variables separately, the best explanatory variable was sediment POC ( $\rho = 0.413$ ), followed by surface fluorescence ( $\rho = 0.361$ ) and sediment grain size ( $\rho = 0.313$ ). In each basin, the coarse fraction of the grain size was always highest at the deepest site (2800 m depth). Sediment carbon content has more wide fluctuations between areas, with its minimum values in the EM, ranging between 0.26 % and 0.34 %. Surface fluorescence in the WM was  $0.82 \pm 0.09 \text{ RFU}$ ; in the CM it was  $0.65 \pm 0.09 \text{ RFU}$ , and

in the EM, it was  $0.62 \pm 0.07$  RFU. The integrated biomass of microplankton between 0 and 200 m depth was  $6.60 \pm 2.40$  mg m<sup>-3</sup> in the WM,  $4.32 \pm 0.82$  mg m<sup>-3</sup> in the CM and  $3.66 \pm 1.27$  mg m<sup>-3</sup> in the EM. Mesozooplankton biomass in the same depth interval was  $8.14 \pm 3.87$  mg m<sup>-3</sup> in the WM,  $10.16 \pm 2.04$  mg m<sup>-3</sup> in the CM and  $7.13 \pm 2.55$  mg m<sup>-3</sup> in the EM.

## DISCUSSION

Food supply is an essential factor believed to shape deep-waters biodiversity. Most of the deep-sea is considered food-limited, excluding chemosynthetically-driven ecosystems or other particular conditions such as areas beneath upwelling systems or depocenters (Danovaro et al. 2003, Gage 2003). Smith et al. (2008) showed that abyssal ecosystem composition and structure are strongly modulated by the quantity and quality of food sinking from the surface of the ocean. In addition to the food quantity limitations, the deep Mediterranean is also limited in food quality compared with the outer Atlantic Ocean due to the enhanced degradation of the organic matter sinking in the water column (Danovaro et al. 1999). This condition has been shown to influence the abundance and diversity of benthic meiofauna (Danovaro et al. 2008, Lampadariou et al. 2009).

A marked decreasing gradient in benthic biomass was found with depth and along the west-to-east axis, with a total biomass an order of magnitude higher on the CS than in the EM site at the same depths. This bathymetric biomass decrease is in accordance with the general global pattern described by Rex et al. (2006). The most striking differences in biomass are found between the CS and the WM, both in the Western Mediterranean basin, especially at the 1200 m depth. Significant differences between these 2 areas were previously found for fishes assemblages (Moranta et al. 1998).

Sediment particulate organic carbon (POC), surface fluorescence (as an indicator of surface

primary productivity) and sediment grain size were selected as the best descriptors of megafauna distribution in the deep Mediterranean. Primary production in the surface layers and carbon content in deep-sea sediments are known to be spatially and temporally correlated (Billett et al. 1983, Smith & Rabouille 2002). The results presented here further confirm the longitudinal west-to-east decrease in productivity. The eastern Mediterranean basin has been traditionally considered as one of the most oligotrophic ocean areas in the world (Azov 1991), with extreme scarcity of sinking organic matter in the water column. The variability explained by the model thus supports the concept by which surface layer dynamics modulate benthic megafaunal communities.

Relationships between sediment composition and benthic diversity have been previously described for smaller size-classes of macrofauna (Etter & Grassle 1992). For the large-sized species addressed in this study whose biology is still poorly known, it is not clear how the different grain size of the substrate is perceived by the fauna and at what scale of variability it is an influencing factor for their distribution and behaviour. It has been proposed that less-mobile species, such as holothurians, could be directly influenced by sediment grain size and carbon supply on the sea floor (Roberts & Moore 1997). Finer-grained sediments, which were present in the shallower areas of the WM and the CM, present higher POC (Table 1) and thus higher food availability, favouring the settlement of a more abundant and diverse in-fauna (Levin et al. 2001). These areas were also the most diverse in terms of species and the taxonomic composition of the megafauna. The regulation of in-fauna could then also influence the diversity of larger-size classes of benthos as an upward trophic modulation.

Similar results were obtained in a Northern Atlantic deep-sea observatory, where nutrient availability in the substrate was defined as the modulating agent for benthic megafauna, and the degradation processes in the water column were

suggested to be influential (Soltwedel et al. 2009). In the deep Pacific Ocean, observations were also made in accordance with relating POC flux with megabenthos abundances (Smith et al. 2009). The enhanced temperatures in the Mediterranean water column, which augment organic matter degradation, could then be pivotal in determining the real extent of the benthic-pelagic coupling.

It is likely that a combination of other factors is occurring and should be explored in the near future. For instance, according to these results, zooplankton biomass was not significantly correlated with the distribution of megafauna. Zooplankton biomass in oceanic areas is substantially less variable than in neritic zones, but is still considered to be a connecting factor between surface productivity and POC flux to the deep (Gage 2003). Even if slight differences can be observed in zooplankton biomass along the longitudinal axis, it is possible that their influence in the BEST analysis was masked by the gradient of primary productivity. In addition, depth was included as a possible explanatory variable, although the cluster analysis suggested a strong grouping by depth strata. The rationale for our BEST analysis was to directly consider some of the variables that are known to co-vary with depth.

Of the 99 species collected, the only ubiquitous groups found in all samples were Actinopterygii and Crustacea. These groups were also the most abundant and speciose. This dominance is well established for the entirety of the deep Mediterranean (Jones et al. 2003, Company et al. 2004), and represents a major difference with the deep Atlantic sea floor (Billett et al. 2001, Soltwedel et al. 2009), which is colloquially termed the “kingdom of the holothurians” (Kunzig 2000). Also, going from shallow to deep stations and from west to east, a reduction in the number of taxonomic groups can be appreciated. The Macrourids, a dominant actinopterygian family in both the deep Mediterranean and the Atlantic, are known to be adapted to various ranges of food availability by employing generalist and opportunistic foraging strategies (Mauchline &

Gordon 1986). The two dominant decapod species, *Aristeus antennatus* and *Acantheephyra eximia*, can maintain high metabolic rates by having a nektobenthic behaviour (Company & Sardà 1998) and thus being able to live along a wide depth range while feeding in more favourable areas like the mesopelagic water column. Only fish and crustacean species seem then to be completely adapted to bathyal and abyssal conditions in the Mediterranean Sea. What remains to be confirmed is whether their presence in the deep basin is stable or just a spread effect of a shallower-based population.

At abyssal depths in the Central Mediterranean basin (3300 and 4000 m) fewer species were collected than found in a previous study in the same area (Tecchio et al. 2011). In the present study, only Agassiz samples were available for those depths, and the species collected were all present also in the bathyal sites with higher abundances. This result further supports the hypothesis of deep-spreading populations. As these were the only published data on megafauna, along with those by Tecchio et al. (2011), they serve as a confirmation of species presence, providing a motivation to establish a permanent sampling station in the central Mediterranean abyssal plain.

In this study, 2 different samplers were used simultaneously, in order to complement each other and give an integrated description of the benthic community. The OTMS was fitted with more flotation devices than usual and a roller line on the footrope to reduce the risk of the net getting caught on the bottom by collecting too much mud. The dynamic of an OTMS haul is such that nektobenthic species are positively selected (Sardà et al. 1998). In contrast, the Agassiz dredge has a smaller opening, and it maintains firm contact with the substrate, permitting the retrieval of strictly benthic species. By combining these two different “sections” of the benthic domain, it was possible to compile the comprehensive faunal list presented in this work. In addition, a general higher variability in gear

behaviour was observed for the OTMS than for the Agassiz dredge; again, this is caused by the differences in the gear designs and concepts. The Agassiz has proven to be usable where bottom conditions did not permit sampling with the OTMS (e.g., semi-volcanic bottoms in the Central Mediterranean) causing the OTMS to break or be lost completely. Thus, the 2 samplers were shown to be complementary in the exploration of deep benthic megafauna. However, with the reduced number of samples retrieved, the resulting diversity patterns are far from being definitive.

The WM presented a slight, non-significant decrease in diversity along the depth axis, which is consistent with previous results with the same OTMS sampler (Tecchio et al. 2011). The Agassiz samples collected in the same area showed a clear decrease in diversity between 2000 and 2800 m depth. The diversity differences found in the western basin between the two sampled communities suggest that the dynamics of strictly benthic species may be different from those of nektobenthic megafauna. In the deep Mediterranean, the biomass of nektobenthic fauna is known to decrease below 1500 m depth (Sardà et al. 2009). Benthic invertebrates are more dependent on the organic matter found in the sediment than fishes and crustaceans, which could explain their significant decrease at the deeper station. No clear diversity patterns were found in the CM. However, the sampling difficulties found when studying these benthic area resulted in a limited number of successful OTMS and Agassiz trawls, not allowing for any definitive conclusions.

In the EM, there is a clear and steep decline in species diversity from 1200 m to 3000 m in both the OTMS and Agassiz samples. The increased oligotrophy of this basin may cause diversity to sharply decrease with depth, whereas in the other areas, this decrease might be partially masked by the more favourable trophic conditions in the deeper layers. Moreover, the sampled zone in the lower depths may represent a patch of reduced diversity on an otherwise homogeneous sea floor. Logistics impaired the retrieval of replicates at

2000 m, and no OTMS sample was available at 2800 m depth; thus, the possibility of heterogeneous habitat cannot be discarded. In recent years, several studies have shown the importance of habitat heterogeneity in shaping deep benthic communities (Levin & Dayton 2009, Ramírez-Llodra et al. 2010b, Vanreusel et al. 2010). Further studies in the bathyal and abyssal Mediterranean, including the use of imaging instruments such as Remote Operated Vehicles (ROVs) or Autonomous Underwater Vehicles (AUVs), are necessary to describe in detail the deep Mediterranean expanse and to identify potential habitat characteristics that could influence heterogeneity.

$\beta$ -diversity was high throughout the Mediterranean, in striking resemblance to the results from other benthic compartments such as meiofauna (Danovaro et al. 2008). This suggests that most of the deep Mediterranean biodiversity is regional rather than local and that even if basin differences are evident and have to be considered, the whole Mediterranean Sea should be considered as a wide-ranging species pool. In addition, the low overlap in composition between zones may further corroborate the hypothesis that habitat heterogeneity, along with the food supply as addressed in this study, is a major structuring factor in deep benthic areas (Levin et al. 2001). Among other reasons, this heterogeneity could be related to different water masses at the bottom. The western and central areas of the Mediterranean are known to be very dynamic and affected by periodic local cascading events (Canals et al. 2006, Company et al. 2008), whereas the hydrodynamics of the deep eastern basin south of Crete are less variable, as cascading waters from the Aegean Sea are blocked by the island mass (Bethoux et al. 1999). Overall, those observations point to an effective heterogeneity of conditions, rather than to a physical heterogeneity of habitats and structures.

## Conclusions

The results from this study suggest that surface layer production differentially influences the



benthic standing stock and diversity of the deep-sea Mediterranean benthos. Food supply imported from the photic zone or advected from the adjacent deep seafloor and deposited in the sediments is one of the main factors regulating the distribution of megafauna in the Mediterranean Sea over large spatial scales. With their heterogeneous distribution and composition, and with their west-east gradient in the deep Mediterranean, these food sources could be one of the key factors determining megafaunal community structure in these habitats. In addition, the hypothesised environmentally-driven bathymetric displacement of communities along the west-east axis of the Mediterranean should be reconsidered.

Two other important processes might be explored in the near future: 1) the relationship between the flow of water masses and larval drift, which may explain some of the species boundaries found in this and previous studies, and 2) the importance of small-scale physical events that may locally trigger high quantities of available organic matter at the bottom, the response to which has not yet been evaluated.

This study was the first attempt to sample the deep megafauna over the entire longitudinal axis of the Mediterranean while simultaneously investigating the possible environmental drivers. The data on deep Mediterranean megafauna diversity are still limited, but the results obtained in this study present an initial basin-wide picture of these deep ecosystems, providing a baseline from which to build a sound understanding of deep Mediterranean biodiversity and ecosystem functioning. This knowledge is also essential for predicting changes in the deep communities in the face of potential anthropogenic impact and, especially, climate change and for developing sustainable management and conservation options.

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## LITERATURE CITED

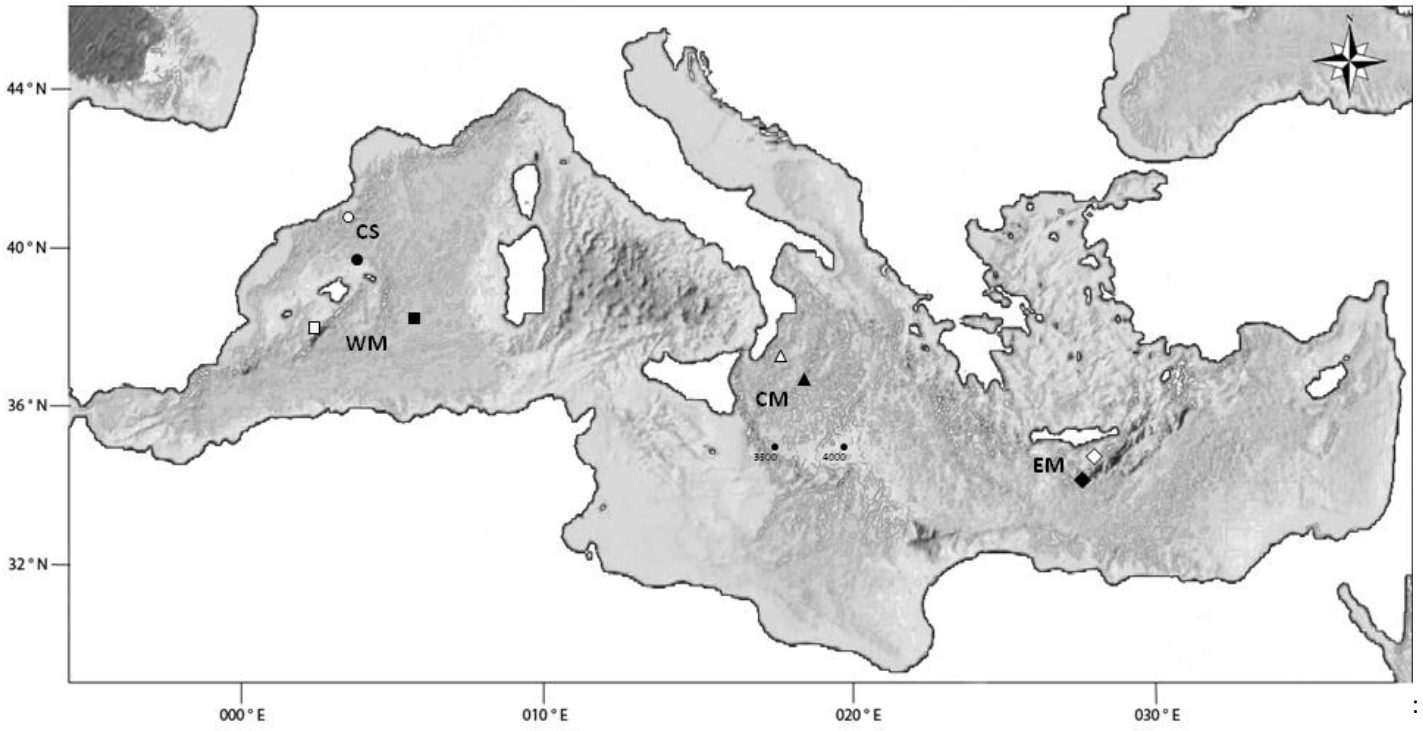
- Azov Y (1991) Eastern Mediterranean - a marine desert? *Mar Pollut Bull* 23:225-232
- Bethoux JP, Gentili B, Morin P, Nicolas E, Pierre C, Ruiz-Pino D (1999) The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. *Prog Oceanogr* 44:131-146
- Billett DSM, Bett BJ, Rice AL, Thurston MH, Galéron J, Sibuet M, Wolff GA (2001) Long-term change in the megabenthos of the Porcupine Abyssal Plain (NE Atlantic). *Prog Oceanogr* 50:325-348
- Billett DSM, Lampitt RS, Rice AL, Mantoura RFC (1983) Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature* 302:520-522
- Canals M, Puig P, Durrieu de Madron X, Heussner S, Palanques A, Fabres J (2006) Flushing submarine canyons. *Nature* 444:354-357
- Carney RS (2005) Zonation of deep biota on continental margins. *Oceanogr Mar Biol Annu Rev* 43:211-278
- Clarke KR, Gorley RN (2006) *Primer v6: User Manual / Tutorial*. PRIMER-E Ltd., Plymouth, UK
- Compagno L, Dando M, Fowler S (2005) *Sharks of the world*. Princeton University Press, Princeton and Oxford
- Company JB, Maiorano P, Tselepides A, Politou C-Y, Plaity W, Rotllant G, Sardà F (2004) Deep-sea decapod crustaceans in the western and central Mediterranean Sea: preliminary aspects of species distribution, biomass and population structure. *Sci Mar* 68S3:73-86
- Company JB, Puig P, Sardà F, Palanques A, Latasa M, Scharek R (2008) Climate influence on deep sea populations. *PLOS ONE* 3:e1431
- Company JB, Sardà F (1998) Metabolic rates and energy content of deep-sea benthic decapod crustaceans in the western Mediterranean Sea. *Deep-Sea Res I* 45:1861-1880

- Corliss BH, Brown CW, Sun X, Showers WJ (2009) Deep-sea benthic diversity linked to seasonality of pelagic productivity. *Deep-Sea Res I* 56:835-841
- D'Onghia G, Politou C-Y, Bozzano A, Lloris D, Rotllant G, Sion L, Mastrototaro F (2004) Deep-water fish assemblages in the Mediterranean Sea. *Sci Mar* 68S3:87-99
- Danovaro R, Company JB, Corinaldesi C, D'Onghia G and others (2010) Deep-sea biodiversity in the Mediterranean Sea: the known, the unknown, and the unknowable. *PLOS ONE* 5(8):e11832
- Danovaro R, Della Croce N, Dell'Anno A, Pusceddu A (2003) A depocenter of organic matter cycling at 7800-m depth in the South Pacific Ocean. *Deep-Sea Res I* 50:1411-1420
- Danovaro R, Dinet A, Duineveld G, Tselepides A (1999) Benthic response to particulate fluxes in different trophic environments: a comparison between the Gulf of Lions–Catalan Sea (western-Mediterranean) and the Cretan Sea (eastern-Mediterranean). *Prog Oceanogr* 44:287-312
- Danovaro R, Gambi C, Lampadariou N, Tselepides A (2008) Deep-sea nematode biodiversity in the Mediterranean basin: testing for longitudinal, bathymetric and energetic gradients. *Ecography* 31:231-244
- Etter RJ, Grassle JF (1992) Patterns of species diversity in the deep sea as a function of sediment particle size diversity. *Nature* 360:576-578
- Gage JD (2003) Food inputs, utilization, carbon flow and energetics. In: Tyler PA (ed) *Ecosystems of the world (Ecosystems of the Deep Ocean)*, Elsevier, Amsterdam, p 315-382
- Gage JD, Tyler PA (1991) *Deep-sea biology: a natural history of organisms at the deep-sea floor*. Cambridge University Press, Cambridge
- Grassle JF, Maciolek NJ (1992) Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *Am Nat* 139:313-341
- Gray JS (2000) The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *J Exp Mar Biol Ecol* 250:23-49
- Jones EG, Tselepides A, Bagley PM, Collins MA, Priede IG (2003) Bathymetric distribution of some benthic and benthopelagic species attracted to baited cameras and traps in the deep eastern Mediterranean. *Mar Ecol Prog Ser* 251:75-86
- Kunzig R (2000) *Mapping the deep: the extraordinary story of ocean science*. W. W. Norton, New York and London
- Lampadariou N, Tselepides A, Hatziyanni E (2009) Deep-sea meiofaunal and foraminiferal communities along a gradient of primary productivity in the eastern Mediterranean Sea. *Sci Mar* 73:337-345
- Levin LA, Dayton PK (2009) Ecological theory and continental margins: where shallow meets deep. *Trends Ecol Evol* 24:606-617
- Levin LA, Etter RJ, Rex MA, Gooday AJ, Smith CR, Pineda J, Stuart CT, Hessler RR, Pawson D (2001) Environmental influences on regional deep-sea species diversity. *Annu Rev Ecol Evol Syst* 32:51-93
- Macpherson E (2011) A new squat lobster of the genus *Munidopsis* (Crustacea: Decapoda: Munidopsidae) from the Mediterranean Sea. *Sci Mar* 75(3):525-532
- Magurran AE (2004) *Measuring biological diversity*. Blackwell Science, London
- Mauchline J, Gordon JDM (1986) Foraging strategies of deep-sea fish. *Mar Ecol Prog Ser* 27:227-238
- Mercader L, Lloris D, Rucabado J (2001) *Tots els peixos del mar català: diagnòsi i claus d'identificació*. Institut d'Estudis Catalans. Arxius de la Secció de Ciències, 128: 350 pp., Barcelona
- Moranta J, Stefanescu C, Massutí E, Morales-Nin B, Lloris D (1998) Fish community structure and depth-related trends on the continental slope of the Balearic Islands (Algerian basin, western Mediterranean). *Mar Ecol Prog Ser* 171:247-259
- Péres JM (1985) History of the Mediterranean biota and colonization of the depths. In: Margalef R (ed) *Western Mediterranean*, Pergamon Press, Oxford, p 198-232
- Ramírez-Llodra E, Brandt A, Danovaro R, De Mol B and others (2010a) Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* 7:2851-2899
- Ramírez-Llodra E, Company JB, Sardà F, Rotllant G (2010b) Megabenthic diversity patterns and community structure of the Blanes submarine canyon and adjacent slope in the Northwestern Mediterranean: a human overprint? *Mar Ecol* 31:167-182
- Rex MA, Etter RJ, Morris JS, Crouse J and others (2006) Global bathymetric patterns of standing stock and body size in the deep-sea benthos. *Mar Ecol Prog Ser* 317:1-8
- Roberts D, Moore HM (1997) Tentacular diversity in deep-sea deposit-feeding holothurians: implications for biodiversity in the deep sea. *Biodivers Conserv* 6:1487-1505
- Saiz-Salinas JI (1993) *Fauna Ibérica. Sipuncula*. Ed. CSIC, Madrid
- Sardà F, Calafat A, Flexas M, Tselepides A, Canals M, Espino M, Tursi A (2004a) An introduction to Mediterranean deep-sea biology. *Sci Mar* 68S3:7-38
- Sardà F, Cartes JE, Company JB, Albiol A (1998) A modified commercial trawl used to sample deep-sea megabenthos. *Fish Sci* 64:492-493
- Sardà F, Company JB, Rotllant G, Coll M (2009) Biological patterns and ecological indicators for

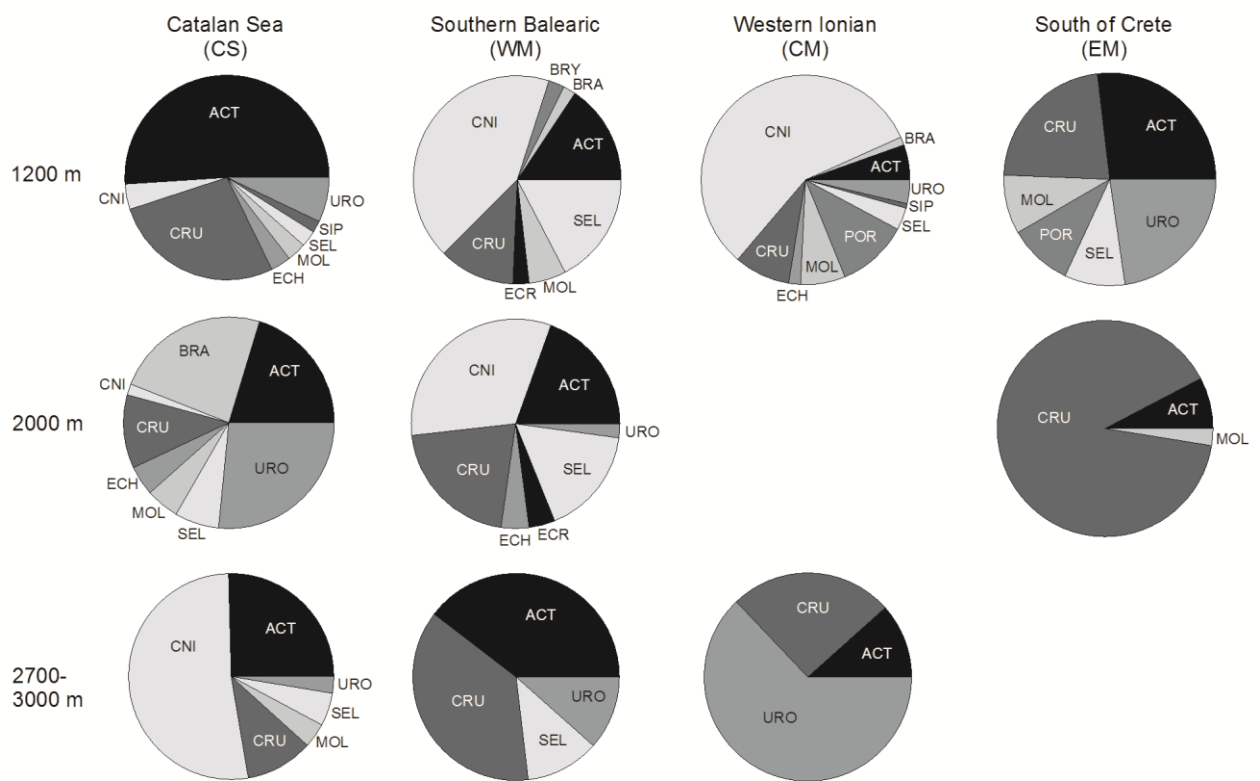
- Mediterranean fish and crustaceans below 1,000 m: a review. *Rev Fish Biol Fish* 19:329-347
- Sardà F, D'Onghia G, Politou C-Y, Tselepides A (eds) (2004b) *Mediterranean deep-sea biology*, Vol 68S3. Scientia Marina
- Smith CR, De Leo FC, Bernardino AF, Sweetman AK, Martinez Arbizu P (2008) Abyssal food limitation, ecosystem structure and climate change. *Trends Ecol Evol* 23:518-528
- Smith CR, Rabouille C (2002) What controls the mixed-layer depth in deep-sea sediments? The importance of POC flux. *Limnol Oceanogr* 47:418-426
- Smith KL, Ruhl HA, Bett BJ, Billett DSM, Lampitt RS, Kaufmann RS (2009) Climate, carbon cycling, and deep-ocean ecosystems. *Proc Natl Acad Sci USA* 106:19211-19218
- Soltwedel T, Jaekisch N, Ritter N, Hasemann C, Bergmann M, Klages M (2009) Bathymetric patterns of megafaunal assemblages from the arctic deep-sea observatory HAUSGARTEN. *Deep-Sea Res I* 56:1856-1872
- Tecchio S, Ramírez-Llodra E, Sardà F, Company JB (2011) Biodiversity of deep-sea demersal megafauna on western and central Mediterranean basins. *Sci Mar* 75:341-350
- Tittensor DP, Mora C, Jetz W, Lotze HK, Ricard D, Vanden Berghe E, Worm B (2010) Global patterns and predictors of marine biodiversity across taxa. *Nature* 466:1098-1101
- Tortonese E (1965) *Fauna d'Italia. Echinodermata*. Ed. Calderini, Bologna
- Tyler PA (2003) The peripheral deep seas. In: Tyler PA (ed) *Ecosystems of the world (Ecosystems of the Deep Ocean)*, Elsevier, Amsterdam, p 261-293
- Vanreusel A, Fonseca G, Danovaro R, da Silva MC and others (2010) The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity. *Mar Ecol* 31:6-20
- Villanueva R (1992) Deep-sea cephalopods of the north-western Mediterranean. *J Zool Lond* 227:267-276
- Wei C-L, Rowe GT, Escobar-Briones E, Boetius A and others (2010) Global patterns and predictions of seafloor biomass using Random Forests. *PLOS ONE* 5(12):e15323
- Zariquiey R (1968) *Crustáceos Decápodos Ibéricos*. *Invest Pesq* 32:510 pp.

**Table 1.** List of OTMS and Agassiz dredge hauls analysed for this study and the environment variables used in the BEST analysis. Numbers in parentheses indicate the total number of hauls conducted, including the ones where no sample was retrievable.

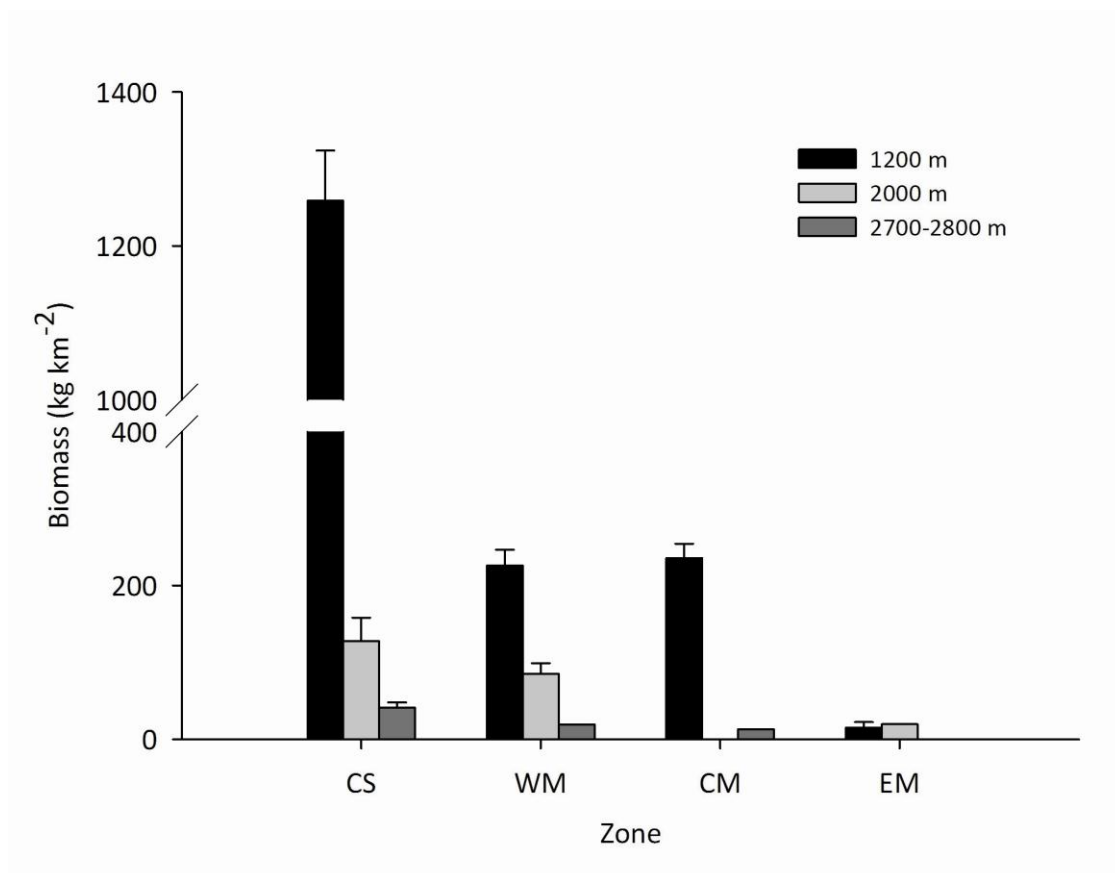
	Zone:	Catalan sea (OS)			Southern Balearic (WM)			Western Ionian (CM)					South of Crete (EM)		
	Depth (m):	1200	2000	2700	1200	2000	2800	1200	2000	2800	3300	4000	1200	2000	3000
<b>BIOFUN 1: 05-29-2009 - 06-30-2009</b>															
OTMS					2 (2)	2 (2)	2 (3)	3 (3)	0 (2)	1 (2)			3 (3)	1 (2)	0 (1)
Agassiz					3 (3)	1 (1)	2 (3)		1		1 (1)	1 (1)	3 (3)	1 (1)	2 (2)
<b>BIOFUN 2: 10-24-2009 - 10-31-2009</b>															
OTMS		2 (2)	2 (2)	2 (3)											
Agassiz		1 (1)	1 (1)												
<b>Environment variables:</b>															
	Benthic temperature (°C)				13.07	13.21	13.32	13.73	13.78	13.95			13.86	13.91	13.92
	Benthic salinity				38.47	38.47	38.47	38.74	38.73	38.73			38.78	38.77	38.74
	Benthic dissolved oxygen (mg l <sup>-1</sup> )				5.70	5.82	5.83	5.73	5.83	5.86			5.53	5.60	5.72
	Benthic turbidity (FTU)				0.77	0.035	0.04	0.072	0.059	0.028			0.04	0.037	0.032
	Sediment grain size (% coarse)				1.72	7.64	16.87	1.08	1.19	4.04			10.48	8.61	12.02
	Sediment POC (% of mass)				0.42	0.52	0.33	0.64	0.58	0.28			0.34	0.26	0.29
	0-200 m Microplankton biomass (mg m <sup>-3</sup> )				5.39	9.36	5.05	5.25	3.96	3.74			2.28	4.77	3.94
	0-200 m Mesozooplankton biomass (mg m <sup>-3</sup> )				4.19	11.93	8.3	11.85	10.74	7.89			5.25	10.04	6.11
	Surface primary production (RFU)				0.739	0.927	0.803	0.559	0.657	0.745			0.541	0.641	0.667



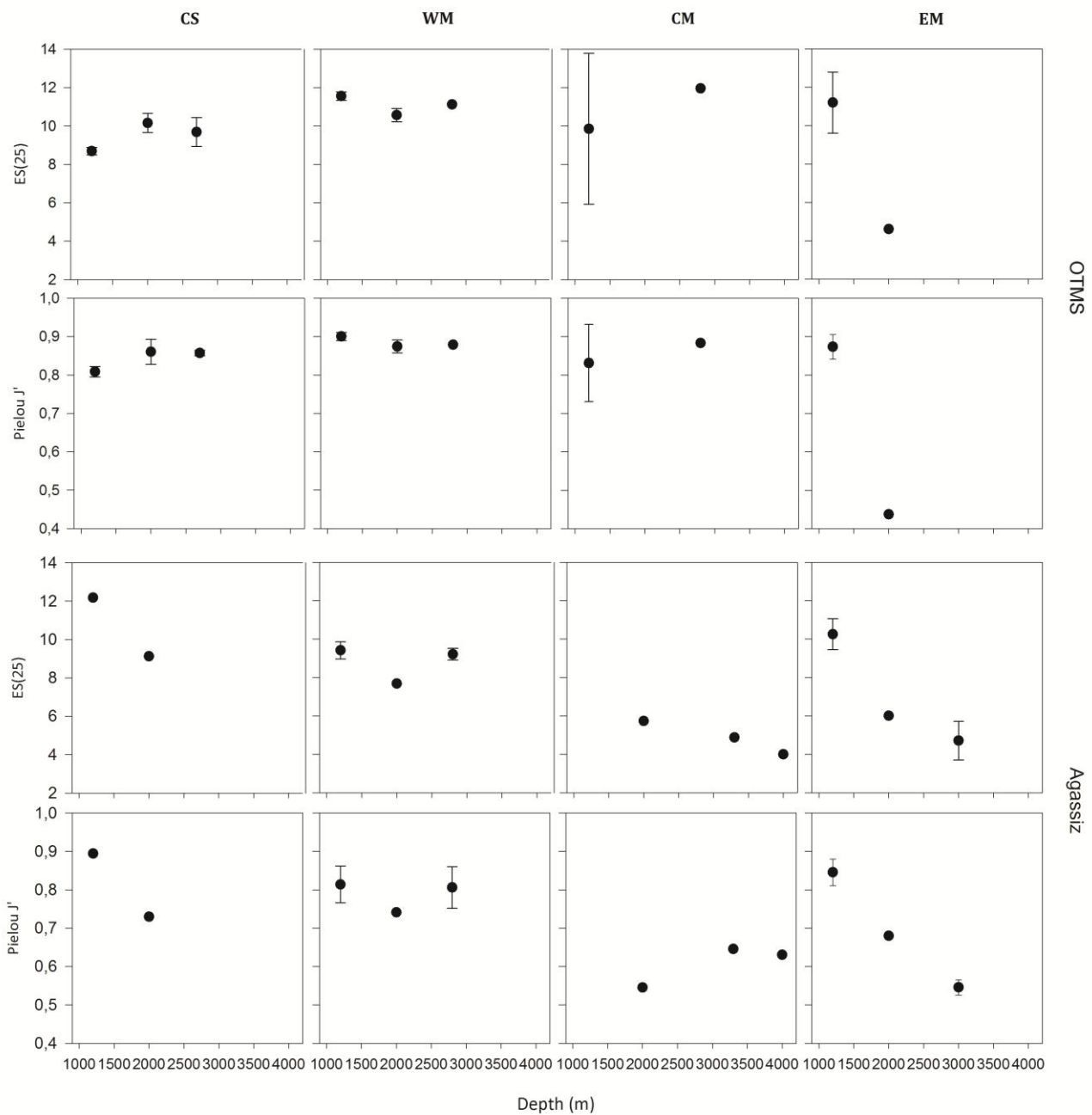
indicate 2700-3000 m depth stations. Abyssal stations at 3300 m and 4000 m depth are also included.



**Figure 2** – Percentage of abundance for taxonomic groups in each zone and depth. ACT: actinopterygians, BRA: brachiopods, BRY: bryozoans, CNI: cnidarians, CRU: crustaceans, ECH: echinoderms, ECR: echiurans, MOL: molluscans, POR: poriferans, SEL: selachians, SIP: sipunculans, URO: urochordata.

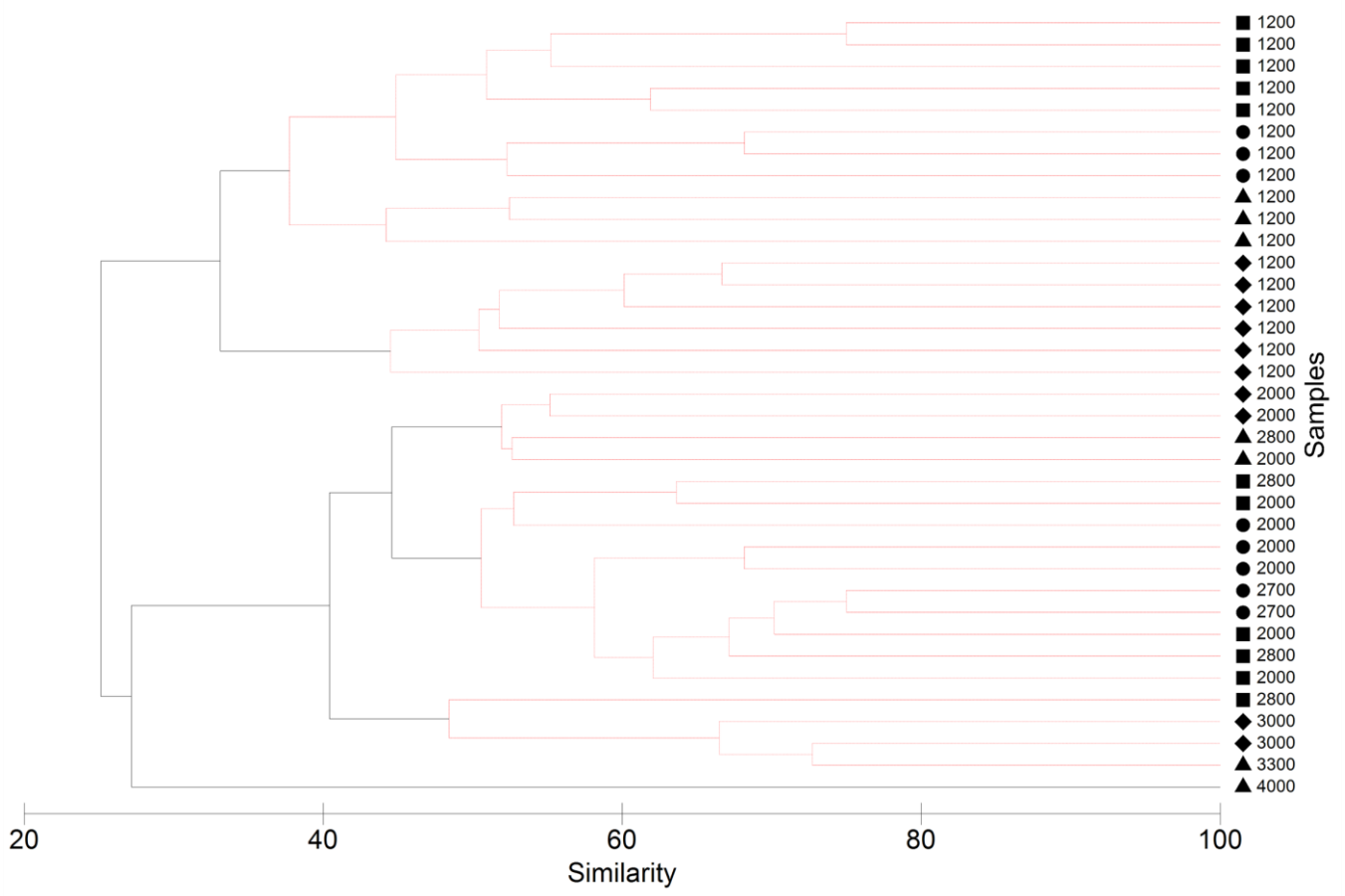


**Figure 3** - Total catch biomass in the four zones, considering standardised biomass data from OTMS samples. Values are Mean  $\pm$  S.E. CS: Catalan slope, WM: Western Mediterranean, CM: Central Mediterranean, EM: Eastern Mediterranean.



**Figure 4** - Expected number of species diversity index ES(25) and Pielou evenness index J' for the otter-trawl Maireta system (OTMS) and the Agassiz dredge trawls at different depths in the 4 study areas. CS: Catalan Sea, WM: southern Balearic, CM: western Ionian, EM: south of Crete. Values are means ( $\pm$ SD).





**Figure 5** – CLUSTER analysis on the abundance of megabenthos from the deep Mediterranean. Numbers alongside samples indicate depth of retrieval. Dashed lines indicate statistically significant groupings according to the SIMPROF routine. ●: Catalan slope, ■: Western Mediterranean, ▲: Central Med., ◆: Eastern Med.