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Spatio-temporal variation of macrofaunal assemblages in the deep Blanes submarine canyon

Tesi di laurea in

Habitat marini: struttura e processi

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A tutta la mia famiglia (grazie di tutto, di cuore)

e a chi si è appena affacciato sullo straordinario cammino del mondo…

Summary

Abstract

The spatio-temporal variations in diversity and abundance of deep-sea macrofaunal assemblages (excluding meiofaunal taxa, as Nematoda, Copepoda and Ostracoda) from the Blanes Canyon (BC) and adjacent open slope are described. The Catalan Sea basin is characterized by the presence of numerous submarine canyons, which are globally acknowledged as biodiversity hot-spots, due to their disturbance regime and incremented conveying of organic matter. This area is subjected to local deep-sea fisheries activities, and to recurrent cold water cascading events from the shelf.

The upper canyon (~900 m), middle slope (~1200 m) and lower slope (~1500 m) habitats were investigated during three different months (October 2008, May 2009 and September 2009). A total of 624 specimens belonging to 16 different taxa were found into 67 analyzed samples, which had been collected from the two study areas. Of these, Polychaeta, Mollusca and Crustacea were always the most abundant groups. As expected, the patterns of species diversity and evenness were different in time and space.

Both in BC and open slope, taxa diversity and abundance are higher in the shallowest depth and lowest at -1500 m depth. This is probably due to different trophic regimes at these depths. The abundance of filter-feeders is higher inside BC than in the adjacent open slope, which is also related with an increment of predator polychaetes. Surface deposit-feeders are more abundant in the open slope than in BC, along with a decrement of filter-feeders and their predators. Probably these differences are due to higher quantities of suspended organic matter reaching the canyon. The multivariate analyses conducted on major taxa point out major differences effective taxa richness between depths and stations.

In September 2009 the analyzed communities double their abundances, with a corresponding increase in richness of taxa. This could be related to a mobilizing event, like the release of accumulated food-supply in a nepheloid layer associated to the arrival of autumn. The highest abundance in BC is detected in the shallowest depth and in late summer (September), probably due to higher food availability caused by stronger flood events coming from Tordera River. The effects of such events seemed to involve adjacent open slope too. The nMDS conducted on major taxa abundance shows a slight temporal difference between the three campaigns samples, with a clear clustering between samples of Sept 09.

All depth and all months were dominated by Polychaeta, which have been identified to family level and submitted to further analysis. Family richness have clearly minimum at the -1200 m depth of

BC, highlighting the presence of a general impact affecting the populations in the middle slope. Three different matrices have been created, each with a different taxonomic level (All Taxa "AT", Phylum Level "PL" and Polychaeta Families "PF"). Multivariate analysis (MDS, SIMPER) conducted on PL matrix showed a clear spatial differences between stations (BC and open slope) and depths. MDSs conducted on other two matrices (AT and PF) showed similar patterns, but different from PL analysis. A 2nd stage analysis have been conducted to understand differences between different taxonomic levels, and PL level has been chosen as the most representative of variation.

The faunal differences observed were explained by depth, station and season.

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1   INTRODUCTION

1.1   The deep sea

Our planet is covered by 70% from water and the name "Water" would be more appropriate than "Earth".

The oceanic environment is vertically divided into five realms, depending on light intensity in clear oceanic water; they are the epipelagic (from the surface to 200 m), the mesopelagic (from 200 to 1000 m), bathypelagic (from 1000 to 4000 m), abyssopelagic (from 4000 to 6000 m) and hadopelagic (below 6000 m) (Fig. 1) . The photic zone is the zone of the epipelagic realm where the daylight is sufficient to support primary production by photosynthesis (max 150 - 200 m). Even in the mesopelagic zone there is some dim light, but it's not energetic enough to excite the electrons in the chlorophyll to start the photosynthesis process. Below the mesopelagic there is the aphotic zone, where the waters are perpetually dark and all life depends from secondary production processes (e.g., degradation by detritivorous organisms) or from some special type of primary production (e.g., chemosynthesis at hydrothermal vents).

The role of oceans in both ecological and climatic global processes is fundamental to maintain the equilibrium into nature's functions and services; they are the most valuable biome in the world, evenly more than their terrestrial counterparts (Costanza et al., 1997). A large part of this importance is due to the extension of the deep seafloors which represent 65% of total ocean basins. The percentage of waters deeper than 1 km is 88% and this, together with their volume and geological age of seabeds (more than 200 millions of years), makes them the most ancient, connected and stable ecosystems of today world (Herring, 2002). Recently, however, this stability has emerged as only relative, with the deep environments being affected by various processes (such as catastrophic events, waste disposal or climate changes) that may strongly influence the dynamics of such fragile ecosystems (Gooday, 2002; Danovaro et al., 2010). However few studies have addressed this issue.

The common feature of deep-sea habitats is the lack of primary production endowed by photosynthesis due to shielding of external light provided by the water column. Therefore most of the deep-sea communities entirely depend from the primary production of the surface layers (Rowe, 1981; Billett et al., 1983; Gooday, 2002; Rex et al., 2006; Smith et al., 2008) with few

exceptions (e.g. hydrothermal vents, cold seeps or organic fall communities) that are just very small parts of the puzzle of all types of deep-sea habitats. Because of this craving, the abundance of life below the photic zone is much less abundant than in shallowest layers (typically 5-10 times fewer), however recent research changed the general perception of deep-sea benthic environments as a species-poor habitat to one that is rich in species (Snelgrove and Smith, 2002). Moreover the circumstances in deep-sea could be changed by some local environmental conditions capable to convoying some sort of energy replenishment from upper layers through changes in thermoaline circulations (McGraw & Hill, 2055), like in submarine canyons.

Submarine canyons

The connection between continental shelf and deep-sea floor occurs through the continental slope (Fig. 2). The angle and extent of the slopes vary with locality, averaging from a gradient of about 7% up to 50% (like a drop of 500 m in 1 km horizontal distance) (Tain and Dipper, 1998). Despite their extension (only 8% of the ocean's surface area) the continental shelves are globally the most productive part of ocean (Sherman and Duda, 1999; Levin et al., 2010). During past glaciations the sea level was shallowest than nowadays and most shelvers were exposed. Their surface was then eroded by rivers and glaciers and these "scars" are now the submarine canyons which are nothing else but the old beds submerged again by the sea, after rising its level later the melting of all glaciers. They cut the continental slopes perpendicular to coastal line and channel sediments from continental shelf to the deep-sea floor, particularly because some of them are still closely connected to major river outflows (Griggs et al., 1969; Gardner, 1989; Sampere et al., 2008; De Leo et al., 2010).

During cold seasons the upper waters of the neritic zone became colder due to the loss of heat of the surface layers raising their density and begin to flow down the continental slope, convoying a large quantity of suspended particles from the upper areas to the deepest through a process named "cascading" (Pérès, 1985; Thistle, 2003). These events can greatly modify the deep-sea fertility by increasing the energy availability along their paths with a massive redistributions of nutrients coming from shallowest realms (Rowe et al., 1982; Josselyn et al., 1983; Epping et al., 2002).

When the water above the continental shelf contains a large quantity of suspended particles it can give rise to "turbidity currents", strong flows of high density fluids (water + suspended sediments) that flow down along the slopes with a high speed, fast enough to excavate their own submarine canyon (Vitorino et al., 2002; de Stiger et al., 2007). At the end of their runs, a large

amount of sediments are deposited, creating huge fan-shaped areas than can reach even the abyssal plain.

These two processes may change the distribution of organisms through modifying the local conditions, e.g. generating hotspots of benthos production (Vetter and Dayton, 1999, De Leo et al, 2010) by enhancing food availability, which depends on several mechanisms: (i) accelerated currents (Rowe, 1971; Shepard et al., 1974), huge concentration of dense layers of krill during their downward vertical migrations, (iii) increased sedimentation rates or (iv) accumulation of macrophyte-detritus (Vetter, 1994,1995). In addition the channeling can be seasonal, depending on atmospheric climatic conditions too (Vetter, 1998) and its effects can change deep-sea assemblages by modifying the local recruitment of some taxa (Fannelli et al., 2011).

Deep-sea fauna

In the aphotic zone, in absence of photosynthesis and with communities being "fertilized" by the flowing (or "raining) of organic matter from surface zones, the abundance of organisms is generally low. There are some "hot spots" (like those of hot waters lifts near oceanic dorsal systems). However, in general, the oligotrophic conditions prevent the rising of huge concentrations of faunal biomasses (Fig. 3). The species living there are adapted to extreme life conditions (e.g., high pressures, very low temperatures). The organisms are generally smaller than the shallower ones. However, there are some documented cases of "deep-sea gigantism" (especially in the glacial deep waters, where the temperature of the more superficial water layers is as low as that of the deepest ones, favoring the nutrient mixing and increasing the energy availability to compensate the cold adaptations) (Thiel, 1979; McClain et al., 2006).

Despite these difficulties, recent studies indicate that the deep sea may be the most biologically diverse environment on earth. Until now the researchers have been unable to collect and identify every species present in all deep-sea habitats due to the difficulties in adequately sampling these environments. Based on "species accumulation curves" (SAC) that analyze how often new species are found when collecting progressively more individuals, has been estimated that 1 million (and possibly more than 10 million) species live on the deep-sea floor, making it as or more diverse than rain forests and coral reefs habitats (Fig. 4). (Coll et al., 2010; Danovaro et al., 2010)
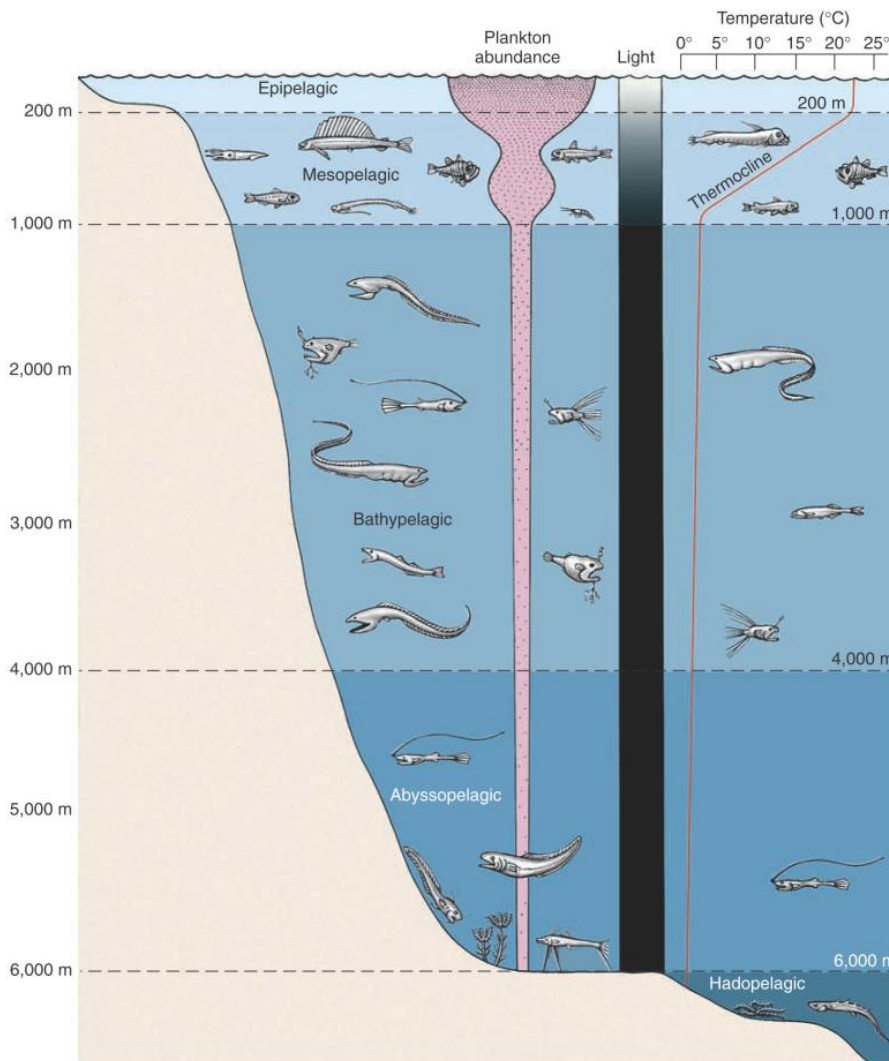
Fig. 1 Life in mesopelagic and deep sea is closely linked to the abundance of plankton and light intensity in the water column. (From McGraw and Hill, 2005)
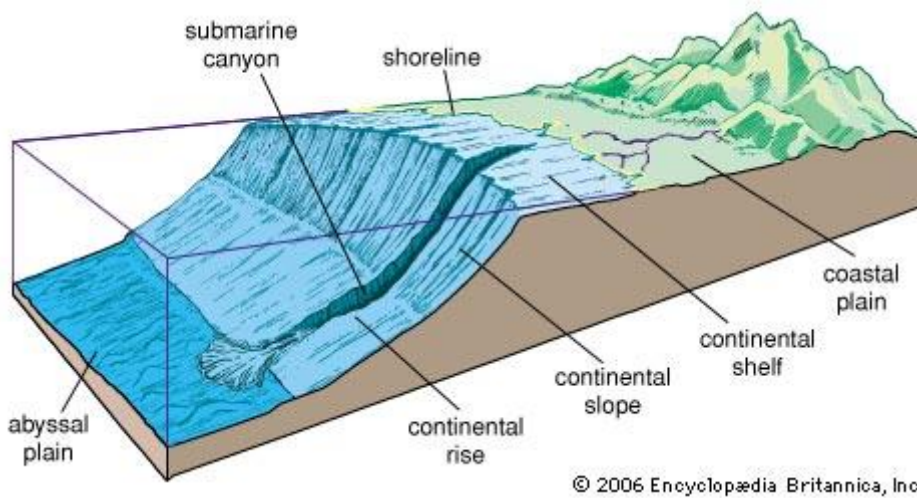
Fig. 2 Scheme of a continental slope lined by a simple submarine canyon. (From Encyclopedia Britannica Inc., 2006)

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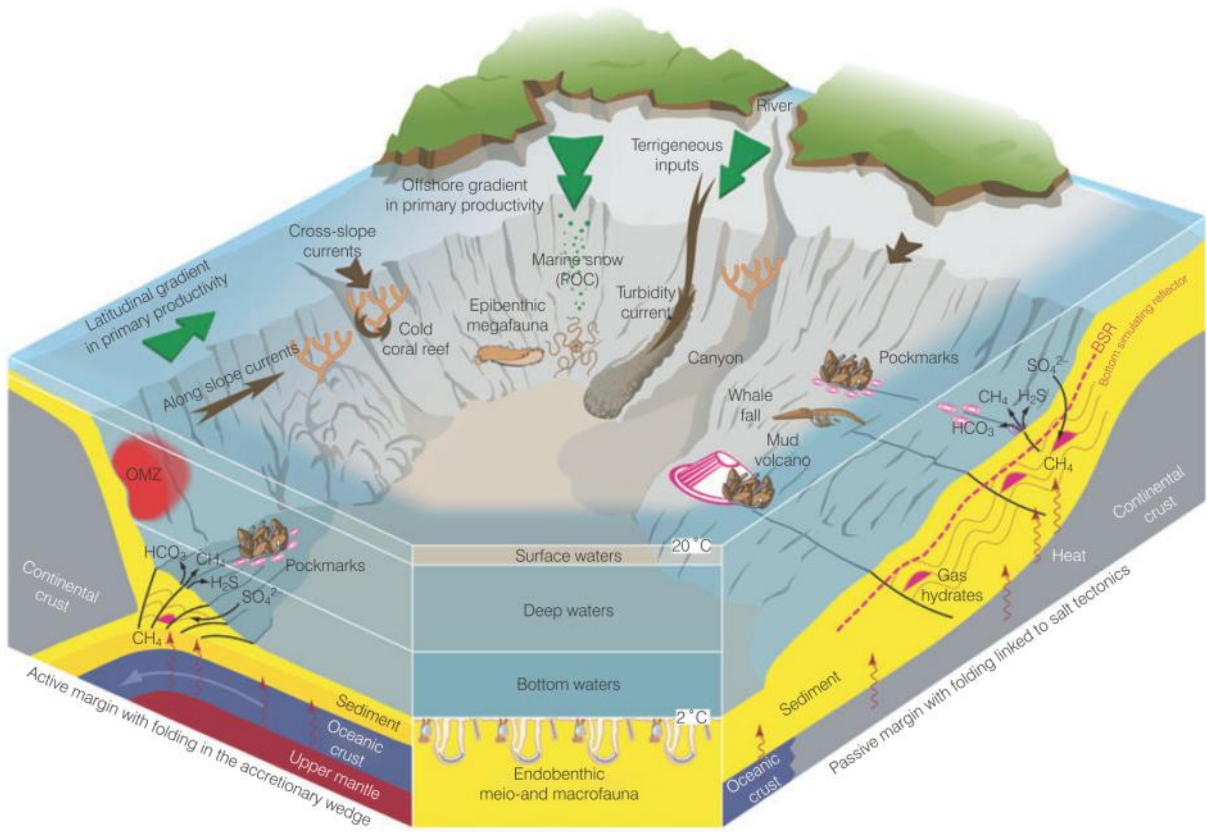
Fig. 3 Diagram summarizing the main geological, hydrological, and biological factors driving habitat heterogeneity on active and passive continental margins. All these features create a complex mosaic of influences shaping margin biodiversity. (From Menot et al., New Perceptions of Continental Margin Biodiversity, 2010)

Fig. 4 Species accumulation curves from the deep-sea and shallow water benthos off the coast of New England. (From McGraw and Hill, 2005)

1.2   Macrofauna

An approach to investigate about the general conception that considers deep-sea diversity low (Longhurst, 1998; May, 1994) is to study the benthic organisms, which are the most comparable to the terrestrial fauna, especially those living within the sediments as we may use similar sampling methods and statistics on the obtained data sets.
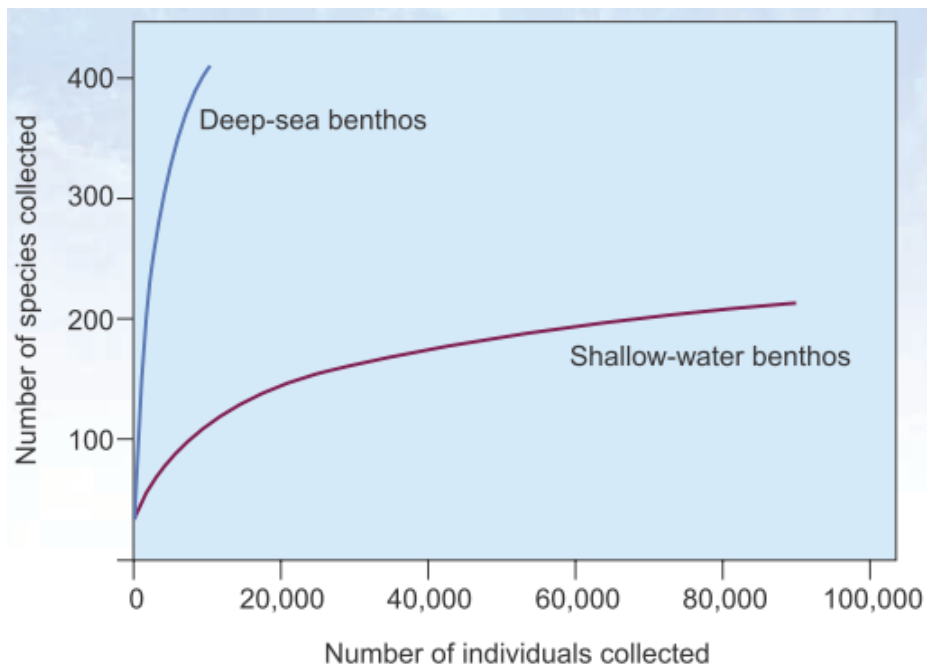
Most of the deep-sea floor is covered by fine, muddy sediment and the animals living among these particles are often divided into two main size categories: meiofauna (from 44 μm to ~300) and macrofauna (>300 μm).

Soft-bottom macrobenthic communities in the deep sea are most often composed by infaunal organisms (i.e. living inside the sediment), mainly including polychaetes, amphipod and decapods crustaceans, bivalve mollusks, and echinoderms. Its density, biomass and trophic structure generally decrease with increasing water depth and increasing distance from shore (Sanders et al., 1965; Rowe, 1981; Rowe et al., 1982; Houston and Haedrich, 1984; Flach and Heip, 1996) so, along with depth, progressively large volumes of sediments are required to have an accurate determination of the most commonly used macrofaunal descriptors (Danovaro, 2009).

The distribution and diversity of deep macrobenthos have mainly been related with depth gradients at several spatial scales, as well as with sediment size (Tselepides and Elefhterious, 1992; Stora et al., 1999). However the highest diversification is most often associated to small-scale changes in sediment structure and in food availability over sedimentary bottoms (Sanders et al., 1965; Gage and Tyler, 1991).

1.3   The Mediterranean Sea

The deep Mediterranean Sea is characterized by 4 main features: (i) deep-hypersaline anoxic basins created during the Messinian salinity crisis, (ii) cold seepage and "mud volcanism" associated with the release of gas from deep-sea sediments, (iii) the role of catastrophic events (like landslides), which increase considerably the topographic complexity of the seafloor, and (iv) volcanism and its influence on the topographic features and the creation of seamounts. These characteristics are all present in the western basin, which consists in two main deep areas: the Algero Provençal basin and the Tyrrhenian Sea (Sardà et al, 2004).

The general water circulation is highly complex: the surface waters come from the Atlantic and turn into intermediate waters in the Eastern Mediterranean. Low-salinity Atlantic waters enter the Mediterranean, while dense deep-Mediterranean waters flow beneath the Atlantic waters in the opposite direction into the Atlantic Ocean. Deep and bottom currents are largely unexplored, but episodic intensification of current speed up to 1 m s-1 has been documented (Canals et al., 2006). So, deep-water turnover is roughly 50 years and it's heavily influenced by the relative small average depth of this sea, that is about 1,450 meters (much shallower than the average of the world oceans, about 3.850 m).

This conditions makes the Mediterranean deep-sea ecosystems highly vulnerable to climate change and deep-water warming, also in conjunction with others main hydrological features: (a) high homeothermy from roughly 300–500 m to the bottom (temperatures of about 12.8°C to 13.5°C in the western basin); (b) high salinity, from about 38 to 39.5 by the stratification of the water column, (c) limited freshwater inputs (the freshwater deficit is equivalent to about 0.5–0.9 m y-1, compensated by the Atlantic inflow of surface water), (d) a microtidal regime, (e) high oxygen concentrations, and (f) oligotrophic conditions, with strong energetic gradients and low nutrient concentrations in the eastern basin (Danovaro et al., 1999).

1.4   The North-Western Mediterranean

The NW Mediterranean is heavily influenced by the presence of the Gulf of Lion. The strongest winds of this district, Tramontane and Mistral, rise here and generate the greatest fetches of entire Mediterranean Sea (Fig. 5). In winter this amount of low temperature air causes a huge turbulent mixing in the water column up to its lowest layers and creates dense water formations that cascade down the slope to SW direction. These cold flows run along the Catalan continental margin following the Western Mediterranean Deep Water (WMDW), originated in the Ligurian Sea under severe winter conditions. Its path describes a general cyclonic form (Millot, 1999) and have a width of 30-50 km and a vertical velocity profile about 30 to 50 cm s-1 at surface, decreasing approximately linearly with depth to speeds of a few centimeters per second at several hundred meters depth (Lapouyade and Durrieu, 2001). The other main current of this area is the Levantine Intermediate Water (LIW). Originated in the Eastern Mediterranean basin and coming from the Aegean Sea passing through the Sicily Channel, the LIW follows the Italian continental slopes due to the Coriolis effect and reaches the NW Mediterranean, where it is forced to deflect into a SW direction (Fig. 6).

The Catalan Sea is the continental area of the Balearic Sea, which extends from the Cap de Creus (north) to the Ebro River delta (south). Along this coast, the continental shelf is narrow and the shelf break occurs at around 150 m depth. This coast is characterized by the presence of numerous canyons that reach depths up to 2000 m and thus play an important role in convoying the northern dense shelf waters to the deep-sea ecosystems.

The Catalan Sea is particularly impacted by mass tourism and fishery activities, which generate many sources of disturbance highly influencing the marine environment (Sardá et al., 2009). Beyond this, several rivers flow into the area, many of them subjected to the typical Mediterranean regime seasonal flooding, often linked to episodes of heavy rain (Canals et al., 2006). All these factors may heavily stress the living communities, even causing the collapse of the ecosystem services that they support.
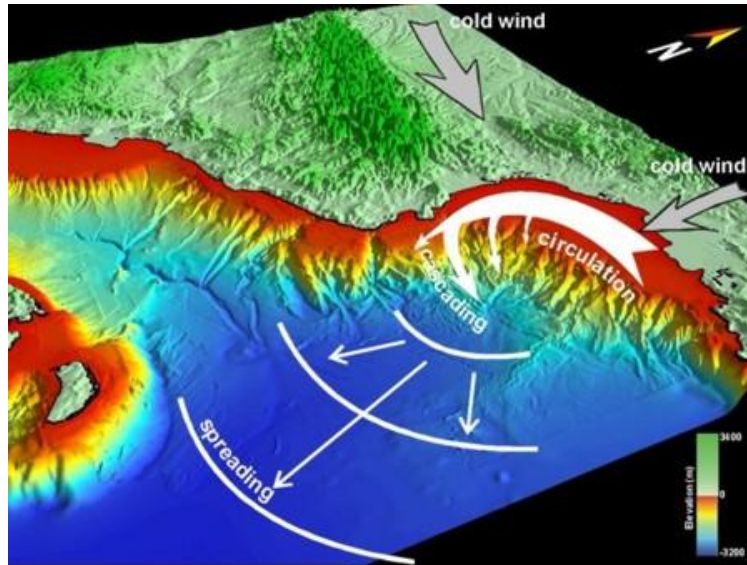
Fig. 5 Representation of the currents flowing along the Catalan margin. (from site: http://www.ub.edu/hermes/prometeo/Goals.html)
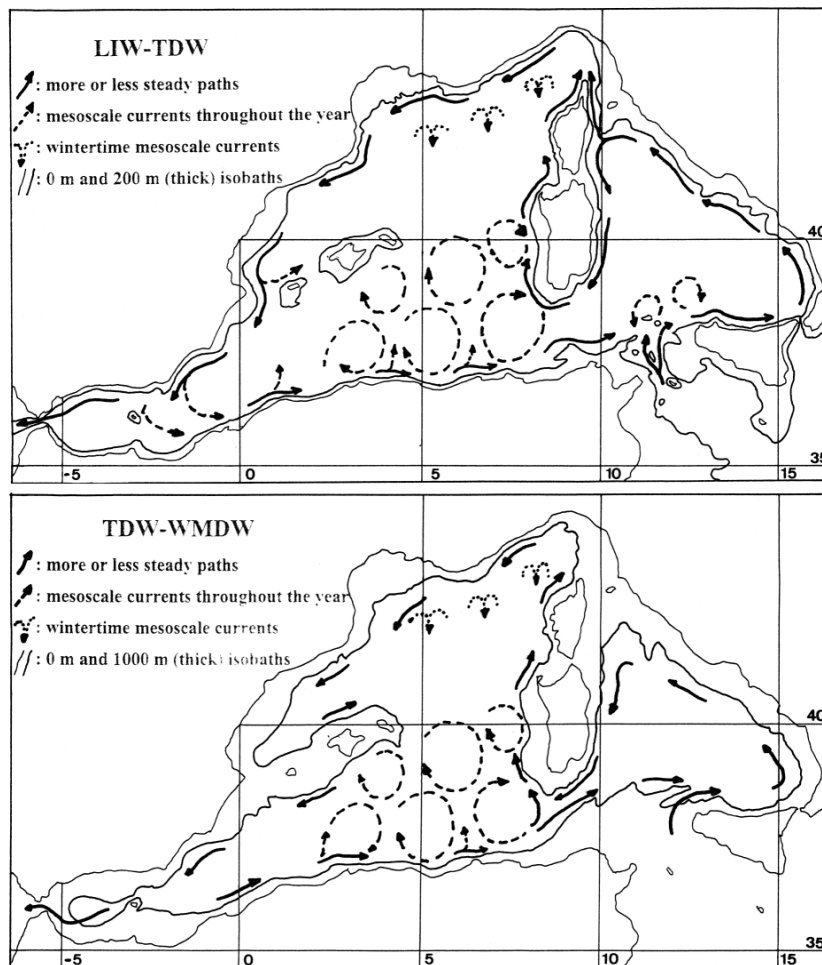

Fig. 6 Circulation of the Levantine Intermediate Water (LIW), Tyrrhenian Dense Water (TDM) and Western Mediterranean Deep Water (WMDW). (From Millot, 1999)

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1.5 Thesis' objective

Submarine canyons are generally recognized as organically enriched environments in comparison with the open slope at similar depths (Pusceddu et al., 2010). Therefore it is not surprisingly that the biomass or density of benthic assemblages are higher there than at the open slopes at comparable depths (Gage et al., 1995; Vetter, 1995; Vetter and Dayton, 1998; Duineveld et al., 2001). The high diversity of the topography of a canyon often creates a patchiness on sediment grain size distribution along their path (de Stigter et al., 2007; Oliveria et al., 2007; Arzola et al., 2008). These different substrata, together with the high disturbance of the canyons systems, allow the settlement of a wide variety of organism (Thistle et al., 1985; Thistle and Wilson, 1996), and the assemblages are not an exception. Canyons are also reported as yielding characteristic and unique faunas, at least in their upper sections (Rowe, 1971; Gage et al., 1995; Rogers et al., 2002; Curdia et al., 2004). However, these statement may only be fully assessed by using the same taxonomic resolution between the different analyses as well as a full assessment of biodiversity and endemism of the different systems (Gage et al., 1995; Escobar Biornes et al., 2008).

The aim of this thesis is to analyze the spatial and temporal variations of the macrofaunal assemblages in the Blanes Canyon (BC), one of the largest ones of the Catalan system of deep-sea submarine system canyon and to compare them variations to those of the macrofauna of the adjacent open slope. This will also permit to understand if (and how) the possible differences in macrofaunal distribution and density may be related to variations of abiotic variables and conditions inside BC and its adjacent open slope. This work is based in part of the benthic samples collected within the framework of the wider, multidisciplinary project PROMETEO.

1.6   The Spanish PROMETEO project

PROMETEO stands for "Estudio Integrado de Cañones y Taludes PROfundos del MEdiTErráneo Occidental: un hábitat esencial", which presents an extraordinary opportunity to investigate the impact on the bathyal populations of meio-, macro- and megafauna, and in particular on the deep red shrimp *Aristeus antennatus*, of the events of massive transport of sediment and fresh organic matter towards the deep margin and basin associated to cold water cascading from the shelf, the implications of which have recently been described by Canals et al. (Nov. 2006) in Nature. The precise relationship between the deep fauna and such cascading events is mostly unknown. In the study area, and in particular in the western side of the Gulf of Lions, such events happen with mid- to low intensities almost every year in late winter and/or early spring, and with a high intensity, such as in spring 2005, every 10-12 years.

The combination of abiotic and biotic factors make the canyons and deep slopes of the Western Mediterranean essential habitats in which occur well structured communities characterized by a clear faunal zonation and trophic web of low energetic level. This fragile equilibrium is highly vulnerable to anthropogenic and natural perturbations (Canal et al, 2006) focused by the submarine canyons.

The participation in PROMETEO of a number of experts from various disciplines (geology, biology, physical oceanography, biogeochemistry, live resources, environment sciences, marine sciences and engineering) and institutions (ICM and CEAB from CSIC, UB, PCB and FBG from the UB Group, UPC, and CEFREM from CNRS-Univ. Perpignan), with a tremendous experience on the topics addressed and in the study area, ensures the pluri- and multidisciplinary character of the project and the achievement of scientific results of the highest relevance on the Western Mediterranean deep canyons and slopes.

This project provided a unique opportunity to reinforce the scientific investigation of the deep Mediterranean Sea.

1.6.1 Project structure

The PROMETEO project is structured in two workpackages based on two main research axes and a third one as a complementary research.

The main elements of the PROMETEO Project from the point of view of scientific outputs, their interdependencies and their potential contribution to the management of biodiversity and natural

resources in the deep Mediterranean canyons and slope as related to subprojects SP1 and SP2 is shown graphically. The figure highlights the scientific outputs and their potential contribution to management tasks.

This work here presented places itself within WP2 which main objective is to analyze the relation between abiotic conditions and the populations of key deep-sea organisms (Fig. 7).

In the last two decades our knowledge of the biology of several species and the living communities of the lower continental slope has substantially increased (Gage and Tyler, 1991). The Institutes of the Spanish Council for Scientific Research (CSIC) have pioneered this type of studies in the Mediterranean Sea. The results obtained have placed the NW Mediterranean as one of the five regions in our planet where deep-sea biology has been better described (Sardà et al., 2004 and references therein). Nonetheless, the investigation of the deep sea is in a preliminary phase, mainly because of the logistics and economical costs involved. Most of the studies so far have been descriptive and the processes that occur at great depths and the natural and anthropogenic factors that drive them are still unknown. Despite the fact that PROMETEO focus particularly on one of the main target species of the fisheries of the region, the red shrimp *Aristeus antennatus* (Risso, 1816), which plays a central role in the ecology of the mid- and lower continental slope. Many efforts have also bee addressed to the understanding of other biological components of the canyon system, including the benthic macro- and meiofaunal organisms, which are the main trophic resource available for the exploited populations of the red shrimp. It was hypothesized that the availability of trophic resources could contribute to explain the large concentrations of certain species (mainly fish) at relatively narrow bathymetric ranges, which is found between 1150 and 1250 m depth (Stefanescu et al., 1993; Moranta et al., 1998; Company et al., 2004; D'Onghia et al., 2004). All these aspects reinforce the value of the multidisciplinary approach provided by the PROMETEO project and provides an excellent framework to develop the study here proposed.
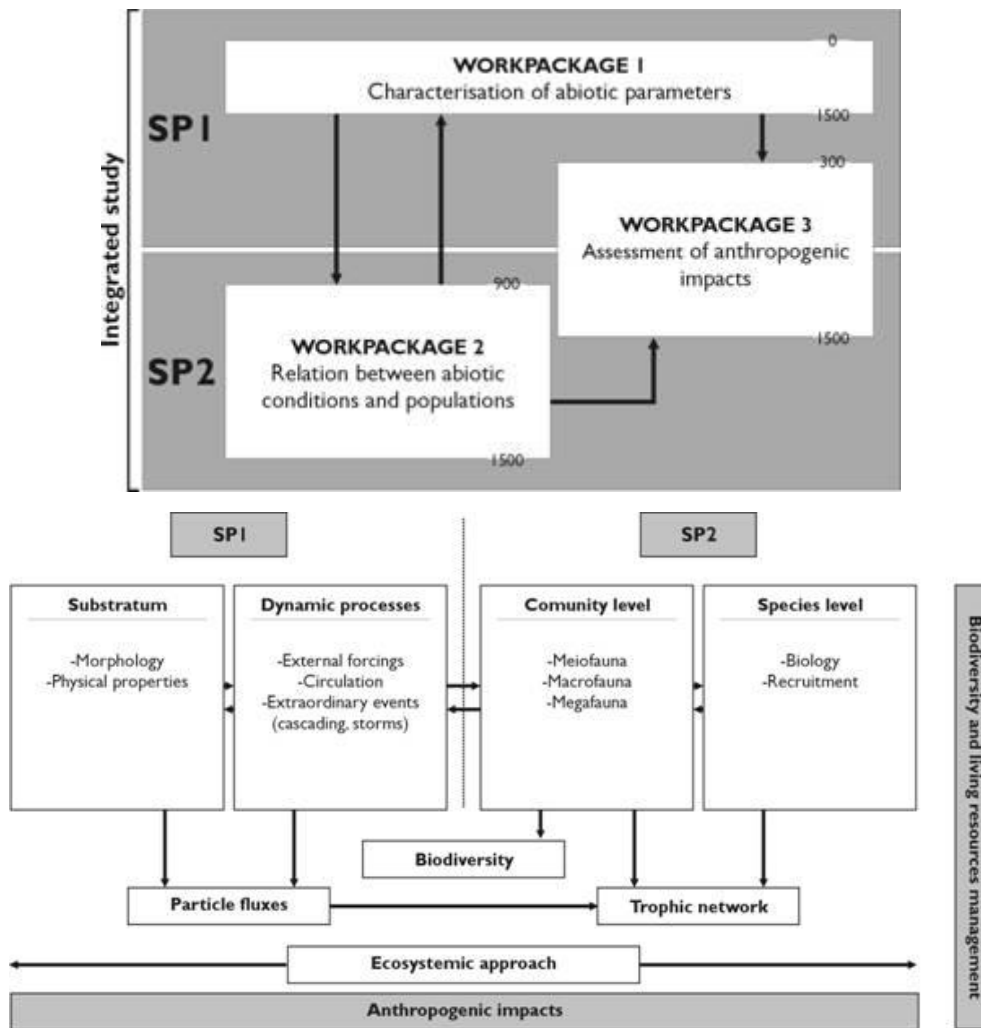
Fig. 7 Framework of PROMETEO project. (from site: http://www.ub.edu/hermes/prometeo/)

2 MATERIALS AND METHODS

2.1 Study area

The investigated area has been the submarine Blanes Canyon (from now referred as BC) and its adjacent open slope in the Northwestern Mediterranean. The head of the canyon is embedded in the continental shelf 60 m depth at less than 4 km offshore (Díaz and Maldonado, 1990) and it has a length of ca. 2km (Fig. 8); the head, in conjunction with the mouth of the canyon, form the "upper canyon". Its offshore continuation is referred to as the "lower canyon" reaching down to 2000 m with a breadth of 20 km (Canals et al., 1982, Flexas et al., 2008).
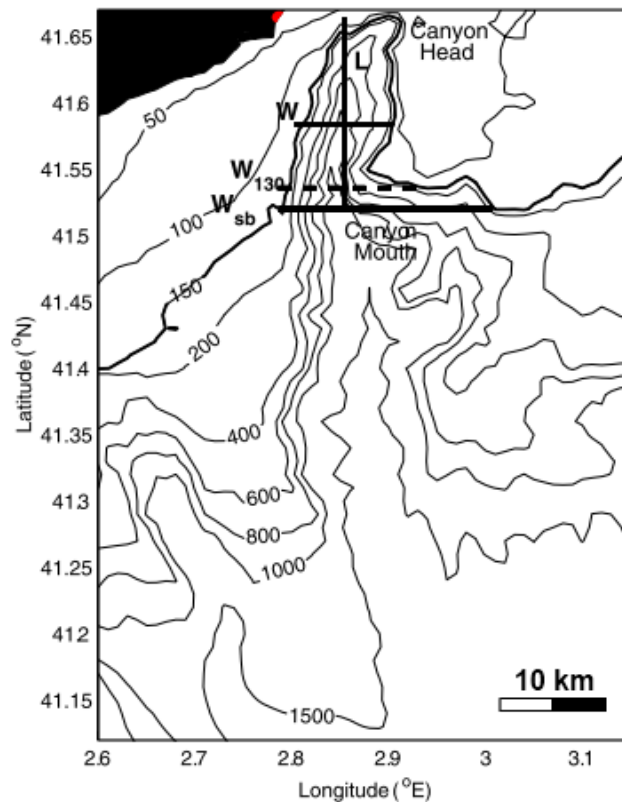
Fig. 8: Representation of the seafloor bathymetries in the area of the Blanes Canyon. The thick bold lines indicate the length (L) and the width (W) of BC's mouth (BCM). W130 indicate that there the BCM is 130 m deep and Wsb indicates where is the shelf break. The red point is the position of Blanes city. (from Flexas et al., 2008; modif.)

Within the framework of the project PROMETEO (PR) a total of five cruises (PR-I to PR-V) were carried out in 2008/2009 around BC (Fig. 9) and on board the oceanographic vessel García del Cid, which belongs to the CSIC of Spain and has the Port de Barcelona as a base. The main characteristics of the vessel are shown in Table 1.

Table 1: Principal characteristics of the O/V García del Cid (for more information visit: http://www.utm.csic.es/Garcíadelcid.asp)

Length	37.2 m	Width	8.40 m
Gross tonnage (GT)	285.5 tons	Max speed	10 knots
Crew	26 members tots. (max 12 scientists)	Launch	1977

The **1st Work-Package** (WP1)of the project focused on the characterization of abiotic parameters of the drainage basin of BC through (i) the study of data recorded by some institutional Catalonian agencies on the environmental physic parameters; (ii) the analysis of the seafloor's sediments through direct sampling and visual validation using ROVs; (iii) the direct monitoring of water flows/sediment rates through moorings equipped with particle traps (see Fig. 1).

The data presented are part of the **2nd Work-Package** (WP2) that focused on the analyses of the relation between abiotic conditions and populations through: (i) identifying the main communities; (ii) determining the life cycles of the key species with the aim to describe spatio-temporal variations in reproductive patterns, population structure and distribution in relation with the variability of physico-geochemical parameters and the temporal variations of food availability; (iii) relating spatio-temporal variations in the composition, structure and biological processes of the main benthic communities with the abiotic characteristics of the system studied in WP 1.
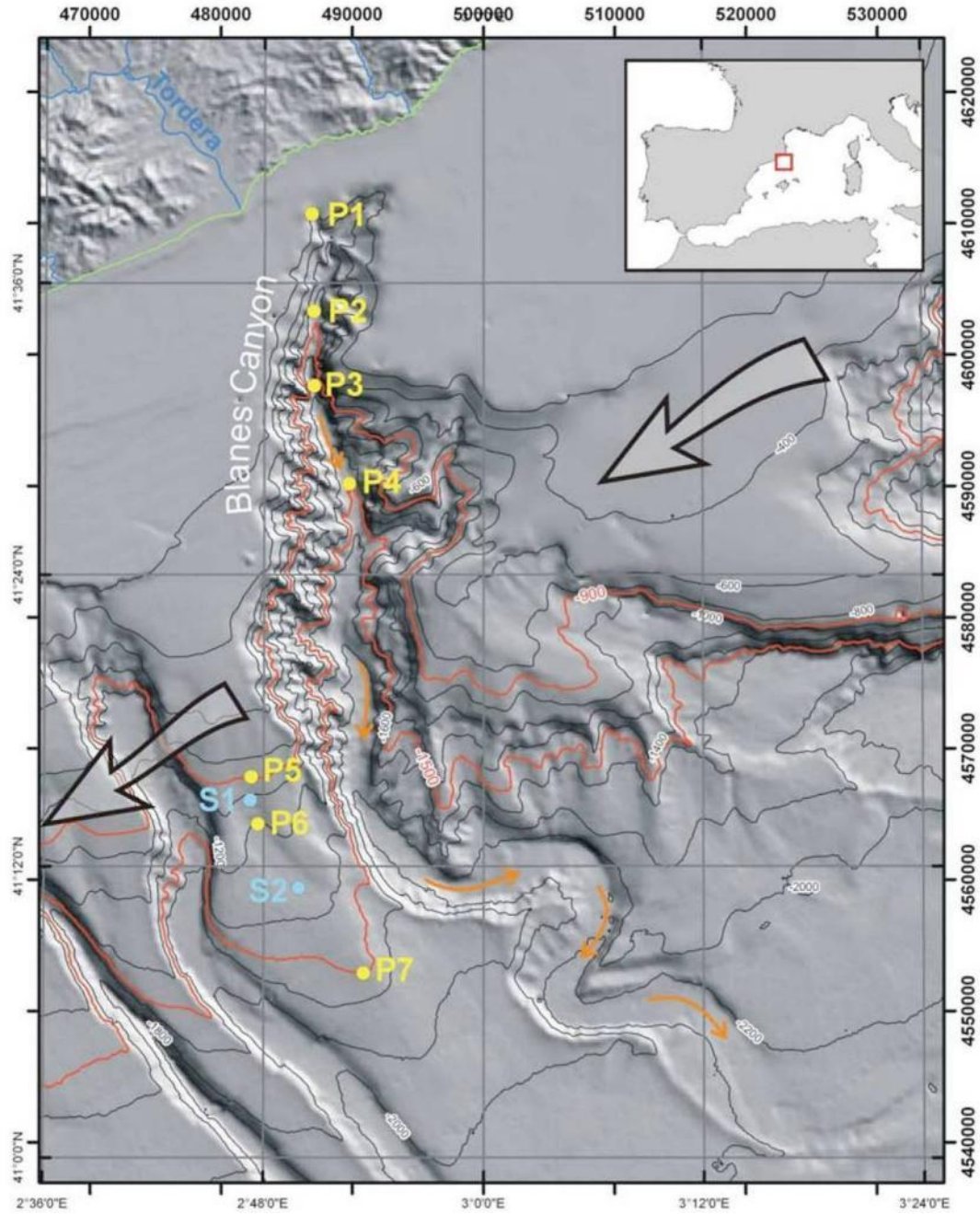
Fig. 9: Map showing the PROMETEO study area. The 900 m and 1500 m depth contours indicated in red correspond to the depth range where a large part of the research activities have been concentrated. The mooring lines and the sampling sites have also been indicated (P1 to P7, and S1 and S2) in the canyon axis and on the open slope to the south of the canyon. Large arrows correspond to regional circulation while small orange arrows follow the axis of the Blanes Canyon.

2.2 Sampling design
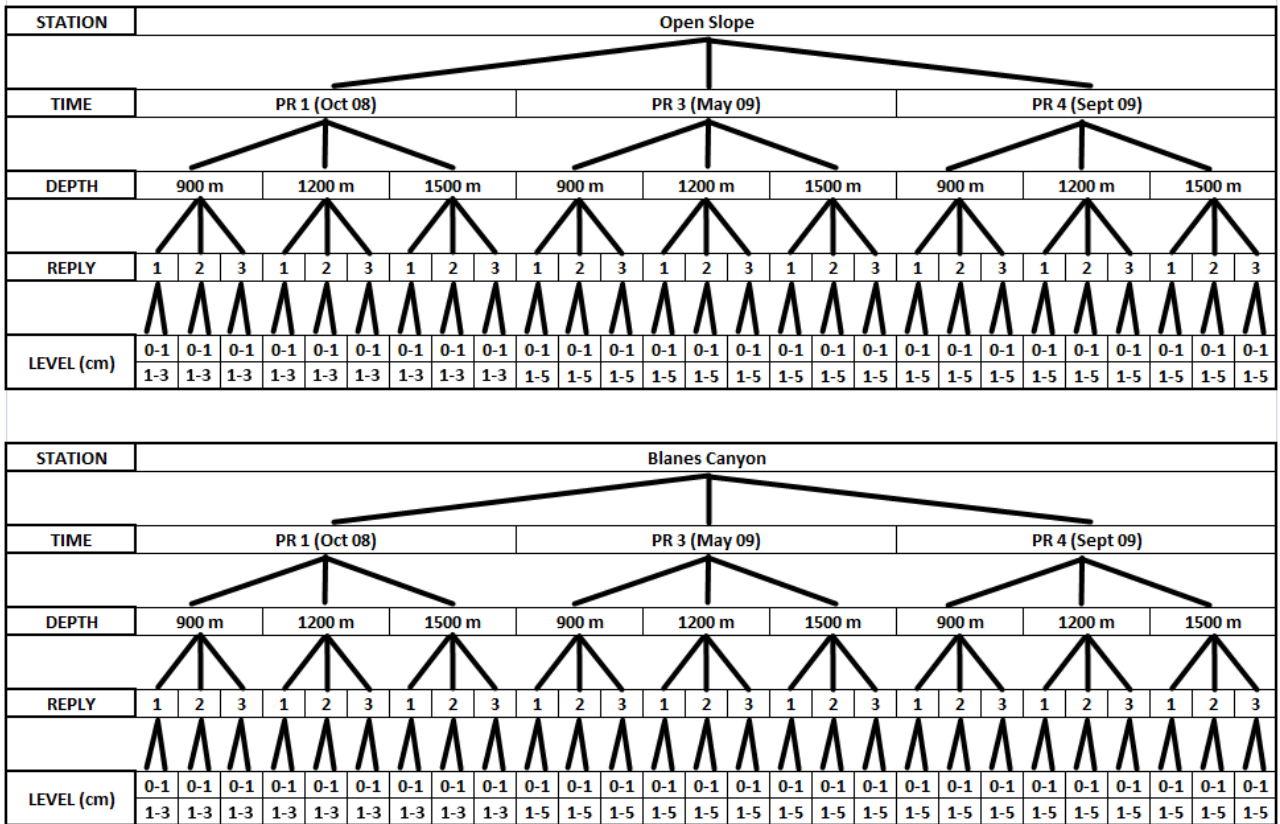
Fig. 10: Scheme of followed sampling design in PROMETEO I, III and IV campaigns.

The planned sampling design to analyze the variations of the deep-sea macrofaunal assemblages in BC was a three-ways model (Fig. 10), based on two variables (space and time):

- 1st fixed factor: "Station", with two levels (BC and adjacent open slope);
- 2nd fixed factor: "Campaign", orthogonal compared to "Station", with three levels (PRI, PRIII and PRIV);
- 3rd fixed factor: "Depth", orthogonal compared both to "Station" than "Campain", with two levels (-900 m, -1200 m and -1500 m).

During PR-I (October 2008), the sediment corers were sliced in two layers (0-1 and 1-5 cm), while in PR-III (May 2009) and PR-IV (September 2009), the three layers were collected (0-0.5, 0.5-1 and 1-5 cm). Only 67 of all collected samples have been analyzed (as shown below).

ProMeTeo I (october 08) — Open slope

Campain	ProMeTeo I (october 08)						
Station	Open slope						
Depth	900 m			1200 m			1500 m
Core	1	2	3	1	2		1
Level (cm)	0-1	0-1	0-1	0-1	0-1		0-1
	1-5	1-5	1-5	1-5	1-5		1-5

ProMeTeo I (october 2008) — Blanes canyon

Campain	ProMeTeo I (october 2008)				
Station	Blanes canyon				
Depth	900 m			1200 m	1500 m
Core	1	2	3	1	1
Level (cm)	0-1	0-1	0-1	0-1	0-1
	1-5	1-5	1-5	1-5	1-5

ProMeTeo III (may 2009) — Open slope

Campain	ProMeTeo III (may 2009)					
Station	Open slope					
Depth	900 m	1200 m				1500 m
Core	1	1	2	3	4	1
Level (cm)	0-0.5	0-0.5	0-0.5	0-0.5	0-0.5	0-0.5
	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1
	1-5	1-5	1-5	1-5	1-5	1-5

ProMeTeo III (may 2009) — Blanes canyon

Campain	ProMeTeo III (may 2009)			
Station	Blanes canyon			
Depth	900 m	1200 m	1500 m	
Core	1	1	1	2
Level (cm)	0-0.5	0-0.5	0-0.5	0-0.5
	0.5-1	0.5-1	0.5-1	0.5-1
	1-5	1-5	1-5	1-5

ProMeTeo IV (september 2009) — Open slope

Campain	ProMeTeo IV (september 2009)		
Station	Open slope		
Depth	900 m	1200 m	1500 m
Core	1	1	1
Level (cm)	0-0.5	0-0.5	0-0.5
	0.5-1	0.5-1	0.5-1
	1-5	1-5	1-5

ProMeTeo IV (september 2009) — Blanes canyon

Campain	ProMeTeo IV (september 2009)		
Station	Blanes canyon		
Depth	900 m	1200 m	1500 m
Core	1	1	
Level (cm)	0-0.5	0-0.5	
	0.5-1	0.5-1	
	1-5	1-5	

Fig. 11: Scheme of all analyzed samples.

2.3   Sample collection and processing

The samples were collected during PR-I. PR-II ad PR-IV, thus corresponding to October 2008 (autumn), May 2009 (full spring) and September 2009 (late summer) (Table 2), which allows to assess the temporal variations of the biological communities in BC and its adjacent open slope.

Table 2: Features of analyzed cruises.

Name of cruise	O/V	Period	
		from	to
PROMETEO I	García del Cid	23/10/2008	3/11/2008
PROMETEO III	García del Cid	6/05/2009	16/05/2009
PROMETEO IV	García del Cid	3/09/2009	10/09/2009

The samples consisted on eight pooled multicore samples (0.011 m^2 each), which were gently homogenized with the help of native seawater, filtered on a 250 µm pore size mesh, transferred into tagged HDPE (high density polyethylene) bags and preserved with 5% buffered formalin. Prior to sorting, a solution of 1% Rose Bengal was added to each sample to permit an easy recognition of the organisms (Snider, 1984; Danovaro, 2009).

Together with the samples from the sledge and sediment traps, the multicore samples will provide information on the structure, composition, biomass and energy of the trophic resources locally available to the red shrimp and fish BC and its adjacent open slope, both horizontally (aggregation) and vertically (in the sediment column). The present study is thus, a contribution to a wider study of the deep-sea bentic community of the region.

In the laboratory, all the samples were kindly homogenized and then washed through a 250 µm pore sieve. The whole procedure has been fulfilled by wearing filtering-mask and protective clothing (gloves & lab coats) to prevent the contact with highly toxic formalin fumes . The material retained by the filter was transferred to Petri dishes and sorted under a stereomicroscope.

For every sample, all the organisms belonging to the same taxa (Brusca & Brusca, 2003) were preserved into glass tubes filled with a 70% alcohol solution. Every tube was tagged as:

- Campain (ProMeTeo I/III/IV)
- Station (Blanes Canyon/Open slope)
- Depth (-900/-1200/-1500 m)
- Core (tube 1/2/3/etc.)
- Level (0-0.5/0,5-1/0-1/1-3/1-5 cm)
- Taxa name.

2.4 Taxonomic analyses

The taxonomic analyses has been fulfilled by consulting international scientific books, dichotomous keys and different scientific literature on deep-sea and Mediterranean taxa (see bibliography).

2.4.1 Polychaeta

The order Polychaeta, belonging to the Phylum Annelida, comprises more than 16.000 species, which are commonly known as "segmented worms". They they are characterized by a segmented bodyplan, which gives them an evolutionary "plasticity" to colonize different niches. Each segment is repeated in series, and so entire body derived from a serial homology (metamerism). They are triploblastic coelomate bilateralia with an elongate body (usually cylindrical). The head is composed of a prostomium and a peristomium, which holds the mouth. These parts often bear appendages (e.g. palps, antennae, tentacles, cirri), which may be greatly modified depending on the different species adaptation. Typically, most trunk segments hold many chitinous chaetae, organized in ndles holded by lateral digitations (called parapodia). The terminal segment is the pygidium, which bears the anus. The principal external diagnostic characters to classify families of benthic polychaetes are: prostomium's structure, type, number and location of the anterior appendages end, type and quantity of body chaetae, presence of elytra or gills, proboscis' shape, presence of a proventricle, jaws' presence and form , pygidium shape, among others.

Some of the most relevant families found during this study are hre briefly characterized both in terms of morphology and ecology (from Fauchald, 1977, and Fauchald & Jumars, 1979):

2.4.1.1 Family Paraonidae (subclass Scolecida)

Body long and slender with lateral parapodia. Prostomium with a single antenna or antennae absent. Branchiae present on a limited number of median setigers in most species. All setae simple, including capillaries and various, usually postbranchial, hooks and otherwise modified setae (Fig. 12).

They are non-selective, burrowing deposit-feeders or surface-feeders that live in muddy environments, especially in deep-sea habitats (where they feed foraminiferans and small crustaceans).
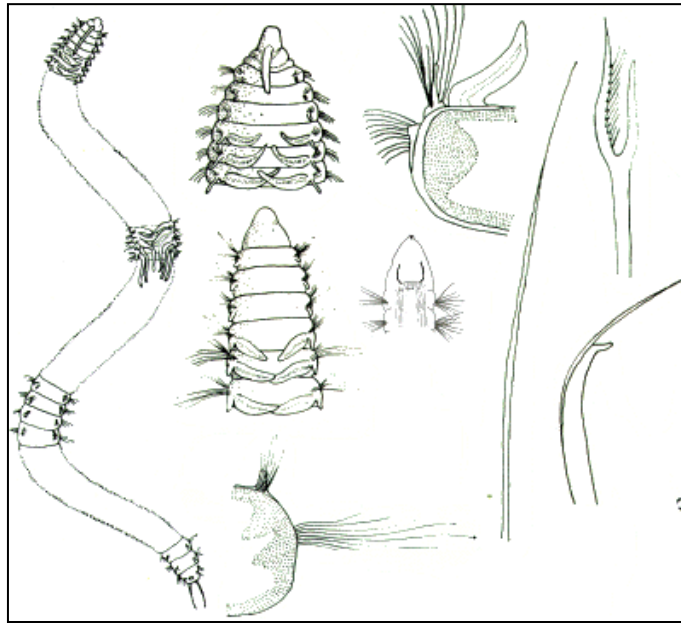
26

Fig. 12. Family Paraonidae (from J. H. Day, 1967. Polychaeta of Southern Africa. British Museum Natural History)

2.4.1.2 Family Spionidae (subclass Canalipalpata, order Spionida, suborder Spioniformia)

Spioniforms with elongated body. Protostomium anteriorly blunt, with frontal horns, or pointed; an occipital papilla may be present, other appendages absent. Palps at the postectal corners of the prostomium. Parapodia biramous, parapodial lobes cirriform or foliose, never serrated. All setae simple, including capillaries and bi- or multidentate, hooded or non-hooded hooks (Fig. 13).

They are mud-tubicolous worms capable of leaving their tubes to build a new one when necessary. They are considered surface deposit-feeders, which use their ciliated palps to select food particles from the surrounding medium or filter feeders, often alternating between these two modes depending on the available food.
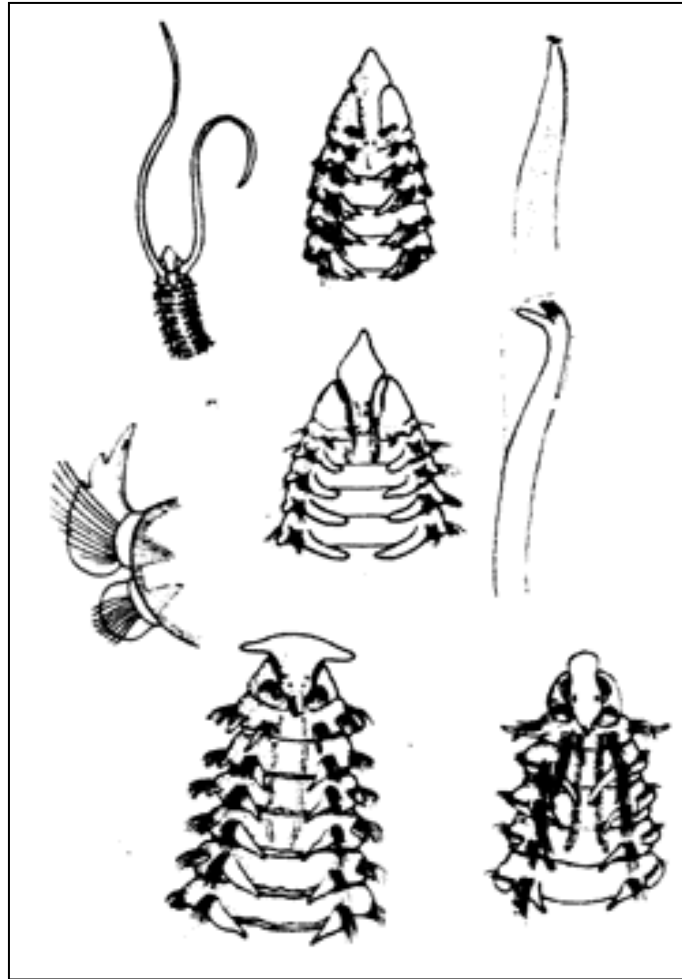
Fig. 13. Family Spionidae (from J. H. Day, 1967. Polychaeta of Southern Africa. British Museum Natural History)

2.4.1.3 Family Syllidae (subclass Aciculata, order Phyllodocida, suborder Nereidiformia)

Small to medium-sized nereidiform polychaetes with usually slender bodies (sometimes dorsoventrally flattened). Three antennae and simple palps present, the latter sometimes fused to each other. Two pairs of tentacular cirri. Eversible pharynx armed with a single tooth or a circlet of smaller teeth or unarmed. Proventricle always present (but often seldom visible through the body wall). Parapodia uniramous, dorsal cirri usually conspicuous, setae simple or composite (Fig. 14).

They have eversible, cylindrical, often-armed pharynges, followed by strongly muscular pumping structures called proventricles. They pierce the surface of their prey and suck out the content with the help of the proventricle. Often they feed on hydroids, bryozoans, and other colonial invertebrates, often living in close association with them.

Fig. 14. Family Syllidae (from J. H. Day, 1967. Polychaeta of Southern Africa. British Museum Natural History)

2.4.1.4 Family Glyceridae (subclass Aciculata, order Phyllodocida, suborder Glyceriformia)

Glyceriform polychaetes with long, slender bodies and conical prostomia. Eversible pharynx with four jaws in a cross. Paraopodia either all biramous or all uniramous. Neurosetae composite; notosetae, when present, simple (Fig. 15).

They have big pharynges tipped by four jaws made of tanned proteins impregnated with iron and copper compounds. They are mostly characterized as carnivores, but some species can feed on faecal pellets or absorp dissolved organic matter.
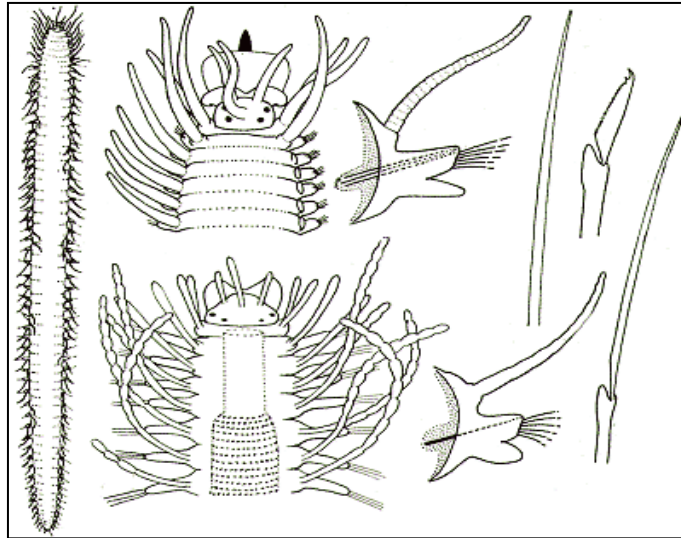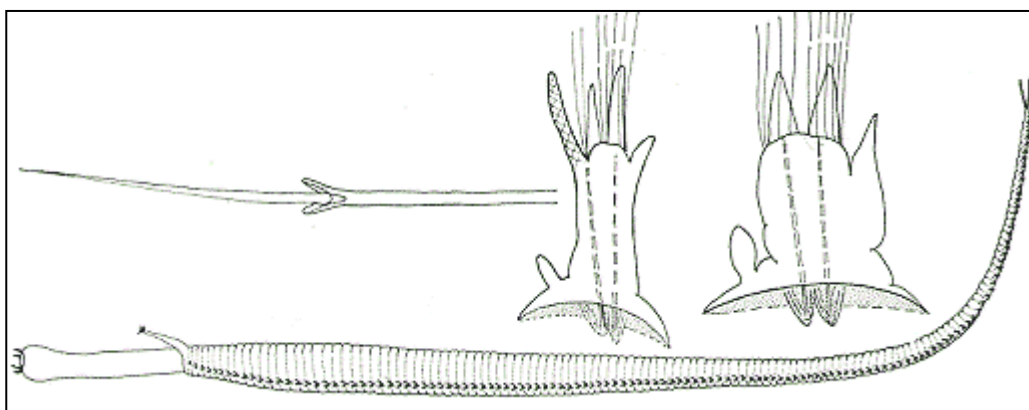

Fig. 15. Family Glyceridae (from J. H. Day, 1967. Polychaeta of Southern Africa. British Museum Natural History)

2.4.1.5 Family Lumbrineridae (subclass Aciculata, order Eunicida)

Eunicea without prostomial appendages, but sometimes with one to three nuchal papillae emerging from a pocher between the pro- and peristomium. Maxillary carriers short, third carrier absent. Maxilla I smooth and curved. Notopodia absent or represented by small, button-shaped projections, sometimes with internal acicula. Setae include limbate setae, simple and composite hooks. Subacicular hooks and pectinate setae are absent (Fig. 16).

They have large eversible jaw complexes that consist of a pair of mandibles and four pairs of maxillae, so they are considered carnivores or carrion-feeders.

Fig. 16. Family Lumbrineridae (from J. H. Day, 1967. Polychaeta of Southern Africa. British Museum Natural History)

2.4.1.6 Family Cirratulidae (subclass Canalipalpata, order Terebellida, suborder Cirratuliformia)

Body cylindrical. Prostomium conical or blunt; peristomium fused with at least two segments. Parapodia reduced; slender filiform or clavate branchiae present on at least some setigers. All setae simple including capillaries and curved or excavate hooks (Fig. 17).

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Most species are free living, but some cirratulids live in tubes (mud-covered or calcareous). They are non-selective deposit-feeders that use their palps for food collecting.
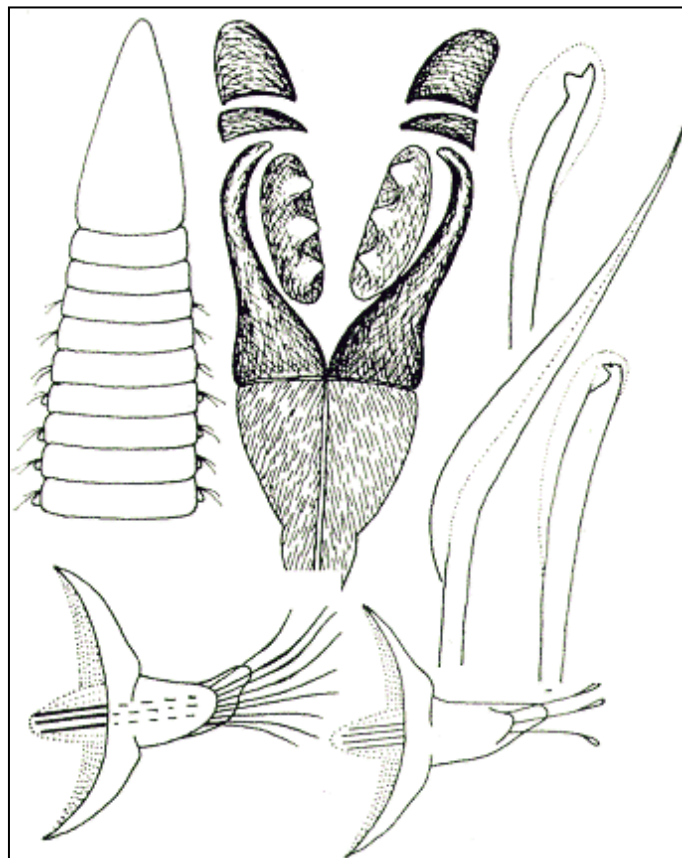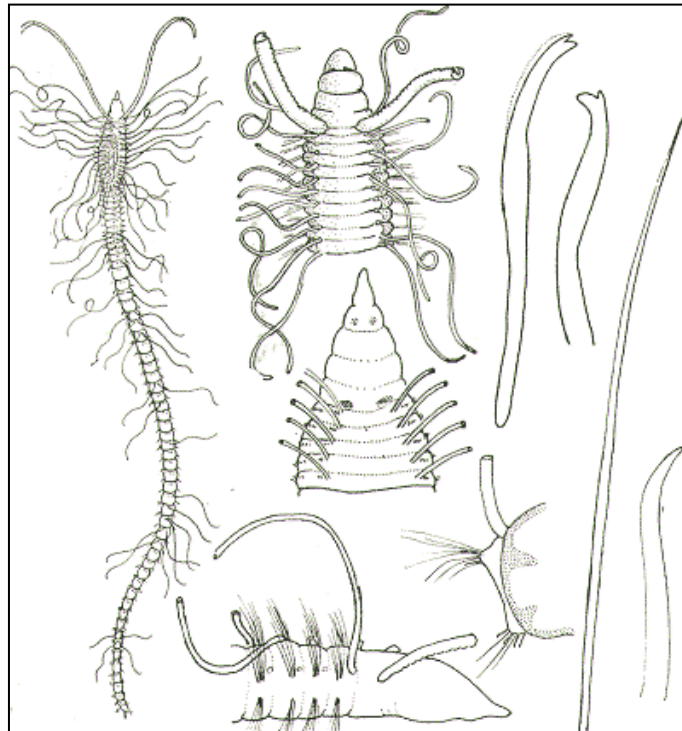

Fig. 17. Family Cirratulidae (from J. H. Day, 1967. Polychaeta of Southern Africa. British Museum Natural History)

2.4.2 Crustacea

This Subphylum of the Arthropoda Phylum is the most abundant (both in number of species than in covered habitats) of the Animalia. The Crustacea can be considered as "sea-insects" due to their diffusion in all marine habitats. Their classification is one of the hardest, because they present many different forms, even some aberrant. The general bodyplan consists in a body composed of a 5-segmented cephalon (head), a multisegmented postcephalic trunk (divided in thorax and abdomen) and a tail fan. The head and the thorax may be fused together to form cephalothorax, which can be covered by a single carapace. On the head there are five pairs of appendages: first and second antennae (or antennuale), mandibles and maxillae. Each somite (or trunk segment) bears bi-ramous limbs, which can have an feed function (maxillipedae) or serving to move (pereopods). Pleopods are the posterior limbs, which mainly serve to swim.

The principal external diagnostic characters used to their classification, are: body shape, presence of compound eyes, structure and size of head's appendages, aspect of somites, comparison between pleopods and pereopods, tail's form.

Some of found specimens are shown below (Fig. 18, 19, 20):

Fig. 18: Photo of one of found specimen belonging to order Isopoda (class Malacostraca, subclass Eumalacostraca, superorder Peracarida).
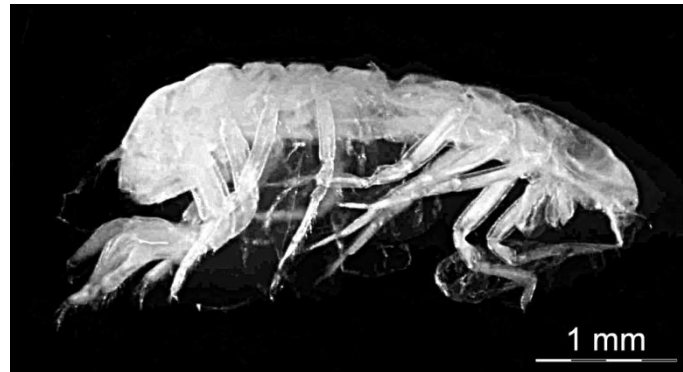

Fig. 19: Photo of one of found specimens belonging to order Amphipoda (class Malacostraca, subclass Eumalacostraca, superorder Peracarida).
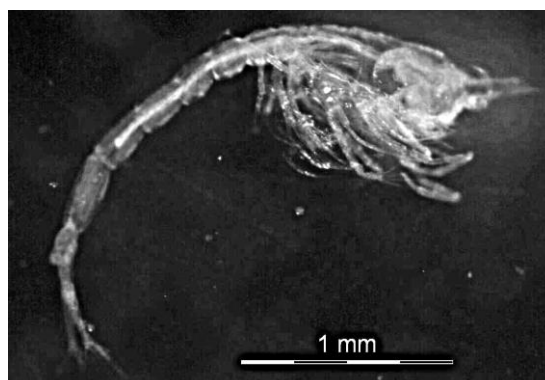
Fig. 20: Photo of one of found specimens belonging to order Cumacea (class Malacostraca, subclass Eumalacostraca, superorder Peracarida).

2.4.3 Mollusca

They are coelomate protostomes, with an unsegmented body and a bilateral symmetry (or secondarily asymmetric). Their body is covered by thick epidermal-cuticular sheet of skin (the mantle), which forms a cavity (the mantle cavity) in which are housed the ctenidia, osphradia, nephridiopores, gonopores, and anus. The mantle cells secrete calcareous material, which is used to form spicules or shells.

In our samples, we have found specimens of the classes Bivalvia and Aplacophora.

Bivalves are microphagous or suspension feeders, with body laterally compressed and typically comprised into two valves hinged together dorsally by elastic ligament and shell-teeth.

Aplacophora are benthic vermiform mollusks, with a shell-less body covered by aragonite (calcareous) spicules or scales (Fig. 21).
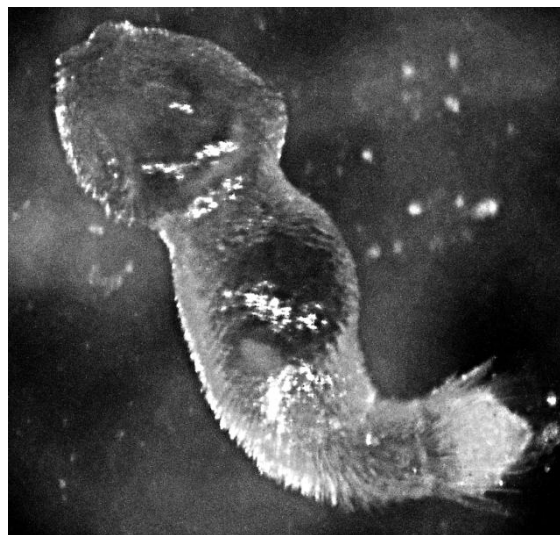
Fig. 21. Photo of one of found Caudofoveata (Mollusca, Aplacophora).

2.4.4 Sipunculida

This phylum includes about 250 species in 17 genera. Their bodyplan resembles that of Annelida, but they show no evidence of segmentation (Fig. 22). The body consists into a thicker trunk with a retractable introvert. The mouth and feeding tentacles lie at the anterior end of the introvert. The tentacles are of two types: peripheral (around the mouth) and nuchal (around the nuchal organ).
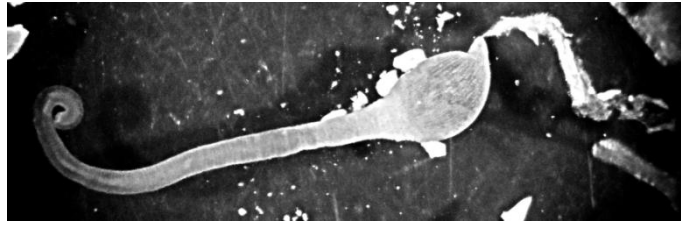
Fig. 22. Photo of one of found Sipunculida.

2.4.5 Phoronida

The Phoronida are deuterostomes belonging to the clade Lophophorates. They are benthic suspension-feeding organisms, living in chitinous tubes, which often have sand aggregates. They comprise only 20 species of solitary of gregarious worms (exclusively marine), with a tripartite body divided into a flaplike epistome (prosome), a middle mesosome (which bears lophophore), and a posterior elongate trunk (metasome). All found specimens were in larval stage (actinotroch) (Fig. 23).
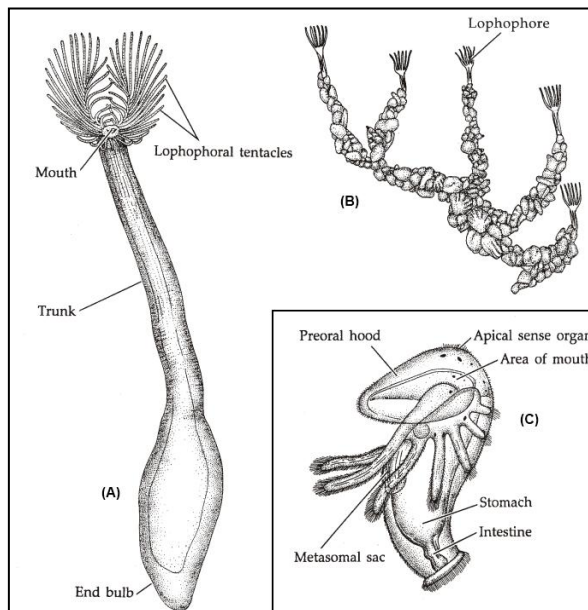
Fig. 23. Representation of Phoronida: adults (A),(B) and larval stage actinotroch (C). (from Brusca & Brusca, 2003; adapted)

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2.5   Data analysis

2.5.1   Univariate analyses.

Univariate analysis collapses all data information into a single coefficient. To characterize the community structure we have used the total number of individuals (N), and the total number of species (S). Also we have estimated the diversity using the Shannon index (H^i):

$$H^i = - \sum p^i \log(p^i)$$

where p^i is the proportion of the total count arising from the ith species. Its values goes to $H^i_{min} = 0$ (one single species present) to $H^i_{max} = \log S$ (when all species are equally represent by same number of individuals). Finally, we estimated the equitability using the Pielou's evenness index (J):

$$J^i = H^i / H^i_{max} = H^i / \log S$$

where H^i_{max} is the maximum possible value of Shannon index.

2.5.2   Multivariate analyses.

To analyze the community structure in a synthetic way, we performed non-metric multi-dimensional scaling (MDS) ordination based on a Bray–Curtis similarity matrix, after different transformations of the abundance data, which were previously organized into a sample vs. taxa matrix. SIMPER analysis (Similarity Percentages–species contributions) was performed to indicate the percentage contributions of each major taxa to the similarity within and dissimilarity between groups of samples. PRIMER v6 software (Clarke and Gorley, 2006) was used to perform these analyses.

2.6   Taxonomic sufficiency

The difficulties to study deep-sea fauna are not only dealing with sampling, but also with the identification of each individual specimens, which often tend to be smaller than their shallow-water relatives. Sampling the deep-sea is not so common, and a high-level taxonomic resolution is often necessary to achieve initial contributions to the knowledge of sampled given area. From an ecological point of view, such an approach may be enough to highlight fine differences between assemblage (Olsgard et al., 1998), which may respond in different ways to the same factor. Full identifications work may be are more labur-intensive and expensive than those done on shallow-water communities (Ferraro et al., 1989), anf often requires specific expertise on deep-sea taxa.

Few studies recent have have approached the possibile of using different taxonomic resolutions to study deep-sea macrobenthic fauna(e.g. Narayanaswamy et al., 2003). However, this does not prevent to the use of a lower taxonomic-sufficiency to determine the response of benthic communities to environmental gradients, as demonstrated in shallow-water studie (Warwick, 1988). However, the use of high taxonomic levels may mask changes in species replacement, which may indicate significant responses to disturbance events. Thus, it is often recommended to conduct additional studies comparing the community responses using different taxonomic levels (Gray et al. 1990, Warwick et al. 1990, Vanderklift et al. 1996, Olsgard et al. 1997).

According to Somerfield and Clarke (1995) a practical approach consist in comparing different similarity matrices based on the same dataset, but using different taxonomic resolutions. Therefore, the taxa obtained in this study were aggregated into different levels: (i) All taxa (AT) = matrix with all taxonomic levels, from Polychaeta species to higher taxa, (ii) Phylum level (PL) = matrix arranged at phylum level, with all lower levels collapsed into them, (iii) Polychaeta Families (PF) = matrix with the polychaete families only. Each matrix has been treated by using four different transformations, which imply different homogenizations of the dataset: (a) no transformation at all, (b) square-root, (c) 4th-root and (d) log(X+1). The ranked matrices of similarity are based on Bray-Curtis similarity (Bray & Curtis, 1957). The Spearman rank correlation (p) has been computed between the corresponding elements of each pair of matrices, and the significance of the correlation has been determined by a permutation procedure (using the RELATE routine of the PRIMER v6 software) (Clarke & Warwick, 1994). Then, all these matrices have been plotted into different nMDS to made evident any possible variations between them. Also, a 2nd-stage nMDS has allowed us to visualize the interrelationships between the different similarity matrices, where the distance between different transformed matrices reflects the degree to which they are correlated.

3   RESULTS

3.1   Major taxa

A total of 867 organisms have been found, belonging to 17 different taxa. Traditional maiofaunal taxa (Nematoda, Copepoda and Ostracoda) have been excluded from the analyses to allow proper comparisons with similar scientific studies on deep-sea macrobenthic communities. Thus, the 624 specimens belonging to 16 different taxa were finally considered (Fig. 24).
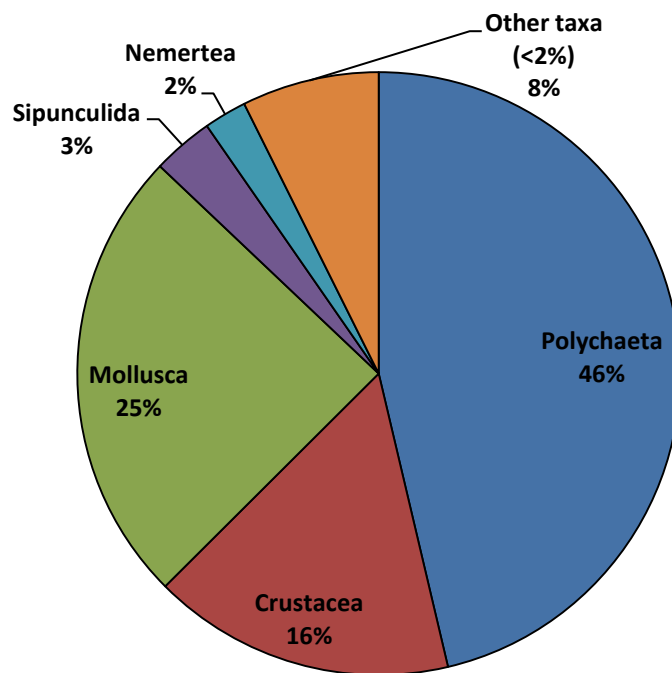
Fig. 24: Percentages of macrofaunal taxa. Other taxa (<2%): Cnidaria, Phoronida, Porifera, Oligochaeta, Echinodermata, Bryozoa, Archianellida, Insecta, Acari, Platyhelmintes, Tunicata.

Table 3 Number of classified, specimens, divided by each found taxa.

	Polychaeta	Crustacea	Mollusca	Sipunculida	Nemertea	Cnidaria	Phoronida	Porifera	Oligochaeta	Echinodermata	Bryozoa	Archiannellida	Insecta	Acari	Platyhelmintes	Tunicata
N	**283**	**99**	**150**	**20**	**14**	**11**	**10**	**6**	**4**	**4**	**3**	**2**	**2**	**1**	**1**	**1**
%	46,3	16,2	24,5	3,3	2,3	1,8	1,6	1,0	0,7	0,7	0,5	0,3	0,3	0,2	0,2	0,2

The most abundant taxa were Polychaeta, Mollusca and Crustacea, which represent over the 87% of all classified individuals, followed by Sipunculida and Nemertea (which represent about 5%). However, it must be pointed out that all phoronids were larval stage (Table 3).

3.1.1 Univariate analyses.
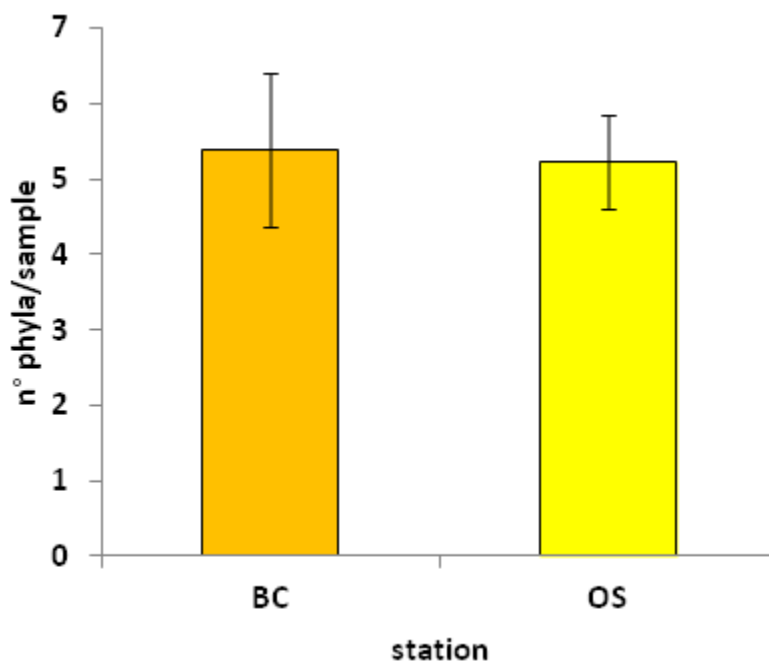
Fig. 25: Average number of phyla per sample ± e.s. (n = 8 on BC; n = 9 on OS) both in the Blanes Canyon (BC) and adjacent open slope (OS).

Both sampled stations showed the same number of phyla per sample (Fig. 25).
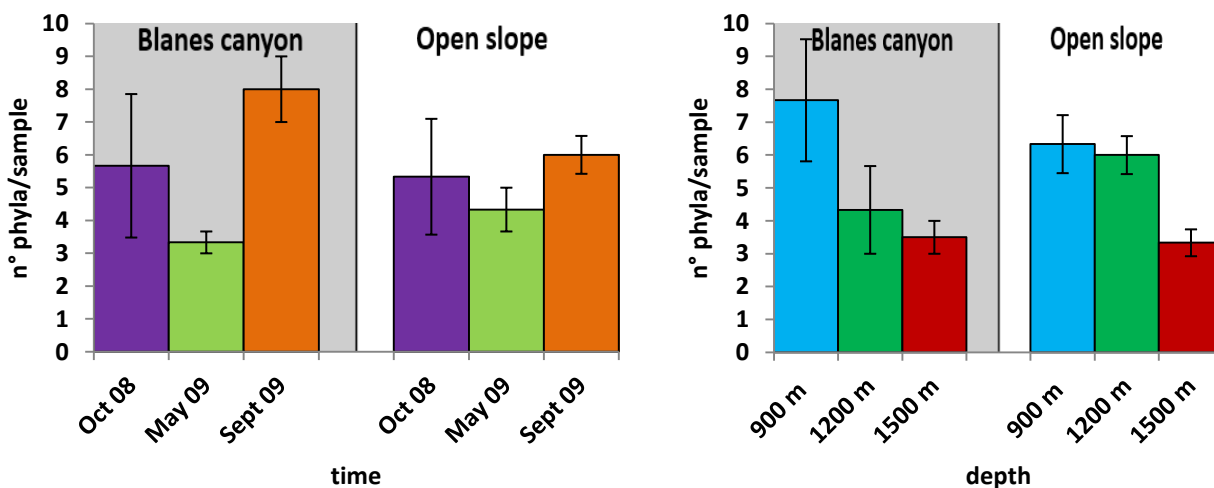
Fig. 26: Comparison between average number of phyla ± e.s. (n = 2 on PR IV Blanes Canyon and on 1500 m BC; n = 3 on all others) both in the Blanes Canyon and open slope, ordered by time and sampled depths (-900 m; -1200 m; -1500 m).

The average number of phyla decreased in May 09 (PROMETEO III) and reached the maximum values in Sept 09 (PROMETEO IV). Also, it decreases together with the increasing depth. In turn, BC showed the same phyla as open slope only in Oct 08, which lower in May 09 and higher in Sept 09 (Fig. 26).

With depth, there were several different patterns: at -900 m depth BC was richer than the open slope, just the contrary that at -1200 m depth. Finally, at 1500 m depth both zones showed the same average number of phyla (which was also the lowest among all depths).
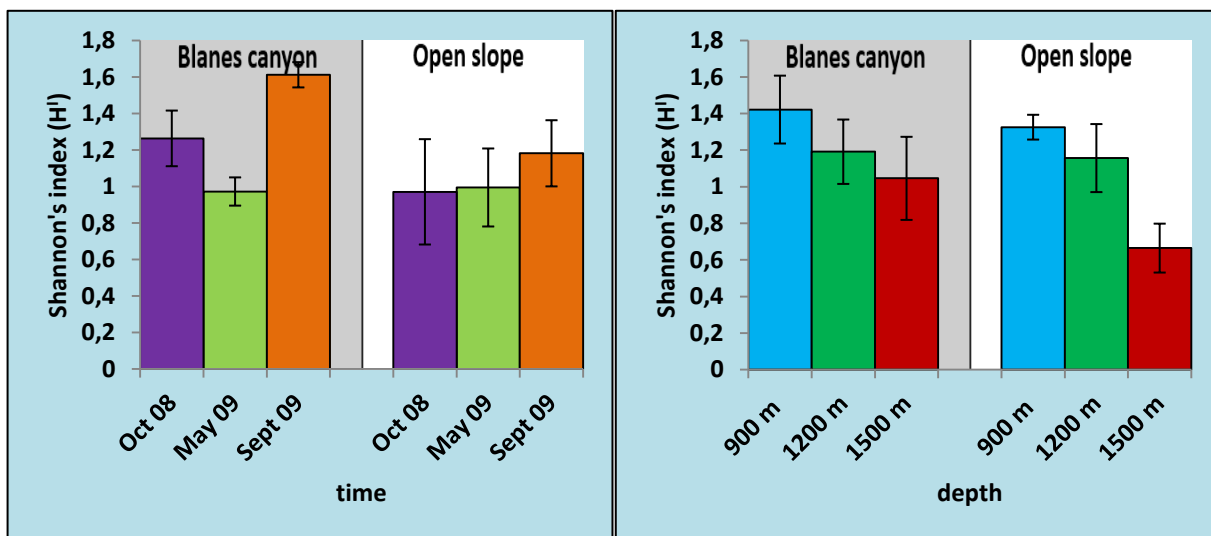
Fig. 27: Comparison between Shannon's index ± e.s. (n = 2 on 1500 m Blanes Canyon and on PR IV Blanes canyon; n = 3 on all others) both in the Blanes Canyon and open slope, ordered by time and sampled depths (-900 m; -1200 m; -1500 m)
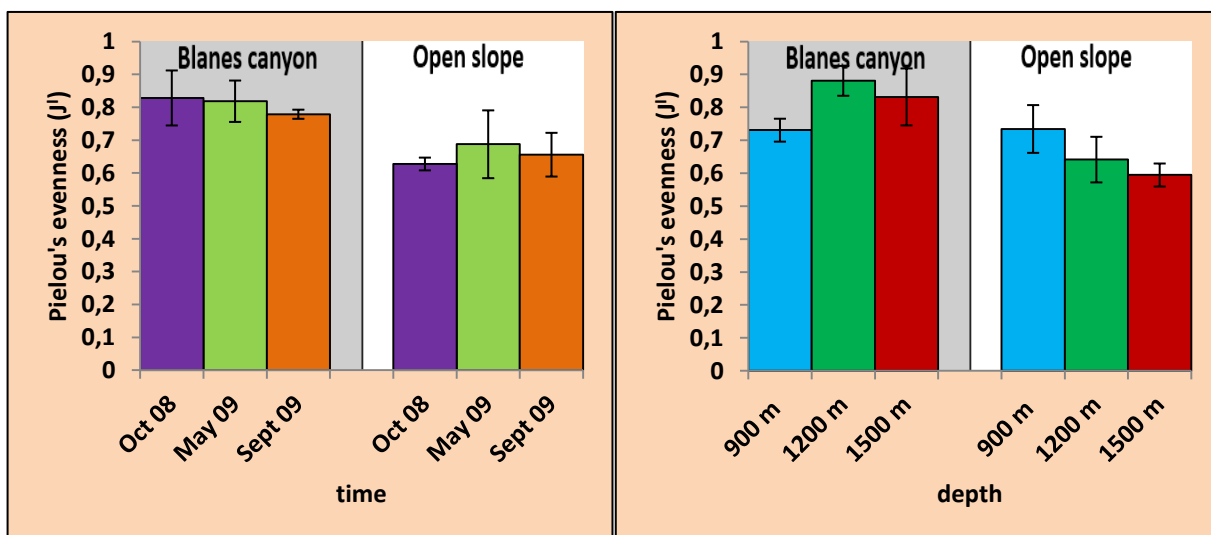
Fig. 28: Comparison between Pielou's index of evenness ± e.s. (n = 2 on 1500 m Blanes Canyon and on PR IV Blanes canyon; n = 3 on all others) both in the Blanes Canyon and open slope, ordered by time and sampled depths (-900 m; -1200 m; -1500 m).

In BC, the species richness slightly decreased in May 09, and was followed by an increase leading to a maximum in Sept 09. In turn, the evenness was similar and high all along all the sampled periods. In the open slope, the richness slightly increased in Sept 09 (as in BC), but the evenness' trends differed from that in BC by showing a maximum in May 09 (Fig. 27, Fig. 28).

The species richness decreased with the increase of depth (both in BC and the open slope). The evenness was high at all depths, but it was higher at -1200 m depth, slightly lower at -1500 m depth and much lower at -900 m depth in BC. In the open slope, the evenness always decreased along depth, having always lower averages than in BC (Fig. 28).

3.1.1.1 Percentage analysis.
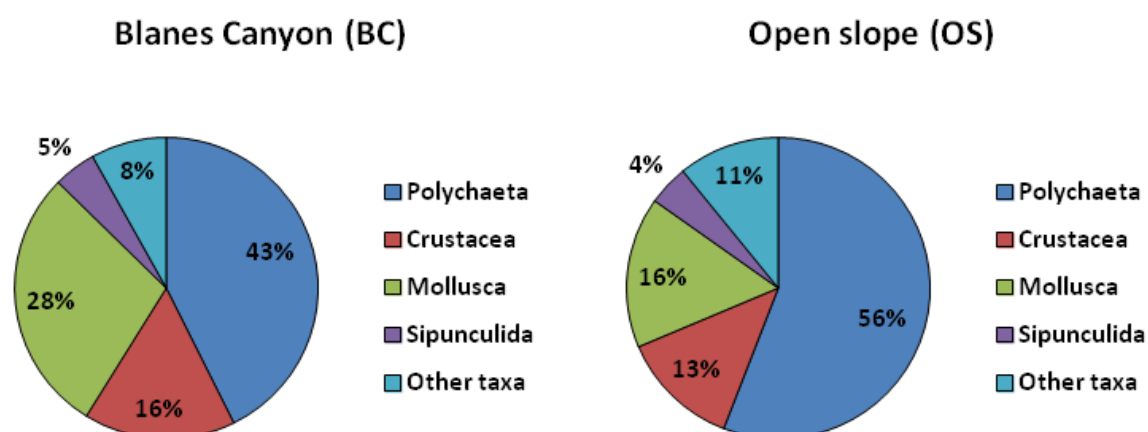
Fig. 29: Comparison between taxa's percentages both in the Blanes Canyon and the adjacent open slope. Other taxa (<2%): Nemertea, Cnidaria, Phoronida, Porifera, Oligochaeta, Echinodermata, Bryozoa, Archianellida, Insecta, Acari, Platyhelmintes, Tunicata.

BC and its adjacent open slope were both dominated by Polychaeta, Mollusca and Crustacea (Fig. 29). However, there was a major relative contribution of Polychaetes in the open slope, compared to BC (56% versus 43%), while the two other major taxa, Mollusca and Crustacea tended to decrease (28%-16% in BC versus 16%-13% in the open slope, respectively).

Among the minor taxa: the Archianellida and Platyhelmintes appear as exclusive of BC, while the Oligochaeta and Acari were found in the open slope only. In turn, the most abundant taxa were Nemertea and Cnidaria, while Phoronida showed the highest difference between BC and the open slope.

3.1.1.1.1 Depth view.
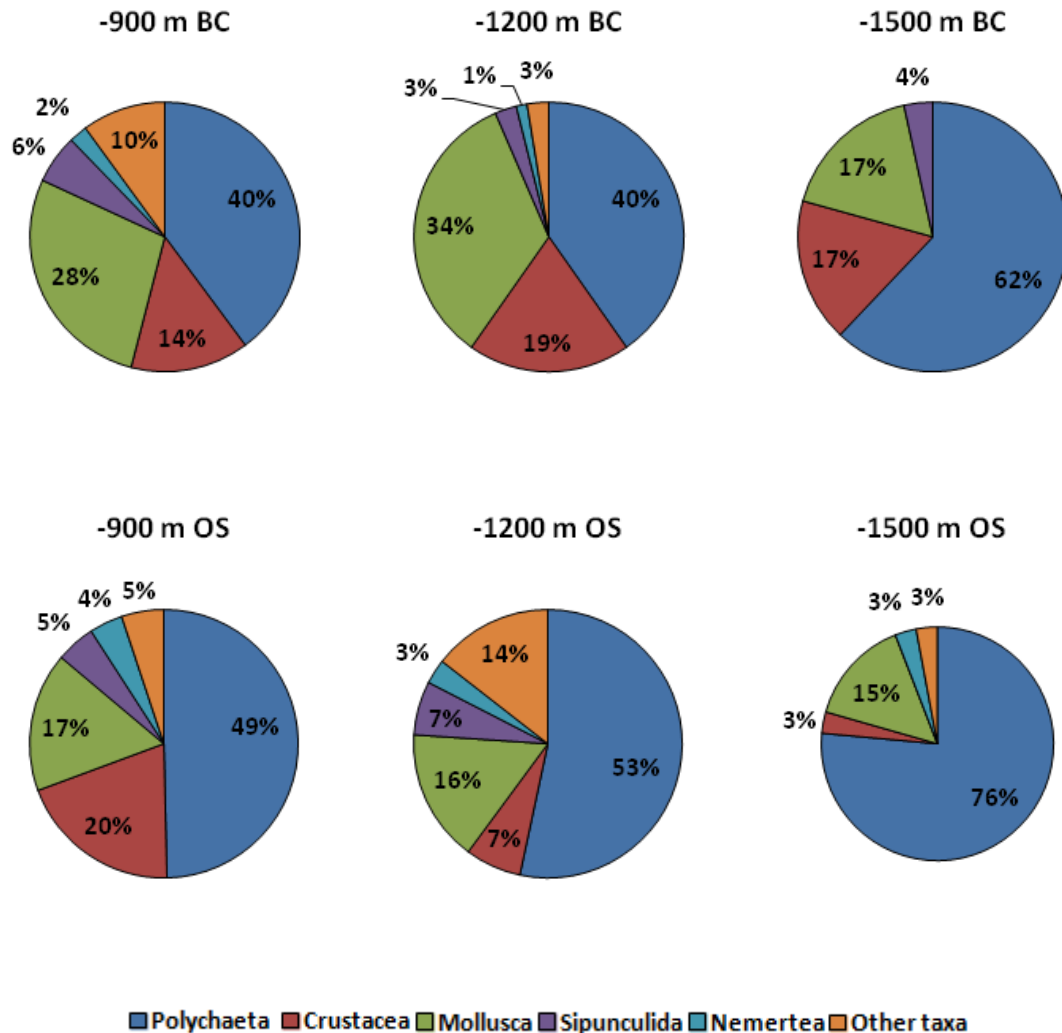
Fig. 30: Comparison between percentages of major taxa, divided by station/depth.

Both BC and the adjacent open slope were dominated by Polychaeta in all depths, especially in the deepest ones (Fig. 30). In BC, the Crustacea maintained similar abundances along all depths, while in the open slope they quickly decreased with the increase of depth. Mollusca was lesser abundant in the open slope than in BC, mainly in the shallowest depths. Other taxa were not found in BC at -1500 m depth, while minor taxa represented up to 10% of the specimens found in BC at -900 m depth and the open slope at -1200 m depth.

The Cnidaria were progressively more abundant along depth, reaching the maimum at -1200 m depth, where they were dominant among the minor taxa. The phoronideans were found only in the shallowest depth, and two Insecta larvae were remarkably found at -900 m depth (

Table 5).

3.1.1.1.2 Temporal view.
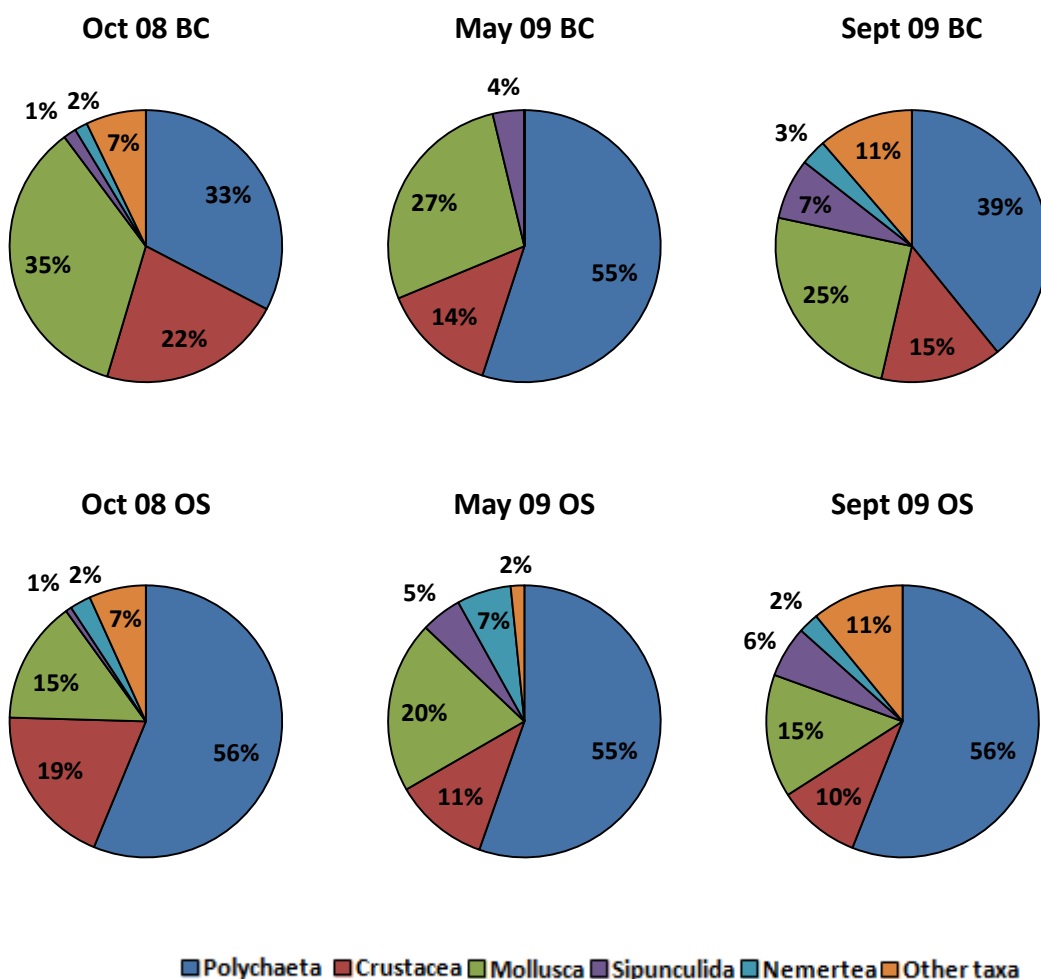
Fig. 31: Comparison of percentages of major taxa, dived by campaign/station. (BC = Blanes Canyon; OS = Open slope)

The Polychaeta, Mollusca and Crustacea were always dominant over the whole sampling period (Fig. 31). In BC, the Mollusca were abundant in all months, while the Crustacea slightly decreased in later months. In the open slope, the percentage of minor taxa increased through time, together with a general decrease of Crustacea abundance.

In PR I (Oct 2008), the percentage of Mollusca was more than half lower in the open slope than in BC, while the Polychaeta were much more abundant the former and the Crustacea had always similar abundances. In PR III (May 2009), the major taxa had similar percentages both in BC and in the open slope, with the Mollusca being slightly more abundant in BC. In PR IV (Sept 09), the Polychaeta dominate. And the Mollusca were more abundant in BC than in the open slope.

The percentages of all taxa were always similar for the Oct 08/Sept 09 annual periods, but were different in May 09.

The Cnidaria were absent only in PR I (Oct 08), and was always present in all the other sampled times. Phoronida were more abundant in BC than in the adjacent open slope. In May 09, the minor taxa were absent from the canyon (Fig. 31; Table 4).

3.1.1.2 Abundance analyses.
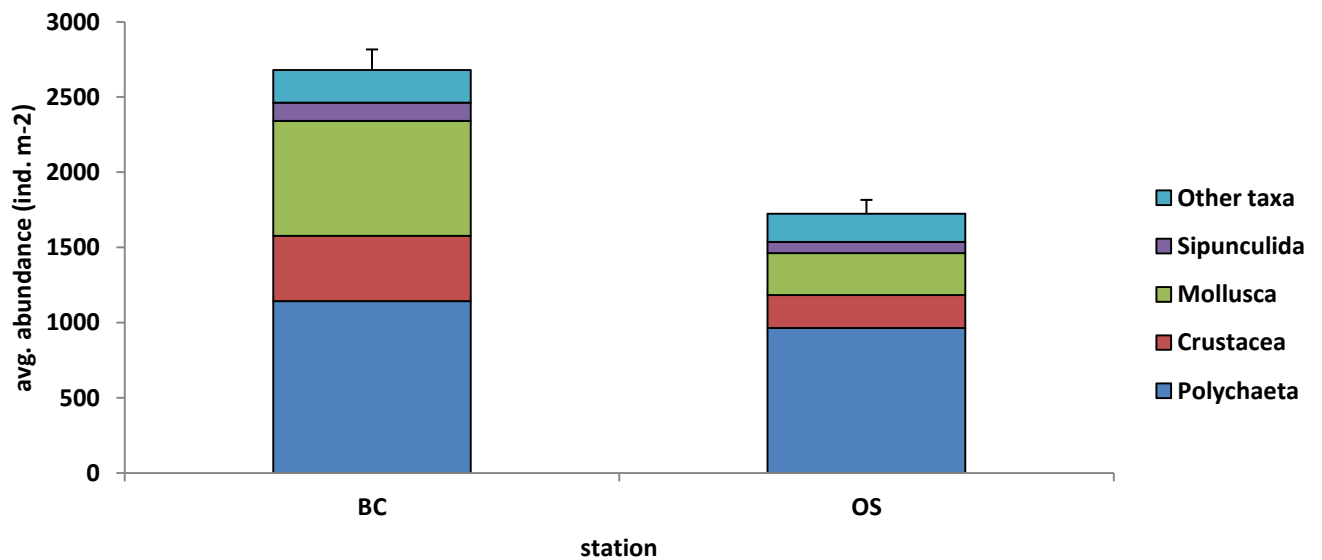
Fig. 32: Comparison between average abundances ± e.s. (n = 8 on BC; n = 9 on OS) both in the Blanes Canyon (BC) and open slope (OS).

In BC, the average abundance was higher than in the adjacent open slope, mainly due to Mollusca and Crustacea (Fig. 32).
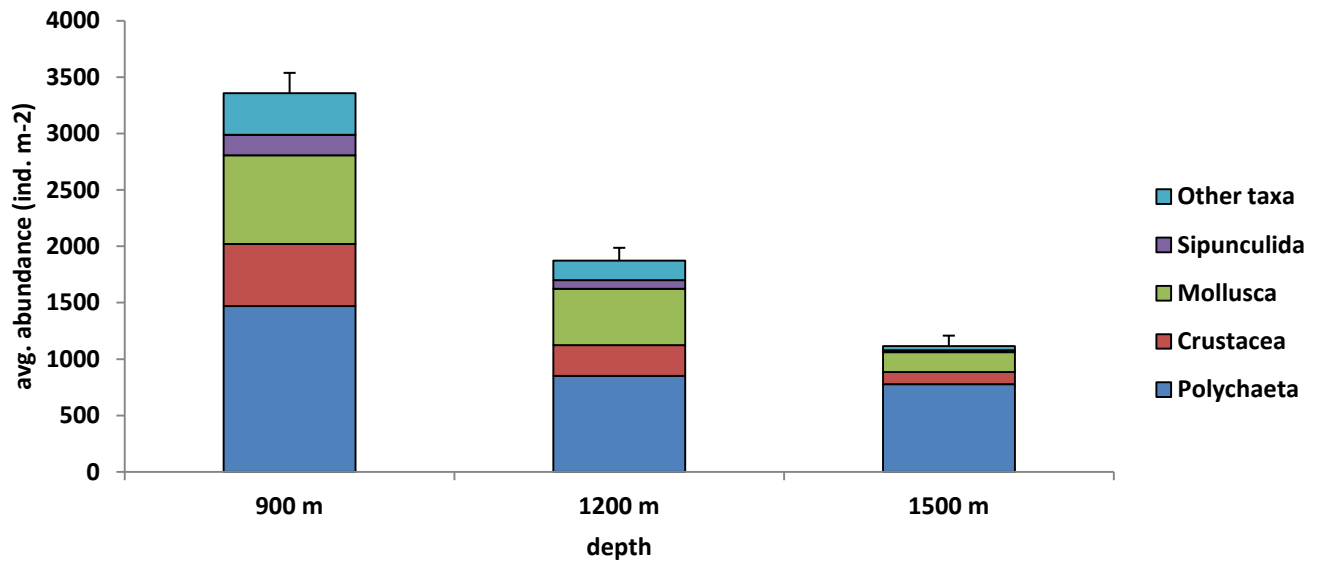
Fig. 33: Average macrofaunal abundances in the three sampled depths ± e.s. (n = 9 on -900 and -1200 m; n = 8 on -1500 m).

The average abundance was different in the three sampled depths (Fig. 33). It decreased along depth, reaching a minimum at -1500 m depth, where the abundances was almost half that at -900 m depth (Table 5). This decrease was similar for all taxa but was more evident for Mollusca and Crustacea. In turn, the Other taxa almost disappeared at -1500 m depth.
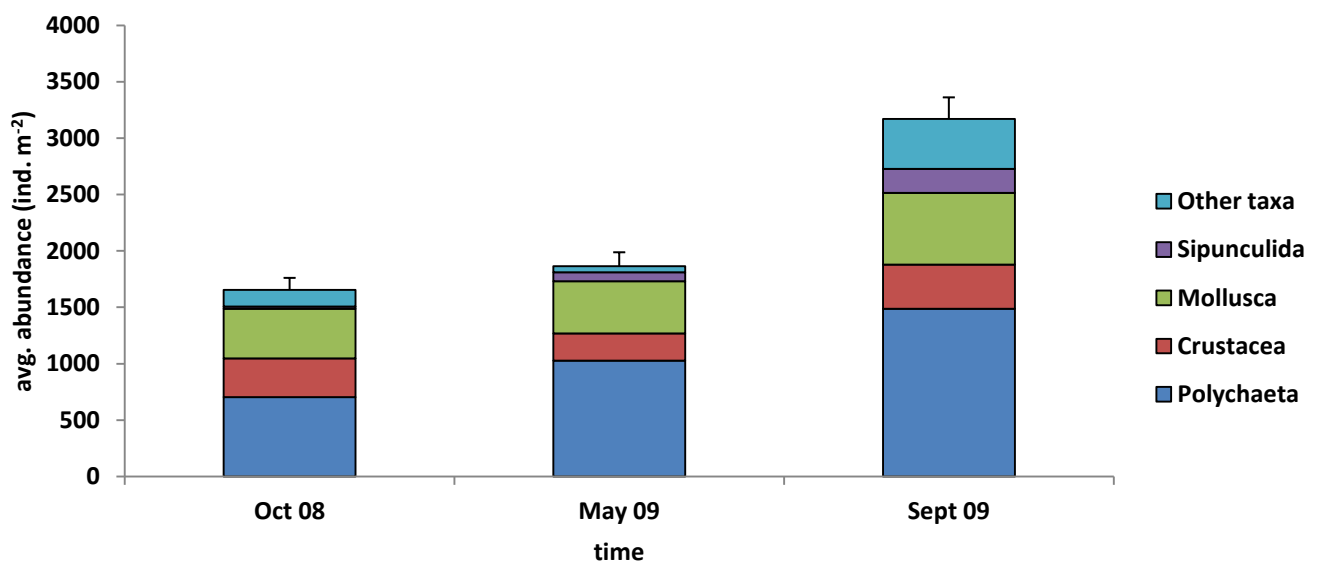
Fig. 34: Average macrofaunal abundance in the three campaigns ± e.s. (n = 5 on Sept 09; n = 6 on Oct 08 and May 09).

The average abundance increased through time within the same annual period, reaching the double from Oct 08 to Sept 09. This increment was similar for all taxa (Fig. 34; Table 6).

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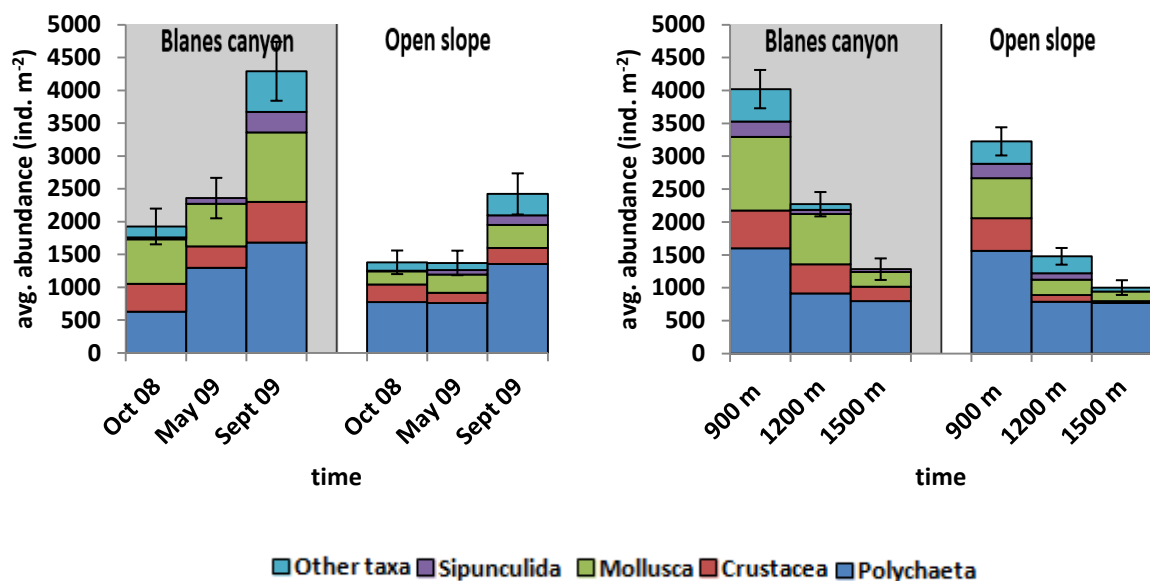
Fig. 35: Comparison between average abundances (individuals/m2) ± e.s. (n = 2 on 1500 m BC and on PR IV Blanes canyon; n = 3 on all others) both in the Blanes Canyon and Open slope, ordered by time (PROMETEO I = Oct 08; PROMETEO III = May 09; PROMETEO IV = Sept 09) and sampled depths (-900 m; -1200 m; -1500 m).

In BC, the macrofaunal abundances increased seasonally, reaching the maximum in Sept 09. In the open slope, the abundance increased between May 09 and Sept 09, and was lower but similar in Oct 08 and May 09 (Fig. 35).

The Polychaeta were always the most abundant taxa, followed by Mollusca and Crustacea. The polychaetes increased through time, reaching maximum in Sept 09 in BC, while in the open slope their abundance slightly increased between May 09 and Sept 09. The same trend was observed for Mollusca and Crustacea in BC, while in the open slope their patterns differed from that of polychaetes in showing a relatively stable abundance. At -1500 m depth, all minor taxa showed high contributions to the abundances, almost doubling it to the same period in the previous year (703 ind./m^2 on Oct 08 to 1519 ind./m^2 on Sept 09).

The abundance showed an overall clear decrease with the increasing depth, particularly in the open slope, and dealing with Mollusca and Crustacea. The Polychaeta showed an initial decrease, while the abundance 1200 m and -1500 m deep was similar (~768 ind./m2). In BC, the pattern of polychaetes was the same as in the open slope, while mollusks and crustaceans have their highest abundances at the shallowest depths. The Other taxa group almost disappear at -1500 m depth, but it quickly recovered the abundance in BC rather than in the open slope (Fig. 35).

Table 4: Number of individuals per m^2 and relative contribution (as percentage) of each taxa in BC (Blanes Canyon) and OS (Open slope). (S = total number of taxa). Grey cells highlight the taxa absent in the respective samples.

	Polychaeta	Crustacea	Mollusca	Sipunculida	Nemertea	Cnidaria	Phoronida	Porifera	Oligochaeta	Echinodermata	Bryozoa	Archianellida	Insecta	Acari	Platyhelmintes	Tunicata	tot	S
BC	1143	435	763	122	44	33	63	22	0	26	7	7	11	0	4	0	2693	13
%	42,43	16,15	28,33	4,52	1,64	1,23	2,33	0,82	0,00	0,96	0,27	0,27	0,41	0,00	0,14	0,00		
OS	964	220	278	75	61	52	3	15	20	10	10	0	3	3	0	10	1725	14
%	55,89	12,76	16,14	4,32	3,51	3,04	0,19	0,85	1,19	0,57	0,57	0,00	0,19	0,19	0,00	0,57		

Table 5: Number of individuals per m2 and relative contribution (as percentage) of each taxa in each station (BC = Blanes Canyon: OS = Open slope) for each depth (-900 m; -1200 m; -1500 m).

	Polychaeta	Crustacea	Mollusca	Sipunculida	Nemertea	Cnidaria	Phoronida	Porifera	Oligochaeta	Echinodermata	Bryozoa	Archianellida	Insecta	Acari	Platyhelmintes	Tunicata	S
-900 m BC	1602	570	1121	236	88	59	167	59	0	39	20	20	29	0	10	0	13
%	39,9	14,2	27,9	5,9	2,2	1,5	4,2	1,5	0,0	1,0	0,5	0,5	0,7	0,0	0,2	0,0	
-900 m OS	1337	531	452	128	108	10	10	29	39	0	29	0	10	10	0	0	12
%	49,6	19,7	16,8	4,7	4,0	0,4	0,4	1,1	1,5	0,0	1,1	0,0	0,4	0,4	0,0	0,0	
-1200 m BC	914	442	767	59	29	29	0	0	0	29	0	0	0	0	0	0	7
%	40,3	19,5	33,8	2,6	1,3	1,3	0,0	0,0	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	
-1200 m OS	789	100	236	96	44	147	0	15	22	0	0	0	0	0	0	29	9
%	53,4	6,8	16,0	6,5	3,0	10,0	0,0	1,0	1,5	0,0	0,0	0,0	0,0	0,0	0,0	2,0	
-1500 m BC	796	221	221	44	0	0	0	0	0	0	0	0	0	0	0	0	4
%	62,1	17,2	17,2	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
-1500 m OS	767	30	147	0	29	0	0	0	0	29	0	0	0	0	0	0	5
%	76,4	3,0	14,7	0,0	2,9	0,0	0,0	0,0	0,0	2,9	0,0	0,0	0,0	0,0	0,0	0,0	

Table 6: Number of individuals per m^2 and relative contribution (as percentage) of each taxa in each station (BC = Blanes Canyon: OS = Open slope) for each campaign (PR I = Oct 08; PR III = May 09; PR IV = Sept 09).

	Polychaeta	Crustacea	Mollusca	Sipunculida	Nemertea	Cnidaria	Phoronida	Porifera	Oligochaeta	Echinodermata	Bryozoa	Archianellida	Insecta	Acari	Platyhelmintes	Tunicata	S
Oct 08 BC	**629**	**423**	**678**	**29**	**29**	**0**	**49**	**29**	**0**	**10**	**20**	**20**	**0**	**0**	**10**	**0**	**11**
%	32,6	21,9	35,2	1,5	1,5	0,0	2,6	1,5	0,0	0,5	1,0	1,0	0,0	0,0	0,5	0,0	
Oct 08 OS	**777**	**265**	**202**	**10**	**34**	**25**	**10**	**15**	**25**	**0**	**0**	**0**	**10**	**10**	**0**	**0**	**11**
%	56,2	19,2	14,6	0,7	2,5	1,8	0,7	1,1	1,8	0,0	0,0	0,0	0,7	0,7	0,0	0,0	
May 09 BC	**1297**	**324**	**649**	**88**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**
%	55,0	13,8	27,5	3,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
May 09 OS	**759**	**155**	**280**	**66**	**88**	**15**	**0**	**0**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**
%	55,4	11,3	20,4	4,8	6,5	1,1	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	
Sept 09 BC	**1681**	**619**	**1062**	**310**	**133**	**133**	**177**	**44**	**0**	**88**	**0**	**0**	**44**	**0**	**0**	**0**	**10**
%	39,2	14,4	24,7	7,2	3,1	3,1	4,1	1,0	0,0	2,1	0,0	0,0	1,0	0,0	0,0	0,0	
Sept 09 OS	**1356**	**240**	**354**	**147**	**59**	**118**	**0**	**29**	**29**	**29**	**29**	**0**	**0**	**0**	**0**	**29**	**11**
%	56,0	9,9	14,6	6,1	2,4	4,9	0,0	1,2	1,2	1,2	1,2	0,0	0,0	0,0	0,0	1,2	

3.1.2 Multivariate analyses.
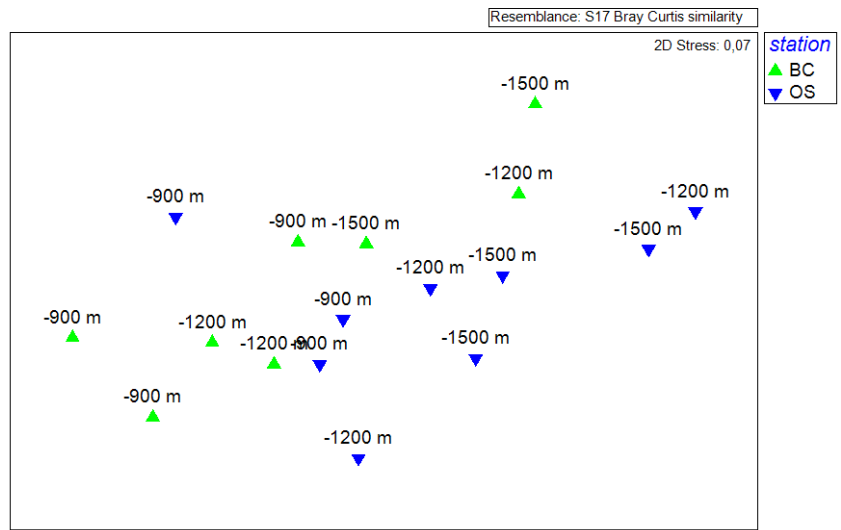
Fig. 36: nMDS obtained through Bray-Curtis index conducted on absolute macrofaunal abundance data (not transformed). (BC = Blanes Canyon; OS = open slope), ordered by station.
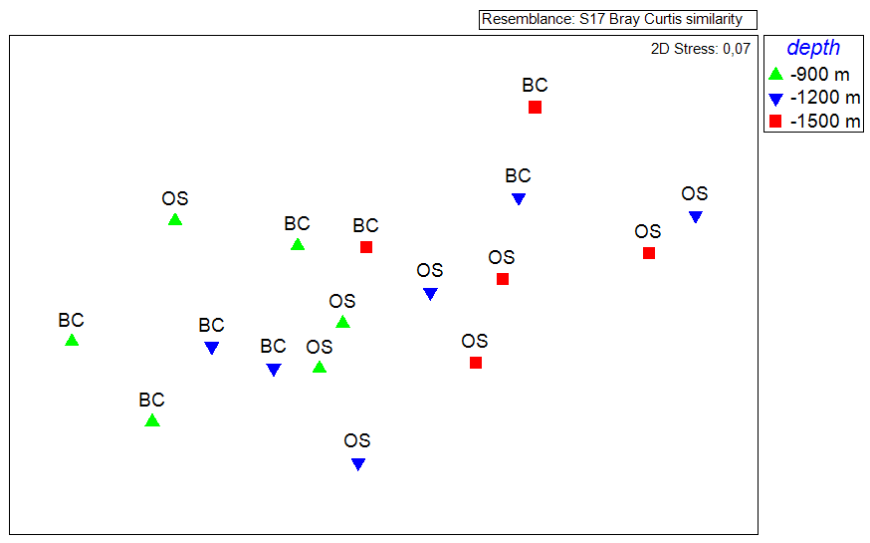
Fig. 37: nMDS obtained through Bray-Curtis index conducted on absolute macrofaunal abundance data (not transformed). (BC = Blanes Canyon; OS = open slope), ordered by depth.
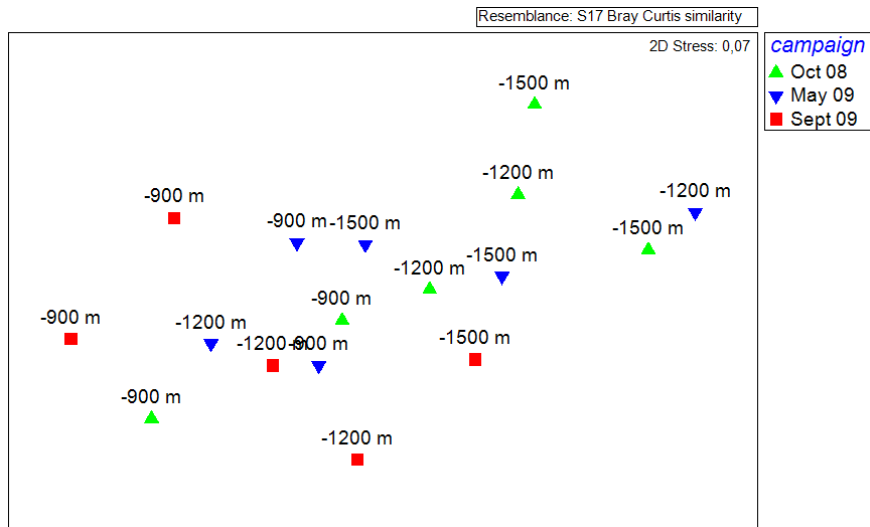
Fig. 38: nMDS obtained through Bray-Curtis index conducted on absolute macrofaunal abundance data (not transformed), ordered by campaign.

The samples corresponding to the two locations, BC and the open slope, are clearly separated between them. Even the samples concerning the 3 different sampled depths are well grouped among themselves, with -900 m and -1500 m depths clearly separated; -1200 m depth samples are interspersed between the two groups. Samples of PR IV are better grouped than samples of other campaigns, with an evident separation between depths. PR III's samples follow a profundity trend (Fig. 38).

3.1.2.1 SIMPER analysis.

BC and open slope macrofaunal communities showed an average dissimilarity of 43,23%, mainly due to annelids, mollusks and crustaceans, which shows a clear decrease in average abundance between BC and the open slope. In turn, there was an increase of the remaining minor phyla.

Groups BC & OS
Average dissimilarity = 43,23

	Group BC	Group OS				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	1142,66	984,57	13,66	1,36	31,60	31,60
Mollusca	763,00	278,50	12,26	1,33	28,36	59,96
Arthropoda	446,01	223,42	8,21	1,74	19,00	78,96

Annelida, Mollusca and Crustacea (as shown by SIMPER analyses) were major responsible for dissimilarities between the 3 sampled depths and between the 3 campaigns.

Groups -900 m & -1200 m
Average dissimilarity = 42,52

	Group -900 m	Group -1200 m				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	1489,15	862,53	13,36	1,19	31,43	31,43
Mollusca	786,35	501,30	11,35	1,42	26,69	58,12
Arthropoda	570,10	271,08	7,35	1,49	17,29	75,42

Groups -900 m & -1500 m
Average dissimilarity = 50,37

	Group -900 m	Group -1500 m				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	1489,15	778,49	16,71	1,43	33,16	33,16
Mollusca	786,35	176,93	12,76	1,43	25,33	58,49
Arthropoda	570,10	106,36	10,41	2,01	20,66	79,15

Groups -1200 m & -1500 m
Average dissimilarity = 39,52

	Group -1200 m	Group -1500 m				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	862,53	778,49	12,68	1,34	32,09	32,09
Mollusca	501,30	176,93	11,06	1,20	27,99	60,08
Arthropoda	271,08	106,36	7,52	1,51	19,02	79,10

Groups Oct 08 & May 09
Average dissimilarity = 40,18

	Group Oct 08	Group May 09				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	715,09	1032,08	14,23	1,45	35,42	35,42
Mollusca	439,86	464,44	12,04	1,23	29,97	65,39
Arthropoda	348,94	239,59	7,53	1,42	18,74	84,12

Groups Oct 08 & Sept 09
Average dissimilarity = 48,41

	Group Oct 08	Group Sept 09				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	715,09	1503,89	16,50	1,34	34,09	34,09
Mollusca	439,86	636,94	10,88	1,46	22,48	56,58
Arthropoda	348,94	409,54	8,06	1,61	16,64	73,22

Groups May 09 & Sept 09
Average dissimilarity = 41,04

	Group May 09	Group Sept 09				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Anellida	1032,08	1503,89	12,97	1,33	31,61	31,61
Mollusca	464,44	636,94	9,21	1,35	22,44	54,05
Arthropoda	239,59	409,54	7,09	1,65	17,27	71,31
Sipunculida	77,41	212,31	3,48	1,37	8,48	79,79

3.2 Polychaeta taxa (phylum Anellida)

An important contribution to the variability in the studied samples is given by the Polychaeta. Therefore, they have been identified to the lower possible taxonomic level (family). This analysis may allow to compare the overall variations of deep-sea macrofauna with other scientific studies, especially under a trophic point of view.
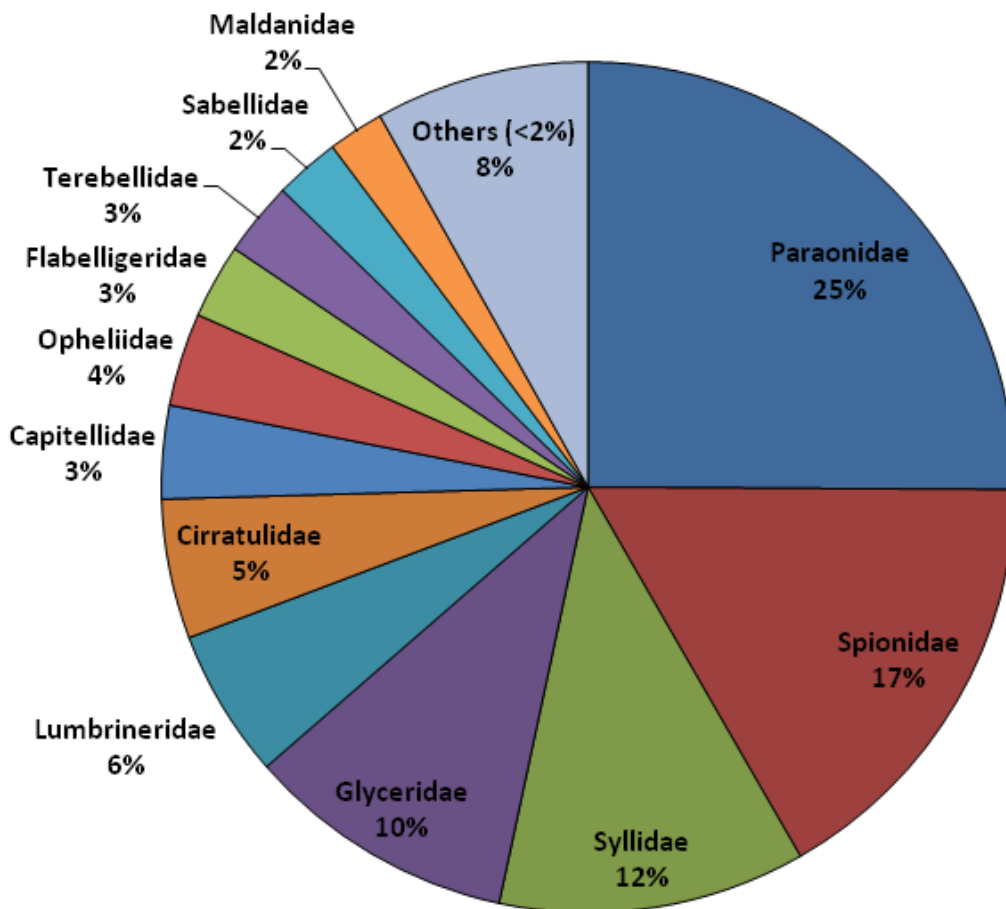

Fig. 39: Percentages of all Polychaeta families found in examined samples.

A total of 283 polychaete specimens were found, belonging to 26 different families (Tables 5, 6). Twelve of them were more than 2% abundant, and 14 families were below this percentage (with only few specimens each one of them).

The most abundant families were the Paraonidae (71 specimens), Spionidae (47 spcs.), Syllidae (33 spcs.) and Glyceridae (29 spcs.), which represent more than 60% of all identified polychaete especimens (Fig. 39) (Table 7, 8).

Table 7: Number of specimens of each Polychaeta family found, with corresponding percentages.

	Paraonidae	Spionidae	Syllidae	Glyceridae	Lumbrineridae	Cirratulidae	Capitellidae	Opheliidae	Flabelligeridae	Terebellidae	Sabellidae	Maldanidae	Others (<2%)
N	**71**	**47**	**33**	**29**	**16**	**15**	**10**	**10**	**8**	**8**	**7**	**6**	**23**
%	25,1	16,6	11,7	10,2	5,7	5,3	3,5	3,5	2,8	2,8	2,5	2,1	8,1

Table 8: Number of specimens of minor found Polychaeta families.

	Nereididae	Onuphidae	Sigalionidae	Hesionidae	Acrocirridae	Trichobranchidae	Cossuridae	Eunicidae	Scalibregmatidae	Polynoidae	Lacydonidae	Alciopidae	Phyllodocidae	Poecilochaetidae
N	**4**	**3**	**2**	**2**	**2**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
%	1,3	0,9	0,9	0,6	0,5	0,4	0,4	0,2	0,2	0,2	0,2	0,2	0,2	0,1

3.2.1 Univariate analyses.

Fig. 40: Number of polychaete families found by sample ± e.s. (n = 2 on 1500 m Blanes Canyon; n = 3 on all others).

In BC, the number of families by sample decreased at -1200 m depth and then increased at -1500 m depth, although the internal variability at this depth was much higher, as expressed by its high e.s. In the open slope there was an evident decrease along depth, with a minimum at -1500 m depth. The open slope had a higher family richness at shallowest depths, while BC was richer at the deepest (Fig. 40).

Fig. 41: Comparison between two different diversity indexes based on Polychaeta abundances: Shannon's abundance index (left) and Pielou's evenness index (right) ± e.s. (n = 2 on 1500 m BC; n = 3 on all others).

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Both in BC and its adjacent open slope, the polychaete family richness was higher at -900 m depth than in all other depths (Fig. 41). Also, the evenness was high (>0.9), pointing out that all families were similarly abundant.

In BC, the family diversity decreased with a concurrent increment in evenness at -1200 m depth. Thus, there was a decrease in the number of families with an increase of the respective relative abundance. At -1500 m depth, both richness and evenness decreased. Although there were more families at this depth than at -1200 m, they had relatively less individuals each one.

In the open slope, the diversity decreased with the increasing depth and the Pielou's index was also higher at -900 m than at -1200 m, indicating that the decrease in number of families was concurrent with a decrease in the respective. At -1500 m depth, the decrease in diversity was related to an increase in evenness so that despite the lower number of families, their respective relative abundances were higher.
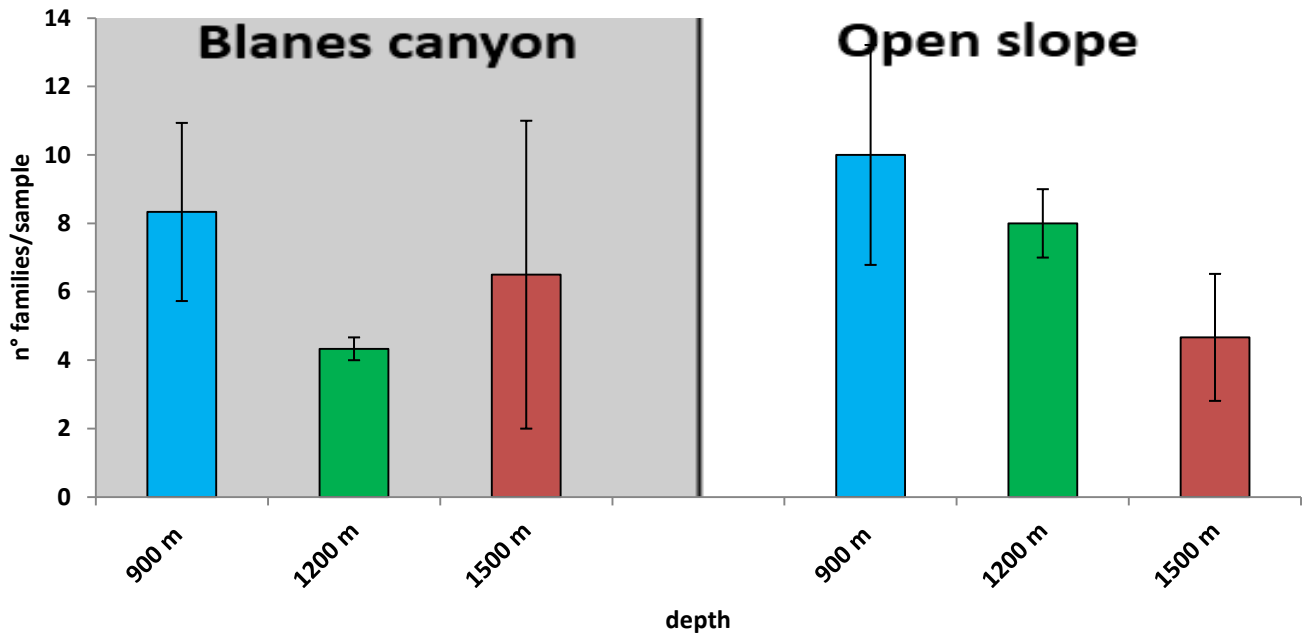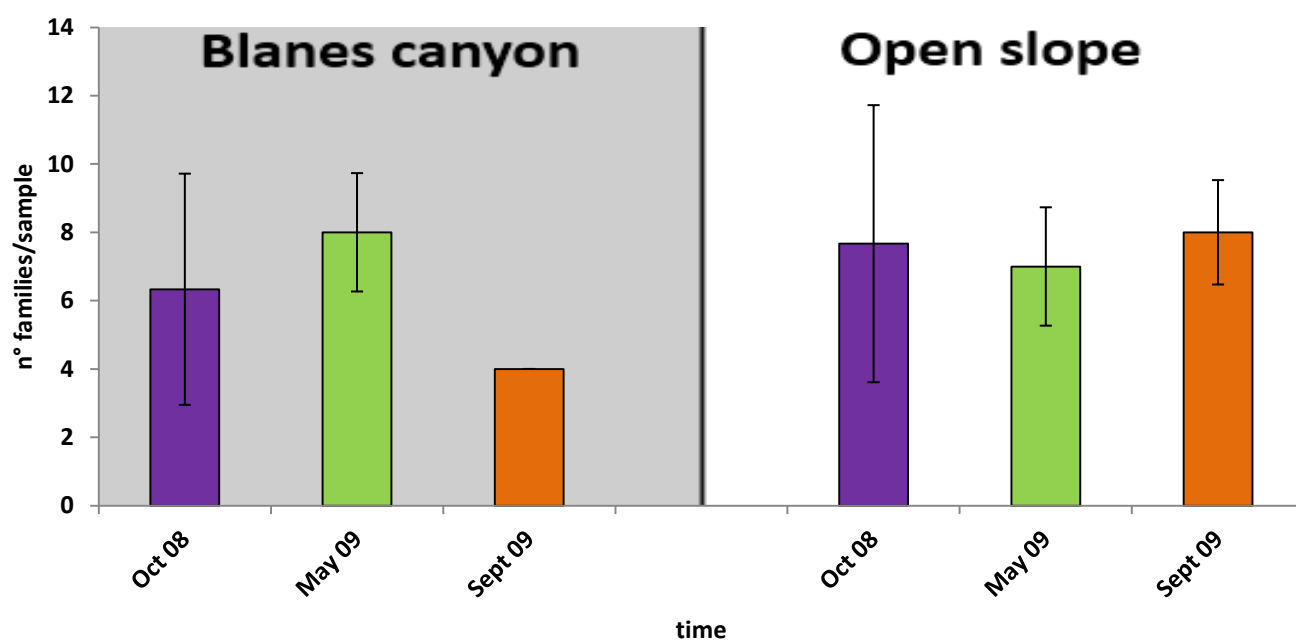

Fig. 42: Average number of Polychaeta families found by sample ± e.s. (n = 2 on Sept 09 Blanes canyon; n = 3 on all others).

In BC, the number of families increased on May 2009 (PR III) with respect to October 2008 (PR I), and the minimum occurred in September 2009 (PR IV).

In the open slope, all three campaigns showed similar number of families per sample, with a slight decrease in May 09 (Fig. 42).
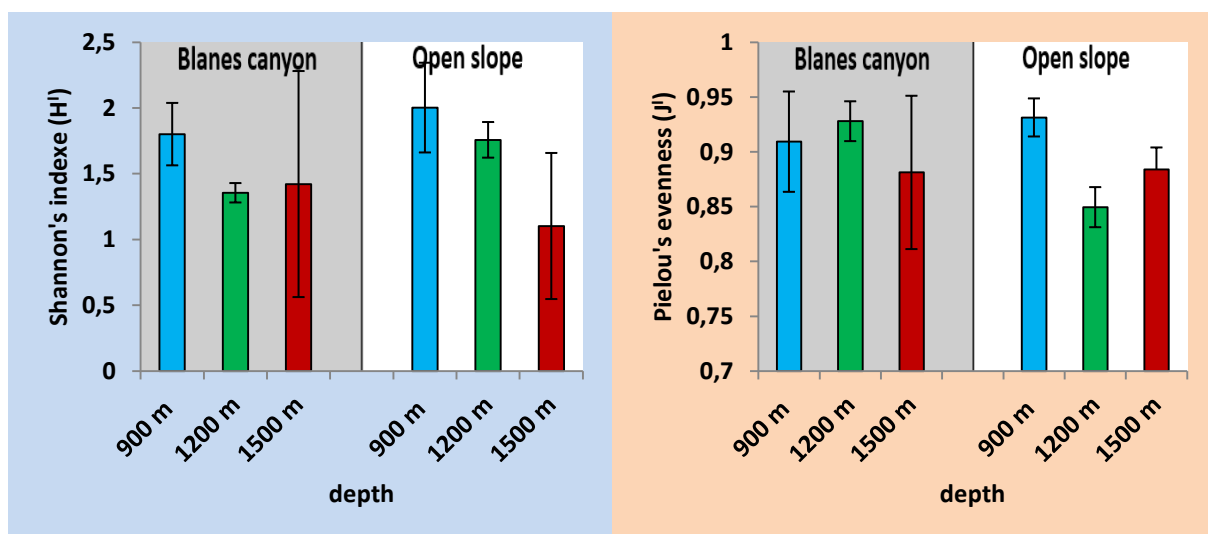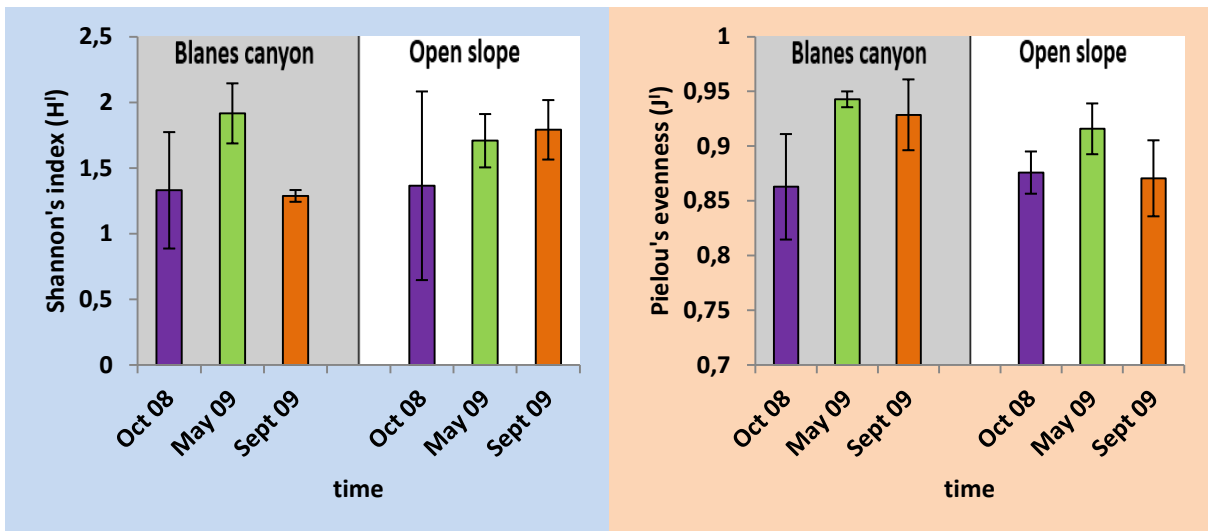
Fig. 43: Comparison between two different diversity indexes based on Polychaeta abundances: Shannon's abundance index (left) and Pielou's evenness index (right) ± e.s. (n = 2 on Sept 09 Blanes Canyon; n = 3 on all others).

In BC, the family richness was maximum in May 09. Few families have been found in Oct 08, with low abundances each. In May 09, there were many families, any of them being dominant. Then, in Sept 09 there was a decrease in the number families, but some of them were dominant (Fig. 43).

In the open slope, the richness showed a clear seasonal increment (with a maximum in Sept 09).

The evenness was minimum in May 09, both in BC and in the open slope, while it reached a maximum in May 09. In Sept 09, the evenness was lower in comparison to that in May 09, but was never lower than in Oct 08 (sampled one year before).

3.2.1.1 Percentage analysis.

Blanes canyon   **Open slope**
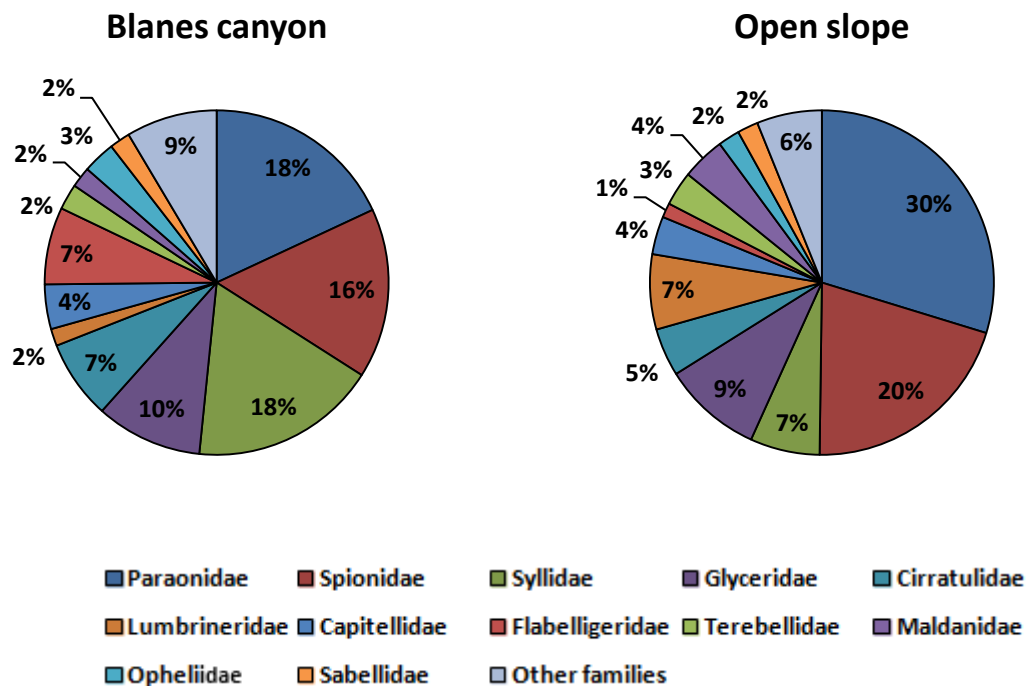
Fig. 44: Percentages of most representative Polychaeta families.

BC was dominated by Paraonidae, Spionidae, Syllidae and Glyceridae (Fig. 44). The first two were also abundant in the open slope, where Syllidae contribution markedly decreased. The Glyceridae, in turn, showed the same percentage in both locations, while the Cirratulidae slightly decreased in the open slope. The Flabelligeridae were much more abundant in BC than in the open slope, while the Lumbrineridae showed just opposite trend (Table 9).

Table 9: Abundance (individuals/m^2) of major Polychaeta families, divided by station. (BC = Blanes Canyon; OS = Open slope).

	Paraonidae	Spionidae	Syllidae	Glyceridae	Lumbrineridae	Cirratulidae	Capitellidae	Opheliidae	Flabelligeridae	Terebellidae	Sabellidae	Maldanidae	Other families	S
BC	258	203	162	147	93	48	31	44	31	40	36	12	6	13
%	22,6	17,8	14,1	12,9	8,2	4,2	2,7	3,9	2,7	3,5	3,2	1,0	0,5	
OS	233	128	51	54	55	49	37	31	44	18	18	31	18	13
%	24,1	13,3	5,3	5,6	5,7	5,1	3,8	3,2	4,6	1,9	1,9	3,2	1,9	

3.2.1.1.1 Depth view
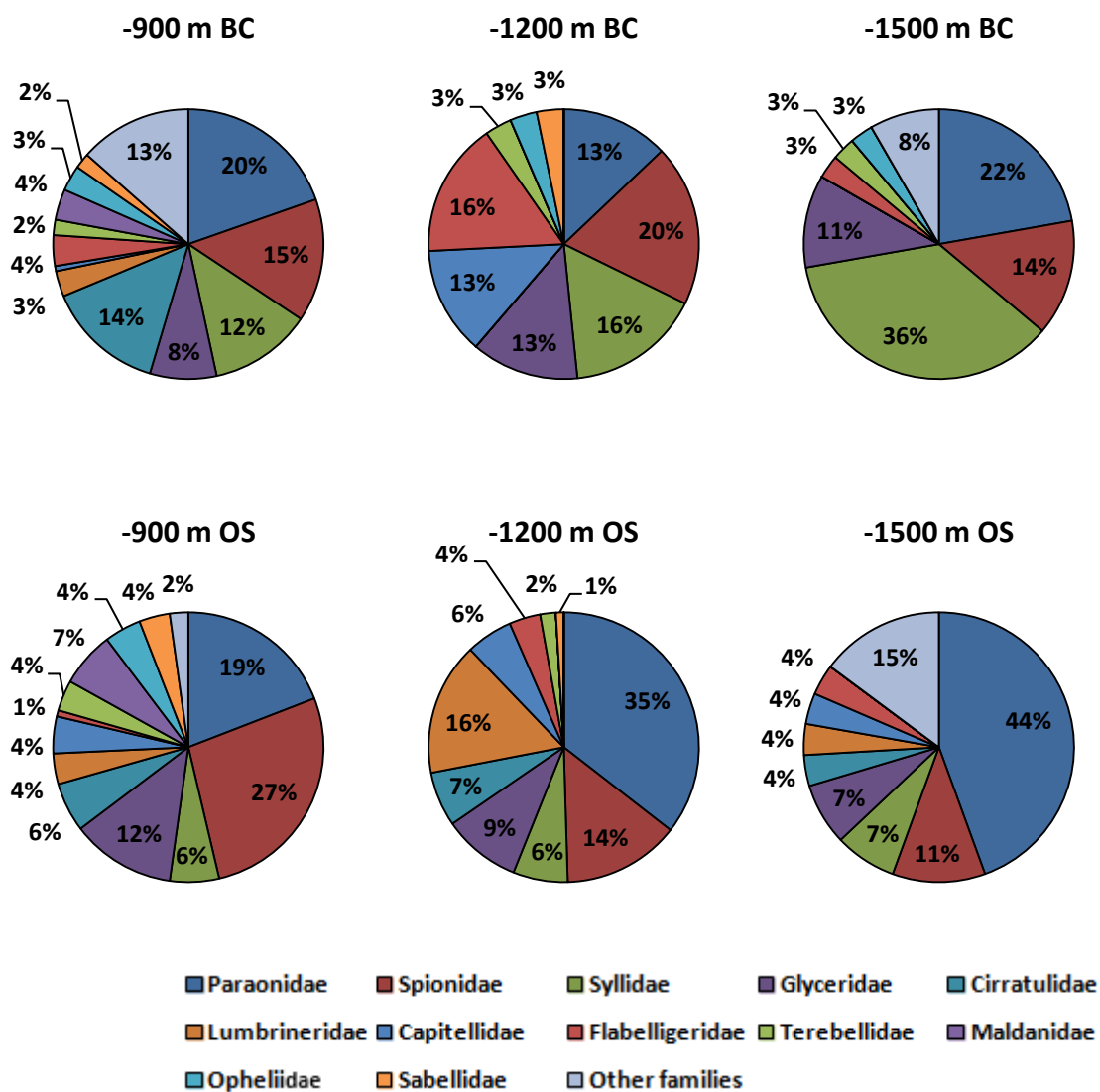
Fig. 45: Percentage of Polychaeta families at each depth of each station. (BC = Blanes Canyon; OS = Open Slope).

In BC, there was a decrease of Polychaeta families with the increasing depth (Table 10). The Paraonidae dominated at -900 m and -1500 m depth, but they halved at -1200 m depth. The Syllidae increased at the intermediate depth and became dominant at -1500 m depth. The Glyceridae increased its abundance at -1200 m depth and then maintained its abundance even at -1500 m depth. The Spionidae remained always abundant and they were dominant at -1200 m depth. The Flabelligeridae highly increased at -1200 m depths. At the intermediate depth, these above mentioned increments seemed to be related to the absence of all minor families (Fig. 45).

In the open slope there was a general decrease of family richness. The Paraonidae were more dominant than in BC, especially at -1200 and -1500 m depth. At -900 m depth, they shared their dominance with the Spionidae and Glyceridae. The Lumbrineridae highly increased at -1200 m depth, and became the second most abundant family at that depth. The Syllidae had a similar abundance along all depths. The Cirratulidae had a low abundance at all depths. In the open slope, the percentages of all minor families highly increased at -1500 m depth, while they were absent at the intermediate depth.

3.2.1.1.2 Temporal view.
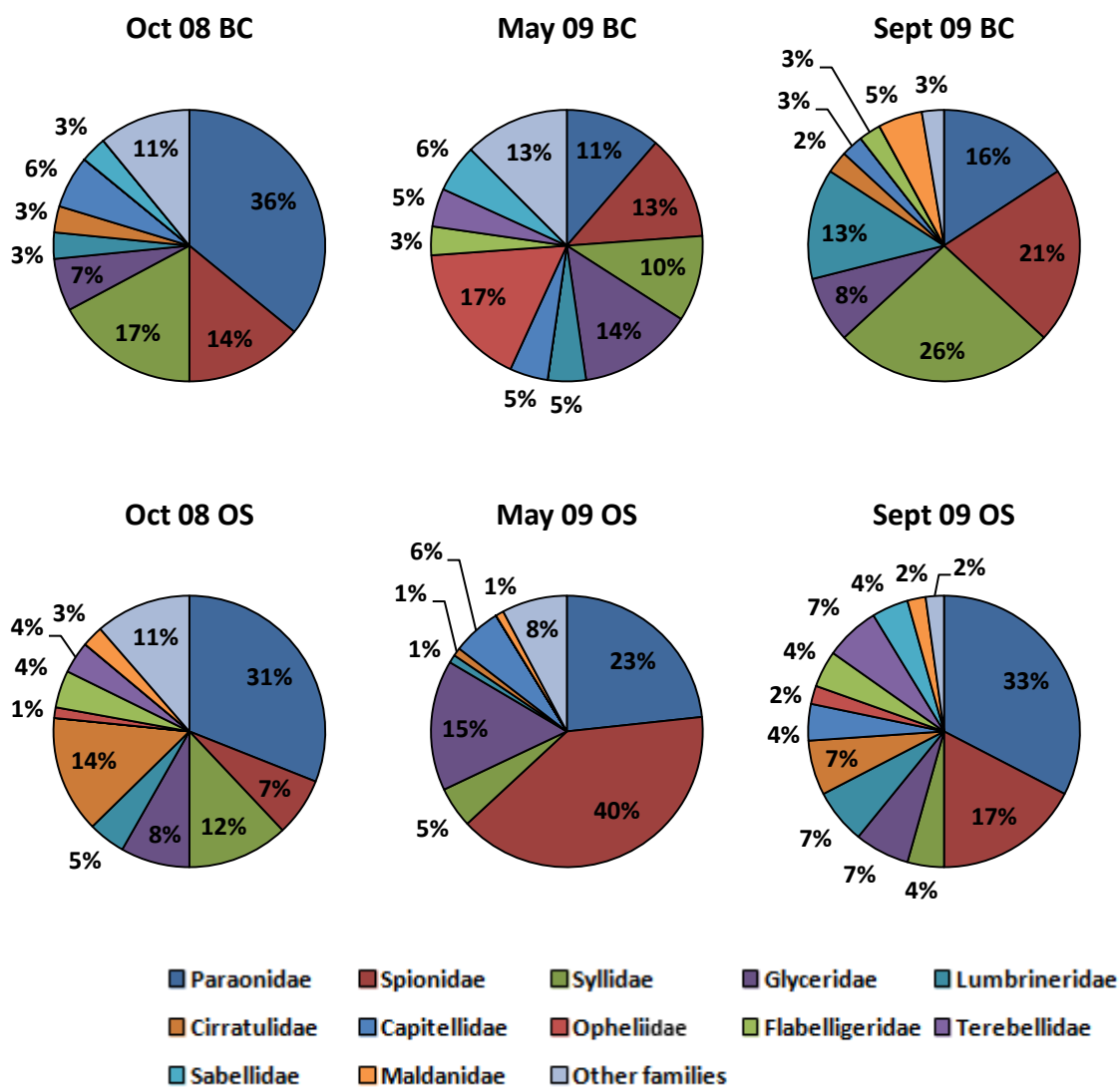
Fig. 46. Percentage of Polychaeta families in each campaign, divided by station. (BC = Blanes Canyon; OS = Open slope)

The Paraonidae dominated in October 08 both in BC and the open slope. In BC, their dominance decreased in May 09. In late spring, the Opheliidae highly increased together with the disappearance of Cirratulidae. In September 09, the Syllidae showed the higher percentage of polychete specimens, together with the Spionidae. Also, there was a clear increase in the number of Lumbrineridae in Sept 09, when the total number of polychete families decreased. The lowest number of families occurred in Oct 08 and May 09, as in the open slope (Fig. 46).

In the open slope, the Paraonidae and Spionidae were dominanti. The Spionidae highly increased in May 09 (almost doubling the Paraonidae abundance). The Syllidae had a minor role than in BC, showing a decrease through time. The Glyceridae were more abundant in May 09, while they were less relevent in the other two sampled periods. The number of families decreased in May 09 (only 9), while they were 13-14 in the other sampled periods (Table 11).

3.2.1.2 Abundance analysis.
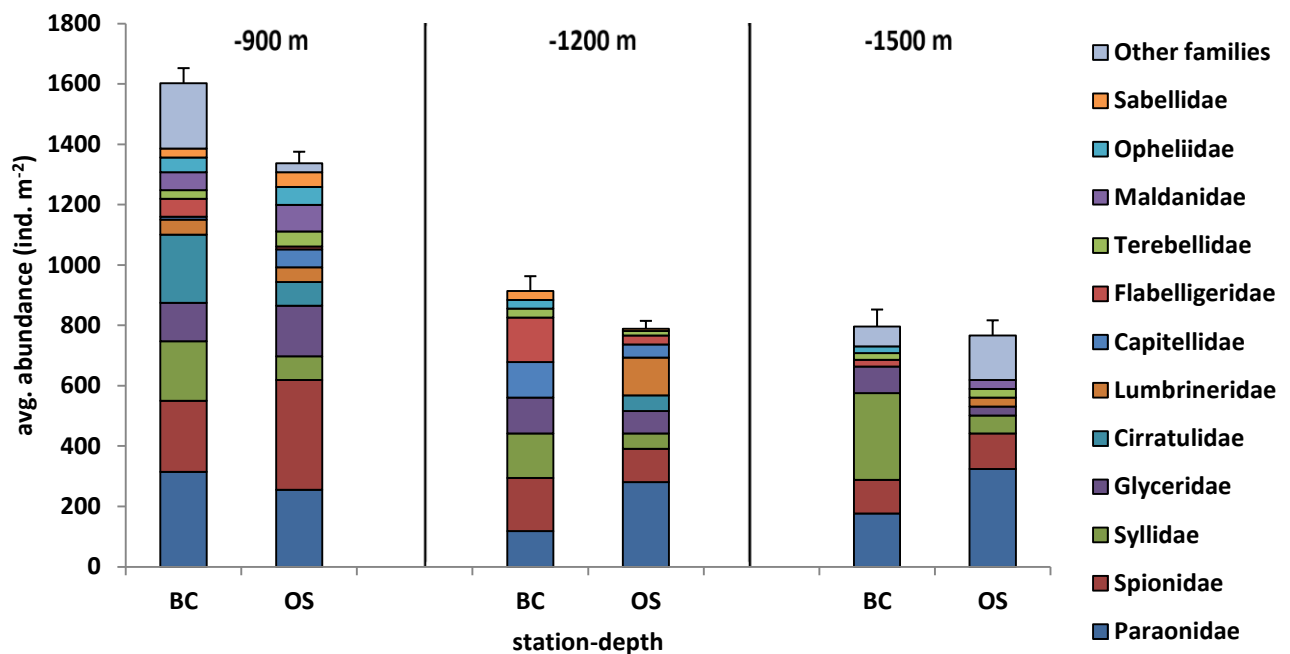
Fig. 47. Polychaeta families average abundances (individuals/m2) ± e.s. (n = 2 on BC -1500m; n = 3 on all others), ordered by depth. (BC = Blanes Canyon; OS = Open slope)

The Polychetes were more abundant in BC than in the open slope. This difference was reduced with an increasing depth, and the abundance was almost the same at -1500 m depth. As previously reported, the Paraonidae, Spionidae, Syllidae, Glyceridae and Cirratulidae were the families that most contributed to the variation in abundances (Fig. 47).
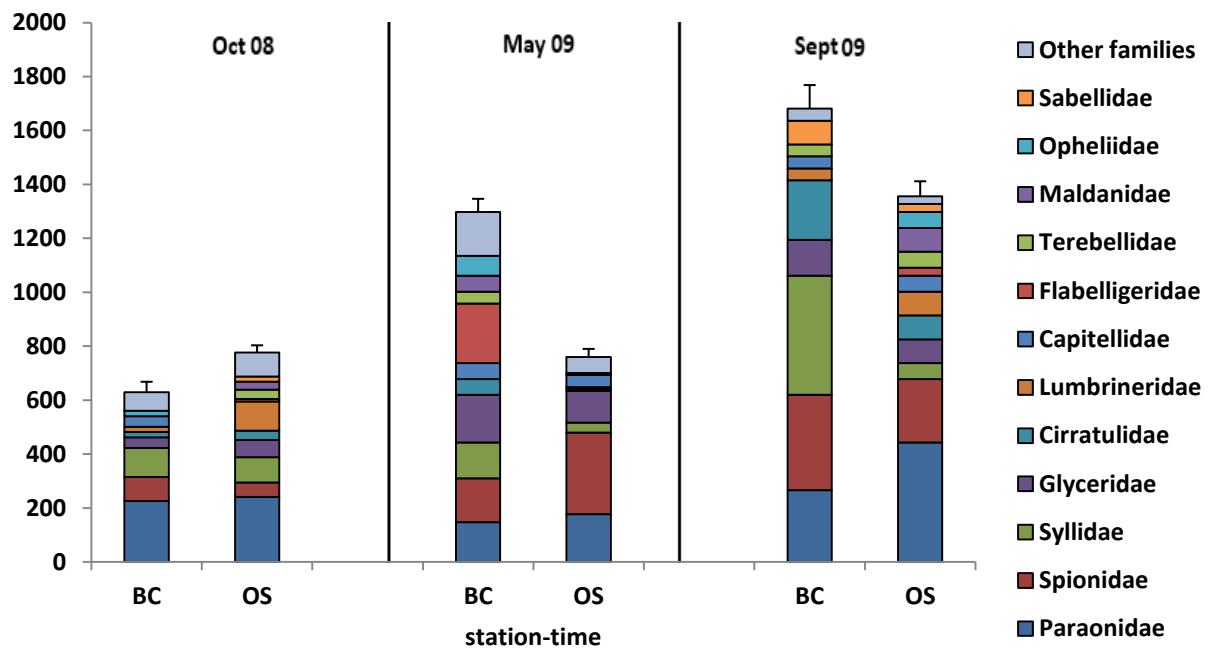
Fig. 48: Polychaeta families average abundances (individuals/m2) ± e.s. (. (n = 2 on BC Sept 09; n = 3 on all others). (BC = Blanes Canyon; OS = Open slope)

Only in Oct 08, the Polychaeta were less abundant in BC than in the open slope (Fig. 48). In BC, the abundances highly increased through time, being triple in Sept 09 than in Oct 08. This increment was mainly due to the Spionidae and Syllidae. The Paraonidae decreased in May 09 (both in BC and in the open slope) and then increased in Sept 09 (as the other major families).

3.2.2 Multivariate analyses.
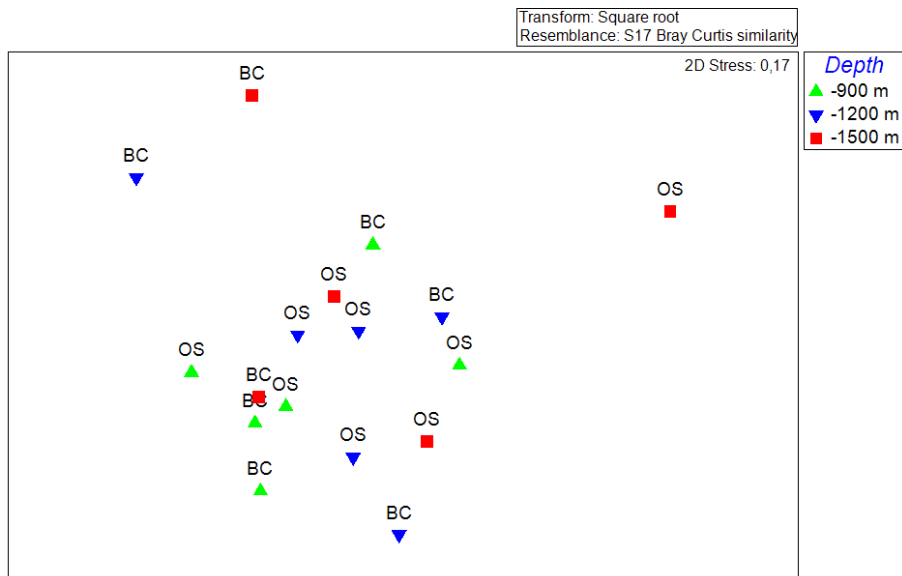
Fig. 49: nMDS obtained through Bray-Curtis index conducted on Polychaeta abundance data (square-root transformed). (BC = Blanes Canyon; OS = Open slope).

There isn't any clear distinction between the samples from BC and the open slope in the nMDS (Fig. 49). However, the samples from -900 m and -1200 m depth were more clustered that those from -1500 m depth. The 2D stress is quite high (0.17), pointing out the possibility of an incorrect interpretation of represented similarity distances.
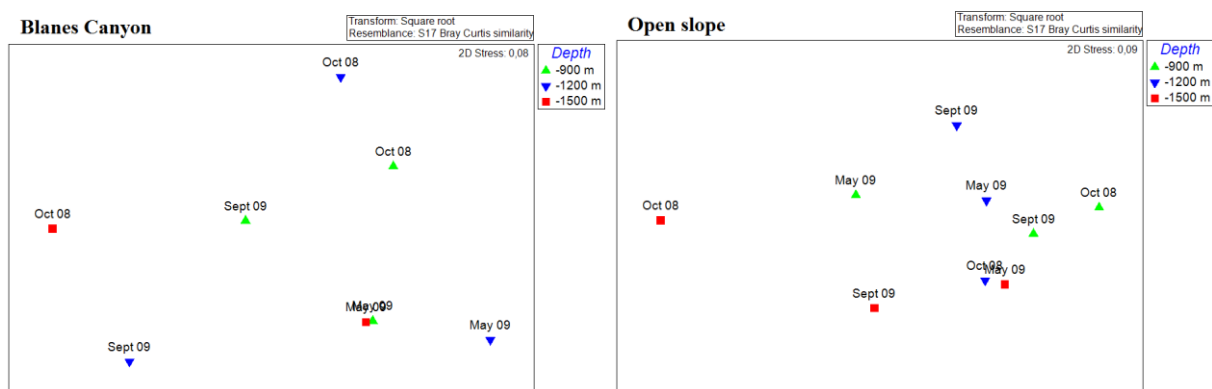
Fig. 50: nMDS obtained through Bray-Curtis index conducted on absolute Polychaeta abundance data (square-root transformed), divided by each station.

Analyzing separately the two samples areas (Fig. 50), it can be seen that the -900 m samples are more clustered together than those from other sampled depths in BC, while in the open slope the best clustered were the samples from -1200 m (which also follow a clear temporal trend).

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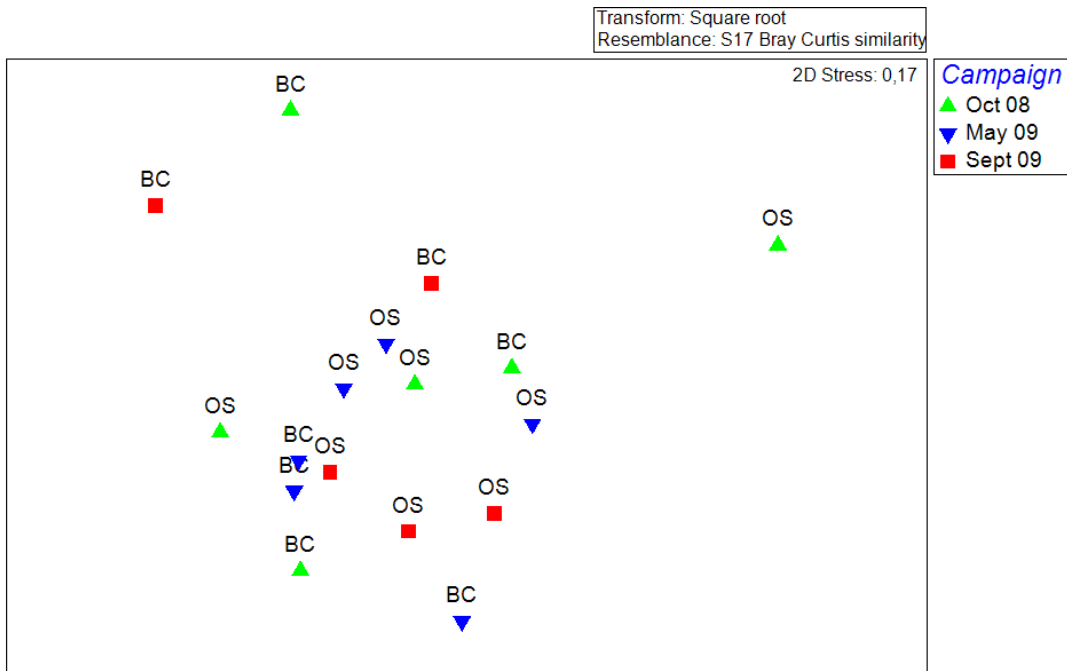
Fig. 51: nMDS obtained through Bray-Curtis index conducted on Polychaeta abundance data (square-root transformed). (BC = Blanes Canyon; OS = open slope)

The samples from May 09 are better clustered than those collected in the other two periods (Fig. 51).
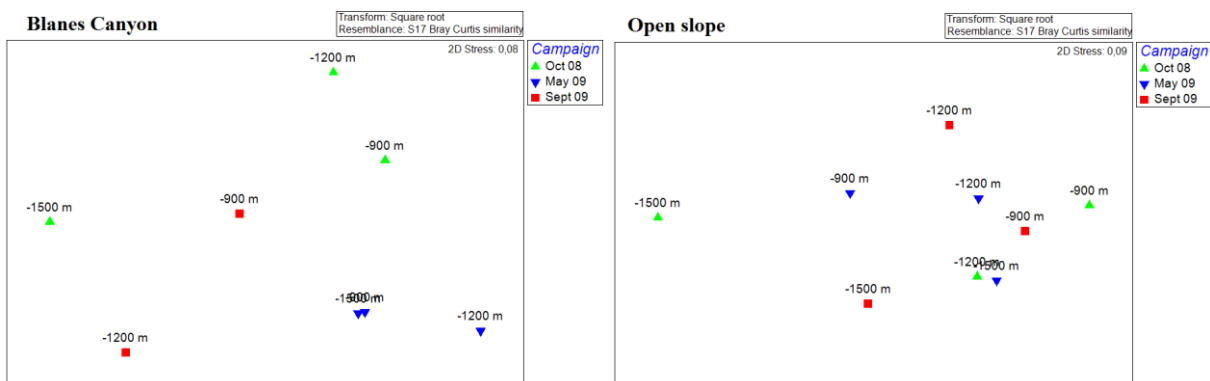
Fig. 52: nMDS obtained through Bray-Curtis index conducted on Polychaeta abundance data (square-root transformed), divided by each station. (BC = Blanes Canyon; OS = open slope)

Analyzing separately the two sampled areas (Fig. 52), BC shows a similar pattern that that of the general analysis with the May 09 showing the best clustering, while in the open slope, May 09 and Sept 09 samples are the best clustered. In turn, the May 09 samples show clear depth pattern.

Table 10: Number of individuals per m2 and relative contribution (as percentage) of each Polychaeta family in each station. (BC = Blanes Canyon; OS = open slope)

	Paraonidae	Spionidae	Syllidae	Glyceridae	Cirratulidae	Lumbrineridae	Capitellidae	Flabelligeridae	Terebellidae	Maldanidae	Ophelidae	Sabellidae	Neridae	Onuphidae	Sygalionidae	Hesionidae	Acrocciridae	Trichobranchidae	Cossuridae	Eunicidae	Scalibragmatidae	Polynoidae	Lacydonidae	Alciopidae	Phyllodocidae	Poecilochaetidae	S	TOT
-900 m BC	315	236	197	128	226	49	10	59	29	59	49	29	20	0	0	0	20	59	10	29	29	10	0	10	29	0	**21**	**1602**
%	19,63	14,73	12,27	7,98	14,11	3,07	0,61	3,68	1,84	3,68	3,07	1,84	1,23	0,00	0,00	0,00	1,23	3,68	0,61	1,84	1,84	0,61	0,00	0,61	1,84	**0**		
-900 m OS	256	364	79	167	79	49	59	10	49	88	59	49	0	0	10	10	0	0	0	0	0	0	0	0	0	10	**15**	**1337**
%	19,11	27,20	5,88	12,50	5,88	3,68	4,41	0,74	3,68	6,62	4,41	3,68	0,00	0,00	0,74	0,74	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,7**		
-1200 m BC	118	177	147	118	0	0	118	147	29	0	29	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**914**
%	12,91	19,36	16,13	12,91	0,00	0,00	12,91	16,13	3,23	0,00	3,23	3,23	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0**		
-1200 m OS	280	111	52	74	52	125	44	29	15	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**10**	**789**
%	35,51	14,02	6,54	9,34	6,54	15,88	5,61	3,74	1,87	0,00	0,00	0,93	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0**		
-1500 m BC	177	111	288	88	0	0	0	22	22	0	22	0	22	0	22	0	0	0	0	0	0	0	22	0	0	0	**10**	**796**
%	22,23	13,89	36,12	11,11	0,00	0,00	0,00	2,78	2,78	0,00	2,78	0,00	2,78	0,00	2,78	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,78	0,00	0,00	**0**		
-1500 m OS	354	88	59	59	29	29	29	29	0	0	0	0	0	88	0	29	0	0	0	0	0	0	0	0	0	0	**10**	**796**
%	44,45	11,11	7,41	7,41	3,70	3,70	3,70	3,70	0,00	0,00	0,00	0,00	0,00	11,11	0,00	3,70	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0**		

Table 11: Number of individuals per m^2 and relative contribution (as percentage) of each taxa in each station (BC = Blanes Canyon: OS = Open Slope) for each campaign (PR I = Oct 08; PR III = May 09; PR IV = Sept 09).

	Paraonidae	Spionidae	Syllidae	Glyceridae	Lumbrineridae	Cirratulidae	Capitellidae	Ophelidae	Flabelligeridae	Terebellidae	Sabellidae	Maldanidae	Nereididae	Onuphidae	Sigalionidae	Hesionidae	Acrocirridae	Trichobranchidae	Cossuridae	Eunicidae	Scalibregmatidae	Polynoidae	Lacydonidae	Alciopidae	Phyllodocidae	Poecilochaetidae	S	TOT
Oct 08 BC	226	88	108	39	20	20	39	0	0	0	20	0	20	0	0	0	20	59	10	0	0	10	0	10	0	0	14	688
%	35,94	14,06	17,19	6,25	3,13	3,13	6,25	0,00	0,00	0,00	3,13	0,00	3,13	0,00	0,00	0,00	3,13	9,38	1,56	0,00	0,00	1,56	0,00	1,56	0,00	0,00		
Oct 08 OS	241	54	93	64	34	108	0	10	34	29	0	20	0	29	10	39	0	0	0	0	0	0	0	0	0	10	14	777
%	30,99	6,96	12,02	8,22	4,43	13,92	0,00	1,27	4,43	3,80	0,00	2,53	0,00	3,80	1,27	5,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,27		
May 09 BC	147	162	133	177	59	0	59	221	44	59	74	0	15	0	15	0	0	0	0	29	29	0	15	0	0	0	15	1238
%	11,37	12,50	10,23	13,64	4,55	0,00	4,55	17,05	3,41	4,55	5,68	0,00	1,14	0,00	1,14	0,00	0,00	0,00	0,00	2,27	2,27	0,00	1,14	0,00	0,00	0,00		
May 09 OS	177	302	37	118	7	7	44	0	0	0	0	7	0	59	0	0	0	0	0	0	0	0	0	0	0	0	9	759
%	23,31	39,82	4,86	15,54	0,97	0,97	5,83	0,00	0,00	0,00	0,00	0,97	0,00	7,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00		
Sept 09 BC	265	354	442	133	221	44	44	0	44	0	0	88	0	0	0	0	0	0	0	0	0	0	0	0	44	0	10	1681
%	15,79	21,05	26,31	7,89	13,16	2,63	2,63	0,00	2,63	0,00	0,00	5,26	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,63	0,00		
Sept 09 OS	442	236	59	88	88	88	59	29	59	88	59	29	29	0	0	0	0	0	0	0	0	0	0	0	0	0	13	1356
%	32,62	17,40	4,35	6,52	6,52	6,52	4,35	2,17	4,35	6,52	4,35	2,17	2,17	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00		

3.2.2.1 SIMPER analyses.

The SIMPER analysis indicates which families are responsible of the differences observed in the nMDS.

The Spionidae, Syllidae, Paraonidae, Glyceridae and Cirratulidae are the responsible of most of the average dissimilarity between BC and the open slope.

Groups BC & OS
Average dissimilarity = 58,63

Species	Group BC Av.Abund	Group OS Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Paraonidae	234,06	233,45	10,23	1,16	17,45	17,45
Spionidae	66,35	221,98	9,60	1,31	16,37	33,82
Syllidae	95,84	108,12	7,35	0,91	12,53	46,35
Glyceridae	64,51	110,58	5,26	1,13	8,96	55,32
Cirratulidae	84,78	41,77	4,66	0,76	7,95	63,27
Lumbrineridae	29,49	50,79	2,93	1,02	5,00	68,27
Capitellidae	14,74	45,87	2,50	1,07	4,26	72,53

Analyzing the differences in depth, the contribution of the Lumbrineridae is the most relevant in explaining the dissimilarity between -900/-1200 m depth and -1200/-1500 m depth.

```
Groups -900 m  &  -1200 m
Average dissimilarity = 50,09

                Group -900 m Group -1200 m
Species             Av.Abund     Av.Abund Av.Diss Diss/SD Contrib%  Cum.%
Spionidae              11,90         9,27    5,05    1,13    10,07  10,07
Cirratulidae            9,12         3,92    4,88    1,00     9,74  19,81
Syllidae                9,50         5,35    4,37    1,18     8,72  28,53
Lumbrineridae           6,34         3,49    3,58    1,21     7,15  35,68
Glyceridae              9,12         9,32    3,52    0,95     7,02  42,70
Capitellidae            3,75         5,35    3,07    1,07     6,14  48,84
Terebellidae            5,06         0,00    2,77    0,93     5,54  54,37
Paraonidae             15,24        16,30    2,72    1,11     5,42  59,80
Sabellidae              5,06         0,00    2,61    0,95     5,21  65,00
Maldanidae              3,14         2,68    2,53    0,86     5,05  70,05


Groups -900 m  &  -1500 m
Average dissimilarity = 55,66

                Group -900 m Group -1500 m
Species             Av.Abund     Av.Abund Av.Diss Diss/SD Contrib%  Cum.%
Cirratulidae            9,12         3,76    5,97    0,89    10,72  10,72
Syllidae                9,50         8,90    5,91    1,11    10,63  21,35
Paraonidae             15,24        10,94    5,69    1,03    10,23  31,58
Glyceridae              9,12         4,18    5,49    1,02     9,87  41,45
Spionidae              11,90         9,86    4,40    0,85     7,91  49,36
Lumbrineridae           6,34         1,88    3,75    1,17     6,74  56,10
Terebellidae            5,06         3,76    3,41    1,00     6,13  62,23
Flabelligeridae         4,04         5,64    3,31    1,00     5,95  68,18
Sabellidae              5,06         0,00    2,79    0,93     5,00  73,18


Groups -1200 m  &  -1500 m
Average dissimilarity = 59,61

                Group -1200 m Group -1500 m
Species              Av.Abund     Av.Abund Av.Diss Diss/SD Contrib%  Cum.%
Paraonidae              16,30        10,94    7,83    1,01    13,14  13,14
Syllidae                 5,35         8,90    7,19    1,11    12,07  25,20
Glyceridae               9,32         4,18    6,54    1,13    10,98  36,18
Spionidae                9,27         9,86    6,30    0,95    10,57  46,75
Capitellidae             5,35         1,88    4,45    1,04     7,46  54,22
Flabelligeridae          1,11         5,64    3,88    1,20     6,51  60,73
Cirratulidae             3,92         3,76    3,77    0,93     6,32  67,06
Lumbrineridae            3,49         1,88    3,00    0,78     5,03  72,09
```

Finally, all sampling periods show dissimilarities at least 50%. The differences are mostly explained by six families: Paraonidae, Syllidae, Glyceridae, Spionidae, Cirratulidae and Terebellidae.

Groups Oct 08 & May 09
Average dissimilarity = 57,10

	Group Oct 08	Group May 09				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Syllidae	7,78	8,49	5,89	0,99	10,32	10,32
Glyceridae	6,91	11,24	5,41	0,90	9,47	19,79
Cirratulidae	1,28	7,05	5,32	1,25	9,32	29,11
Spionidae	11,53	8,35	5,32	0,94	9,31	38,42
Paraonidae	11,60	13,64	4,91	0,90	8,59	47,01
Maldanidae	0,00	5,81	4,49	1,16	7,86	54,87
Lumbrineridae	2,56	5,06	3,53	0,99	6,18	61,06
Flabelligeridae	2,01	4,70	3,40	1,01	5,95	67,00
Capitellidae	4,86	2,68	3,27	1,04	5,73	72,74

Groups Oct 08 & Sept 09
Average dissimilarity = 58,43

	Group Oct 08	Group Sept 09				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Paraonidae	11,60	18,49	7,05	0,91	12,07	12,07
Cirratulidae	1,28	9,41	6,86	1,06	11,73	23,80
Syllidae	7,78	7,20	5,60	1,00	9,59	33,40
Spionidae	11,53	11,41	5,50	0,92	9,41	42,81
Glyceridae	6,91	4,54	4,52	1,12	7,73	50,54
Capitellidae	4,86	3,76	3,45	1,04	5,90	56,44
Lumbrineridae	2,56	4,54	3,14	0,94	5,38	61,82
Terebellidae	1,28	4,54	2,99	0,83	5,12	66,94
Flabelligeridae	2,01	3,76	2,80	0,89	4,79	71,73

Groups May 09 & Sept 09
Average dissimilarity = 49,06

	Group May 09	Group Sept 09				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Glyceridae	11,24	4,54	5,66	1,29	11,54	11,54
Syllidae	8,49	7,20	4,84	1,13	9,87	21,41
Spionidae	8,35	11,41	4,78	1,08	9,74	31,15
Cirratulidae	7,05	9,41	3,77	0,93	7,69	38,84
Paraonidae	13,64	18,49	3,71	1,29	7,57	46,41
Maldanidae	5,81	1,88	3,70	1,15	7,53	53,94
Lumbrineridae	5,06	4,54	3,52	1,07	7,18	61,12
Terebellidae	3,14	4,54	3,10	0,96	6,31	67,44
Flabelligeridae	4,70	3,76	3,07	0,95	6,26	73,70

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3.3   Taxonomic sufficiency

To test whether the changes in taxonomic resolution would affect the corresponding analyses of the community structure, MDS analyses have been conducted on three different square-root transformed datasets:

- All taxa (AT) = matrix with all taxonomic levels, from Polychaeta species to higher taxa;
- Phylum level (PL) = matrix arranged at phylum level, with all lower levels collapsed into them;
- Polychaeta Families (PF) = matrix with the polychaete families only.
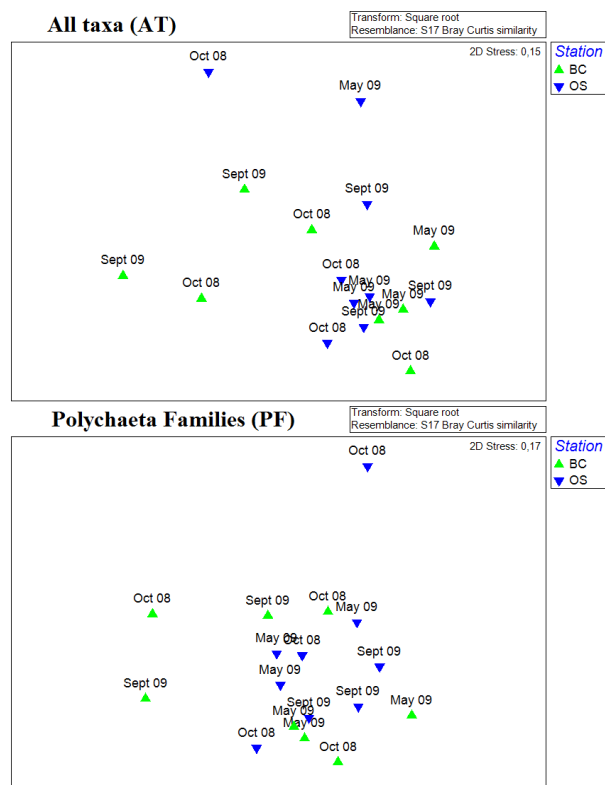

Fig. 53: nMDS obtained through Bray-Curtis similarity index conducted on square-root transformed abundance data of two distinct matrix: All taxa (matrix with all taxonomic levels) and Polychaeta (matrix with only Polychaeta families). (BC = Blanes Canyon; OS = Open Slope)
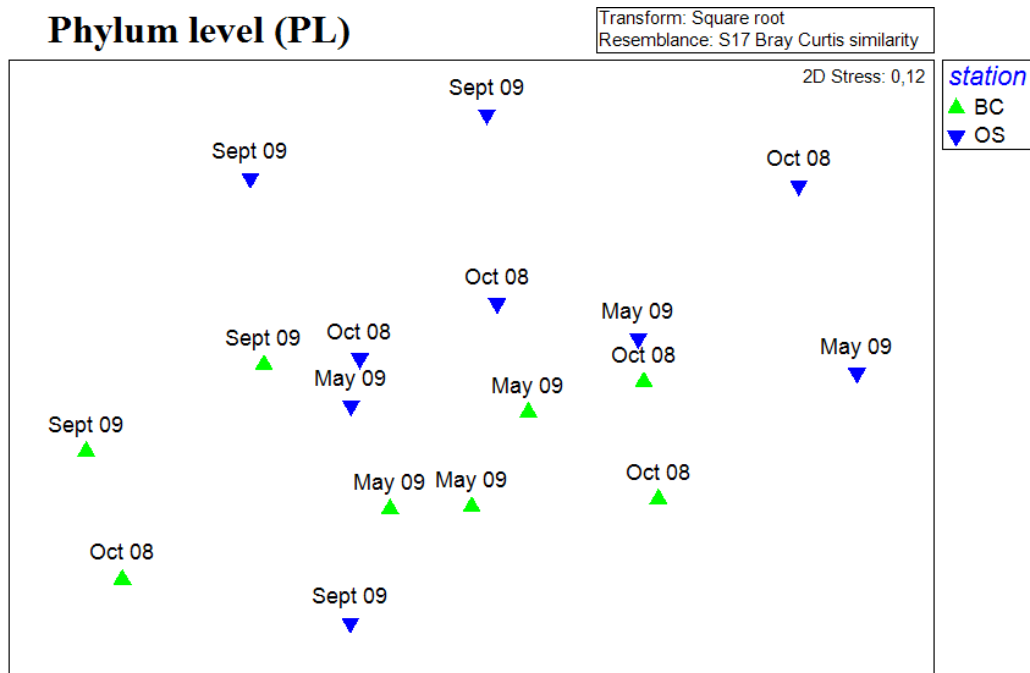
Fig. 54: nMDS obtained through Bray-Curtis similarity index conducted on square-root transformed abundance data of Polychaeta families. (BC = Blanes Canyon; OS = Open slope)

The AT and PF nMDS are not significantly different, showing a similar pattern of sample distribution, where BC and the open slope are not clearly separated (Fig. 53). In turn, the PL nMDS shows a more evident segregation between the BC and adjacent open slope stations, except for Sept 09 from the open slope (Fig. 54).

The RELATE analysis conducted on the three similarity matrices shows highly significant correlation between AT and PF (Rho = 0.71; $p<<0.05$) , while PL is not correlated neither of the two (PL vs AT: Rho = 0.11, $p>0.05$. PL vs PF: Rho = 0.10, $p>0.05$).

Fig. 55: 2nd stage MDS obtained through comparison between Bray-Curtis similarity matrix conducted on abundance data of 3 different matrix, each with 4 types of transformation (no transformed, square-root transf., 4th-root transf., log(x+1) transf.). (All = matrix with all taxonomic levels; Major = matrix with only phyla; Poly = matrix with only polychaetes families)

To see how much the multivariate pattern changes as a results of various data transformation, has been conducted a 2nd stage MSD on similarity matrix with different levels of data transformation. This routine uses p value of each matrix as its corresponding similarity measure to conduct a new MDS (of MDSs!). The result is showed in Fig. 55. In spite of increasing in data transformations, the relationships between the three taxonomic matrix didn't change.

5 DISCUSSION AND CONCLUSIONS

Submarine canyons are biodiversity hot-spots, due to their disturbance regime and incremented conveying of organic matter. They host huge quantities of deposit and filter-feeders (Buhl-Mortensen et al., 2009), and their predators (Haedrich et al., 1980; Buzas & Martin, 1978). The Catalan Sea is one of the most interesting site to study these habitats in the Mediterranean area. Its basin is characterized by the presence of numerous submarine canyons and a heavy influence of the energy inputs from the adjacent terrestrial districts (Sardà et al., 2009; Zuniga et al., 2009). Many anthropic activities are conducted in this area, heavily impacting local marine assemblages.

For instance, the red shrimp *Aristeus antennatus* (Risso, 1816) is one of most exploited prey by local fisheries. Its catching grounds are localized at depths between -600 m and -900 m (Sardà et al., 2009), and its fishing period is from late summer to mid winter. Its life cycle strongly depends on the canyon's environmental conditions and the topography, which originate different habitat conditions that are used in different ways by the red shrimp, as the different life cycle phases may be found in different areas of the canyon depending on the period of the year (Sardà et al., 2009). Also, the persistence of their populations is strongly related with cold-water "cascading" events, which completely deplete the annual stock in recurrent episodes within each 5 to 7 year period (Company et al. 2008).

Benthic macrofaunal assemblages are at the basis of the trophic chain for this highly valuable resource and, also, it is well known that the variability of these assemblages reflect in an integrative mode the whole functioning of the system, including the intensity of anthropogenic impact at these depths. Therefore, by studying the macrobenthic assemblages, is is possible to improve the identification of the main operative task to restore compromised equilibriums.

Therefore, this study has been conceived to understand if (and how) deep-sea macrofaunal assemblages are influenced by living inside submarine canyons, by comparing variation in their abundances with trends of communities living at same depth in adjacent open slope (Rex, 1973, 1976, 1981; Sanders, 1968; Sander et al., 1969). Some variation patterns may be traced through studies concerning benthic macrofaunal taxa, depending on analyzed factor and taxonomic level used

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(Narayanaswamy et al., 2003). This work focuses also on the identification at different taxonomic levels in order to construct a framework useful for further analyses.

All assemblages studied were always dominated by Polycheata, Mollusca and Crustacea, both in the Blanes Canyon (BC) and in open slope. This is a common characteristic of many soft-bottom macrofaunal assemblages (Vetter et al., 1998; Cardell et al., 1999; Sorbe, 1999) and supports the presence of complex trophic-nets in these habitats (Danovaro et al., 1999). The abundance of filter-feeders was higher inside BC than in the adjacent open slope, which was also related with an increment of predator polychaetes. Surface deposit-feeders were more abundant in the open slope than in BC, along with a decrement of filter-feeders and their predators. Probably these differences are due to the higher quantities of suspended organic matter reaching the canyon, which may cause the increment of the different organisms exploiting this resource and, thus, of their predators. The relevance of the relatively higher contents of organic matter inside the canyon is also reflected in the pattern of mollusks' abundance along depth, which decreased slower in BC than in the open slope. The multivariate analyses conducted on the abundance of major taxa point out major differences effective taxa richness between depths .

Many different phyla have been identified, and both BC and its adjacent open slope had similar diversity (number of phyla per sample). As expected, relative abundances differed both in depth and in temporal trends. The highest abundance in BC was detected in the shallowest depth and in early fall (September-October), probably due to higher food availability caused by stronger flood events coming from Tordera River (Zuniga et al., 2009). High concentrated fluxes may be caused also by cold water masses coming from Gulf of Lion district during cold seasons (Ferré et al., 2005; Company et al. 2008).). Taxa diversity and abundance were higher in the shallowest depth and lowest at 1500 m depth. This is probably due to different trophic regimes at these depths (Levin et al., 1994; Levin & Gage, 1998), which may intensify the typical Mediterranean conditions leading to a general oligotrophy in the deepest zones (Cartes & Sardà, 1993). The nMDS conducted on major taxa abundance also pointed out differences between BC and open slope samples, which are consistent with the zonation of the bathyal zone (200-2.500 m) proposed by Pèrés (1985), with a upper zone dominated by euribathic species, a middle zone with high species diversity and a lower

zone with lower diversity and the presence of deep-water species not found in the shallower zones.

Interestingly, the macrofaunal abundance doubled between October 2008 and September 2009, mainly due to increments of all minor taxa and polychetes. In BC, Spionidae and Syllidae highly increase from one year to other, as many other minor taxa (e.g. Nemertea, Cnidaria and Phoronida). This could be related to a mobilizing event, which may drag huge quantitities of nutrients to these environments, sufficient to stress/support benthic communities. The effects of such an event seemed to involve adjacent open slope too, where Paraonidae and Spionidae also highly increased (although at a different magnitude than in BC). One possible explanation may be the formation of a nepheloid layer during the water stratification characteristic of the warmer season, which deprived May communities of organic supply and released all accumulated food-supply with the water mixing associated to the arrival of autumn (Puig & Palanques, 1998; Puig et al., 2001). The nMDS conducted on major taxa abundance shows a slight temporal difference between the three campaign samples, with a clear clustering between samples of Sept 09.

Polychaeta family richness have clearly a minimum at the 1200 m depth of BC. This poverty is also related with a decrease in diversity (Shannon index) and an increase of evenness (Pielou index). All polychaete families decreased in the same way, highlighting the presence of a general impact affecting their populations in the middle slope. This area is the most impacted by commercial bottom trawling (Demestre, 1990), which constantly disturbs the benthic communities through fishing gears. Furthermore, diversity of other taxa also decreased at the 1200 depth, with a corresponding increase in evenness. Even abundance highly decreased between 900 m and 1200 m depths both in BC and in the adjacent open slope, but with different gradients between Polychaeta and other taxa, likely because Mollusca and Crustacea may have a higher survival potential due to their shells or their highly motility, the latter also taking advantage of the organic matter coming from animals damaged by fishing gears (Ramírez-Llodra et al., 2007).

To conclude, this study demonstrates that both TIME and DEPTH affected the macrofaunal assemblages in the deep submarine Blanes canyon, which often contras with the patterns of the adjacent open slope. The whole area is constantly subjected to

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anthropogenic activities, particularly in the fishing grounds, and this is well known to have long lasting impacts on deep-sea communities. In turn. the coupling between river discharges and marine currents conveys large amounts of suspended sediments to deep-sea zones, impacting also the local ecosystems. As this work is framed within a project currently in progress, it has not been possible to have access to the corresponding data set of environmental factors. Thus the identification of possible correlations between biotic variations here reported and the concurrent environmental factors, will be a matter of further work. However, this study is a contribution to the building up of a general oveview of the deep-sea assemblages of the region, which includes the identification of all found taxa and a test to assess the taxonomic level that could be the most efficient for the purposes of this type of studies (time spent on identification/ obtained variations of information)

Threating the seep-sea would affect the equilibrium of its functions and services as a habitat, on which many human activities in the Mediterranean region depend. Therefore, mitigation of sources of disturbance is nowadays one of the main research fields in marine ecology . Particularly, the Mediterranean Sea, where the investigation of the deepest-bottoms have just started still requires many further studies . Many efforts must also be addressed to any topic providing key issues in these directions, among them, to avoid any long lasting endangering of the ecological properties of the deep-sea assemblages, benthic ones includes, and their population dynamics.

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