Mediterranean deep-sea ecosystems: Biodiversity, functioning and vulnerability

Ecosistemes profunds de la Mediterrània: Biodiversitat, funcionament i vulnerabilitat

Samuele Tecchio

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MEDITERRANEAN DEEP-SEA ECOSYSTEMS: BIODIVERSITY, FUNCTIONING AND VULNERABILITY

ECOSISTÈMES PROFUNDS DE LA MEDITERRÀNIA: BIODIVERSITAT, FUNCIONAMENT I VULNERABILITAT

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Preface: three paths

In offering this book to the public the writer uses no sophistry as an excuse for its existence. The hypocritical cant of reformed (?) gamblers, or whining, mealymouthed pretensions of piety, are not foisted as a justification for imparting the knowledge it contains. […] *It may caution the unwary who are innocent of guile, and it may inspire the crafty by enlightenment on artifice.* […] *But it will not make the innocent vicious, or transform the pastime player into a professional; or make the fool wise, or surtail thae annual crop of suckers, but whatever the result may be, if it sells it will accomplish the primary motive of its author, as he needs the money.*

- **S. W. Erdnase**, *The Expert at the Card Table* (1902)

As I write this, I am staring at the twilight sun, inexorably approaching the silvered coat of *our sea* for one time more. In front of me, behind the blinking lights of the port, and hidden somewhere behind the horizon, stand the coasts of my home country. How did I get here?

Many paths led to this particular conclusion. I will talk about three of them.

First, there is the path of discovery. Science, at least the healthy one, revolves around discoveries. Sometimes the discovery is just a small, although worthy, step towards a better understanding. Sometimes the discovery is considerable, and makes a quantum leap in how we understand a particular topic. As Erdnase expressed in his citation above, this book will not transform a layman into a deep-sea ecologist, nor will radically change the vision we have of those fascinating, obscure expanses.

But indeed, there is discovery, and there is understanding.

Then, there is the path of trial and error. And hope. During my years as an undergraduate student in Padova, I had the unlucky idea of getting in love with science, and at the same time, the lucky occasion of being negatively selected. That environment attempted to quell me down to their standards, and failed miserably. But at one go, it taught me those directions in research one should not pursue.

Fortunately, genuine isles do remain. And resist. And this gives me hope about the future of marine research in my home country.

The third path is one of motivation and purpose. One that is hardly fulfilled by choosing to work on the deep sea. This branch of ecology is mainly vocational: the good-willing student who is searching for achievement in a plethora of data points will have to look elsewhere. I found my motivation in distilling a handful of samples into the highest number of drops possible. Four of them are included here, with my great satisfaction, for the reader to appreciate. But even greater satisfaction, and of course conscience of purpose, came from the smile and laughs with my colleagues, shoulder to shoulder in the cramped lunch room of the ship, after a successful sampling day.

As always, you get the most from the people nearest to you. Which leaves me with the unavoidable but entertaining task of acknowledging them. A rather large list of people who, willing or unwilling, have contributed to the work you have in your hands.

I would like to start by Saša Raicevich and Federico Lauro, known as the **initiators**. Their timely suggestions and support were those which, back in 2007, sparked my decision of having a peek at the world outside.

I want to warmly thank Antonio Zuccaro, Martina Zancan, Marie Le Guitton, Michele Grego, Francesco Cozzoli, Alessia Moneta, Lara Pozzato, Ricardo Amoroso, Krista Greer, Lisa Boonzaier, and Laurenne Schiller, known as the **outside supporters**, who made me feel at home in any part of this planet I did land on. I will also share an enormous hug with the whole *De Kêête* family, remembering their wonderful and warm applauses after my card magic performances.

The completion of this thesis has been, of course, made possible by all my colleagues during these years, who I will call the **main actors**. Thus, at the Institute of Marine Sciences in Barcelona I had the luck of working alongside David Costalago, Ulla Fernández de Arcaya, Samuel Koenig, Valerio Sbragaglia, Noelia Díaz, Àngel López, Ariadna Mechó, Jacopo Aguzzi, Joan Navarro, Marta Coll, and Isabel Palomera.

During my frequent stays at the NIOZ in Yerseke, the Netherlands, I was kindly helped and supervised by Leon Moodley, Karline Soetaert, Dick van Oevelen, and Peter van Breugel.

Prof Villy Christensen of the UBC Fisheries Centre in Vancouver, Canada, has been a great host and his help on ecosystem modelling was invaluable. I will also thank Deng Palomares and Shawn Booth, with whom I had interesting conversations on the model details.

My two **promoters**. Before starting this adventure, I had a vague idea of what a thesis supervisor would be and luckily, I was wrong. They inspired me with their enthusiasm, passion for discovery, and attention to detail. They gave me ideas, they gave me smiles. And as I acknowledge their momentous duality, a vivid image comes to my mind. The moment when we knew I would be the new Ph.D. student for the project, they shouted:

Eva: "*Tenim becari!*"

Siscu: "*Pobret!*"

Turned out that both of you were right. Thank you Eva, thank you Siscu, for helping me shape my future in such an amazing way.

A **special mention** will go to Joan B. Company. All the great and enlightening conversations we had in these years can be condensed into one single conclusion, a rather flamboyant one, but one that he would appreciate nonetheless:

While everybody was dressing me up with every kind of possible neckties, he taught me how to tie the knot. Thank you, Batis.

Last, but certainly not the least, come the **cornerstones**. Those who more than any other deserve an acknowledgement for having supported me throughout these years, and in ultimate instance, made this book possible. To Fulvio, for sharing with me all those joyful chats on physics, and all those glasses of wine. To Monica, for helping me see the life of the emigrant in a different way. To my great friend Fabio, for the countless conversations, the numerous hugs during difficult moments, and for forgiving me.

To my mother and to my father, for teaching me to be a good person, to be curious, to always pose questions, to strive for more. To Sandra, my beloved wife, for being "life, rarer than a quark and unpredictable beyond the dreams of Heisenberg; the clay in which the forces that shape all things leave their fingerprints most clearly".

These were my great inspirations along the three paths. Some of them conveniently unmentioned, some of them were luckily left behind. But to each and every one of them goes my greatest acknowledgement for having shaped my last four years, with the hope that in a not so distant future, our lives may cross again.

Samuele Tecchio

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Introduction

1

The deep sea is the portion of the ocean that stands below 200 m depth, both in the water column and in the benthos (Gage & Tyler 1991). It is the largest biome of planet Earth, with about 90% of the ocean area being deep sea (Ramírez-Llodra *et al.* 2010a). In the past decades, researchers have loved to refrain that "we know more about the surface of the Moon than about our deep oceans". To date, this still holds true. The deep sea is considered impenetrable, difficult and expensive to reach and observe.

The deep sea is also defined as the region starting below the continental shelf-slope break (Pérès 1985). Thus, it is formed by a large part of the continental margin and by the deep basin proper. Another definition that applies to the deep-sea habitat regards the definition of bathymetric zones. The *bathyal* zone includes all the continental margin, from 200 to 3000 m depth, while the *abyssal* zone embraces most of the deep seafloor between 3000 and 6000 m depth. The *hadal* zone, the deepest part of the oceans, is found in trenches and reaches a maximum depth of just over 11 km at the Mariana's trench.

All the above to realize that to date, the definitions applied on the deep sea are varied and not always overlapping. This is also because the realization that the deep sea was a separate biome – with its peculiar environmental conditions – happened only in recent years (Gage & Tyler 1991).

Environmental characteristics

Sunlight provides the energy for the sustainment of the majority of life on Earth. Sunlight penetration in seawater, however, is damped with increasing depth due to light absorption and scattering in the water particles (Jerlov 1976). It follows that below approximately 1000 m, photon penetration is absent. What is considered the deep sea is an expanse almost totally devoid in light. This also means that, descending into the depths, we arrive at a level below which photosynthesis may not be performed with enough efficiency to sustain life. This level, called *compensation depth*, is found at about 150-200 m in the most clear ocean waters and, logically, is shallower in more turbid waters. The region delimited by the compensation depth on the upper part, and the twilight zone end on the lower part, is called the *disphotic zone*. Below the disphotic zone, lies the *aphotic zone*, i.e. the zone with total absence of light (Jerlov 1976).

Water temperatures in the deep sea are only marginally affected by surface atmospheric oscillations and, during the whole year, are subjected to minimum variations. In the bathypelagic habitat and below, seawater temperature is stable between 0 to 3 ºC, except in the Mediterranean Sea (13 ºC) and in the Red Sea (21.5 ºC). Below the polar and sub-polar regions, temperature can be even lower due to the compensation effect of pressure. Similarly, water salinity also presents very stable conditions below 1000 m depth, both in spatial and temporal scales.

With the exceptions of *oxygen minimum zones*, the almost totality of the deep sea is not limited by dissolved oxygen, even considering that the interaction of the deep-water masses with the atmosphere is null. The present amount of oxygen in the deep waters was dissolved into them when these masses were at the surface, and oxygen depletion in the deep is unlikely to achieve due to the extremely low densities of organisms and therefore, oxygen consumption (Nybakken & Bertness 2005).

Pressure is a factor that reaches extreme magnitudes in the deep sea. Environment pressure linearly increases at a rate of approximately 100,000 Pa every 10 m of water depth. The effects of pressure on deep-sea living organisms is still far from being understood in detail, with unclear theories on the extent of possible pressure effects (Childress 1995, Carney 2005).

The deep benthic environment is heterotrophic (i.e. the food web depends upon organic matter ultimately produced in the surface waters by photosynthesis) and therefore extremely food limited, in the sense that only a small fraction of the production (primary or secondary) of the surface layers arrives to the seafloor (Gage 1992, 2003). During its fall in the water column, faecal pellets, particulate organic matter (POM) from dead planktonic animals, and terrestrial input particles, get progressively degraded by bacteria (Azam *et al.* 1983). Larger particles are directly ingested by feeding mesopelagic animals. POM can form larger aggregates, increasing its descent rate, and further contributing to this constant but extremely scarce rain of food particles called *marine snow* (Gage 2003). Once on the seafloor, marine snow entrains the benthic food web, and is thus essential in regulating how species interact with food sources and between them (Levin *et al.* 2001, Smith *et al.* 2008).

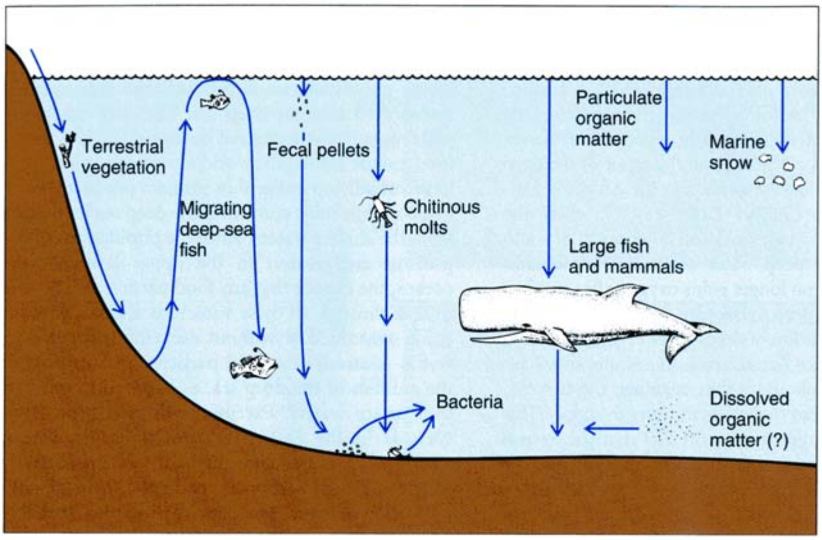


Figure 1.1. Schematic representation of the various food sources for the deep sea. Source: Nybakken and Bertness (2005).

A major topic over the past decades has been the definition of the various possible pathways and processes that may drive food from the surface layers to the deep benthos (Gage 2003). As the deep sea in an extremely open system, this definition is not trivial. In Figure 1.1, some of the possible pathways are represented. It is important to mention the immigration and emigration of organic matter performed by vertically-moving fish and crustacean species, and the occasional import by large carcasses such as those of marine mammals. Another process of organic matter input is the lateral advection from adjacent deep seafloor, as dissolved organic matter (DOM). At present, the relative importance of each of these sources is still matter of evaluation.

Finally, the temporal variability of the above mentioned parameters has started to be addressed from the 1980s, although only in recent years with detail. The major topic in this regard is the variation of organic matter arrival at the deep sea over various short-term (i.e. not geological) time scales. Downward vertical fluxes of organic matter, used as food by a part of the benthos, provide a constant baseline rain of food particles (with seasonal fluctuations), while also presenting time-concentrated pulses such as landing of animal carcasses of any size (Tyler 1988, Smith & Baco 2003, Aguzzi *et al.* 2012). The study of how deep-sea animals react to these falls and how they adapt to the scarce, albeit constant, rain of marine snow, is one of the pivotal themes in marine ecology, from which new inclusive theories on food web functioning will be hopefully synthesized.

The Mediterranean deep-sea habitat

The present thesis will focus on the fine-mud benthos habitat of the Mediterranean Sea, on its bathyal and abyssal zones. The Mediterranean Sea is an enclosed seawater basin formed, on a general sense, by the convergence of the north-drifting African-Arabian and the more stable European tectonic plates (Stanley & Wezel 1985). It is formed by two main depressions, which are usually classified into three basins: the western, the central, and the eastern (or Levantine) basins (Figure 1.2). The *mare nostrum* has been the cradle of ancient civilizations that sailed on its coastal waters and, in recent centuries, dwelled far in the open sea. Little they knew how far the waters extended below them: a first sounding of the depths came only in the 1850s (Kunzig 2000). The general observation on the wide extent of deep-sea areas in the globe holds true in the Mediterranean: it is composed mainly by deep-sea habitats, with a mean depth of 1500 m and a maximum depth of 5267 m, found in the Calypso Deep in the eastern Ionian Sea.

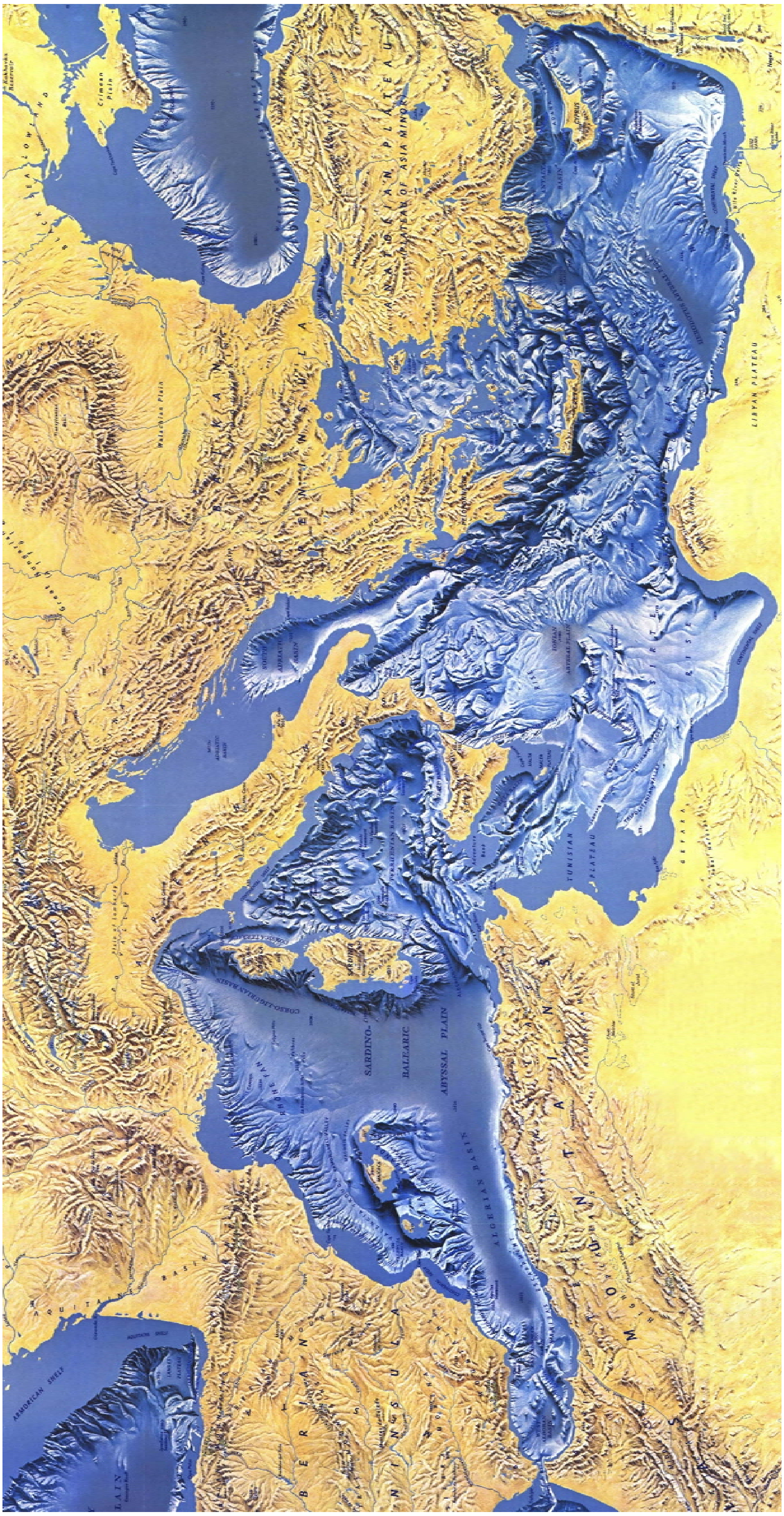


Figure 1.2. Map of the Mediterranean Sea (scale 1:4371000). Vertical scale is greatly exaggerated, permitting to appreciate the complex bathymetric structures along the whole basin. Modified from: *"The Mediterranean Seafloor"*, National Geographic Society.

In the Mediterranean Sea, there exists a particular temperature condition in deep waters: below 200 m depth, seawater temperature is stable and higher than in the outer Atlantic Ocean, standing at approximately 12 to 14 ºC. Regarding salinity and the salt balance, the Mediterranean is considered a concentration basin, where the amount of water lost by evaporation is higher than the amount of water introduced by riverine input and by precipitation. It follows that salinity in the Mediterranean is higher than that of the outer Atlantic Ocean, and in the deep water it varies from 38.4 to 38.7, depending on basin (Miller *et al.* 1970). Oxygen is also not limiting, if we exclude specific areas such as the anoxic brine lakes in the central basin. Primary production levels in the Mediterranean follow a longitudinal gradient, decreasing from west to east. In Figure 1.3, the concentration levels of surface chlorophyll-a are presented, as measured by the SeaWiFS ocean colour satellite, for the month of June 2009. The figure clearly shows a marked difference in surface primary productivity between the western and eastern basins, while the central part of the Mediterranean presents transitional characteristics.

The Mediterranean Sea can thus be considered an excellent natural laboratory for benthic ecologists, due to its peculiar environmental conditions, its broad-scale gradients, and its deep-sea fauna (Bethoux *et al.* 1999).

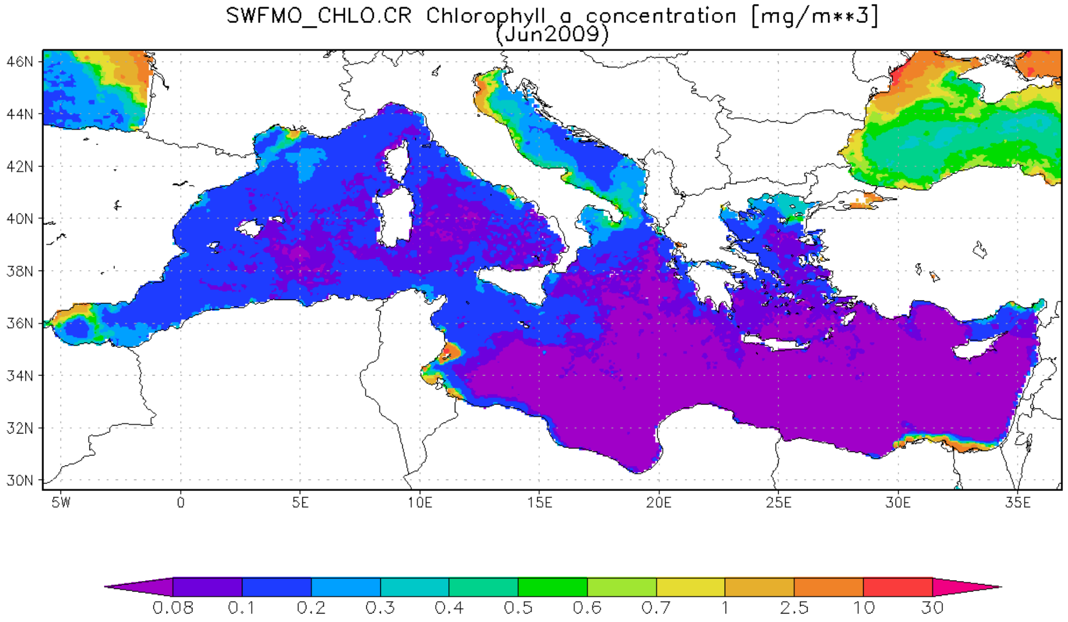


Figure 1.3. Surface chlorophyll-a concentration in the Mediterranean Sea, used as an estimator of surface primary production, expressed as mg·m^{-3}. Data was retrieved from the SeaWiFS satellite online database (http://oceancolor.gsfc.nasa.gov) and integrates all measurements from June 2009, when the trans-Mediterranean cruise BIOFUN was conducted.

The research framework

This thesis will focus on megafauna, i.e. that fraction of the benthos whose animals are easily caught by deep-sea dredges and trawls (Gage & Tyler 1991). The marine fauna of the Mediterranean Sea is relatively young relative to the fauna of the outer Atlantic Ocean, and compared to that habitat it is reduced in density and diversity (Fredj & Laubier 1985, Tyler 2003). The main reason is the catastrophic event that marked the history of Mediterranean marine animal populations, the Messinian salinity crisis. In this event, dated at approximately 5.6 million years ago, tectonic movements provoked the closing of the Gibraltar sill. The Mediterranean basin thus remained separated from the Atlantic Ocean, and the prevailing evapo-transpiration processes caused a reduction of its sea level of more than 1 kilometre, producing Lago Mare remnants in each basin. At 5.33 million years ago, further tectonic processes opened again the Gibraltar strait, provoking a catastrophic event known as the Zanclean flood, during which waters from the Atlantic Ocean massively entered the Mediterranean basin (Garcia-Castellanos *et al.* 2009). During and right after this event, species from the northern Atlantic Ocean entered the Mediterranean basin, re-colonizing it. Thus, it is logical to conclude that, at least in the case of the Mediterranean, the species than now inhabit the deep continental slopes and abyssal basins are originally species that lived above 320 m depth (i.e. the maximum depth of the Gibraltar sill) and re-colonized the Mediterranean basin after the Zanclean flood. This is reflected, for instance, in the general eurybathicity and widespread distribution of the majority of the fish species that live below a depth of 1000 m (Moranta *et al.* 1998, D'Onghia *et al.* 2004) and for some crustacean species as well (Company *et al.* 2004).

The fauna on the continental margins of the Mediterranean Sea is known to present a strong zonation pattern along depth gradients. Moving downward from the shelf break, distinct upper-, middle- and lower-slope communities can be identified (Pérès 1985). These zones have been confirmed, with only slight variations, across the whole Sea (Abelló *et al.* 1988, Cartes & Sardà 1993, Stefanescu *et al.* 1993, D'Onghia *et al.* 1998, Kallianiotis *et al.* 2000, Maynou & Cartes 2000, Follesa *et al.* 2009). The most represented groups in terms of abundance and diversity are bony fishes (Actinopterygii) and decapod crustaceans, and indeed these were the considered groups in the above mentioned literature. Non-crustacean invertebrates, although presenting high speciosities, are not abundant in the deep Mediterranean (Ramírez-Llodra *et al.* 2008, Tecchio *et al.* 2011), although a great effort is being carried out in these last years to increase the taxonomic knowledge of these groups, so neglected in the previous decades (Mechó *et al.* in preparation).

In 2001, an international research project endeavoured to study the western and central Mediterranean deep basins. The original focus of the program was to collect data on the stocks (both exploited and virgin) of a highly commercial deep-sea shrimp species, the red shrimp *Aristeus antennatus*. The project, named DESEAS, revolved around a deep-sea oceanographic cruise conducted in June 2001, with researchers from Spain, Italy, and Greece. The results of the DESEAS project have been put together in a comprehensive volume spanning both geological and biological areas ("Mediterranean deep-sea biology", edited by Sardà *et al.* 2004b). In more recent years, these results have also been analysed putting them in an even wider context of biodiversity patterns and general ecosystem functioning (Sardà *et al.* 2009b, Tecchio *et al.* 2011). They found that under 1500 m depth, a general sharp decrease in abundance, biomass, body size and diversity occurs. This limit is now considered a pivotal breakpoint in the depth transect, below which environmental conditions do not seem to permit the settling of an abundant and diverse fauna.

The topic of biodiversity in the deep sea is being addressed in numerous sites of the oceans and, in the Mediterranean Sea, is of particular interest because of the peculiar environmental characteristics of the habitat. Every species, and especially the most abundant ones, perform a specific function in the ecosystem, and contribute to its general functioning (Loreau 2008). Highly-diverse ecosystems can draw from a panoply of different adaptations, when confronted with a sudden environmental change, while low-diversity systems – with less available "answers" – may be more prone to collapse (Gray 2001). The same patterns extends to the type and quality of services the ecosystem is able to provide, which in recent years has been subject to a considerable reduction across the world oceans (Worm *et al.* 2006). A thorough knowledge of the biodiversity patterns in the deep Mediterranean is therefore required, especially in a broader, regional, scale of observation. Moreover, a major issue will be to determine the extent of basin-wide processes in determining the biogeography of the benthic species. This is facilitated when studying the deep sea, because of the higher degree of stability of environmental parameters of the habitat, as described in the previous sections.

Trophic relations in the deep sea have only in recent years started to be evaluated in detail. The initial idea of the deep-water trophic webs postulated a series of low-abundance species, dwelling around and rapidly exploiting the rare food falls (Marshall 1980). We now know this was an oversimplification and various other processes occur, such as the vertical movements of mesopelagic species that periodically enter the deep benthic habitat (Smith *et al.* 1994, Gartner *et al.* 1997). Although studies on dietary habits of Mediterranean deep benthic species have been conducted abundantly over the last decades, they all addressed a specific site, being broad regional studies almost absent for the Mediterranean Sea. This is mainly caused by the difficulties in retrieving a sufficient number of samples: the stomach of fishes get everted during retrieval due to decompression shock. Large-scale studies present also another advantage: they will permit a more thorough definition of the trophic function of each species in the food web and, more generally, in the ecosystem.

The human species continuously increase its demand for resources. In the oceans, this means a continuous and growing search for minerals, crude oil, and fishing resources (Thiel 2003, Ramírez-Llodra *et al.* 2011). It has been shown that megafauna possess less recovery capabilities from direct impact than the other size classes of benthos (Jones *et al.* 2012). In the Mediterranean Sea, the most important exploitation activity is commercial fishing, which implied a continuous expansion of fleets along depth gradients, distance from the coast, and technical improvements, over the last decades (Bas *et al.* 1985). All these processes have substantially increased the capacity of retrieving commercially-important species, while coping with the decrease of efficiency due to biomass removal (Myers & Worm 2003, Pauly *et al.* 2005). Exploitation of fish resources has been defined as a constant "ploughing" and in some cases, the definition has shown to be more than just figurative (Puig *et al.* 2012). Present data points to an increase of fishing pressure in ocean living resources in the future, with the increased risk of not being able to determine the unimpacted baseline situation (Thiel 2003). This problem is particularly accentuated in the deep sea because of the scarcity of available data. The establishment of solid baselines is then fundamental to address future changes in light of possible anthropogenic and global processes, and to evaluate how these resources could respond to a possible future exploitation.

In this thesis, the following hypothesis is brought forward for the entire Mediterranean Sea:

> H_0: the structure and functioning of the ecosystems in the deep Mediterranean Sea are not related with geographic position and depth.
>
> H_1: the structure and functioning of the ecosystems in the deep Mediterranean Sea are related with either geographic position and depth, or both.

The testing of this hypothesis is innovatively carried out by considering gradients over large spatial scales, i.e. a whole enclosed basin. This is the first study of its kind in the Mediterranean Sea.

Moreover, a distinctive characteristic of the continental slopes in the western Mediterranean Sea is the presence of numerous submarine canyons indented in the continental shelf. These canyons act as preferential conduits for suspended particulates in the shelf waters, facilitating their descent to the deep basin (Canals *et al.* 2006). Canyons seem to act as hotspots for local faunal diversity and biomass, providing strong habitat heterogeneity, augmented food availability, and refuge areas for motile species (De Leo *et al.* 2010, McClain & Barry 2010). The same assumptions seem to be valid for other canyons in the Mediterranean Sea, although data in this sense are still scant (Danovaro *et al.* 2010). The following additional hypothesis is thus brought forward:

H_0: the submarine canyon environment in the Mediterranean Sea does not form a separate habitat (and is thus only a particular geological structure within the continental slope).

H_1: the submarine canyon environment in the Mediterranean Sea is in itself an habitat separated from the outer continental slope.

To test this second hypothesis, the habitats of the Blanes submarine canyon and the adjacent slope in the Catalan continental margin (north-western Mediterranean) were sampled and analysed for their biodiversity and community composition. Results from the two areas were then compared.

OBJECTIVES

The present study was conducted in the framework of two research projects which spanned from 2008 to 2011: the BIOFUN project ("BIOdiversity and ecosystem FUNctioning in contrasting southern European deep-sea environments: from viruses to megafauna") of the European Science Foundation EuroDEEP, and the Spanish national project PROMETEO ("Integrated study of deep western Mediterranean canyons and slopes: an essential habitat").

According to the hypotheses, the main objective of the present thesis is to describe and characterize the deep habitats of the Mediterranean Sea, relating their biodiversity and ecosystem functioning with the varying environmental conditions along the geographic, bathymetric, and temporal axes. The specific objectives were:

- To describe the patterns of distribution, biomass and abundance of bathyal and abyssal megafauna in the Mediterranean Sea, in relation with environmental variables, along longitudinal and bathymetric gradients;
- To describe the seasonal fluctuations of the above mentioned parameters as proxies of ecosystem structure and functioning in the temporal axis;
- To characterize the trophic patterns of the benthic megafauna to determine how energy flows between sources, benthopelagic, and benthic compartments;
- To integrate the whole dataset into a comprehensive ecotrophic model, evaluating the general ecosystem functioning as well as the degree of vulnerability of these deep ecosystems.

Following from this introduction, the thesis is thematically divided into two parts. The first part, comprising chapters 2, 3 and 4, covers the topic of biodiversity, while the second part includes chapters 5 and 6 and addresses trophic relations and the food-web modelling issues. In detail:

- **Chapter 2** describes the sampling plan and the oceanographic cruises conducted within this thesis work. It also describes the general methods used to collect deep-sea megafauna samples for biodiversity analyses, and the main diversity indices that were applied.
- **Chapter 3** explores biodiversity patterns in the deep Mediterranean Sea over two large spatial scales, longitudinal and bathymetric, and attempts to relate the differences in community composition with a series of environmental variables.
- **Chapter 4** is a highly replicated, multi-period study in the lower continental slope of the Catalan margin (north-western Mediterranean Sea). The focus was on characterizing the essential habitat lying between 900 and 1500 m depth, describing the bathymetric patterns of biomass and diversity, the seasonal movements of the benthos along that depth range, and defining an adjacent submarine canyon as a biodiversity hotspot.
- **Chapter 5** is a study on trophic relations of deep-sea fauna, over the same large spatial transects considered in Chapter 3, by means of natural-abundances stable isotopes analyses and the use of novel statistical methods to analyse heavy datasets all in once.
- **Chapter 6** describes the set-up and parameterization of the first food-web model of a deep Mediterranean Sea ecosystem. Detailed network analysis was performed once the model was balanced, along with a simulation of fishery expansion.
- **Chapter 7** is a collection of conclusions summarizing the results, putting them into a wider context of deep-sea exploration and postulating new ecological perspectives for the deep-sea biome.

Materials and methods

2

Figure 2.1. Edward Forbes FRS, FGS (Isle of Man 1815 – Edinburgh 1854)

In 1842, professor and naturalist Edward Forbes (Figure 2.1) embarked on a geological and biological exploration cruise on board *H.M.S. Beacon*. When he came back, he reported on the extreme scarcity of fauna in the depths of the Levantine basin and postulated that below approximately 550 m depth, no life would be present at all (the "Azoic" theory, Forbes 1844). The most famous – and, might I dare, the most overcited – error in the history of ocean exploration, had two main causes. It turned out that Prof Forbes sampled an extremely poor zone in the eastern Mediterranean basin, which is by itself a region with reduced abundances than the western and central basins. Moreover, Prof Forbes used a sampler which typically underestimated the fauna: a rudimentary fixed-mouth dredge with scarce capabilities of staying in contact with the substrate (Kunzig 2000). In this chapter, the sampling plan and the sampling equipment used nowadays will be described.

Benthic trawling in the Mediterranean

Ever since the start of ocean exploration, the deep sea has remained impervious to sampling. To have a sampler reach the seafloor and work correctly is an arduous task, often implying feeding out kilometres of steel cable to sample poorly explored grounds, and thus exposing the gear to a high risk of damage or failure (Gage & Bett 2005, Danovaro 2010). Meso-pelagic nets, even if designed to work specifically in mid-water, also present some problems because the vessel heaves, forcing the net to haul in an almost sinusoid, rather than linear, pattern. During moments of strong weather, it is impossible to retrieve a correctly closed corer from the bottom. However, the most difficult, time-consuming and dangerous type of sampling the deep sea is, without doubt, benthic trawling.

The Mediterranean Sea has a long tradition of exploration of its deep seafloor by means of trawling with a flexible-mouth net. The pioneers in the region were the group of the Marine Sciences Institute (ICM-CSIC) in Barcelona, who in 1985 started carrying out a large sampling plan using the Semi-Balloon Otter Trawl (OTSB; Rucabado *et al.* 1991, Stefanescu *et al.* 1992). A first description of the OTSB appeared in print in the 1980s (Sulak 1982). The OTSB (also called "Marinovich-type trawl") is a one-warp benthic otter-trawl with a 7-9 m by 1.2 m mouth opening (horizontal x vertical), approximately. The main characteristic of this net is that the bridles are short, compared to standard commercial trawls, and are doubled between the otter doors and the net wings. This gear has also been deployed in other regions of the Mediterranean Sea, such as the Levantine basin (see e.g. Ilan *et al.* 2003, Galil 2004).

Starting from the 1990s, a completely new gear was developed at the ICM-CSIC in order to improve catches of the Mediterranean red shrimp *Aristeus antennatus*, for which a series of studies on population distribution were being conducted (e.g. Sardà *et al.* 1997). The new equipment was called Otter-Trawl *Maireta* System (OTMS), in honour to the name of the fishing vessel where the first trials were conducted, and it was registered as a Spanish national utility model (n. 9200614, ICM-CSIC). The OTMS yielded a 12.4 m spread by 1.4 m height of front opening, with bridles adapted to work at high depths (length of 25 to 70 m), and a roller line on the footrope to significantly reduce mud catching (Sardà *et al.* 1998). The OTMS can thus be towed at higher speeds than the OTSB: 2.6 to 2.8 knots, approximately. This also implies that the OTMS maintains less contact with the substratum, catching reduced quantities of the strictly benthic species while collecting higher numbers of nektobenthic species, with respect to the OTSB catches. Since its development, the OTMS went on to be applied in a large number of studies on megafaunal community composition and structure (Sardà *et al.* 1994, Ramírez-Llodra *et al.* 2008, Ramírez-Llodra *et al.* 2010b), biodiversity (Tecchio *et al.* 2011), life histories (Company & Sardà 1998, 2000, Fernandez-Arcaya *et al.* 2012), marine litter evaluation (Ramírez-Llodra *et al.* in press) and contamination (Koenig *et al.* in press) along the whole Mediterranean basin, and it has proven its reliability down to 4000 m depth. At shallower depths, the OTMS has also been validated as a commercially-usable gear, because it yielded statistically identical catches as the two-warp trawling nets operated by fishermen in the area (Sardà *et al.* 2002).

Another benthic sampler widely used to study the deep-sea megabenthos is the Agassiz dredge (Gage & Tyler 1991). It is a fixed-mouth dredge, with a 2.5 m by 1 m of front opening approximately, with slight variations used by different research teams. Because of its rigidity and overall resistance, it is preferred in case of unknown seafloors or when the ground presents rocky structures or outcrops (Danovaro 2010). The Agassiz dredge is one of the first samplers used in the deep Mediterranean Sea, especially in the Eastern basin where it has been extensively deployed (Klausewitz 1989, Galil & Zibrowius 1998, Galil 2004), and it has also been applied elsewhere in the oceans in recent years (Lavaleye *et al.* 2002). It is usually hauled at a speed of approximately 2.0 knots, thus slower than the OTSB and the OTMS procedures.

Study areas and cruises

The target areas for this study were the fine-mud benthos habitats at four sites of the Mediterranean Sea: the Catalan continental margin in the north-western basin, the southern Balearic margin in the western basin, the western Ionian margin in the central basin, and the Cretan margin in the eastern basin (Figure 2.2).

A 1 month trans-Mediterranean cruise (BIOFUN I) was conducted in June 2009 aboard the R/V *Sarmiento de Gamboa*. This cruise visited and sampled the southern Balearic, the western Ionian and the southern Cretan seas at 1200, 2000 and 3000 m depth, plus an additional abyssal sampling site at 4000 m depth in the central basin.

Five oceanographic cruises (named PROMETEO I, II, III, IV and V) were conducted in the western Mediterranean, on the northern Catalan continental margin outside the city of Blanes, to sample the Blanes canyon and the adjacent continental slope, during the biennium 2008-2009 on board the R/V *García del Cid*. The sampling plan was laid out along five stations located at intervals of 150 m depth from 900 m to 1500 m on the open slope. Two additional sampling stations were positioned in the Blanes canyon, one in the canyon head at 900 m depth and the other, deeper in the canyon wall at 1500 m depth. During the fifth cruise, two samples of opportunity were also collected at 1750 m depth in the open slope area.

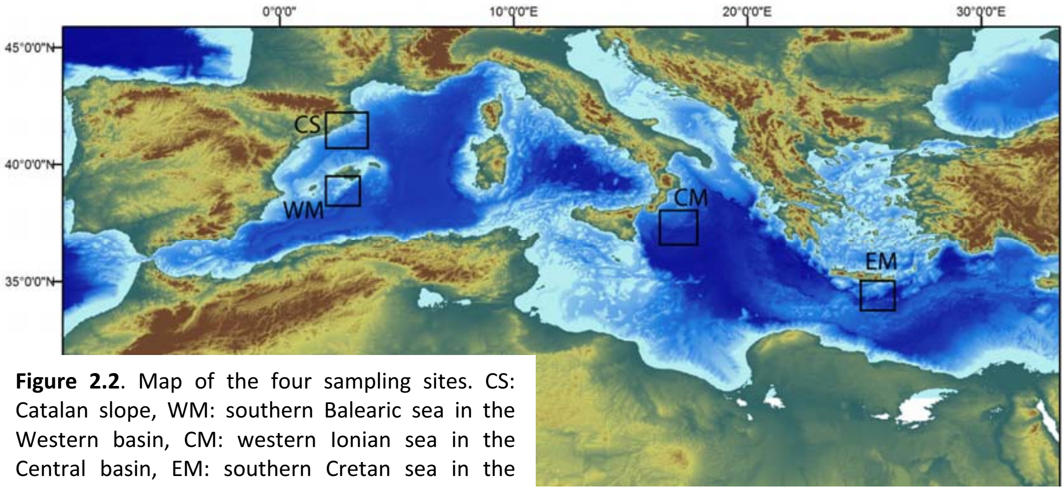


Figure 2.2. Map of the four sampling sites. CS: Catalan slope, WM: southern Balearic sea in the Western basin, CM: western Ionian sea in the Central basin, EM: southern Cretan sea in the Eastern basin.

During these cruises, multidisciplinary samplings were carried out using a series of different sampling gears, with the objective of characterizing the benthic habitat and the overlying water column, both biotic and abiotic domains. Table 2.1, at the end of this chapter, contains the full list of samples considered in the present study.

Sampling for benthic and nektobenthic megafauna was conducted by means of the OTMS and the Agassiz dredge, described in the previous section. Trawling lanes were positioned as parallel to isobaths as possible to maintain a constant depth during the haul. Scanmar sensors were used down to 1200 m depth to estimate net geometry and times of arrival and departure from the bottom. The net horizontal opening was estimated at 12.7 ± 1.3 m, and the vertical opening at 1.4 m with substantially less variability. For trawls deeper than 1200 m, the same values for the opening geometry were assumed, whereas the bottom times were calculated as the period between the end of cable paying out and the start of cable retrieval. Trawls were conducted at 2.6 to 2.8 knots. The Agassiz dredge had a 2.5 m horizontal opening and 1.2 m vertical opening, a net mesh size of 12 mm, and was trawled at 2.0 knots. Haul bottom times were estimated using the same method as for the OTMS. The total trawled area for each haul was then estimated using the following equation:

$$Area = \frac{S \cdot BT \cdot H \cdot 1852}{10^6},$$

where S is the mean speed (in knots), BT is the bottom time (in decimal hours), and H is the horizontal opening of the net (in meters). The resulting area is expressed in km^2, and it is then used to standardize catch data.

Complementarity of the two trawl samplers

In marine biodiversity studies, the objective is to retrieve the widest part of the compartment that is the target for that particular study (in this case, megafauna). This is especially true when working in the deep-sea, because the majority of its biodiversity is still undescribed (Danovaro *et al.* 2010, Ramírez-Llodra *et al.* 2010a) and is mostly present in the form of singletons (i.e. uncommon species with a few or only one individual per sample). Sampling with inappropriate gear would result in significant loss of these uncommon species – the tail of the species/abundance distribution – and would lead to a skewed perception of the communities.

Another pivotal topic is the definition of the domain that is being observed. Throughout the world oceans, and also in the Mediterranean Sea, we observe an increase of zooplankton biomass and diversity while approaching the seafloor, contrasting with its general scarcity along the water column (Wishner 1980, Vereshchaka 1995). This has been identified as the *benthic-boundary layer* and is now considered to be a specific compartment of the marine ecosystems (McCave 1976, Angel & Boxshall 1990, Dauvin & Vallet 2006). Since the deep Mediterranean benthic communities are dominated by swimming fish and crustacean species, it is imperative to be able to catch those species along with the strictly benthic species of the substrate. Nektobenthic species, which possess high movement capability, participate significantly in the dynamics of the benthic boundary layer from various point of view such as trophic interactions, rhythmic migrations and dispersion processes (Mees & Jones 1997). In the case of this work, the objective was to describe the community in the continental slope and in the deep basins, which as previously said it is mainly constituted by species that move along the benthic-boundary layer. Neither of these two samplers, considered alone, would have been able to screen that full compartment.

In this study, every individual was identified to species level whenever possible, then counted and grouped by species. The Scanmar data were used to standardize species abundances into species density (N km^{-2}). The species × samples matrix was transformed into a similarity matrix using Bray-Curtis measure, and a non-metric multidimensional scaling (nMDS) was performed on the whole dataset. This analysis is an iterative procedure that minimizes the difference between ranked Bray-Curtis similarity values and 2-dimensional distance between each pair of samples. The MDS analysis was reiterated 1000 times with a minimum stress value of 0.01.

In the following figure, the same nMDS configuration was plotted four times using abundance data from the Catalan continental slope (i.e. all cruises except BIOFUN 1), separated by taxonomic groups, to compare the general catch patterns between the OTMS and the Agassiz dredge (Figure 2.3).

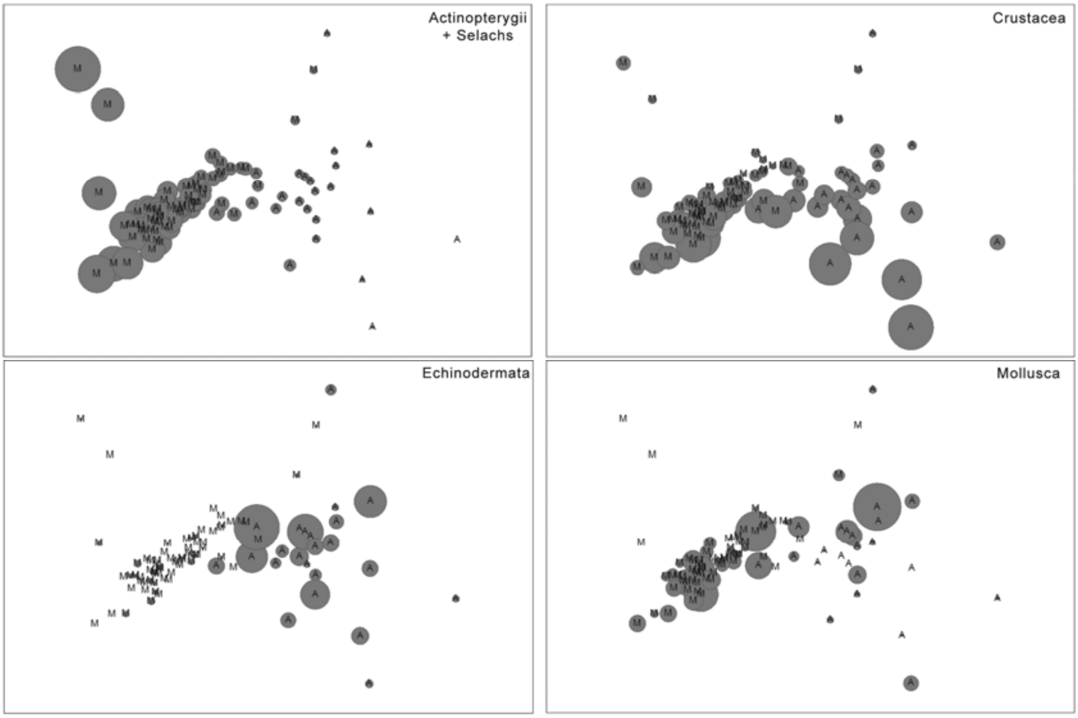


Figure 2.3. Non-metric multidimensional scaling plots of all samples collected in the Catalan margin and considered in the present thesis. Each letter and surrounding circle represent one sample of the otter-trawl Maireta system (M) or the Agassiz dredge (A). Area of circles is proportional to the abundance of the 4 most relevant taxonomic groups collected within that sample.

Almost no Echinodermata species were caught by the OTMS trawl, which was expected considering the behaviour of the net: the OTMS would simply have passed above the individuals, possibly damaging them in the process, but without the capacity to lift them from the seafloor and collect them. This might also have happened in the case of sessile molluscs (e.g. *Abra longicallus*, *Antalis* sp., *Aporrhais serresianus*). The invertebrates caught by the OTMS were mainly swimming molluscs that perform slow migrations along the bottom – i.e. cephalopods such as *Opistoteuthis calypso*, *Bathypolipus sponsalis*, and *Todarodes sagittatus* – or natantian decapod crustaceans. For similar reasons, the Agassiz dredge could not collect efficiently neither Actinopterygii nor Selachians, most probably because of their high escape capabilities. No species of sponges (Porifera) were retrieved by the OTMS. This is a general known issue of the use of trawling for deep-sea megafauna: sponges are either crumbled by the roller line or their size is too small to be caught in the cod-end mesh. This was solved for the OTSB by adding a fine-meshed zooplankton net atop of the trawl, and permitted the collection of small-sized Porifera in the eastern Mediterranean basin, most of which were new to science (Ilan *et al.* 2003). By using the Agassiz dredge, this problem can still be solved because this sampler can collect sponge species, as data from the present work will show.

Deep-sea explorations are difficult, expensive, and often result in a large effort to retrieve a relatively reduced number of valid samples. It is therefore imperative to maximize efficiency and retrieve samples that can be analysed with the highest number of possible points of view. Here, it has been shown that the combined use of the OTMS and the Agassiz dredge – if standardized procedures are followed – is complementary in the study of deep-sea biodiversity and provides an integrated image of the megafaunal compartment. The results can be explored within various contexts, including biodiversity, population and community structure, species zonation, and temporally changing processes.

Biodiversity measures

In Chapters 3 and 4, various measures of diversity will be applied, and is thus necessary to introduce them briefly. Traditionally, ecologists have identified three types of diversity, depending on the scale of observation and the ecological context of the study (Magurran 2004). The most straightforward of the diversity concepts is α diversity, or point-diversity, and it is defined as the species diversity of a sample. The α diversity of a particular site may be then calculated as the mean value of all samples collected within that site. The total diversity of species in a region is called γ diversity, or regional diversity, and it is usually calculated as the total number of different species found within a site, when repeated samplings are conducted and no further species are encountered. The degree of variation of α diversity between sites of a broader regional species pool (γ diversity) is called β diversity, and it is usually defined as $\beta_W = \gamma / \bar{\alpha}$ (Whittaker 1972). However, the concept behind it is more complicated, and β diversity can be defined either as *turnover* diversity or as *variation* diversity. The turnover diversity defines the rate of change in species composition along a gradient, while the variation diversity expresses how much a sampling unit is contained in the regional species pool (Anderson *et al.* 2011). In the case of a gradient analysis, multivariate dissimilarity measures can be applied as good estimators of β diversity (Mumby 2001) and this is indeed what was applied in Chapter 3.

All α diversity indices applied in this study are nonparametric, *i.e.* they do not assume any particular species abundance model. They all begin their calculation by considering the N total number of individuals in each sample, distributed in S species.

The first calculated index is the Shannon information statistic, more commonly known as the Shannon-Wiener index. It integrates the information of species richness and of their distribution, resulting in a general purpose measure of diversity, which is commonly used nowadays (Shannon 1948, Magurran 2004). It begins by calculating the proportions of individuals found for each species, calculated as $p_i = {}^{n_i}/_{N}$. The value of the index is then obtained from the information theory, as:

$$H' = -\sum_{i=1}^{S} p_i \log p_i$$

The second considered index is a measure of evenness in species abundances, and it has been first described by Pielou (1966). It describes the ratio between the observed Shannon-Wiener diversity (H') and the maximum possible diversity for the considered sample:

$$J' = \frac{H'}{\log S}$$

The values from this index can range between 0 and 1, with higher values assumed for samples where the species are more evenly distributed (i.e. at J = 1 all species are equally represented). Note that in both Shannon-Wiener and Pielou indexes, the logarithms can be calculated with any base, but it is common and widespread practice to use natural logs. This also allows for more straightforward comparisons between different datasets and studies.

Of increasing use among deep-sea ecologists is the expected number of species (ES) and, more broadly, the rarefaction analysis. The index ES(n) is defined as the expected number of species in a reduced sample of n individuals (Hurlbert 1971). For example, ES(50) index expresses the number of species that would be found in a sample, if it was composed of only 50 random individuals. In the present work, and because of the low megafaunal densities that are found in the deep Mediterranean Sea, the ES(25) index was calculated. The interpretation of this index is similar to that of the Shannon-Wiener index, since they are both richness- and distribution-integrative. The use of ES index has also the advantage to be useful in the case of non-quantitative samplers.

Rarefaction curves are constructed to evaluate the degree of sampling completeness of a site (Magurran 2004). Briefly, the number of new species found in each sample is plotted cumulatively against the sample numeric count, and points are then connected forming a curve. Ideally, if a community has been sampled completely, observing all the species that are present, the slope of the curve would end up being horizontal (i.e. no new species are to be found by taking subsequent replicas). In addition, it is possible to directly compare the overall local diversity of two sites, by plotting their respective rarefaction curves in the same plot. The sample which curve stands higher on the ordinate with respect to the other would present higher species diversity. This technique has been directly applied in Chapter 4.

In the following chapters, additional details on the materials and methods will be given, relative to the specific analyses and calculations conducted therein.

Future directions in deep-sea exploration

The advances in our knowledge of the deep sea travelled in parallel to the improvements in the techniques employed to observe these habitats, so difficult to reach. The timeline of deep-sea samplers went from frame dredges (Agassiz, Macer-Giroq, etc.), to baited traps, to flexible-mouth trawl nets (bottom otter-trawls). At each step, a plethora of new observations were made, and in addition, scientists could retrieve large quantities of deep-sea organisms to study their taxonomy, reproduction, diet, and other biological characters. However, deep trawling is still expensive, extremely time-consuming, and prone to a series of gear failures. Video cameras operated from vessels, Remotely Operated Vehicles (ROVs), research submersibles or cabled observatories offer unique opportunities of observing the deep-water environment, but their capacity of retrieving physical samples is still limited. In my opinion, flexible-mouth benthic trawling, if carried out with standardised and accurate procedures, is still an efficient method of observing community composition, diversity, and other ecological parameters, on spatial and temporal transects, and both at small and large scales.

After describing the biodiversity techniques, a more direct question may arise: how then we interpret a possible difference between two sites of increasing biodiversity? Over the last decades, it has been pointed out that an ecosystem richer in species may possess a wider margin of adaptability in response to external stimuli or disturbances. In a wider species pool, there is a higher probability that one or more of them would be able to perform the function required to maintain stability. The levels of ecosystem functioning, which can also be related to the quality of ecosystem services provided, are known to be positively correlated with biodiversity, and this is especially true for benthic deep-sea habitats (Danovaro *et al.* 2008a).

This is why ecologists are particularly concerned with low-diversity ecosystems, such as the Arctic marine food web, which hosts a low number of highly abundant species. It is unlikely that the current ecosystem services provided by that system will be able to cope with the increasing human impact exerted including, but not limited to, fishing activities, human-induced warming, and drilling for hydrocarbons (Elmqvist *et al.* 2003). The same philosophy will be applied in the discussion of results of the present work.

Concluding, I wonder what would have thought Prof Forbes if he looked at the sampling gear we use nowadays. He is known also for his *Song of the Dredge*, frequently sung to his friends and colleagues:

> *Hurrah for the dredge, with its iron edge*
> *And its mystical triangle,*
> *And its hided net with meshes set*
> *Odd fishes to entangle!*

Table 2.1. Comprehensive list of samples collected and studied in the present thesis, indicating the sampling gear (A: Agassiz dredge, M: otter-trawl Maireta system) and the geomorphological site (OS: open continental slope, BC: submarine canyon). Coordinates are expressed in decimal degrees.

					Latitude		Longitude	
Sample code	**Gear**	**Date**	**Geomorphology**	**Depth (m)**	**Start**	**End**	**Start**	**End**
BF1A01	A	30/05/2009	OS	1200	38,4195	38,4080	1,7727	1,7887
BF1A02	A	30/05/2009	OS	1200	38,4175	38,3937	1,7743	1,8080
BF1A03	A	30/05/2009	OS	1200	38,3912	38,4142	1,8053	1,7770
BF1A04	A	01/06/2009	OS	2000	38,0410	38,0567	1,9082	1,9550
BF1A05	A	03/06/2009	OS	3000	38,6855	38,6865	5,5488	5,5945
BF1A06	A	09/06/2009	OS	2000	37,6842	37,6731	16,5895	16,5725
BF1A07	A	10/06/2009	OS	3300	36,1502	36,1398	16,4773	16,4803
BF1A08	A	17/06/2009	OS	1200	34,5010	34,5397	25,7743	25,7832
BF1A09	A	17/06/2009	OS	2000	34,6072	34,5678	25,8717	25,8683
BF1A10	A	19/06/2009	OS	3000	34,6483	34,6212	26,0922	26,0742
BF1A11	A	19/06/2009	OS	3000	34,6315	34,6557	26,6315	26,0995
BF1A12	A	20/06/2009	OS	1200	34,5785	34,5480	25,7742	25,7843
BF1A13	A	20/06/2009	OS	1200	34,5283	34,5537	26,7800	25,7812
BF1A14	A	22/06/2009	OS	4000	35,9897	35,7140	18,5150	18,5237
BF1A15	A	24/06/2009	OS	2000	38,1837	38,1587	17,5143	17,4880
BF1A16	A	27/06/2009	OS	2850	39,5238	39,4665	5,8775	5,8047
BF1M01	M	30/05/2009	OS	1200	38,3975	38,3543	1,8042	1,8600
BF1M02	M	30/05/2009	OS	1200	38,3867	38,4340	1,8183	1,7498
BF1M03	M	01/06/2009	OS	2000	38,0380	38,0758	1,9027	2,0087
BF1M04	M	01/06/2009	OS	2000	38,0967	38,0482	2,0532	1,9357
BF1M05	M	03/06/2009	OS	2800	38,6888	38,6802	5,6883	5,5718
BF1M06	M	04/06/2009	OS	2800	38,6883	38,7050	5,6998	5,7953
BF1M07	M	04/06/2009	OS	2800	38,6855	38,7050	5,6618	5,7953
BF1M08	M	07/06/2009	OS	1200	38,2453	38,2477	16,6663	16,6710
BF1M09	M	07/06/2009	OS	1200	38,2475	38,2417	16,6790	16,6130
BF1M10	M	08/06/2009	OS	1200	38,2470	38,2502	16,6625	16,7185
BF1M11	M	09/06/2009	OS	2000	37,6848	37,7217	16,6100	16,6713
BF1M12	M	10/06/2009	OS	3300	36,1367	36,1617	16,4683	16,4208
BF1M13	M	15/06/2009	OS	1200	34,5633	34,5265	25,7733	25,7782
BF1M14	M	16/06/2009	OS	1200	34,5438	34,5908	25,7783	25,7758
BF1M15	M	16/06/2009	OS	1200	34,5387	34,5008	25,7800	25,7753
BF1M16	M	17/06/2009	OS	2000	34,6128	34,6503	25,8723	25,8837
BF1M17	M	17/06/2009	OS	2000	34,5715	34,5345	25,8723	25,8733
BF1M18	M	18/06/2009	OS	2700	34,0527	34,0640	25,2890	25,3242
BF1M19	M	23/06/2009	OS	2800	37,3235	37,3300	17,4927	17,5283
BF1M20	M	23/06/2009	OS	2800	37,3253	37,3080	17,4578	17,3728
BF1M21	M	24/06/2009	OS	2200	38,0713	38,0378	17,5963	17,5840
PR1A01	A	03/11/2008	OS	900	41,2562	41,2583	2,7902	2,8087
PR1M01	M	30/10/2008	OS	900	41,2500	41,2667	2,7833	2,8333
PR1M02	M	30/10/2008	OS	1500	41,1167	41,1222	2,8500	2,9063
PR1M03	M	01/11/2008	OS	1200	41,2457	41,2717	2,8337	2,8388
PR1M04	M	01/11/2008	OS	1500	41,1242	41,1258	2,8682	2,9200

PR1M05	M	02/11/2008	OS	1200	41,2287	41,2645	2,8367	2,8423
PR2A01	A	04/03/2009	OS	900	41,2643	41,2692	2,8123	2,8303
PR2A02	A	04/03/2009	OS	1075	41,2503	41,2367	2,8193	2,8075
PR2A03	A	04/03/2009	OS	1350	41,1725	41,1627	2,8102	2,7658
PR2A04	A	08/03/2009	OS	1500	41,1283	41,1220	2,8628	2,9082
PR2A05	A	08/03/2009	OS	1200	41,2342	41,2598	2,8345	2,8390
PR2A06	A	08/03/2009	OS	900	41,2630	41,2575	2,8047	2,7732
PR2A07	A	09/03/2009	OS	1500	41,1268	41,1215	2,8700	2,9070
PR2A08	A	09/03/2009	OS	1200	41,2377	41,2675	2,8343	2,8395
PR2M01	M	28/02/2009	OS	900	41,2588	41,2575	2,7900	2,7732
PR2M02	M	28/02/2009	OS	1050	41,2477	41,2177	2,8225	2,7855
PR2M03	M	01/03/2009	OS	1200	41,2342	41,2673	2,8345	2,8420
PR2M04	M	01/03/2009	OS	1350	41,1667	41,1843	2,7915	2,8513
PR2M05	M	01/03/2009	OS	1500	41,1233	41,1225	2,8865	2,9213
PR2M06	M	02/03/2009	OS	1350	41,1678	41,1838	2,7845	2,8460
PR2M07	M	02/03/2009	OS	1500	41,1248	41,1202	2,8760	2,9230
PR2M08	M	02/03/2009	OS	900	41,2603	41,2737	2,7970	2,8408
PR2M09	M	02/03/2009	OS	1050	41,2535	41,2343	2,8247	2,7980
PR2M10	M	02/03/2009	OS	1500	41,1188	41,1175	2,9277	2,9370
PR2M11	M	03/03/2009	OS	1350	41,1685	41,1833	2,7975	2,8468
PR2M12	M	03/03/2009	OS	1200	41,2300	41,2667	2,8327	2,8402
PR2M13	M	03/03/2009	OS	1200	41,2343	41,2597	2,8470	2,8405
PR2M14	M	03/03/2009	OS	1200	41,2343	41,2082	2,8337	2,8305
PR2M15	M	03/03/2009	BC	1500	41,4348	41,4518	2,8840	2,8778
PR2M16	M	04/03/2009	OS	900	41,2582	41,2730	2,7870	2,8395
PR3A09	A	26/05/2009	OS	900	41,2555	41,2685	2,7903	2,8242
PR3A10	A	27/05/2009	OS	1500	41,1260	41,1208	2,8388	2,9040
PR3M17	M	11/05/2009	OS	900	41,2593	41,2743	2,7892	2,8437
PR3M18	M	11/05/2009	OS	1050	41,2490	41,2322	2,8202	2,7978
PR3M19	M	12/05/2009	OS	1200	41,2335	41,2673	2,8328	2,8432
PR3M20	M	12/05/2009	OS	1200	41,2310	41,2757	2,8335	2,8453
PR3M21	M	12/05/2009	OS	1350	41,1798	41,1840	2,8298	2,8605
PR3M22	M	12/05/2009	OS	1500	41,1270	41,1203	2,8648	2,9245
PR3M23	M	12/05/2009	OS	1500	41,1247	41,1330	2,8830	2,8295
PR3M24	M	13/05/2009	OS	1200	41,2282	41,2690	2,8323	2,8420
PR3M25	M	13/05/2009	OS	1050	41,2492	41,2312	2,8208	2,7972
PR3M26	M	13/05/2009	OS	1050	41,2468	41,2722	2,8140	2,8448
PR3M27	M	13/05/2009	OS	1350	41,1678	41,1848	2,7973	2,8545
PR3M28	M	13/05/2009	OS	1500	41,1267	41,1193	2,8693	2,9257
PR3M29	M	13/05/2009	OS	900	41,2580	41,2668	2,7835	2,8255
PR3M30	M	14/05/2009	OS	1050	41,2497	41,2723	2,8160	2,8457
PR3M31	M	14/05/2009	OS	1200	41,2298	41,1962	2,8335	2,8292
PR3M32	M	14/05/2009	OS	1350	41,1678	41,1583	2,7973	2,7492
PR4A09	A	11/09/2009	BC	900	41,5933	41,5725	2,8500	2,8497
PR4A10	A	11/09/2009	BC	1500	41,4518	41,4318	2,8778	2,8898
PR4A11	A	12/09/2009	OS	1500	41,2277	41,2277	2,8398	2,8398
PR4A12	A	12/09/2009	OS	1350	41,1717	41,2753	2,8118	3,1050
PR4A13	A	12/09/2009	OS	1200	41,2383	41,2753	2,8343	3,1050
PR4A14	A	12/09/2009	OS	1050	41,2475	41,2360	2,8170	2,8005

PR4A15	A	12/09/2009	OS	900	41,2598	41,2642	2,7957	2,8262
PR4M33	M	07/09/2009	OS	900	41,2640	41,2767	2,8110	2,8487
PR4M34	M	07/09/2009	OS	1050	41,2537	41,2722	2,8203	2,8432
PR4M35	M	08/09/2009	OS	1050	41,2562	41,2722	2,8278	2,8432
PR4M36	M	08/09/2009	OS	1200	41,2348	41,2113	2,8327	2,8260
PR4M37	M	08/09/2009	OS	1350	41,1717	41,1865	2,8040	2,8397
PR4M38	M	08/09/2009	OS	1500	41,1215	41,1207	2,8883	2,9233
PR4M39	M	08/09/2009	OS	1500	41,1215	41,1207	2,8883	2,9233
PR4M40	M	08/09/2009	OS	1500	41,1265	41,1312	2,8680	2,8398
PR4M41	M	08/09/2009	OS	1350	41,1727	41,1787	2,8127	2,8490
PR4M42	M	08/09/2009	OS	1500	41,1215	41,1207	2,8847	2,9277
PR4M43	M	09/09/2009	OS	1200	41,2448	41,2712	2,8355	2,8402
PR4M44	M	09/09/2009	OS	1350	41,2587	41,1910	3,1603	2,8363
PR4M45	M	09/09/2009	OS	1200	41,2507	41,2713	2,8340	2,8440
PR4M46	M	09/09/2009	OS	1050	41,2448	41,2260	2,8150	2,7957
PR4M47	M	09/09/2009	OS	1050	41,2577	41,2760	2,8287	2,8462
PR4M48	M	09/09/2009	OS	900	41,2640	41,2575	2,8087	2,7733
PR4M49	M	09/09/2009	OS	1050	41,2563	41,2727	2,8293	2,8483
PR4M50	M	10/09/2009	OS	900	41,0000	41,2705	2,8132	2,8438
PR4M51	M	10/09/2009	OS	900	41,2632	41,2708	2,8153	2,8460
PR4M52	M	10/09/2009	BC	1500	41,4405	41,4222	2,8855	2,8898
PR4M53	M	10/09/2009	BC	1500	41,4408	41,4218	2,8833	2,8897
PR4M54	M	10/09/2009	BC	900	41,5813	41,5648	2,8500	2,8508
PR4M55	M	10/09/2009	BC	1500	41,4430	41,4215	2,8832	2,8895
PR5A16	A	29/10/2009	BC	900	41,5938	41,5727	2,8503	2,8507
PR5A17	A	29/10/2009	BC	1500	41,4512	41,4333	2,8797	2,8898
PR5A18	A	29/10/2009	OS	900	41,2600	41,2628	2,7957	2,8100
PR5A19	A	29/10/2009	OS	1050	41,2457	41,2445	2,8153	2,8150
PR5A20	A	30/10/2009	OS	1200	41,2542	41,2607	2,8343	2,8418
PR5A21	A	30/10/2009	OS	1350	41,1715	41,1638	2,8107	2,7735
PR5A22	A	30/10/2009	OS	1500	41,1222	41,1207	2,8803	2,9087
PR5A23	A	30/10/2009	OS	1750	41,1377	41,1417	2,9838	3,0090
PR5A24	A	30/10/2009	OS	2000	41,0532	41,0420	3,0702	3,0388
PR5A25	A	31/10/2009	OS	1050	41,2453	41,2342	2,8157	2,7992
PR5M56	M	24/10/2009	OS	1500	41,1217	41,1203	2,8865	2,9282
PR5M57	M	24/10/2009	OS	1350	41,1722	41,1783	2,8090	2,9800
PR5M58	M	24/10/2009	OS	1200	41,2482	41,2757	2,8367	2,8445
PR5M59	M	24/10/2009	OS	1050	41,2492	41,2330	2,8187	2,7995
PR5M60	M	25/10/2009	OS	900	41,2615	41,2705	2,8087	2,8437
PR5M61	M	25/10/2009	OS	900	41,2632	41,2572	2,8080	2,7745
PR5M62	M	25/10/2009	OS	900	41,2617	41,2702	2,8077	2,8435
PR5M63	M	25/10/2009	OS	1050	41,2483	41,2337	2,8182	2,7988
PR5M64	M	25/10/2009	OS	1200	41,2480	41,2752	2,8370	2,8457
PR5M65	M	25/10/2009	OS	1050	41,2570	41,2722	2,8305	2,8485
PR5M66	M	25/10/2009	OS	1350	41,1700	41,1767	2,8097	2,8473
PR5M67	M	25/10/2009	OS	1500	41,1207	41,1187	2,8858	2,9272
PR5M68	M	26/10/2009	OS	1500	41,1258	41,1305	2,8653	2,8375
PR5M69	M	26/10/2009	OS	1350	41,1710	41,1775	2,8087	2,8470
PR5M70	M	26/10/2009	OS	2000	41,0597	40,5168	3,0902	4,8448

PR5M71	M	26/10/2009	OS	2000	41,0583	41,0788	3,0917	3,1500
PR5M72	M	27/10/2009	OS	2700	40,5997	40,6433	4,8532	4,8437
PR5M73	M	27/10/2009	OS	2700	40,5613	40,4897	4,8572	4,8658
PR5M74	M	27/10/2009	OS	2700	40,5325	40,5998	4,8612	4,8557
PR5M75	M	28/10/2009	OS	1750	41,1425	41,1492	3,0138	3,0442
PR5M76	M	28/10/2009	OS	1750	41,1335	41,1273	2,9600	2,9297
PR5M77	M	28/10/2009	BC	1500	41,4413	41,4223	2,8847	2,8905
PR5M78	M	29/10/2009	BC	1500	41,4437	41,4228	2,8837	2,8900
PR5M79	M	29/10/2009	BC	900	41,5840	41,5622	2,8497	2,8540

Drivers along large-scale gradients

3

INTRODUCTION

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

A series of factors have been proposed as potential drivers for the composition and diversity of deep benthic communities (Levin *et al.* 2001, Carney 2005). Most deep-ocean ecosystems, excluding reducing systems such as hydrothermal vents or cold seeps, are heterotrophic, depending on allochthonous organic matter inputs from the surface strata (Gage & Tyler 1991). This input of food to the deep seafloor is, in turn, directly linked to the primary production of the sunlit layers (Billett *et al.* 1983, Corliss *et al.* 2009), river runoff, atmospheric events (Company *et al.* 2008), climate (Smith *et al.* 2009) and, to an unknown extent, human activities (Smith *et al.* 2008). Degradation processes in the water column, which affect organic matter availability on the bottom, have also been proposed to be influential (Soltwedel *et al.* 2009). In this context, it is essential to address the potential relationship between biodiversity and environmental factors to highlight the strengths and weaknesses of these ecosystems, ultimately leading to a better understanding of their functioning and of the possibilities for their management.

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

Variations in physical conditions are known to strongly influence the response of large size classes of benthos such as macro- and megafauna. Conversely, small size classes are more dependent on biotic inter-relationships (Gage & Tyler 1991), although in recent years an effect of the environment has been observed on these biota fractions as well (Danovaro *et al.* 2008b). These physical conditions are known to vary along the west-east axis in the Mediterranean, forming a decreasing gradient of organic matter availability at the seafloor (Azov 1991, Danovaro *et al.* 1999). In addition, since the start of deep-sea exploration in the Mediterranean, it has been known that although the western and central basins host varied and abundant benthic megafauna communities, in the eastern basin (Levantine) all size classes of deep benthos are highly scarce in the whole continental margin (Galil & Goren 1992, Galil & Zibrowius 1998, Basso *et al.* 2004, Lampadariou *et al.* 2009). It could thus be hypothesised that communities at deep-basin depths in the western Mediterranean are structured similarly to communities in the mid-slope of the eastern basin. However, the relatedness of these 2 gradients (environmental and biotic) is unclear and leaves open a number of questions regarding the deep-water megafauna in the Mediterranean.

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

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

MATERIALS AND METHODS

Study areas

The target ecosystem was the fine-mud benthos of the Mediterranean Sea on the continental slope and in the deep basins, between 1200 and 4000 m in depth. Environmental and biological data were collected during two multidisciplinary oceanographic cruises in 2009, in the Southern Balearic Sea in the Western Mediterranean (WM), the Western Ionian Sea in the Central Mediterranean (CM) and the Southern Cretan Sea in the eastern Mediterranean (EM). The sampling plan included three main depths in each zone: 1200, 2000 and 2700-3000 m. In addition, stations at 3300 and 4000 m depth in the Western Ionian were included in the sampling plan. Throughout the text, the acronyms CS, WM, CM and EM will be used to identify the study areas.

Environment variables

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

At each station, plankton was collected using 2 nets: a standard WP2 net with a 200 μm mesh for mesozooplankton and a scaled-down version for microplankton with a 53 μm mesh size. Hauls were vertical from 200 m depth to the surface, at a speed of 1 m s^{-1}. The filtered water volume was estimated using General Oceanics 2030R flowmeters. After sieving through 200 and 2000 μm sieves for micro- and mesozooplankton, respectively, the samples were filtered on board using pre-dried and pre-weighted 25 and 47 mm diameter GF/C glass microfiber filters for microplankton and mesozooplankton, respectively. The filters were then stored at -20 °C. In the laboratory, the filters were freeze-dried for 48 h and weighed (with resolution of 0.0001 g) to retrieve plankton biomass, which was then standardised for filtered water volume.

Sediment samples were collected using a multiple corer at the same stations. Surface sediment samples (the top 1 cm) were used to investigate grain size and elemental composition. A non-homogenised fraction of approximately 1 g of sediment sample was treated with 10% H_2O_2 to oxidise organic matter. A Coulter Counter LS230 laser particle size analyser was used to determine sediment grain size. The results are expressed in percentages of the coarse fraction (>63 μm). Another fraction of sediment was homogenised with an agate mortar for organic carbon analysis. The samples were first decarbonated using repeated additions of 25% HCl with 60 °C drying steps in between until no effervescence was observed, and particulate organic carbon (POC) was measured on a Carlo Erba Flash 1112 elemental analyser at the Scientific Technical Services of the University of Barcelona.

Megafauna sampling and data analysis

A combination of 2 samplers was used to collect the widest range of benthic megafauna: an otter-trawl Maireta system (OTMS) and an Agassiz dredge. The details on the sampling plan and procedures are described in Chapter 2. Every individual collected with the OTMS or the Agassiz dredge was identified to species level or to the lowest taxonomical level possible. A series of taxonomic literature was applied for the identification of fishes (Mercader *et al.* 2001, Compagno *et al.* 2005), crustaceans (Zariquiey 1968) and other invertebrates (Tortonese 1965, Villanueva 1992, Saiz-Salinas 1993). Species names were then cross checked with the World Register of Marine Species, available online (WoRMS, www.marinespecies.org). Individuals were then counted and weighted.

Diversity indices from abundance data were calculated separately for the OTMS and Agassiz samples. For α-diversity, the expected number of species (ES(25)), and Pielou's evenness index (J') were calculated (Hurlbert 1971, Magurran 2004). Total abundance and biomass of each species were standardised for trawled area (in km^2) using swept area data from the Scanmar system. The species × samples matrix was also presence/absence transformed to permit treating the OTMS and Agassiz samples as a whole. A group average CLUSTER analysis using the Sørensen similarity index as resemblance measure was carried out, and a similarity profile test (SIMPROF) permutation routine (1000 restarts) was applied to test for the significance of genuine clustering. Differences in species composition between zones were tested with a single-factor similarity percentages routine (SIMPER) using Bray-Curtis similarity and were used as a β-diversity estimator (Gray 2000, Danovaro *et al.* 2008b). A faunal list was also compiled from the presence/absence matrix.

Environmental variables were normalised, after benthic turbidity was log-transformed to reduce an initial left-skewness. A 1-way ANOSIM test, using Euclidean distance as the measure and basin as the factor, was conducted on the environmental variables set. A BEST (BIOENV) routine was run to identify environmental variables correlated with differences between communities in terms of both species identities and abundances. This routine searches for the best subset of environmental variables, which optimises the match with the biotic patterns (Clarke & Gorley 2006). To avoid the introduction of errors from possible seasonal patterns, only quantitative OTMS data from the June 2009 trans-Mediterranean cruise (BIOFUN I) were considered as response variables. BEST was run with Spearman rank as the correlation method and Euclidean distance as the resemblance measure for environmental variables. A global match test was performed, with 1000 permutations, to test for the significance of selections.

RESULTS

A total of 17 OTMS trawls and 16 Agassiz hauls were conducted successfully and thus considered in the present chapter. Ninety-nine species belonging to 10 different phyla were collected, and are listed in Table 3.2. Of these, one was a new species of decapod crustacean belonging to family Galatheidae, *Munidopsis ariadne*, described by Macpherson (2011) and retrieved at 2000 m depth in the EM. Samples at 2000 m in the CM and at 2800 m in the EM were not retrievable with OTMS even after repeated deployments.

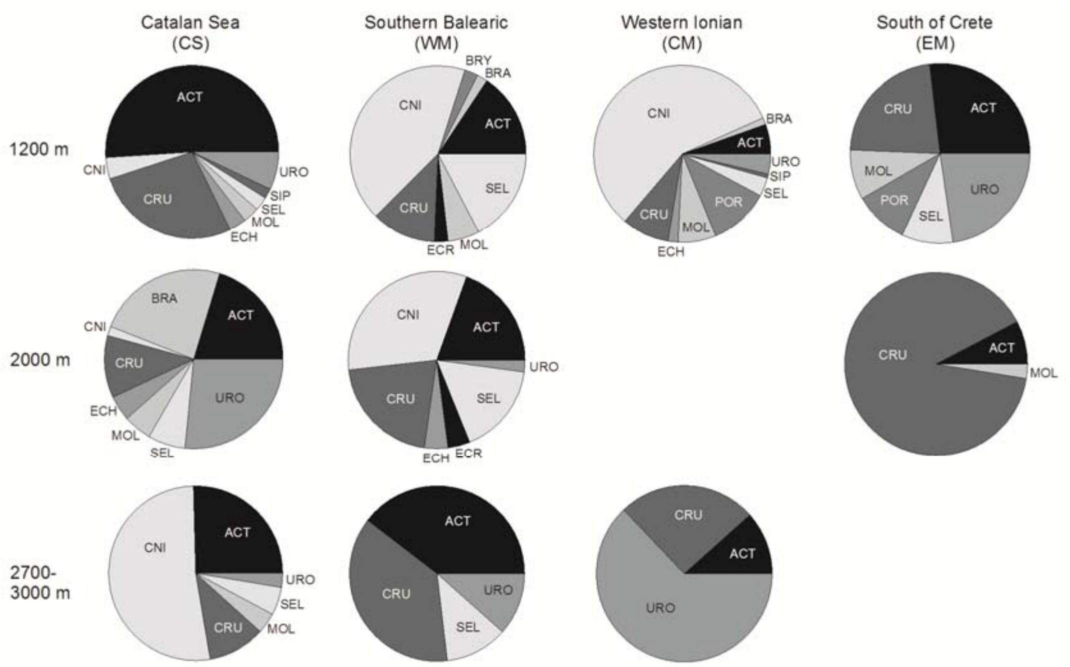


Figure 3.1. Percentage of abundance for taxonomic groups in each zone and at each depth (where samples were retrieved). ACT: actinopterygians; BRA: brachiopods; BRY: bryozoans; CNI: cnidarians; CRU: crustaceans; ECH: echinoderms; ECR: echiurans; MOL: molluscans; POR: poriferans; SEL: selachians; SIP: sipunculans; URO: urochordata

The overall taxonomic composition showed that the most speciose groups were bony fishes (Actinopterygii, 40 species), decapod crustaceans (27 species) and molluscs (19 species, mainly Cephalopoda and Gastropoda). Bony fishes and decapod crustaceans were also the only ubiquitous groups in all areas (Fig. 3.1). The areas with the least number of groups (3) were the 2800 m station in the CM and the 2000 m station in the EM, followed by 4 groups at 2800 m depth in the WM. The maximum number of groups (10) was found in the CM at 1200 m depth. The other areas hosted between 6 and 8 taxonomic groups.

The total biomass (Fig. 3.2) measured at 1200 m depth presented significant differences between areas (global Kruskal-Wallis test, $p = 0.008$). The CS biomass was the highest (mean ± SE, 1258.8 ± 92.2 kg km^{-2}), followed by the WM (226.0 ± 20.2 kg km^{-2}) and the CM (235.6 ± 26.0 kg km^{-2}). The biomass of the catches in the EM was the lowest (15.7 ± 9.1 kg km^{-2}), significantly differing from the CS biomass (Mann-Whitney test, $p < 0.0001$). At 2000 m depth a slight, non-significant (global Kruskal-Wallis test, $p = 0.301$) eastward decrease in biomass was evident. Biomass levels at 2800 m were low in all studied areas, ranging from 41.1 ± 9.6 kg km^{-2} on the CS to 13.0 kg km^{-2} in the CM.

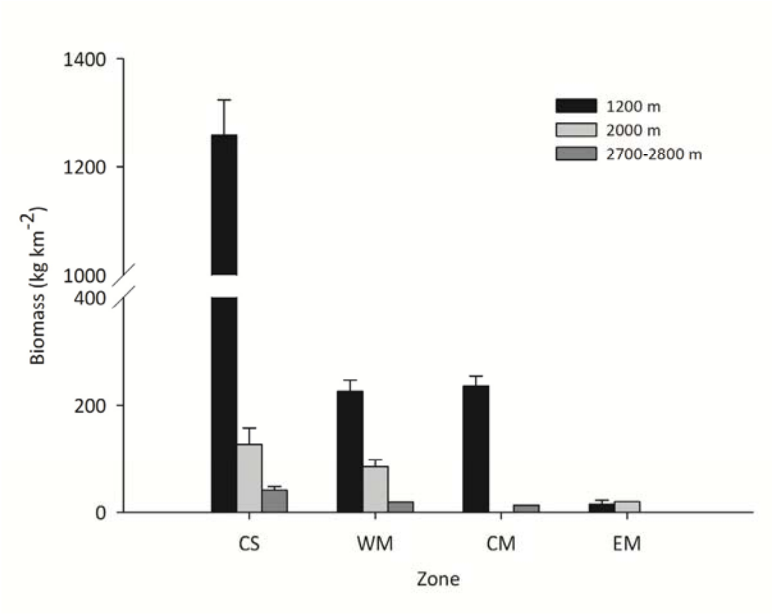


Figure 3.2. Total catch biomass at different depths in the 4 zones, considering standardised biomass data from otter-trawl Maireta system samples. Values are means (± SE). CS: Catalan Sea; WM: southern Balearic; CM: western Ionian; EM: south of Crete

Ordination by CLUSTER with SIMPROF analysis of both OTMS and Agassiz hauls showed a significant grouping by depth and zone (Fig. 3.3). All of the 1200 m samples grouped together, also showing a grouping by zone, whereas at depths of from 2000 to 4000 m the influence of geographic area was less marked. β-diversity estimation by the SIMPER routine showed a relatively low similarity within zones (ranging from 35.9 to 46.2%) and a high turnover diversity between zones (ranging between 61.3 and 74.5%). The zone with the lowest internal similarity was the CM (27.1%), which also included the widest depth range between samples (2800 m of depth gradient between the shallowest and deepest stations).

Diversity estimated by ES(25) on the OTMS samples showed a stable pattern with depth on the CS, ranging from 8.5 to 10.5 (Fig. 3.4). The same pattern was present in the WM, with values ranging between 10.3 and 11.7. The CM showed a similar diversity between the 1200 m and the deep 2800 m stations, although no data were available for the 2000 m depth. In the EM, diversity abruptly decreased between 1200 m and 2000 m. Pielou's index of evenness ranged between 0.36 in the EM at 2000 m depth and 0.95 at 1200 m also in the EM. In all of the other zones, Pielou's evenness was relatively constant across depths, and values ranged between 0.54 and 0.86.

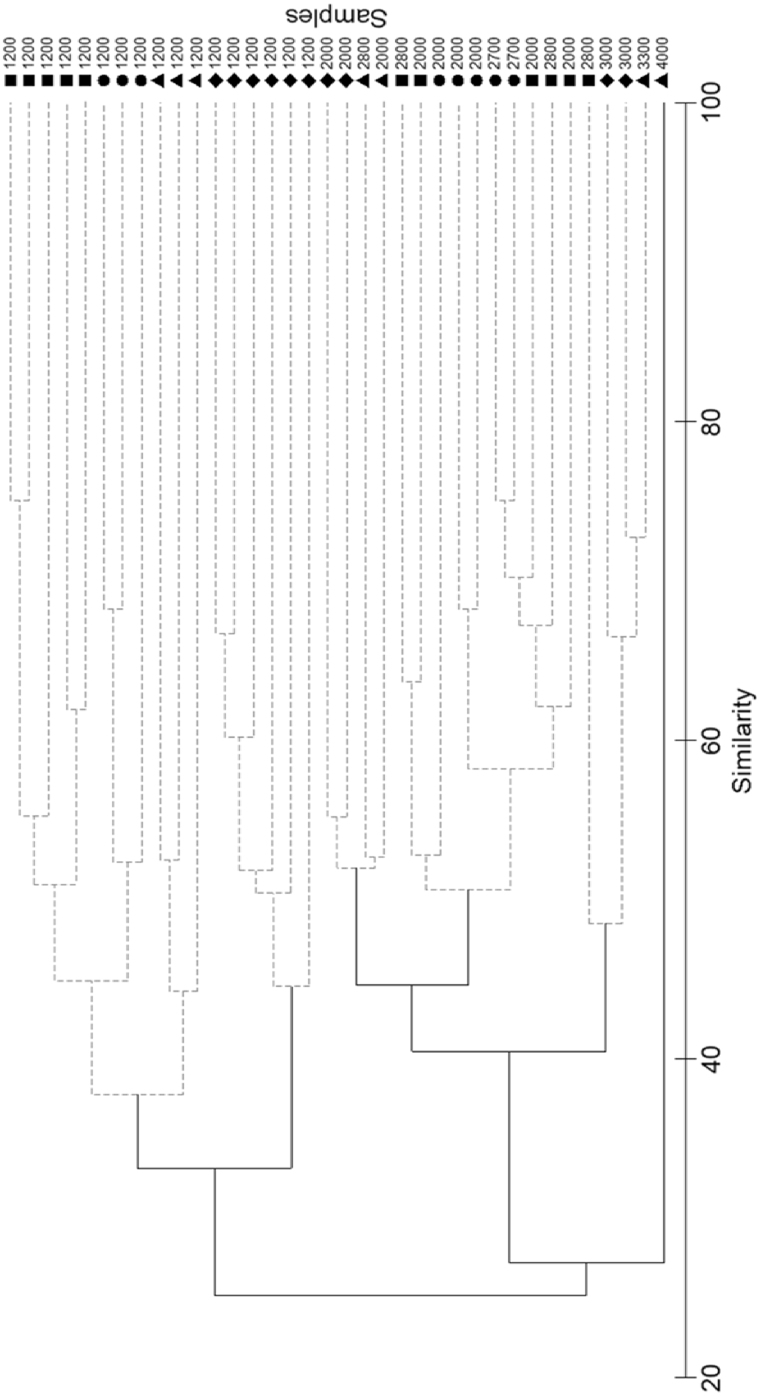


Figure 3.3. Group-average CLUSTER analysis on the abundance of megabenthos from the deep Mediterranean. Numbers alongside samples indicate depth of retrieval. Solid lines indicate statistically significant groupings (SIMPROF). ●: Catalan Sea; ■: southern Balearic; ▲: western Ionian; ◆: south of Crete

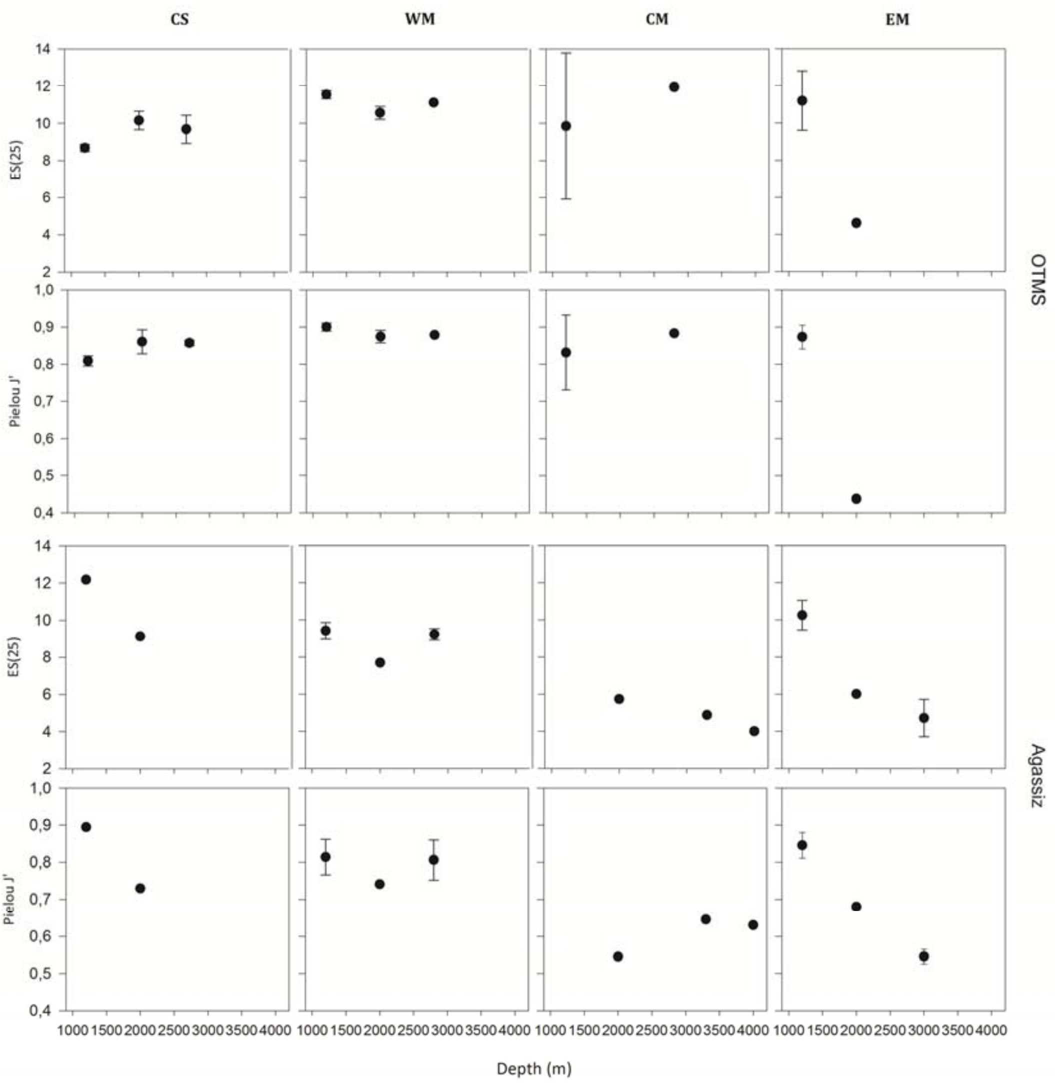


Figure 3.4. Expected number of species diversity index [ES(25)] and Pielou evenness index (J') for the otter-trawl Maireta system (OTMS) and the Agassiz dredge trawls at different depths in the 4 study areas. CS: Catalan Sea; WM: southern Balearic; CM: western Ionian; EM: south of Crete. Values are means (± SD)

Considering the Agassiz samples, ES(25) showed decreasing patterns in the 4 areas (Fig. 3.4). On the CS, diversity decreased from 12.2 at 1200 m depth to 9.1 at 2000 m, although no replicates were available. In the WM, ES(25) decreased between 1200 m and 2000 m, but it remained constant between 2000 m and 2800 m depth. In the CM, no samples were available at 1200 m depth, but diversity decreased slightly between 2000 m and 3300 m and then remained constant down to 4000 m depth. In the EM, diversity decreased steeply with depth.

Evenness followed comparable patterns, except in the CM, where the samples from 3300 m and 4000 m presented higher evenness than that from the 2000 m station. In all 4 study areas, the sample sizes were too small to perform statistical tests to verify the significance of differences.

Table 3.1 shows the environmental variables considered in the study. Significant environmental differences between basins were found by the ANOSIM test (global $R = 0.711$, $p = 0.001$). Pairwise comparisons of WM-CM and WM-EM were also significant ($p = 0.0008$ in both cases), whereas the CM-EM contrast was borderline ($p = 0.029$) but still significant. Results from the BEST routine identified a group of 3 variables that together best explained the community dissimilarity along the longitudinal axis of the Mediterranean: sediment grain size, sediment POC, and surface fluorescence. The results of the global match test ($\varrho = 0.555$, $p = 0.004$) corroborated the significance of that selection. Considering these three variables separately, the best explanatory variable was sediment POC ($\varrho = 0.413$), followed by surface fluorescence ($\varrho = 0.361$) and sediment grain size ($\varrho = 0.313$). In each basin, the coarse fraction of the grain size was always highest at the deepest site (2800-3000 m depth). Sediment carbon content has wider fluctuations between areas, with its minimum values in the eastern zone, ranging between 0.26 and 0.34%. Surface fluorescence in the WM was 0.82 ± 0.09 RFU; in the CM it was 0.65 ± 0.09 RFU, and in the EM it was 0.62 ± 0.07 RFU. The integrated biomass of microplankton between 0 and 200 m depth was 6.60 ± 2.40 mg m^{-3} in the WM, 4.32 ± 0.82 mg m^{-3} in the CM and 3.66 ± 1.27 mg m^{-3} in the EM. Mesozooplankton biomass in the same depth interval was 8.14 ± 3.87 mg m^{-3} in the WM, 10.16 ± 2.04 mg m^{-3} in the CM and 7.13 ± 2.55 mg m^{-3} in the EM.

Table 3.1. Environmental variables measured at different depths (m) and locations in 3 zones in the deep Mediterranean Sea. FTU: formazin turbidity units; POC: particulate organic carbon; RFU: relative fluorescence units. See Chapter 2 for detailed location of sampling sites.

	Southern Balearic (WM)			**Western Ionian (CM)**			**South of Crete (EM)**		
	1200	**2000**	**2800**	**1200**	**2000**	**2800**	**1200**	**2000**	**3000**
Environmental variables									
Benthic temperature (°C)	13.07	13.21	13.32	13.73	13.78	13.95	13.86	13.91	13.92
Benthic salinity	38.47	38.47	38.47	38.74	38.73	38.73	38.78	38.77	38.74
Benthic dissolved oxygen (mg/l)	5.70	5.82	5.83	5.73	5.83	5.86	5.53	5.60	5.72
Benthic turbidity (FTU)	0.77	0.035	0.04	0.072	0.059	0.028	0.04	0.037	0.032
Sediment grain size (% coarse)	1.72	7.64	16.87	1.08	1.19	4.04	10.48	8.61	12.02
Sediment POC (% of mass)	0.42	0.52	0.33	0.64	0.58	0.28	0.34	0.26	0.29
0-200 m microplankton biomass (mg m^{-3})	5.39	9.36	5.05	5.25	3.96	3.74	2.28	4.77	3.94
0-200 m mesozooplankton biomass (mg m^{-3})	4.19	11.93	8.30	11.85	10.74	7.89	5.25	10.04	6.11
Surface primary production (RFU)	0.739	0.927	0.803	0.559	0.657	0.745	0.541	0.641	0.667

DISCUSSION

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

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

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

Relationships between sediment composition and benthic diversity have been previously described for smaller size classes of macrofauna (Etter & Grassle 1992). For the large-sized species addressed in this study whose biology is still poorly known, it is not clear how the different grain size of the substrate is perceived by the fauna and at what scale of variability this becomes an influencing factor for their distribution and behaviour. It has been proposed that less-mobile species, such as holothurians, could be directly influenced by sediment grain size and carbon supply on the sea floor (Roberts & Moore 1997). Finer-grained sediments, which were present in the shallower areas of the WM and CM, present higher percentages of

POC (Table 3.1) and thus higher food availability, favouring the settlement of a more abundant and diverse infauna (Levin *et al.* 2001). These areas were also the most diverse in terms of species and the taxonomic composition of the megafauna. The regulation of infauna could then also influence the diversity of larger size classes of benthos as an upward trophic modulation.

Similar results were obtained in a northern Atlantic deep-sea observatory, where nutrient availability in the substrate was defined as the modulating agent for benthic megafauna, and the degradation processes in the water column were suggested to be influential (Soltwedel *et al.* 2009). In the deep Pacific Ocean, observations were also made in accordance with relating POC flux with megabenthos abundances (Smith *et al.* 2009). The enhanced temperatures in the Mediterranean water column, which augment organic matter degradation, could be pivotal in determining different processes of benthic-pelagic coupling. However, the present findings are not in agreement with results of a large dataset study across all the world oceans. In that study, sea surface temperature (SST) was found to be the only significant descriptor for marine biodiversity over large spatial scales (Tittensor *et al.* 2010), bringing profound implications with respect to future climatic changes and other human impacts. Seawater temperatures in the deep Mediterranean Sea are already high, and this may be the reason for the absence of correlation between temperature and faunal composition. This does not exclude that significant water temperature increases, that are now known to have occurred over the last decades (Levitus *et al.* 2000), could produce considerable alterations of marine life also in the deep Mediterranean.

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

Of the 99 species collected, the only ubiquitous groups found in all samples were Actinopterygii and Crustacea. These groups were also the most abundant and speciose. This dominance is well established for the entirety of the deep Mediterranean (Jones *et al.* 2003, Company *et al.* 2004), and represents a major difference with the deep Atlantic seafloor (Billett *et al.* 2001, Soltwedel *et al.* 2009), which is colloquially termed the "kingdom of the holothurians" (Kunzig 2000). Also, going from shallow to deep stations and from west to east, a reduction in the number of taxonomic groups can be appreciated. The grenadiers (Actinopterygii: Macrouridae), a dominant fish family in both the deep Mediterranean and the Atlantic, are known to be adapted to various ranges of food availability by employing generalist and opportunistic foraging strategies (Mauchline & Gordon 1986). The two dominant decapod species, *Aristeus antennatus* and *Acantephyra eximia*, can maintain high metabolic rates by having a nektobenthic behaviour (Company & Sardà 1998) and thus being able to live along a wide depth range while feeding in more favourable areas like the mesopelagic water column. Only fish and crustacean species seem then to be completely adapted to bathyal and abyssal conditions in the Mediterranean Sea. What remains to be confirmed is whether their presence in the deep basin is stable or just a spread effect of a shallower-based population.

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

In this study, 2 different samplers were used simultaneously, in order to complement each other and give an integrated description of the benthic community (also refer to Chapter 2). The OTMS was fitted with more flotation devices than usual and a roller line on the footrope to reduce the risk of the net getting caught on the bottom by collecting too much mud. The dynamic of an OTMS haul is such that nektobenthic species are positively selected (Sardà *et al.* 1998). By contrast, the Agassiz dredge has a smaller opening, and it maintains firm contact with the substrate, permitting the retrieval of strictly benthic species. By combining these two different 'sections' of the benthic domain, it was possible to compile the comprehensive faunal list presented in this work. In addition, a generally higher variability in gear behaviour was observed for the OTMS than for the Agassiz dredge; again, this is caused by the differences in gear designs and concepts. The Agassiz has proven to be usable where bottom conditions do not permit sampling with the OTMS (e.g., semi-volcanic bottoms in the central Mediterranean) because they may cause the OTMS to break or be lost completely. Indeed, 2 replicates in the CM were lost because of that occurrence. Thus, the 2 samplers were shown to be complementary in the exploration of deep benthic megafauna (see also Chapter 2). However, with the reduced number of samples retrieved, the resulting diversity patterns are far from being definitive.

The WM presented a slight, non-significant decrease in diversity along the depth axis, which is consistent with previous results with the same OTMS sampler (Tecchio *et al.* 2011). The Agassiz samples collected in the same area showed a clear decrease in diversity between 2000 and 2800 m depth. The diversity differences found in the western basin between the 2 sampled communities suggest that the dynamics of strictly benthic species may be different from those of nektobenthic megafauna. In the deep Mediterranean, the biomass of nektobenthic fauna is known to decrease below 1500 m depth (Sardà *et al.* 2009b). Benthic invertebrates are more dependent on the organic matter found in the sediment than fishes and crustaceans, which could explain their significant decrease at the deeper station. No clear diversity patterns were found in the CM. However, the sampling difficulties experienced when studying these benthic areas resulted in a limited number of successful OTMS and Agassiz trawls, not allowing for any definitive conclusions.

In the EM, there is a clear and steep decline in species diversity from 1200 to 3000 m in both the OTMS and Agassiz samples. The increased oligotrophy of this basin may cause diversity to sharply decrease with depth, whereas in the other areas, this decrease might be partially masked by the more favourable trophic conditions in the deeper layers. Moreover, the sampled zone in the lower depths may represent a patch of reduced diversity on an otherwise homogeneous sea floor. Logistics impaired the retrieval of replicates at 2000 m, and no OTMS sample was available at 2800 m depth; thus, the possibility of heterogeneous habitat cannot be disregarded. In recent years, several studies have shown the importance of habitat heterogeneity in shaping deep benthic communities (Levin & Dayton 2009, Ramírez-Llodra *et al.* 2010b, Vanreusel *et al.* 2010). Further studies in the bathyal and abyssal Mediterranean, including the use of imaging instruments such as remote operated vehicles or autonomous underwater vehicles, are necessary to describe in detail the deep Mediterranean expanse and to identify potential habitat characteristics that could influence heterogeneity.

β-diversity was high throughout the Mediterranean, in striking resemblance to the results from other benthic compartments such as meiofauna (Danovaro *et al.* 2008b). This suggests that most of the deep Mediterranean biodiversity is regional rather than local and that even if basin differences are evident and have to be considered, the whole Mediterranean Sea should be considered as a wide-ranging species pool. The Mediterranean deep-sea fauna is unique in the sense that it is relatively young, compared to the fauna of the outer Atlantic ocean, mainly due to the Messinian salinity crisis event. This peculiar process of colonization may have been the reason for the results of beta-diversity analyses. After the re-opening of the Gibraltar strait, Atlantic species would have dispersed throughout the whole basin, and only subsequently the process of selective settling would have taken place.

In addition, the low overlap in composition between zones may further corroborate the hypothesis that habitat heterogeneity, along with the food supply as addressed in the present study, is a major structuring factor in deep benthic areas (Levin *et al.* 2001). Among other reasons, this heterogeneity could be related to different water masses at the bottom. The western and central areas of the Mediterranean are known to be very dynamic and affected by periodic local cascading events (Canals *et al.* 2006, Company *et al.* 2008), whereas the hydrodynamics of the deep eastern basin south of Crete are less variable, as cascading waters from the Aegean Sea are blocked by the island mass (Bethoux *et al.* 1999). Overall, those observations point to an effective heterogeneity of conditions, rather than to a physical heterogeneity of habitats and structures.

Conclusions

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

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

Table 3.2. Presence/absence faunal list of the collected species, including OTMS and Agassiz samples. A = only Agassiz samples were available.

			Catalan slope			**Western Med.**			**Central Med.**					**Eastern Med.**		
			1200	**2000**	**2700**	**1200**	**2000**	**2800**	**1200**	**2000^{A}**	**2800**	**3300^{A}**	**4000^{A}**	**1200**	**2000**	**3000^{A}**
Arthropoda																
Amphipoda																
	Phronimoidea	*Phronima sedentaria*	*						*					*		
Decapoda																
	Aristeidae	*Aristeus antennatus*	*	*	*	*	*	*	*	*				*	*	
	Axiidae	*Calocaris macandreae*					*		*							
	Benthesicymidae	*Gennadas elegans*	*	*	*	*	*	*	*		*			*	*	
	Crangonidae	*Pontophilus norvegicus*	*	*		*			*							
	Galatheidae	*Munida tenuimana*	*	*		*	*									
		Munidopsis ariadne													*	
	Geryonidae	*Chaceon mediterraneus*			*		*	*			*	*				
		Geryon longipes	*			*			*							
	Homolidae	*Paromola cuvieri*	*													
	Inachidae	*Macropodia longipes*	*			*										
	Nematocarcinidae	*Nematocarcinus exilis*		*	*		*	*		*	*	*	*		*	*
	Oplophoridae	*Acantephyra eximia*	*	*	*	*	*	*		*	*	*	*	*	*	*
		Acantephyra pelagica	*	*	*	*	*	*	*	*					*	
	Paguridae	*Pagurus alatus*	*						*							
	Palaemonidae	*Periclimenes sp.*							*							
	Pandalidae	*Plesionika acanthonotus*	*			*			*					*		
	Pasiphaeidae	*Pasiphaea multidentata*	*	*	*	*	*	*						*	*	
		Pasiphaea sivado	*					*		*						
	Polychelidae	*Polycheles typhlops*	*			*			*					*	*	
		Stereomastis sculpta		*	*	*	*	*								
	Portunidae	*Bathynectes maravigna*							*					*		
	Sergestidae	*Sergestes arcticus*	*			*	*	*	*	*	*			*	*	*
		Sergestes corniculum							*	*	*			*	*	
		Sergia robusta	*	*	*	*	*	*	*	*		*		*	*	*
Euphausiacea																
	Euphausiidae	*Meganyctiphanes norvegica*	*			*										
Brachiopoda																
Rhynchonellata																
	Terebratulidae	*Gryphus vitreus*		*		*			*					*		
Bryozoa																
	-	Bryozoa n.id.				*										
Chordata																
Thaliacea																
	Pyrosomatidae	*Pyrosoma atlanticum*	*	*	*			*	*	*	*			*	*	*
	Salpidae	*Salpa sp.*	*	*			*	*								
Chondrichtyes																
	Chimaeridae	*Chimaera monstrosa*	*						*							
	Dalatiidae	*Dalatias licha*	*						*					*		
	Etmopteridae	*Etmopterus spinax*	*			*			*							
	Hexanchidae	*Hexanchus griseus*	*						*							
	Scyliorhinidae	*Galeus melastomus*	*			*			*					*		
	Somniosidae	*Centroscymnus coelolepis*		*	*		*	*								
		Somniosus rostratus												*		
Actinopterygii																
	Alepocephalidae	*Alepocephalus rostratus*	*	*		*	*									
	Bythitidae	*Cataetyx alleni*	*	*												
		Cataetyx laticeps		*	*		*				*				*	*
	Cynoglossidae	*Symphurus ligulatus*	*													
	Epigonidae	*Epigonus telescopus*	*													
	Evermannellidae	*Evermannella balbo*							*							
	Gadidae	*Phycis blennoides*	*						*							
	Gonostomatidae	*Cyclothone braueri*						*		*	*		*	*		
	Ipnopidae	*Bathypterois mediterraneus*	*	*	*	*	*	*	*	*	*			*	*	*
	Lophiidae	*Lophius piscatorius*	*													
	Macrouridae	*Coelorinchus mediterraneus*	*	*		*			*							
		Coryphaenoides guentheri	*	*	*		*	*		*	*					
		Coryphaenoides mediterraneus		*	*	*	*	*		*	*	*			*	*
		Nezumia aequalis	*													
		Nezumia sclerorhyncus				*			*					*		

	Trachyrincus scabrus	*													
Moridae	*Lepidion lepidion*	*	*		*	*		*						*	
	Mora moro	*			*			*					*		
Myctophidae	*Ceratoscopelus maderensis*					*									
	Diaphus rafinesquei												*		
	Hygophum benoiti									*					
	Lampanyctus crocodilus	*	*	*	*	*	*	*	*	*					*
	Lobianchia dofleini						*								
	Myctophidae sp.	*		*	*	*		*							
	Symbolophorus verany		*			*									
Nettastomatidae	*Nettastoma melanurum*	*			*			*					*		
Notacanthidae	*Notacanthus bonaparte*	*	*					*							
	Polyacanthonotus rissoanus	*				*									
Paralepididae	*Arctozenus risso*	*		*	*										
	Paralepis speciosa									*					
Sternoptychidae	*Argyropelecus hemigymnus*	*	*		*	*	*	*		*			*	*	
Stomiidae	*Chauliodus sloani*					*	*	*		*		*	*	*	
	Stomias boa	*						*						*	
Zoarcidae	*Melanostigma atlanticum*									*					
Cnidaria															
Anthozoa															
-	Anthozoa n.id.							*							
Caryophylliidae	*Desmophyllum dianthus*							*							
Hormathiidae	*Actinauge richardi*							*							
Scyphozoa															
Nausithoidae	*Nausithoe sp.*	*	*												
Pelagiidae	*Chrysaora hysoscella*					*									
	Pelagia noctiluca	*	*	*	*	*									
Echinodermata															
Asteroidea															
Brisingidae	*Hymenodiscus coronata*		*						*						
Goniasteridae	*Ceramaster grenadensis*	*	*			*	*						*		
Echinoidea															
Brissidae	*Brissopsis lyrifera*		*												
Holothuroidea															
Synallactidae	*Mesothuria intestinalis*	*			*			*							
Ypsilothuriidae	*Ypsilothuria bitentaculata*	*													
Echiura															
Echiuroidea															
Bonelliidae	Bonelliinae n.id.				*	*									
Mollusca															
Bivalvia															
Pectinidae	*Delectopecten vitreus*							*	*						
Semelidae	*Abra longicallus*							*							
Cephalopoda															
-	Cephalopoda n.id.							*							
Histioteuthidae	*Histioteuthis reversa*	*						*							
Octopodidae	*Bathypolipus sponsalis*	*			*										
Opisthoteuthidae	*Opisthoteuthis calypso*	*	*												
Sepiolidae	*Neorossia caroli*	*													
Gastropoda															
-	Gastropod n.id.								*						
Aporrhaidae	*Aporrhais serresianus*	*					*	*							
Cymbuliidae	*Cymbulia peronii*	*	*	*		*	*	*	*				*	*	
Pterotracheaidae	*Pterotrachea scutata*	*													
Scaphopoda															
Dentaliidae	*Antalis sp.*	*	*		*										
Porifera															
-	Porifera n.id. 1							*							
-	Porifera n.id. 2						*								
-	Porifera n.id. 3												*		
Sipuncula															
Sipunculidea															
Sipunculidae	*Sipunculus norvegicus*	*	*					*	*						

Seasonal and small-scale patterns

4

INTRODUCTION

In the deep ocean, thought for decades to be a stable and invariable environment, variations of biological processes have been documented over both large time scales (i.e. decadal community shifts; Billett *et al.* 2010) and at the 24-h frequency (i.e. day-night migrations; Aguzzi & Company 2010). Photoperiodic adaptations of metabolism seem to contribute in maintaining a seasonal pattern in biological activities (Childress 1995). Some deep-sea benthic species apparently synchronize their reproductive behaviour with the periodic food input from the surface strata caused by phytoplankton blooms, to increase larval survival and hence the fitness of species (Tyler 1988). At a community level, seasonal processes have been identified in terms of fluctuations in the abundance of certain species and trophic compartments. These fluctuations have been documented especially for the Mediterranean continental upper and middle slope (Sardà *et al.* 1994, Kallianiotis *et al.* 2000, Ramírez-Llodra *et al.* 2008), although similar information for other oceans at similar depths is scant (Gage *et al.* 2004). On the other extreme, no seasonal variations were found in the Pacific abyss, at more than 4000 m depth (Bailey *et al.* 2006). This suggests that seasonal processes are progressively damped with increasing depth. However, the ecological drivers responsible of this damping phenomenon are presently unknown, as well as the extension and depth positioning of the boundary at which this seasonal effect ends.

Continental slopes are among the most accessible areas of the deep sea, comprising approximately 11% of the ocean floor, but information on their diversity is still limited with minimal areas explored to date (Ramírez-Llodra *et al.* 2010a). Most importantly, sampling in the deep is still often conducted under exploratory surveys, without considering any temporal scale, e.g. day- or night-time, leading to skewed perceptions of the communities (Aguzzi & Bahamón 2009). Inasmuch, with a sampling limited only to a particular period of the year – as is the case of many large-scale deep-sea surveys – benthopelagic and nektobenthic movements of individuals may occur in relation to seasonal variations in bathymetry for reproduction and moulting (Aguzzi *et al.* 2009). This phenomenon may cause the appearance or disappearance of species from obtained samples.

In the Mediterranean Sea, the megafauna communities in the lower continental slope are diverse and, particularly in the Western basin, present high abundances and standing stocks with respect to the other Mediterranean basins (Stefanescu *et al.* 1993, Tecchio *et al.* 2011). Temporal periodicity in deep sea metazoans has been studied at smaller time scales relating it with factors such as light attenuation with depth, internal currents and pelagic productivity (Corliss *et al.* 2009, Aguzzi & Company 2010), while wide spatial variations have been associated with organic matter arrival at the bottom and its availability in the sediment (Chapter 3, and Danovaro *et al.* 2008b). However, these factors are also strictly related to seasonal changes in sun irradiation and internal tidal effects (Aguzzi *et al.* 2010). This brings the seasonal factor to a particular interest since it could ultimately control a large portion of the variability of the deep benthic fauna.

Diversity in the benthos follows a unimodal bathymetric pattern, peaking in the oceans at 2000-3000 m depth (reviewed by Rex & Etter 2010). As depth increases, so does the stability of the environment. Various hypotheses have been formulated to relate the extremely low environmental variability at abyssal depths with the detected levels in the diversity of benthos. No definitive consensus has been yet reached about the real pattern of species diversity with depth and about the causes that generate it (Snelgrove & Smith 2002). In the case of the enclosed deep Mediterranean Sea, a study of all size classes (prokaryotes, meio-, macro- and megafauna) showed that none of the considered groups followed the hump-shaped curve of diversity with depth (Danovaro *et al.* 2010) and other studies reported a fall of megafaunal diversity starting from 1500 m depth, although that limit is presently considered as fuzzy, since it seems to depend on the geographic location considered and upon local trophic conditions (Sardà *et al.* 2009b, Tecchio *et al.* 2011).

The north-western Mediterranean continental margin is one of the most extensively studied environments in the last two decades. Results of previous studies indicate that atmospheric-forced events concur in shaping benthic communities with enhanced organic matter arrival and periodic disturbances (Puig *et al.* 2001, Company *et al.* 2008). A distinctive characteristic is the presence of numerous submarine canyons indented in the continental shelf. These canyons act as preferential conduits for suspended particulates in the shelf waters, facilitating their descent to the deep basin (Canals *et al.* 2006). Canyons seem to act as hotspots for local faunal diversity and biomass, providing strong habitat heterogeneity, augmented food availability, and refuge areas for motile species (De Leo *et al.* 2010, McClain & Barry 2010). These canyons are known to host diverse faunal assemblages (Stefanescu *et al.* 1994, Ramírez-Llodra *et al.* 2010b) and influence the movements of species of commercial interest, such as the red shrimp *Aristeus antennatus* (Sardà *et al.* 2009a). The same assumptions seem to be valid for other canyons in the Mediterranean Sea, although data in this sense are still scant (Danovaro *et al.* 2010).

Outside these geological structures, the open slope descends gradually from the shelf-slope break (200 m depth) down to the deep basin (2800 m depth approximately). Depths around 1200 m in this zone present a peak of biomass and abundance and host a set of dominant fish species (Stefanescu *et al.* 1993, Sardà *et al.* 2009b), whose recurrent and concentrated presence has not been explained yet.

In 2008 and 2009, a seasonal sampling survey was carried out on the Blanes submarine canyon in the Catalan continental margin (western Mediterranean) and on the adjacent open slope, from 900 to 1500 m. The objective was to study the patterns of species biomass and diversity as a combined function of depth and season. The surveyed depth range encompassed the twilight zone end (i.e. 1000 m depth), as the upper boundary of the deep sea, where ambient sunlight no longer penetrates. This depth is considered of importance for seasonal and day-night regulation of rhythmic biology, distinguishing in that respect between upper/mid continental margin and lower margin/abyssal species (Aguzzi & Company 2010).

In the temporal concept framework of species sampling variations as results of seasonal rhythmic regulation in their biological functioning, the objective of this chapter is to analyze megafaunal diversity and assemblage structure in different depth strata and seasons. The analysis was innovatively carried out by hypothesizing that reported variations in megabenthic species composition and overall diversity are the product of seasonal modifications in species bathymetric distributions in relation to changes in environmental conditions. Beyond the twilight zone, seasonal biomass movements would be only indirectly driven by photic signals received by species inhabiting the shallower slope layers.

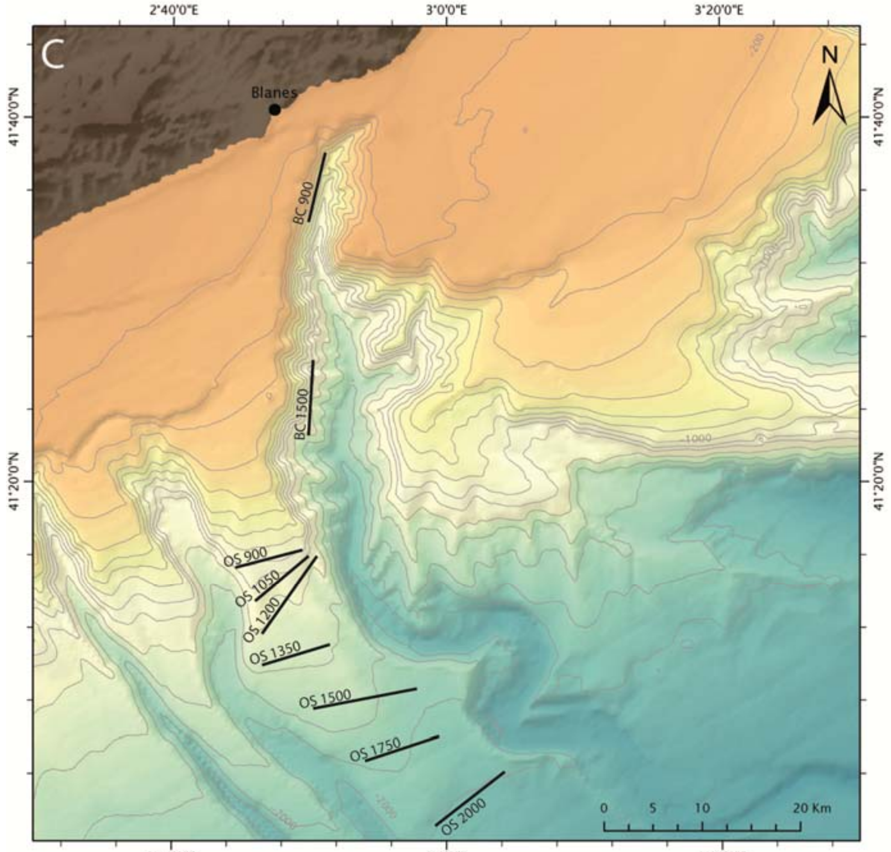


Figure 4.1. Map of the study area in the northern Catalan margin, with detailed bathymetry, showing the locations of the trawling lanes in the open slope (OS) and in the Blanes canyon (BC).

MATERIALS AND METHODS

In this chapter, the data from the five PROMETEO cruises, conducted on the northern Catalan continental margin, will be analysed (see Chapter 2 for details). The sampling of benthic megafauna was carried out with both the otter-trawl Maireta System (OTMS) and an Agassiz trawl, over the same trawling lanes (Fig. 4.1).

Every individual was identified to species level whenever possible, using the available taxonomic literature (Tortonese 1965, Zariquiey 1968, Villanueva 1992, Saiz-Salinas 1993, Mercader *et al.* 2001, Compagno *et al.* 2005), then counted and weighted. Species names were cross-checked with the World Register of Marine Species, available online (WoRMS, www.marinespecies.org). The Hurlbert's rarefaction index ES(25) was calculated for α-diversity (Magurran 2004). Species accumulation curves were calculated separately for the open slope and the canyon, by pooling together OTMS and Agassiz data. Mann-Whitney's non-parametric U-tests (M-W tests) were applied to contrast values of ES(25) for OTMS samples between open slope and canyon areas.

Abundance and biomass of every species were standardized for trawled area (in km^2) using data from the Scanmar system. All species density data were square-root transformed and similarity between all samples was calculated using the Bray-Curtis measure. The ANOSIM permutational routine was used to test for effects of season and depth in species composition (Clarke 1993). Using abundance in species composition data, centroids of samples were then established using a combined Depth × Season factor. Distances among centroids were calculated with the same Bray-Curtis measure, and plotted with a Principal Coordinates Ordination routine (PCO, Anderson *et al.* 2008). The objective of this analysis was to visualize the extent of composition changes along the two considered sampling "transects": seasonal and bathymetric. Ecological analyses were performed with the PRIMER-E 6 and PERMANOVA software (Clarke & Gorley 2006).

In addition, total catch biomass data was compiled from two previous datasets, to obtain a general view on the bathymetric trend of benthic biomass all over the local continental margin, from 100 m to 2000 m depth. Data for this purpose were from the surveying projects NERIT (October 1999 and June 2000) and RECS (years 2003 - 2004; Ramírez-Llodra *et al.* 2010b), carried out with the same OTMS gear and hauling procedures in the same area.

Physical oceanographic data were obtained at the open slope area by deploying near-bottom current meters (Aanderaa RCM9) and temperature-conductivity sensors (Microcat Seabird 37) at 900 m, 1200 m, 1500 m, and 1800 m. Current meters and microcats were located 23 m above the seafloor. All devices were calibrated before use. Temperature and salinity data were corrected using CTD SBE 911 casts performed over the mooring sites during the different oceanographic cruises.

RESULTS

A total of 25276 individuals from 115 different species were collected. The number of identified phyla was 10, comprising a total of 21 classes, 89 families, and 107 genera. The faunal list compiled is presented in Table 4.1, at the end of the chapter. The proportion of unidentified species was low (5.2 % of total species), but it was still possible to discriminate them into different species and were thus included in the biodiversity analyses. The species accumulation curve for the slope nearly reached plateau (Fig. 4.2). On the contrary, the accumulation curve for the canyon is still rising steeply, indicating that the residing assemblage was only sampled partially. Also, the canyon accumulation curve lies constantly above the slope one, suggesting higher levels of biodiversity inside the canyon.

The two samplers captured two different but partially overlapping components of the benthic community and catch results were therefore considered as complementary. Decapod crustaceans (22.6 % of species) and actinopterygians (33.9 % of species) were the dominant groups in the catches, with molluscs at third place (16.5 %). Considering the two samplers separately, decapod crustaceans and bony fishes dominated in the OTMS, while the Agassiz trawl samples were dominated by brachiopods (55 % of total abundance) and included a higher proportion of smaller and less mobile faunal groups such as crustaceans, molluscs and echinoderms (Fig. 4.3).

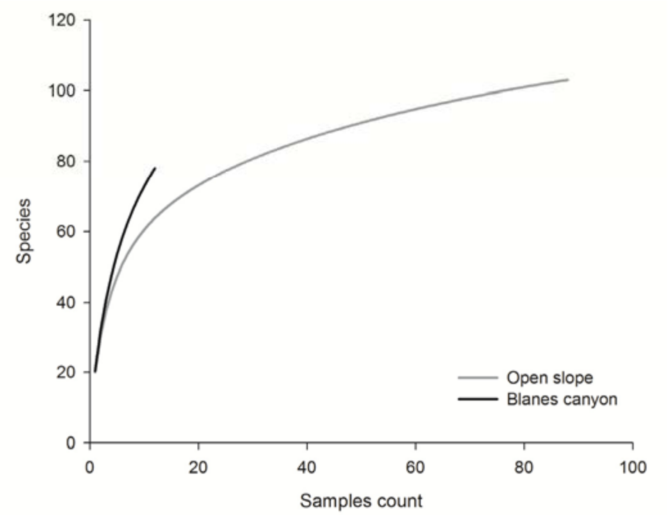


Figure 4.2. Species accumulation curves for megafauna in the Catalan open slope and in the Blanes submarine canyon.

The rarefaction index ES(25) in the slope area ranged from 6.3 to 10.5 for OTMS samples, and from 7.1 to 12.9 for Agassiz samples. In the Blanes canyon, ES(25) diversity ranged 6.4 - 11.8 for OTMS samples, and 5.7 - 13.2 for Agassiz samples. Linear regression analysis between α-diversity in OTMS samples and depth yielded no significant relationships. Considering Agassiz samples, a significant decreasing linear regression of ES(25) with depth was found (corrected $R^2 = 0.589$, $p < 0.0001$). Mobile megafauna sampled with the OTMS was significantly more diverse in the canyon than in the open slope at 1500 m depth (Mann-Whitney test, $p = 0.02$), while it was more diverse in the open slope than in the canyon at 900 m depth (Mann-Whitney test, $p = 0.067$, borderline).

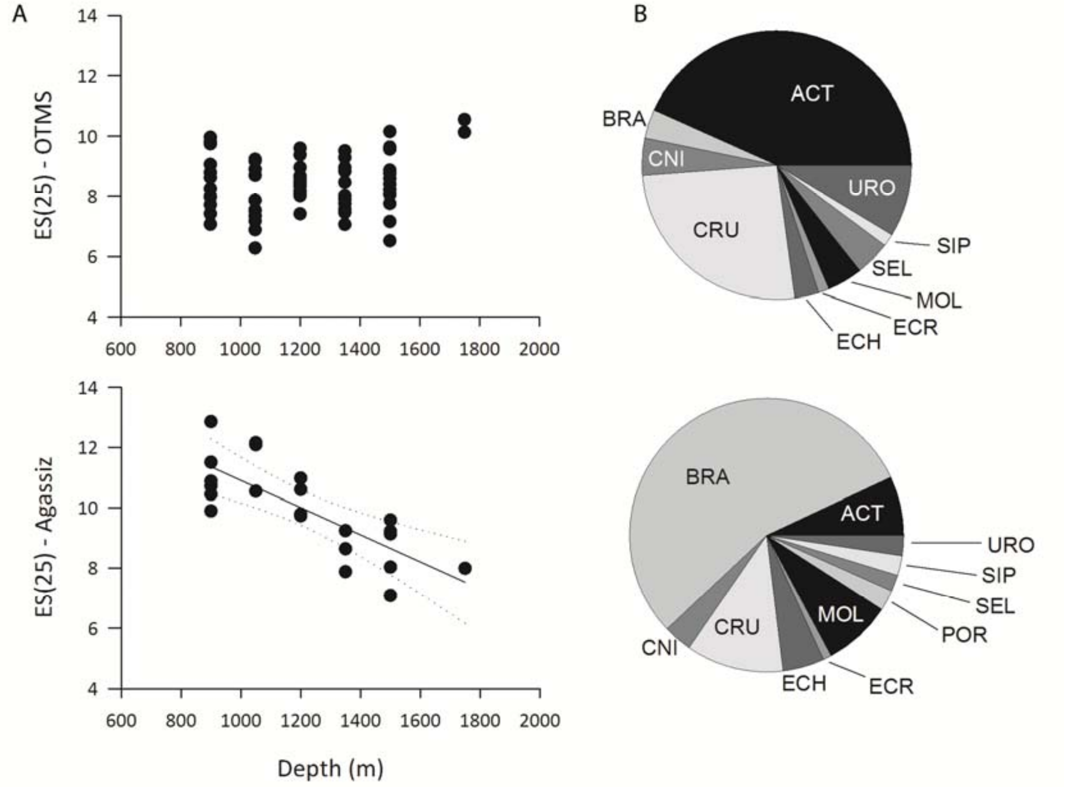


Figure 4.3. (a) Hurlbert's rarefaction index ES(25) for otter-trawl Maireta system (OTMS) and Agassiz samples in the Catalan open slope, as estimators of α-diversity. **(b)** Taxonomic breakdown for OTMS and Agassiz samples, in terms of relative abundance. ACT: actinopterygians, BRA: brachiopods, CNI: cnidarians, CRU: crustaceans, ECH: echinoderms, ECR: echiurans, MOL: molluscans, POR: poriferans, SEL: selachians, SIP: sipunculans, URO: urochordata.

The variation analysis in the biodiversity at different depth strata revealed seasonal changes. The highest values of seasonal diversity were found inside the canyon in September at 900 m depth and in November at 1500 m depth, while in the open slope, diversity was at its maximum in February at 900 m depth and in November at 1500 m depth (Fig. 4.4).

The biomass pattern can be interpreted as a downslope migratory pattern during late autumn and winter, and conversely as an upslope shift during spring-summer (Fig. 4.5). In the deeper layers (1350 m and 1500 m) differences in biomass with season were not appreciable, thus indicating a more temporally stable assemblage. Abundance was high at 1200 m depth throughout the year, while decreasing both upslope and downslope. The general image is of a more dynamic region above and on the twilight zone limit (1000 m), while the lower part of the analysed depth range is more stable throughout the year.

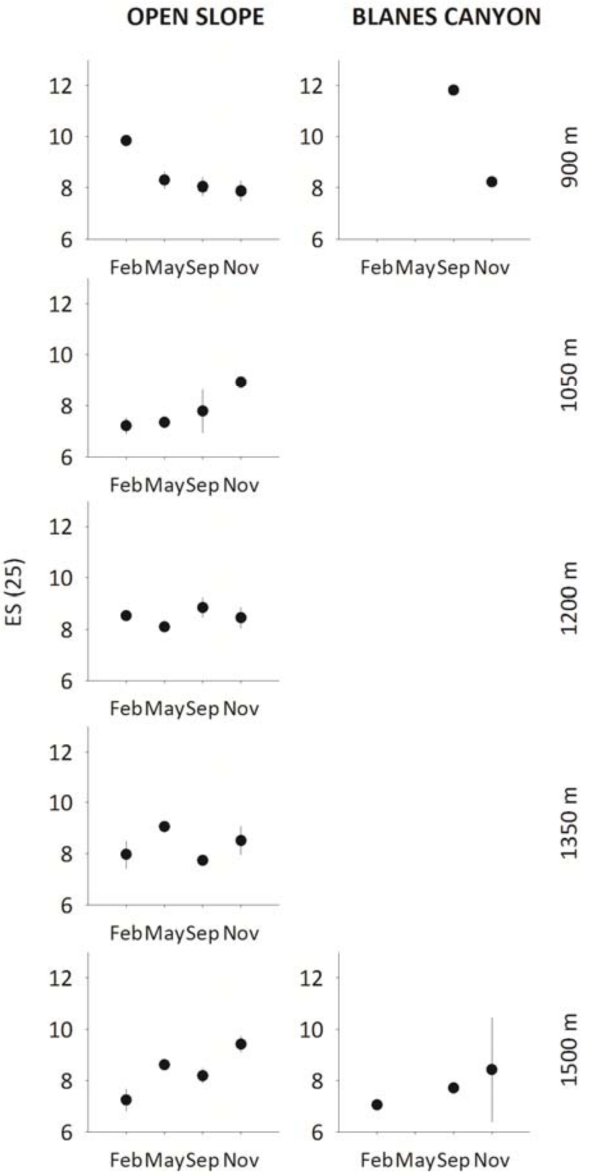


Figure 4.4. Seasonal patterns of species diversity in the OTMS samples on the Catalan open slope and in the Blanes submarine canyon, expressed as ES(25). Values are Mean ± SD

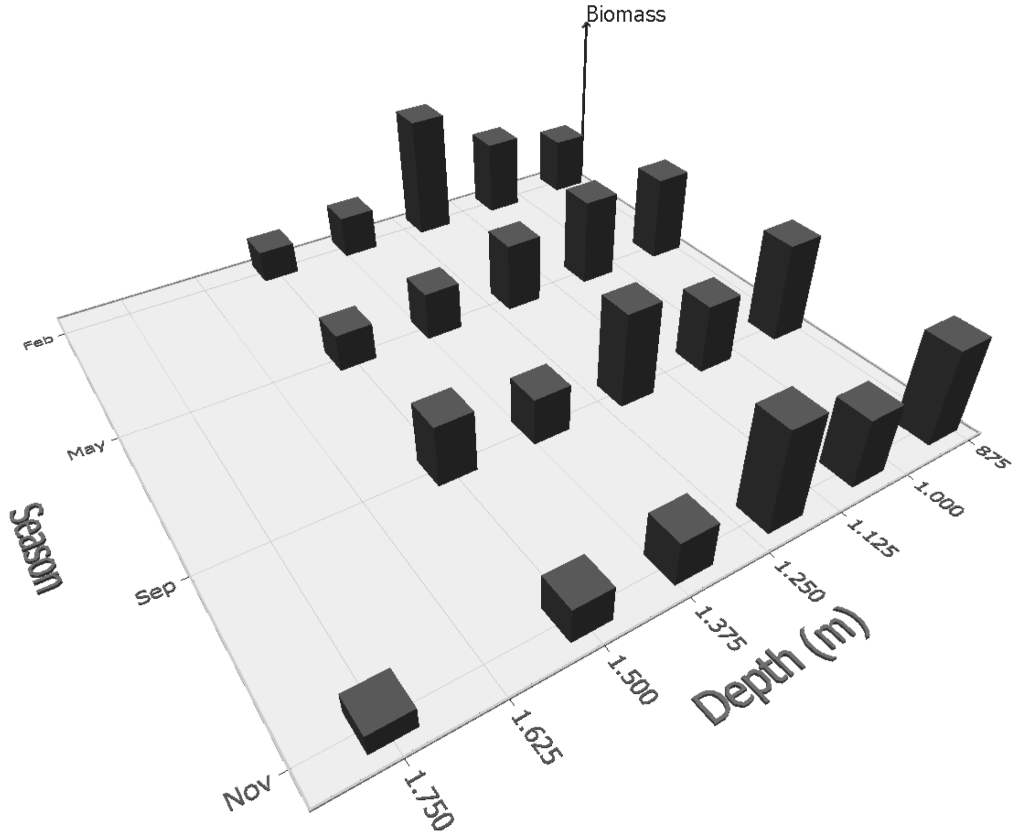


Figure 4.5. Seasonal biomass of benthic megafauna on the Catalan open slope, as retrieved by the OTMS.

Total megafauna abundance at 900 m in the slope was 7470 ± 2241 individuals km^{-2}, while the abundance in the Blanes canyon at the same depth was 5264 ± 3532 individuals km^{-2}. Abundance at 1500 m depth in the slope was 6601 ± 2059 individuals km^{-2} and in the canyon it was 8257 ± 4805 individuals km^{-2}. M-W test comparisons of abundance at these two depths between the slope and the canyon were both non-significant. Considering total biomass, values in the open slope at 900 m depth were 1163.1 ± 493.7 kg km^{-2} and at 1500 m depth they were 575.1 ± 193.4 kg km^{-2}. Biomass in the canyon was 568.1 ± 630.5 kg km^{-2} at 900 m depth, and 440.4 ± 197.8 kg km^{-2} at 1500 m depth. M-W test comparisons for biomass differences were also non-significant.

The first two PCO axes (Fig. 4.6) captured a cumulative 70.2 % of the total variability, while the third coordinate only explained 7.2 % of the variability. The prevalent changes in composition were in February with respect to the other seasons. This analysis also evidenced assemblage changes along a depth gradient, especially between 1050 m and 1200 m and between 1200 m and 1350 m, showing a compacted assemblage composition in the 1200 m area. The second axis of the PCO, which captured 12.2 % of the total variability, fairly represented the seasonal factor.

A clear bathymetric zonation was also evidenced by all ANOSIM contrasts between assemblage compositions at adjacent depths ($p < 0.001$ in all comparisons). The species composition between the slope and the canyon significantly differed in OTMS samples, both at 900 m depth (ANOSIM, $R = 0.997$, $p = 0.01$) and at 1500 m depth (ANOSIM, $R = 0.838$, $p = 0.01$), and the same occurred with Agassiz samples at 900 m depth (ANOSIM, $R = 0.99$, $p = 0.036$) and at 1500 m depth (ANOSIM, $R = 1$, $p = 0.048$).

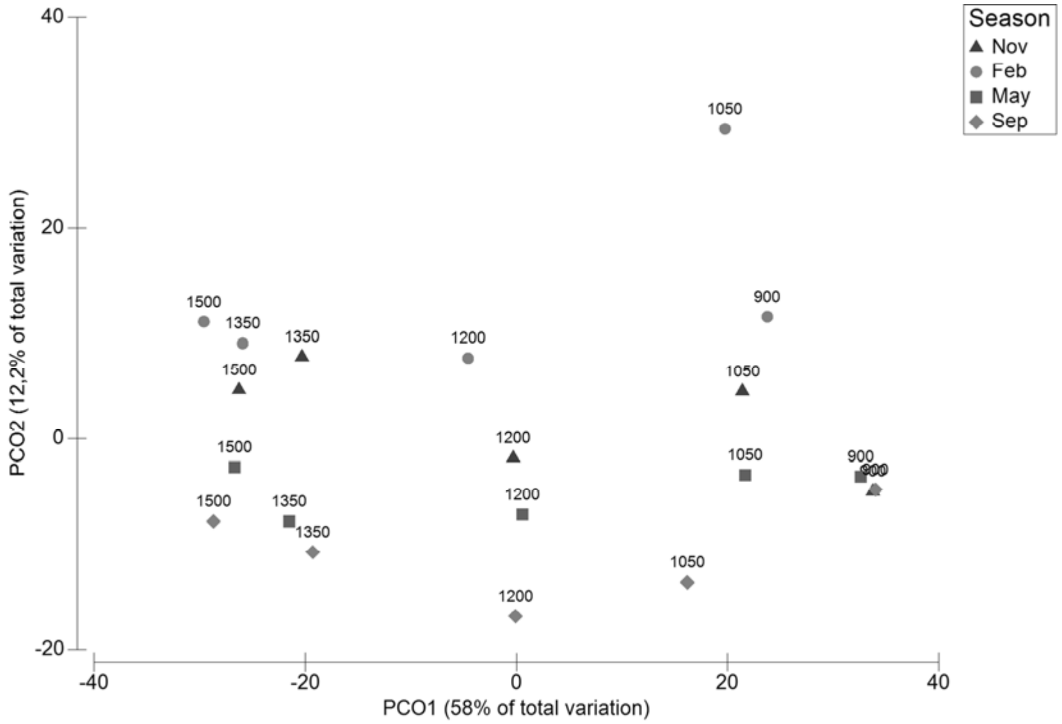


Figure 4.6. Centroid-based Principal Coordinates Ordination (PCO) of the OTMS samples in the open slope, using species abundances as variables.

Current speed at 900 m, 1200 m and 1800 m showed an increase spanning from March to June 2009 (Fig. 4.7). At the same time, the lower temperature and higher salinity recorded the arrival of new Western Mediterranean Deep Water (nWMDW) along the continental slope, and affecting the whole benthic layer from March to early May 2009.

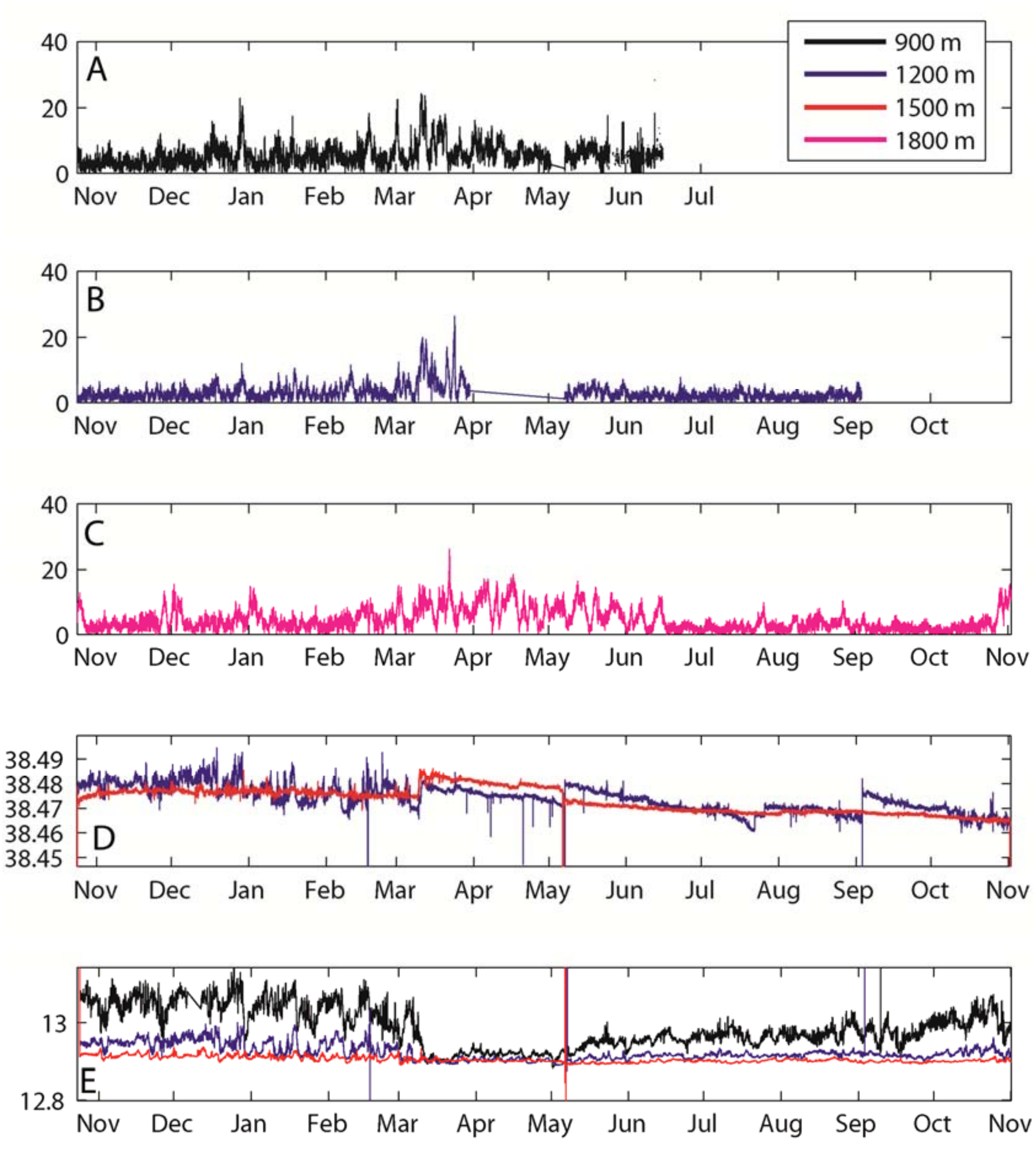


Figure 4.7. Physical characteristics of the water column in the Catalan continental margin. (A-C) Current speed (in cm s^{-1}) recorded at 900 m, 1200 m and 1800 m in the open slope during 2009. (D-E) Water salinity (D) and potential temperature (in °C) (E) recorded during the same period at 900 m, 1200 m and 1500 m.

DISCUSSION

Inter-annual time series in deep waters have been collected starting as early as the 1970s, but intra-annual variability in species diversity is a novel theme around the globe. In the Mediterranean Sea, small scale seasonal/environmental drivers – both temporal and spatial – have been studied previously only in relation with fisheries and single-species population analyses (Sardà *et al.* 1997). Here, the influence of these drivers on total megabenthos diversity is addressed.

The most prominent cause of food input to the deep is the surface primary production, which in Chapter 3 was shown to be influential on species distribution in the deep Mediterranean Sea. Seasonality in the deep-sea has been described as the intra-annual variations of organic matter impulses from the surface strata, already related with diversity in the benthos (Corliss *et al.* 2009). However, laterally advected organic matter due to the seafloor morphology and the local hydrodynamic regime may also be an important source of food to the continental margin. Indeed, lateral transport of organic matter from the continental shelf has been documented in the north Catalan continental slope as being triggered by physical forcing such as dense shelf water cascading (Canals *et al.* 2006, Zuñiga *et al.* 2009) or eastern storms (Martín *et al.* 2006, Sanchez-Vidal *et al.* 2012). The combination of these spatial and temporal processes represents therefore a favourable situation to delve into the understanding of the functioning of deep-sea ecosystems.

Seasonal changes of benthic megafauna

The temporally scheduled use of trawling allows a partial assessment of the movements carried out by individuals at different day-night or seasonal time scales, by associating the presence/absence of species in catches with the same patterns in adjacent areas (Aguzzi & Company 2010). In this sense, the results on the seasonal variation in the sampled community and species biomasses were interpreted as the product of bathymetric shifts in population distributions.

In February, biomass patterns show a single concentration peak at 1200 m depth. From then on, two differing trends were identified: an upslope shift from 1200 m to 900 m during spring that reaches its maximum at late-summer and autumn (September and November), and another part of the assemblage that remains resident at 1200 m depth throughout the year. This is in accordance with previous results from the middle-slope (600-650 m depth) that showed a concentration of biomass at those depths during spring and summer (Sardà *et al.* 1994).

The north Catalan margin presented significant intra-annual variations in hydrological and sediment dynamics during the study period. In late autumn and winter remobilization of shelf sediments by storms triggered the arrival of large quantities of sediments relatively poor in organic matter to the overall slope, while phytoplankton blooming during spring caused an increased settling of particles rich in organic matter from the surface waters (López-Fernández *et al.* in press). In addition, increased near bottom current speeds linked to changes in temperature and salinity in early spring 2009 suggest the arrival of a different water mass coming from deeper layers (identified as the nWMDW) in the study area, that again triggered an increased sedimentation of organic matter due to the resuspension of seafloor sediments. The first observations of the nWMDW occurred in 2005 and later again in 2009 (López-Jurado *et al.* 2005, CIESM 2009). This water mass has a local lower temperature and relatively higher salinity than the typical WMDW, and is formed in extreme winter conditions in the Gulf of Lions / Ligurian Sea region due to important heat loss from the sea surface to the atmosphere (Schroeder *et al.* 2010). Consequently, it may take several days to reach the BC area following the main circulation feature of the NW Mediterranean, the Northern Current.

These enhanced currents reached a speed as high as 20 cm s^{-1} (by contrast, nominal current speeds during the other periods of the year were around 7 cm s^{-1}). While deep-sea fish species may be able to cope with these current levels, it is doubtful that they could actively remain at the 1200 m depth stratum during a couple of months of sustained increased flow. Moreover, current speeds have been considered a factor influencing the presence of deep-sea species relative to seasonality (Sardà *et al.* 2009a).

Anomalies in water masses like the one that occurred in 2009, and presented here, may be pivotal in modulating the behaviour of whole fish and crustacean populations, by forcing them to migrate upslope either by direct physical forcing or by washing away food particles and prey. Processes as basic as the upslope displacement of water masses may exert much more influence on the general ecosystem functioning than previously thought.

Ramírez-Llodra *et al.* (2008) did not find seasonal differences in abundance and biomass of benthic non-crustacean invertebrates in the same western Mediterranean sampling area. Conversely, we found these seasonal differences for the megafauna including the crustaceans in this area. If the downward biomass movement occurring in February is performed by few species with high abundances, the results on diversity should show an exactly inverse pattern with respect to biomass. This pattern can be appreciated in the alpha-diversity results in the open slope (see Fig. 4.4). This suggests that only a group of highly mobile species is seasonally shifting downslope or upslope, not the whole benthic community. In addition, in the lower part of the studied depth range this process starts to get independent from the upper stratum.

Seasonal oscillations in species abundance, even in the deep sea, can lead to biased perceptions of communities and should be taken into account whenever possible. I nevertheless appreciate that spatially wide samplings in the deep sea can hardly be conciliated with this seasonal concept. Still, it is my opinion that studies in the deep should not disregard the inherent variability brought in by seasonality.

Bathymetric pattern

Unlike biodiversity in the Atlantic Ocean, which peaks at intermediate depths forming a well-known hump-shaped curve (Rex 1981), the Mediterranean megafauna diversity clearly decreases with depth, reaching a minimum in the abyssal bottoms of the central region (Tecchio *et al.* 2011). We performed a highly-replicated sampling in a reduced depth range, allowing for accurate description of the pattern in that particular area of the open slope, but not for inclusion of the observed pattern in the whole continental margin setting. In contrast, it was possible to perform a brief collection of benthic biomass data, to visualize its bathymetric trend in the whole Catalan margin slope (see Fig. 4.8). Benthic biomass is an important indicator of the status of an ecosystem, and more generally it is one of the key parameters to evaluate ecosystem functioning (Wei *et al.* 2010). It is evident that the depth range studied in this work represents a maximum of biomass in the whole continental margin, decreasing above 900 m and from 1500 m downward, as partially observed also by Sardà *et al.* (2009b).

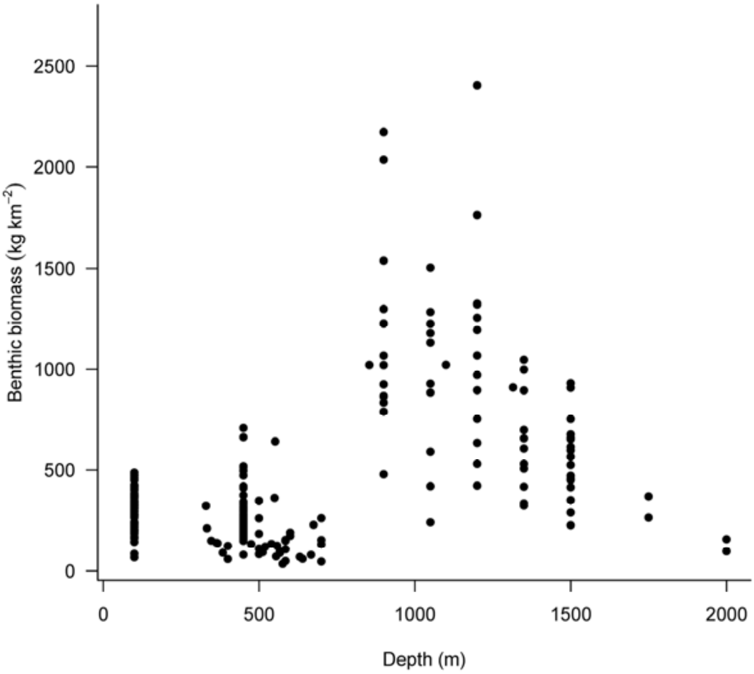


Figure 4.8. Review of data from OTMS trawls conducted in the Catalan continental margin, from different projects, showing the standardized total catch biomass against depth.

The pattern obtained with the Agassiz trawl confirmed the general reduction of diversity with depth. When highly mobile species like fishes are included in the analyses by using trawl data, the alpha-diversity tends not to exhibit any particular pattern with depth. This also underlines the differences between invertebrates with relatively low motility capabilities (e.g. gastropods, bivalves, echinoderms) and the more motile species (e.g. fish, decapod crustaceans, cephalopods) retrieved with the OTMS. The less mobile invertebrates mainly sampled by the Agassiz present a decrease of diversity with depth, possibly caused by their limited or lack of motility and thus their reduced capacity of spatially and seasonally selecting habitats with favourable food conditions. The constant diversity in OTMS samples, mostly composed by highly mobile decapod crustaceans and fishes, is probably caused by a higher transfer of species between depth strata. Another possible explanation is the enhanced coastal input of organic matter, typical of the western Mediterranean, which masks a bathymetric gradient comprising the whole megafauna. This hypothesis has been brought forward by McClain *et al.* (2010) on a Pacific Ocean seamount. Seamounts are, however, highly heterogeneous mid-scale structures (Vanreusel *et al.* 2010); to extend this explanation at the open slope addressed in the present study would require use of imaging instruments to explore the seabed.

Having accounted in the previous section for the temporal shifts of the upper slope biomass, it remains to be explained the fraction of resident biomass at 1200 m depth. The reasons for this peaking are not yet fully understood, being a phenomenon already reported for the western Mediterranean (Sardà *et al.* 1994) but never before on a seasonal basis. Oceanographic causes can be brought forward as possible explanations. At lower slope depths in the Atlantic Ocean, current dynamics favour an increased permanence of surface material, before being washed downslope (Thomsen & van Weering 1998). A similar effect may take place in the Catalan margin, aided by the lateral advection of nutrients from the nearby Blanes canyon. This concentration of downward fluxes around the 1200 m depth range has been recently pointed out, being its generating mechanism under analysis (Palanques *et al.* 2012).

We also propose that this concentration is caused by a combination of ecological forcing between the increasingly stringent ambient conditions and the intrinsic upward expansion of the depth range of deeper species. Those species are naturally adapted to a stable, photon-absent, high-pressure system and, as such, are unable to compete with the more active counterparts living in the middle slope (Haedrich 1996). This could lead to the production of an ecotone-like boundary at depths around 1200 m, sustaining high levels of biomass but not diversity, with respect to the surrounding depths. This condition would be extremely concentrated in space: the horizontal distance between two trawling lanes of adjacent depths was approximately 1.5 – 3 km.

The present hypothesis should be tested in other deep-sea areas of the Mediterranean and other oceans, keeping into account the particular conditions of the deeper basin of the former, where the temperatures are constant above 13 °C throughout the year below 200 m depth. This temperature factor could be pivotal in maintaining the boundary.

As an alternative hypothesis, impact of fisheries may be the cause for this biomass pattern. Fishing activities, especially benthic trawling, have the potential to subtract an important portion of the standing stock biomass in exploited systems (Myers & Worm 2003). Fishing activities in the Catalan continental margin reach depths of approximately 850 m and they have been active during the last 50 years approximately. In a non-exploited region, benthic biomass should decrease from the shelf to the abyss in a typical exponential decay pattern (see e.g. Godbold *et al.* 2013). If this holds true also in the Mediterranean Sea, then the peak observed around 1200 m depth may be the result of this biomass removal at shallower grounds. From the peak at 1200 m down the margin – i.e. in the non-impacted region – the biomass pattern would behave as an exponential decay model.

The submarine canyon environment

Submarine canyons have been identified all over the globe as hotspots of biomass and diversity and as regions of increased hydrodynamics that create heterogeneous habitat patches (Levin & Dayton 2009, De Leo *et al.* 2010, McClain & Barry 2010). Detailed comparative studies between specific canyons and adjacent slopes are however scarce, while these type of analyses should increase in the near future as they would provide a quantitative assessment of the real extent of canyon effects. Ramírez-Llodra *et al.* (2010b) found significant canyon-slope differences in community composition, although at shallower depths (400-700 m) with respect to the present study, suggesting habitat heterogeneity as a regulator of benthic biodiversity. Highly mobile megafauna, taxonomically similar to the one sampled with the OTMS gear, was also strongly divergent between canyon and slopes in the Hawaiian Archipelago, in addition to being more diverse inside the canyon (Vetter *et al.* 2010).

Although sampling in the canyon was limited by the difficulty of using the OTMS in unknown rough terrain, the assemblage in the Blanes canyon could be identified as a separate system from the open slope assemblage, showing higher diversity as well as a distinct composition, but neither abundances nor biomasses appeared to differ between the two areas. Dissimilarities between the canyon and slope communities were more marked considering highly mobile megafauna than strictly benthic species, suggesting also that even species with high movement capabilities manifest habitat preferences. Still, connectivity between these two habitats is likely to occur. Species such as the red shrimp *Aristeus antennatus* are known to perform seasonal migrations along the canyon head, the canyon base and the adjacent open slope, synchronizing their reproduction with the annual cycle (Sardà *et al.* 1997).

The Blanes submarine canyon also hosts some exclusive species with respect to the outer slope. The echinoid *Brissopsis lyrifera*, which dominated the non-crustacean megafauna in the canyon at 1500 m depth, reached densities of as high as 14000 individuals km^{-2}, and was completely absent in the outer slope. Comparable results were obtained at shallower depths both in the same Blanes canyon and in a near canyon site (Ramírez-Llodra *et al.* 2008, Ramírez-Llodra *et al.* 2010b). However, according to fishermen that operate benthic trawling in the area, this echinoid was present in high abundances also in the open slope environment in the previous decades. Commercial benthic trawling has been performed constantly on these grounds down to 700 - 800 m depth since the 1960s, removing benthic biomass and at the same time, reducing seafloor heterogeneity by physical reworking (Puig *et al.* 2012). This suggests that these activities can considerably impact low-mobility species and that the canyon may be acting as a refuge area for those species. Deep-water coral banks were also recognised as possible refuge areas and identified as potential sites for protection, especially inside submarine canyons (Huvenne *et al.* 2011). Specific conservation measures other than the general trawling ban under 1000 m depth currently in force should be endorsed in order to protect these hotspot areas.

Overall, it remains clear that most of the biodiversity of the Blanes canyon is yet to be described and that sampling in the canyon is thus far from exhaustive. Only an intensive, multi-period sampling would disentangle the real drivers that produce and maintain high levels of diversity in submarine canyons all around the globe.

Conclusions

In this chapter, a small-scale observation was conducted on a seasonal basis in a continental slope habitat and in an adjacent submarine canyon, in the north-western Mediterranean Sea, which is a high productivity area compared to other regions of the Mediterranean basin. Specifically, it can be concluded that: (i) a marked bathymetric zonation of benthic megafauna is evident from 900 to 1750 m in the Catalan continental margin, (ii) the depths of 1200-1350 m represent an absolute maximum of benthic biomass of the whole continental margin, being the explanations for this accumulation far from exhaustive, (iii) a fraction of the resident biomass of nektobenthic individuals move from 1200 m depth upslope during spring, possibly driven by the arrival of the nWMDW from the deep basin, and (iv) the environment of the Blanes submarine canyon hosts a more diverse community than in the outer open slope and may represent a refuge area for strictly benthic megafauna species.

Table 4.1. Presence/absence faunal list of the collected species, integrating OTMS and Agassiz samples, in the Catalan continental margin at 6 depths in the 2 zones.

		Open slope						Blanes canyon	
Phylum / Class / Family		**900**	**1050**	**1200**	**1350**	**1500**	**1750**	**900**	**1500**
Porifera									
-	Porifera n.id.	*			*	*			
Cnidaria									
Anthozoa									
Caryophylliidae	*Desmophyllum dianthus*				*	*			*
Epizoanthidae	*Epizoanthus* sp.							*	
Isididae	*Isidella elongata*					*	*		
Scyphozoa									
Coronatae	Coronatae n. id.		*	*	*	*	*		*
Pelagiidae	*Pelagia noctiluca*	*	*	*	*	*	*	*	*
Sipuncula									
Sipunculidea									
Golfingiidae	*Golfingia vulgaris vulgaris*	*							
Sipunculidea									
Sipunculidae	*Sipunculus norvegicus*	*	*	*	*	*			
Echiura									
Echiuroidea									
Bonelliidae	*Bonellia viridis*				*	*			*
Echiuridae	*Echiurus abyssalis*								*
Brachiopoda									
Rhynchonellata									
Terebratulidae	*Gryphus vitreus*	*	*		*	*	*	*	
Bryozoa									
-	Bryozoa n.id.				*				
Mollusca									
Bivalvia									
Limidae	*Limaria hians*					*			
Pectinidae	*Delectopecten vitreus*				*				
Semelidae	*Abra longicallus*	*	*			*		*	*
Cephalopoda									
Cranchiidae	*Galiteuthis armata*				*	*	*		
Histioteuthidae	*Histioteuthis bonnellii*		*			*			
Histioteuthidae	*Histioteuthis reversa*			*					*
Octopodidae	*Bathypolypus sponsalis*	*	*	*	*	*			*
Ommastrephidae	*Todarodes sagittatus*		*						
Opisthoteuthidae	*Opisthoteuthis calypso*	*	*	*	*	*			
Sepiolidae	*Neorossia caroli*	*	*	*	*	*			*
	Sepiolid n.id.		*		*				*
Gastropoda									
-	Gastropod n.id.	*						*	
Aporrhaidae	*Aporrhais serresianus*	*	*	*	*	*		*	*
Cymbuliidae	*Cymbulia peronii*	*	*	*	*	*	*		*
Muricidae	*Pagodula echinata*							*	*
Naticidae	*Lunatia fusca*	*						*	*
Pterotracheaidae	*Pterotrachea scutata*	*	*	*		*			
Scaphopoda									
Dentaliidae	*Antalis sp.*	*	*	*	*	*			
Arthropoda									
Amphipoda									
Phronimoidea	*Phronima sedentaria*			*	*	*			
Decapoda									
Aristeidae	*Aristeus antennatus*	*	*	*	*	*	*	*	*
Axiidae	*Calocaris macandreae*	*			*	*		*	
Benthesicymidae	*Gennadas elegans*	*		*	*	*	*		
Crangonidae	*Pontocaris lacazei*	*						*	
	Pontophilus norvegicus	*	*	*	*	*	*	*	*

Galatheidae	*Munida tenuimana*	*	*	*	*	*	*		*
Geryonidae	*Geryon longipes*	*	*	*	*	*	*		*
Homolidae	*Paromola cuvieri*	*	*	*					
Inachidae	*Macropodia longipes*			*				*	
Nematocarcinidae	*Nematocarcinus exilis*						*		
Nephropidae	*Nephrops norvegicus*								*
Oplophoridae	*Acantephyra eximia*	*	*	*	*	*	*		*
	Acantephyra pelagica		*	*		*	*		*
Paguridae	*Pagurus alatus*	*	*	*				*	*
Pandalidae	*Pandalina profunda*							*	
	Plesionika acanthonotus	*	*	*	*	*			
	Plesionika martia							*	
Pasiphaeidae	*Pasiphaea multidentata*	*	*	*	*	*	*	*	*
	Pasiphaea sivado	*	*	*	*	*		*	*
Polychelidae	*Polycheles typhlops*	*	*	*	*	*		*	
	Stereomastis sculpta				*	*	*		*
Processidae	*Processa canaliculata*							*	
Sergestidae	*Sergestes arcticus*	*	*	*	*	*	*	*	*
	Sergestes corniculum	*							
	Sergia robusta	*	*	*	*	*		*	*
Xanthidae	*Monodaeus couchii*	*						*	
Euphausiacea									
Euphausiidae	*Meganyctiphanes norvegica*	*		*	*	*	*		*
Isopoda									
Cirolanidae	*Natatolana borealis*		*						*
Echinodermata									
Asteroidea									
Brisingidae	*Hymenodiscus coronata*								*
Goniasteridae	*Ceramaster grenadensis*	*	*	*	*	*	*	*	
Echinoidea									
Brissidae	*Brissopsis lyrifera*								*
Echinidae	*Gracilechinus alexandri*								*
Holothuroidea									
Molpadiidae	*Molpadia musculus*	*	*					*	
Synallactidae	*Mesothuria intestinalis*	*	*	*	*	*			
Ypsilothuriidae	*Ypsilothuria bitentaculata*	*	*	*	*				
Chordata									
Ascidiacea									
-	Ascidiacea n.id.					*			
Thaliacea									
Pyrosomatidae	*Pyrosoma atlanticum*	*	*	*	*	*	*		*
Salpidae	*Salpa sp.*	*	*	*	*	*	*		*
Chondrichtyes									
Chimaeridae	*Chimaera monstrosa*			*	*	*			*
Dalatiidae	*Dalatias licha*	*	*	*	*	*			*
Etmopteridae	*Etmopterus spinax*	*	*	*	*	*		*	*
Hexanchidae	*Hexanchus griseus*	*	*	*					
Scyliorhinidae	*Galeus melastomus*	*	*	*	*	*		*	*
Somniosidae	*Centroscymnus coelolepis*				*	*	*		
Actinopterygii									
Alepocephalidae	*Alepocephalus rostratus*	*	*	*	*	*	*	*	*
Bythitidae	*Cataetyx alleni*	*	*	*	*	*	*	*	*
	Cataetyx laticeps						*		
Centrolophidae	*Schedophilus medusophagus*				*				
Congridae	*Conger conger*	*						*	
Cynoglossidae	*Symphurus ligulatus*	*		*					
Epigonidae	*Epigonus denticulatus*							*	
	Epigonus telescopus	*	*	*				*	
Gadidae	*Micromesistius poutassou*	*							
	Phycis blennoides	*	*	*	*			*	
Gonostomatidae	*Cyclothone braueri*	*							
Ipnopidae	*Bathypterois mediterraneus*		*	*	*	*	*		*

Lophiidae	*Lophius piscatorius*			*					
Lotidae	*Gaidropsaurus biscayensis*							*	
	Molva dypterygia							*	
Macrouridae	*Coelorinchus mediterraneus*	*	*	*	*	*		*	*
	Coryphaenoides guentheri			*		*	*		*
	Coryphaenoides mediterraneus					*			
	Hymenocephalus italicus	*						*	
	Nezumia aequalis	*	*	*	*	*		*	
	Trachyrincus scabrus	*	*	*	*	*		*	*
Merluccidae	*Merluccius merluccius*							*	
Moridae	*Lepidion lepidion*	*	*	*	*	*	*	*	*
	Mora moro	*	*	*	*			*	
Myctophidae	*Ceratoscopelus maderensis*	*							
	Lampanyctus crocodilus	*	*	*	*	*	*		*
	Myctophidae sp.	*	*	*	*	*			*
	Symbolophorus verany								*
Nettastomatidae	*Nettastoma melanurum*	*	*	*	*				
Notacanthidae	*Notacanthus bonaparte*	*	*	*	*	*		*	*
	Polyacanthonotus rissoanus	*	*	*	*	*			
Ophidiidae	*Benthocometes robustus*							*	
Paralepididae	*Arctozenus risso*	*	*	*	*	*			
Scophthalmidae	*Lepidorhombus boscii*	*							
Sternoptychidae	*Argyropelecus hemigymnus*	*		*	*	*	*		*
Stomiidae	*Chauliodus sloani*	*	*		*	*	*	*	
	Stomias boa	*		*		*			
Zoarcidae	*Melanostigma atlanticum*	*	*					*	
	Zoarchid n.id.	*							

Trophic dynamics mediated by marine productivity

5

INTRODUCTION

Heterotrophic, bottom-up controlled, food webs are the most abundant ecosystem structure in the planet; i.e. they are the most frequent food webs found in the deep sea, which is the largest biome on Earth (Gage & Tyler 1991, Ramírez-Llodra *et al.* 2010a). Deep-sea systems thrive under a downward flux of particulate organic matter, the *marine snow*, which provides the main food source for the benthos (Polunin *et al.* 2001). Recent advances in benthic ecology identified that deep-sea food webs present a complex trophic structure, with a high number of trophic levels and various processes of niche adaptation (Iken *et al.* 2001, Madurell *et al.* 2008, Jeffreys *et al.* 2009). Organic carbon input and its availability at the seafloor have also been shown to control benthic standing stock, community composition, and diversity (Danovaro *et al.* 2008b, Smith *et al.* 2009). Once arrived at the seafloor, marine snow entrains the benthic food web that begins with benthic deposit feeders and culminates with benthopelagic predators. This process of carbon deposition – its magnitude decreasing with increasing depth – is essential in regulating how species interact with food sources and between them (Levin *et al.* 2001).

The Mediterranean Sea is geographically divided into three basins: the western, central and eastern, with important variations in primary productivity and organic matter availability between them (Danovaro *et al.* 1999). The eastern basin is the most impoverished area in terms of organic matter input to bathyal depths (Azov 1991) while, in the western basin, high fluvial inputs, increased surface productivity and other mesoscale oceanographic events generate a higher quantity of organic matter reaching the deep seafloor (Margalef 1985, Company *et al.* 2008). It follows that the carbon fluxes of the western basin at similar depths are two orders of magnitude higher than in the eastern basin (Danovaro *et al.* 1999). The central basin presents intermediate environmental conditions between the west and the east, and its continental slope hosts a diverse benthic megafaunal community, more similar to the one present in the western basin (Tecchio *et al.* 2011). This gradient spans the entire Mediterranean and thus provides an interesting benchmark to test for macro-ecological patterns in numerous processes such as biodiversity, ecosystem functions, and trophic relationships.

In marine systems, depth is also considered another major driver of benthic processes, such as species distribution (Carney 2005). Depth provides an additional gradient of food availability and quality at the bottom, because the rate of degradation of organic matter during its fall is correlated with the height of the water column (Gage 2003). Generally speaking, water depth thus negatively correlates with energy availability at the seafloor (Levin *et al.* 2001). This offers two possible dimensions of observation (bathymetric and longitudinal) at the same time and, in the case of the Mediterranean, over large spatial scales.

Megabenthic communities of the continental margins and deep basins of the Mediterranean Sea, mainly constituted by fishes and decapod crustaceans, present strong biomass reductions that follow the gradients of productivity and food arrival at the bottom (see Chapter 3). From a trophic interactions point of view, however, how megafauna respond to this gradient is still a matter of speculation. Studies conducted until now have not managed to fully disclose the actual process modulating these adaptations (Carrassón & Cartes 2002, Zintzen *et al.* 2011).

Deep-sea Mediterranean studies on dietary habits, niche overlap, and more generally, trophic dynamics, were conducted traditionally by stomach content analyses (Carrassón & Matallanas 1998, Carrassón & Cartes 2002). However, the collecting methods for megafauna at high bathyal depths do not permit the retrieval of an acceptable number of useful samples, because of the high number of everted stomachs caused by decompression shock. Other issues impair completeness in dietary studies of deep-sea species, namely (i) extremely scant information about ingestion rates, (ii) overestimation of preys that leave hard structures such as fish otoliths or cephalopods beaks, and (iii) relatively low number of samples for diet descriptions to be credible.

In the last decades, the use of stable isotopes (especially $\delta^{13}C$ and $\delta^{15}N$ values) became an important tool in marine ecosystem studies (reviewed by Layman *et al.* 2012). Because of the isotopic discrimination in metabolic processes, the nitrogen isotope ratio increases by 2.3 to 5‰ with each trophic step, while the carbon isotope ratio of a consumer is only slightly enriched (i.e. between 0 and 1.5‰) with respect of that of its food (Fry 2006). The carbon isotope is therefore an indicator of the sources of organic matter, while the nitrogen isotopes ratio inform on the trophic position of the individual in the food web (Peterson & Fry 1987, Post 2002).

As carbon and nitrogen stable isotopes are indicators of both the sources of organic matter and the structure of food-web flows, they provide two dimensions of observation that are linkable with ecological concepts such as the trophic niche (Newsome *et al.* 2007). The trophic niche can be defined as an n-dimensional hyper-volume representing the *role* of a particular species in the trophic web (Hutchinson 1957). Carbon and nitrogen stable isotope ratios are usually plotted in an isotopic bi-space; the variability of the samples has been demonstrated to be an indicator of the trophic niche width of the analysed individuals (Bearhop *et al.* 2004). Quantitative metrics based on the geometric distribution of carbon and nitrogen isotopes ratios in the 2-dimensional space, have been recently developed to evaluate community-wide trophic dynamics such as vertical structuring, species packaging and niche diversification (Layman *et al.* 2007, Jackson *et al.* 2011). Species that adapt by specialising would show a decrease in their occupied area in the bi-space, and an increase of their distance from the other species (i.e. reduced packaging). By contrast, generalisation should be evidenced by a broadening of the carbon isotope signature, and by an increase of their occupied area.

In the present chapter, analyses of carbon and nitrogen stable isotope natural abundances in benthic megafauna were applied, to compare nine sites of the deep Mediterranean Sea with different oligotrophy, i.e. in the three basins and at three different depths. The scientific question addressed was: What are the adaptations, in terms of trophic dynamics, of megabenthic species in the deep Mediterranean when exposed to gradients – depth and longitude – of varying organic matter availability?

MATERIALS AND METHODS

Sampling procedures

All samples were collected in June 2009 in the three basins of the deep Mediterranean Sea (western, central, and eastern basins) at three depths (1200, 2000, and 3000 m) in each of the three basins (see Chapter 2 for details). In the western basin, we sampled the southern Balearic region (code WM), in the central basin we sampled the western Ionian Sea (code CM) while the southern Cretan Sea was considered for the eastern basin (code EM). Nektobenthic and benthopelagic megafauna were captured with the otter-trawl Maireta system (OTMS), while strictly benthic species were captured with the Agassiz dredge (see Chapter 2). Muscle samples without skin (in the case of fishes) and without exoskeleton (in the case of crustaceans) from individuals of all collected species were immediately retrieved after sorting on board and were immediately frozen at -20 °C until their isotopic determination.

At the same sampling stations, pelagic microplankton (size range: 53 - 200 µm) and mesozooplankton (size range: 200 - 2000 µm) samples were also collected by using WP2 plankton nets in vertical hauls from 200 m depth to the surface. Plankton samples were filtered on board, on G/FC glass microfiber filters. Macroplankton from the deep scattering layer (identified by echo sounding) was additionally collected with an Isaaks-Kidd Midwater Trawl (IKMT), and selected species from each sample were pooled together. Similarly to megafauna samples, all micro-, meso- and macro-plankton samples were stored frozen at -20 °C until their isotopic determination.

Isotopic analysis

Stable isotopes analyses were conducted at the dedicated research facility of the Netherlands Institute for Sea Research (NIOZ), in Yerseke, the Netherlands. In the laboratory, all samples collected were freeze-dried for 48 hours and grounded to a fine powder. Drop-by-drop acidification with diluted HCl (0.1 M) was performed only on suprabenthos and plankton samples, with no water rinsing afterwards, to remove the calcium shells (Jacob *et al.* 2005).

Stable isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$, and organic carbon and nitrogen content were measured simultaneously on a Flash EA 1112 coupled to a DeltaV Advantage IRMS (Thermo Electron Instruments). Monitoring of CO_2 (m/z = 44 and 45) and N_2 (m/z = 28 and 29) ion currents of samples against standards with known C and N content allowed accurate measurement of organic carbon and nitrogen contents to determine C/N ratios. $\delta^{13}C_{org}$ and $\delta^{15}N_{tot}$ values are expressed relative to Vienna Pee-Dee Belemnite and air and normalized to $\delta^{13}C$ and $\delta^{15}N$ of USGS40 and USGS41 (Qi *et al.* 2003). All measurements were corrected for blanks, and NIOZ laboratory standards were run alongside each 96-samples plate. Values of $\delta^{13}C$ and $\delta^{15}N$ were pooled by species, and considered separately in each of the sampled sites.

Isotopic metrics and trophic levels

Megafauna was split between fishes and invertebrates (mainly crustaceans) species. The community-wide metrics applied are described and validated in detail by Layman *et al.* (2007). Briefly, the following indices were considered:

- $\delta^{15}N$ range (dNR): expresses the distance between the most ^{15}N-enriched and the most ^{15}N-depleted samples in the community, and is an indicator of its vertical structuring (i.e. trophic length).
- $\delta^{13}C$ range (dCR): the equivalent of dNR considering ^{13}C, provides an indicator of the diversity of basal food resources.
- Total convex hull area (TA): the area of the smallest convex polygon containing all species in the isotopic bi-space. TA is correlated with the total niche amplitude of the food web.
- Mean distance to centroid (CD): the average Euclidean distance of each species to the $\delta^{13}C$ - $\delta^{15}N$ centroid (which is the mean $\delta^{13}C$ and $\delta^{15}N$ value of the entire food web). It correlates positively with the trophic niche amplitude and with the spacing between species.

Community-wide indices were calculated using the *SIAR* package for the R statistical language ("Stable Isotope Analysis in R"; Parnell *et al.* 2010). As sample sizes differed among sites, a Bayesian approach was adopted to propagate uncertainty in the mean values of the metrics using 10^4 randomly calculated communities (Jackson *et al.* 2011). Differences of metrics between sites were tested with Kruskal-Wallis non-parametric tests. Bayesian isotopic ellipses (SEA) for each site were calculated, considering only benthic megafauna, and differences in ellipses areas were analysed with Tukey's HSD tests.

The trophic level ($TL_{consumer}$) of each individual was estimated using the equation:

$$TL_{consumer} = TL_{basal} + {(\delta^{15}N_{consumer} - \delta^{15}N_{prey})}\Big/{\Delta\delta^{15}N}$$

$\delta^{15}N_{prey}$ and $\delta^{15}N_{consumer}$ were, respectively, the isotopic values of microplankton and individual fish or crustacean obtained in the present study (at each site). A basal trophic level (TL_{basal}) of 1.5 was applied, assuming that microplankton (mostly composed by phytoplankton) possesses a trophic level between 1 of the primary producers and 2 of micro- and mesozooplankton (Costalago *et al.* 2012). A value of $\Delta\delta^{15}N$ = 3.5‰ was used as the isotopic discrimination factor for nitrogen (Post 2002).

Environmental variables

A series of environmental variables, collected at exactly the same sites of the megafauna samples, were also recorded. Values for these variables were taken directly from the study in Chapter 3, and the variables considered were: benthic temperature (°C), benthic salinity, benthic turbidity (Formazin turbidity units, FTU), surface fluorescence (relative fluorescence units, RFU), sediment grain size (% of coarse fraction, >63 μm), sediment particulate organic carbon (POC, % of mass), microplankton biomass (mg m^{-3}), and mesozooplankton biomass (mg m^{-3}). The mean values of fluorescence between 0 and 150 m depth were used as an estimator of surface primary production, as the data was collected with the same CTD and protocols throughout the sampling cruise. Biomass of microplankton and mesozooplankton was integrated between 0 and 200 m depth in the water column. A complete description of the collection of these environmental variables is given in Chapter 3.

Statistical analysis

One-way ANOVA tests, followed by pair-wise Tukey's Honestly Significant Difference (HSD) tests, were applied to test for differences between depths and sites in: $\delta^{13}C$ and $\delta^{15}N$ means, and species mean TL values. Non-parametric Kruskal-Wallis tests were used to compare means of isotopic metrics between depths and basins. Spearman rank correlation tests were used to identify correlations between environmental variables and $\delta^{13}C$ and $\delta^{15}N$ means, and isotopic metrics.

RESULTS

The collected megafauna were mainly composed by Actinopterygii fishes and decapods crustaceans, and the number of specimen analysed in each site ranged from 33 to 108. Microplankton and mesozooplankton occupied the lower portion of the bi-isotopic space in all sites, segregated from the species in the benthic domain (Tables 5.1 and 5.2). Mesozooplankton values of $\delta^{15}N$ were higher than microplankton values in the same site, showing the natural isotopic enrichment with trophic level (Figure 5.1). This pelagic enrichment was not visible at the Eastern site at 1200 m depth, which could be caused both by the reduced number of samples and by the sampling position: samples were collected in the north (1200 m depth) and south (2000 and 3000 m depth) of the island of Crete, thus with potentially two differing food input regimes.

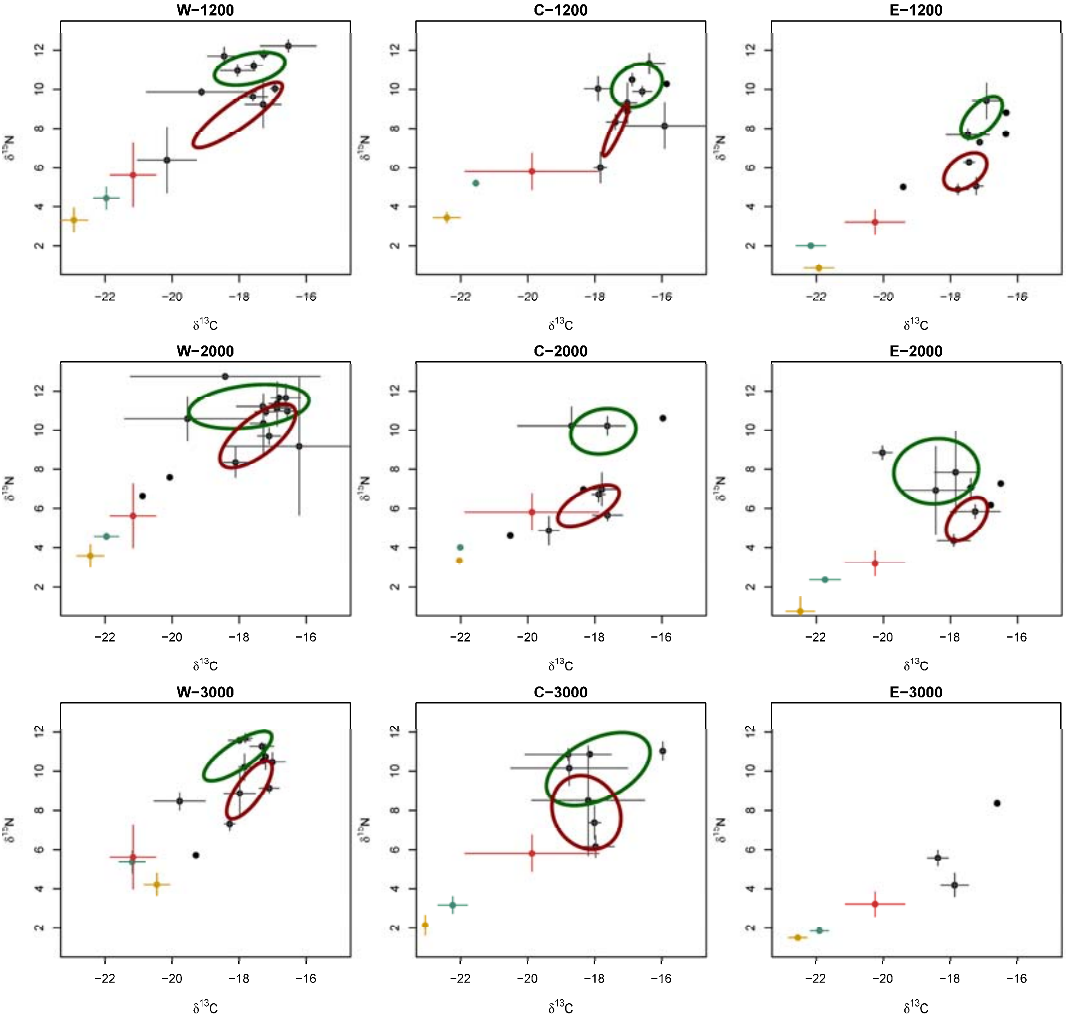


Figure 5.1. Mean $\delta^{13}C$ and $\delta^{15}N$ values for megafauna and plankton components in the 3 basins of the Mediterranean Sea at 3 different depths. Mean Bayesian isotopic ellipses for each site are drawn separately for fishes and crustaceans of benthic megafauna. Colour codes: yellow – microplankton, red – mesozooplankton, green – mesopelagic macroplankton, grey – demersal megafauna.

Carbon isotopic ($\delta^{13}C$) ratios did not show statistical differences between sites, when considering the whole assemblage (1-way ANOVA, $p > 0.05$). By testing against depth, $\delta^{13}C$ ratios showed significant differences between the shallower site (1200 m depth) and the deeper layers of 2000 to 3000 m depth (Tukey HSD, $p = 0.006$), which instead grouped together (Tukey HSD, $p = 0.063$). Considering assemblages as a whole, $\delta^{15}N$ values significantly decreased with longitude (1-way ANOVA, $p < 0.001$), while $\delta^{15}N$ values presented no significant differences between depths over the same site. Crustacean $\delta^{15}N$ mean values were significantly higher in the western basin, than in both the central and eastern basins (Tukey HSD, $p = 0.005$ for W-C and $p < 0.001$ for W-E).

The estimated trophic level (TL) of fish assemblages did not show any significant differences with depth and longitude. On the contrary, the mean TL of the crustacean assemblages from the western and eastern basins was higher mean than those of the central basin crustacean assemblages. When considering the whole assemblage, the central and the eastern basin grouped together (Tukey HSD, $p = 0.836$), with a mean TL for the whole community lower than the mean TL of the western basin community (Tukey HSD, $p = 0.019$).

Table 5.1. Isotopic ratios (mean ± S.D.) for carbon and nitrogen in microplankton, mesozooplankton, and macroplankton of the deep scattering layer (DSL, available only by basin) and values of calculated community metrics for demersal megafauna.

	WM-1200	**WM-2000**	**WM-3000**	**CM-1200**	**CM-2000**	**CM-3000**	**EM-1200**	**EM-2000**	**EM-3000**
Plankton									
Microplankton $\delta^{13}C$	-22.93 ±0.41	-22.44 ±0.40	-20.45 ±0.38	-22.41 ±0.39	-22.04	-23.06 ±0.03	-21.92 ±0.44	-22.48 ±0.42	-22.55 ±0.27
Microplankton $\delta^{15}N$	3.31 ±0.62	3.59 ±0.56	4.21 ±0.57	3.44 ±0.26	3.34	2.13 ±0.50	0.85 ±0.20	0.74 ±0.71	1.49 ±0.08
Mesozooplankton $\delta^{13}C$	-21.96 ±0.38	-21.96 ±0.36	-21.19 ±0.39	-21.54 ±0.09	-22.01 ±0.07	-22.24 ±0.43	-22.16 ±0.44	-21.74 ±0.46	-21.91 ±0.27
Mesozooplankton $\delta^{15}N$	4.44±0.57	4.56±0.14	5.37±0.56	5.21±0.15	4.01±0.04	3.16±0.42	2.01±0.08	2.37±0.01	1.84±0.04
DSL Macroplankton $\delta^{13}C$		-21.16±0.67			-19.88±1.99			-20.52±0.88	
DSL Macroplankton $\delta^{15}N$		5.63±1.63			5.81±0.93			3.21±0.62	
Community metrics									
$\delta^{15}N$ range (dNR)	7.42	6.33	6.13	6.68	7.04	5.98	5.43	6.21	4.94
$\delta^{13}C$ range (dCR)	6.13	7.85	3.83	3.28	4.55	4.94	3.15	3.86	2.03
Total hull area (TA)	24.79	35.87	12.84	12.87	22.95	20.83	8.47	15.71	3.90
Mean distance to centroid (CD)	1.52	1.78	1.60	1.45	2.02	2.28	1.55	1.83	1.19
Mean nearest neighbour distance (NND)	0.43	0.44	034	0.35	0.50	0.72	0.32	0.48	0.86

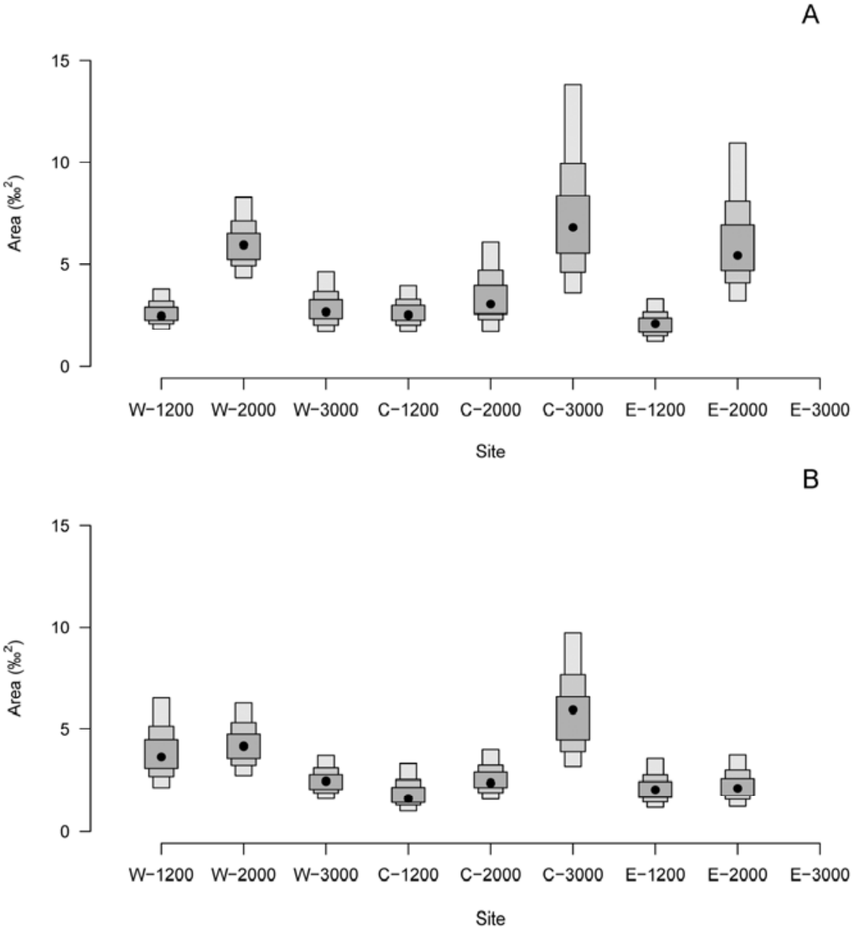


Figure 5.2. Area of isotopic Bayesian ellipses for demersal fishes (A) and crustaceans (B) in the 3 basins of the Mediterranean Sea (W, C, E) at 3 different depths (1200, 2000, and 3000 m). Black dots represent the simulated median. Boxes represent the 50%, 75% and 95% confidence intervals (dark grey to light grey, respectively).

Community-wide indices (Table 5.1) were calculated with both traditional (exact, no variability) and Bayesian methods. Values of all indices obtained by Bayesian estimation showed no significant differences between sites (Kruskal-Wallis test, $p \sim 1.00$ for all contrasts). $\delta^{15}N$ ranges were the highest in the Central Mediterranean deep site (C-deep, dNR = 15.84), while in the other sites they ranged from 5.43 to 7.42.

Ranges of $\delta^{13}C$ for benthic megafauna were comparable between sites, with all ellipses contained between values of -19 and -16.5. Values of dCR ranged from 3.15 to 7.85 across the whole basin. By contrast, the absolute values of $\delta^{15}N$ were lower in the Eastern basin (3.42 – 10.26) than in the other basins (Western basin was 7.00 – 12.95, Central was 4.03 –

19.87). Values of CD calculated exactly were always higher in the deepest sites than in the slope sites, suggesting an increase of the distance of species niches with increasing depth. This can be related to the difference in the TA, which, in the central basin, was the highest of the dataset.

The area of Bayesian isotopic ellipses did not show any appreciable pattern with depth and basin, although when considering fishes alone, areas were the lowest in the shallowest sites of each basin (i.e. at 1200 m depth, Figure 5.2). The same is true for the traditional equivalent, the Total Convex Hull Area (TA). As these values do not include a variance expression, statistical testing was not possible; however, we can consider TA and the area of Bayesian ellipses to convey the same ecological meaning.

Pearson correlation tests yielded no significant relationships between SEA ellipse areas of each depth and locality and any environmental variable. The total hull area of the assemblages (TA) and the range of dCR of each depth and site were positively correlated with both surface microplankton biomass (TA: $R^2 = 0.55$, $p = 0.02$. dCR: $R^2 = 0.58$ and $p = 0.01$) and surface fluorescence (TA: $R^2 = 0.49$, $p = 0.03$; dCR: $R^2 = 0.57$, $p = 0.01$).

DISCUSSION

Studies comprising the whole longitudinal axis of an enclosed deep sea are scant. The Mediterranean Sea presents a distinct environment in each of its three basins, which renders it particularly interesting to test ecological hypotheses across environmental gradients (Azov 1991, Bethoux *et al.* 1999, Danovaro *et al.* 1999). The continental slope areas in the Mediterranean are considered oceanographically dynamic, influenced by local-scale surface events such as river input and coastal atmospheric events (Company *et al.* 2008, Sanchez-Vidal *et al.* 2012). By contrast, the deepest areas considered in the present study (2000 - 3000 m depth) are subject to a considerably lower nutrient input and are thus more oligotrophic (Margalef 1985, Sardà *et al.* 2004a). This factor, coupled with the longitudinal gradient, leads to two axes of food availability, which have been addressed in this study.

The collected megafauna was a fair representation of the majority of the species in the deep Mediterranean Sea, both in terms of richness and in terms of densities and biomasses. The number of specimen analysed in each site was proportional to the decreasing gradient of megafaunal density found in the Mediterranean along both depth and west-to-east axes (see Chapter 3). The species analysed included nektobenthic fishes (such as the Macrourids and Morids), species that perform vertical benthic-pelagic migrations (e.g. the shrimps *Acanthephyra spp.* and *Aristeus antennatus*, and various components of macroplankton such as myctophids and gonostomatiids) and less-mobile and sessile species, which are strictly connected to the sea floor (i.e. the reptantian crustaceans and non-crustacean invertebrates).

Thus, the community analysed in this study can be considered an image of the benthic domain and of the overlying nektobenthic compartment.

The species that perform wide-ranging vertical migrations in the water column (i.e. the benthopelagic fishes and decapod crustaceans) play a major role in what is called the *downward bentho-pelagic coupling*: the transfer processes from the pelagic domain to the benthos, and the responses of the latter (Smith *et al.* 2006). As stated above, and in contrast to the deep waters of the Atlantic Ocean, megafauna assemblages of the deep Mediterranean Sea are mainly constituted by nektobenthic and benthopelagic species, mainly fishes and decapod crustaceans (Massutí *et al.* 2004). Strictly benthic species (such as molluscs and other low-motility non-crustacean invertebrates) are speciose but not abundant in the deep Mediterranean (Ramírez-Llodra *et al.* 2010b, Tecchio *et al.* 2011). The predominance of benthopelagic species may enhance levels of carbon transfer along the water column and towards the deep seafloor, with respect to the outer Atlantic and, thus, strengthen the downward coupling. In addition, the presence of nektobenthic species also increases the quantity of carbon transported laterally from the adjacent deep seafloor (Mees & Jones 1997).

Deep benthic systems in the Mediterranean Sea are strictly linked to surface productivity, both primary and secondary, and to mesopelagic processes (Chapter 3, and Fanelli *et al.* 2009). In deep-basin areas, the main input of nutrients is performed by direct pelagic sinking of organic carbon, while on continental slopes lateral advection and riverine input processes can also contribute significantly to the quantity of carbon reaching the seafloor (Zuñiga *et al.* 2009). In all sites examined in the present study, the isotopic positions of planktonic components (i.e. microplankton, mesozooplankton, and deep macroplankton) were evidently connected. This indicates a strong link, both in carbon sourcing and feeding relations, between the water-column domain and the demersal compartment.

The microplankton fraction, as sampled in this study, is constituted by both phytoplankton and zooplankton species (Costalago *et al.* 2012). The amplitude of benthic community niche and the spectrum of carbon sources were positively correlated with both the microplankton biomass and the surface fluorescence, the latter being an indicator of primary production. Phytodetritus arrival at the deep sea plays a major role in certain areas of the northeast Atlantic Ocean (Billett *et al.* 1983, Lampitt 1985) or the Pacific Ocean (Smith *et al.* 1994, Smith & Druffel 1998). However, this process has not been considered a main factor in the deep Mediterranean because of its high oligotrophy, and megafauna has been observed ignoring patches of phytodetritus that were experimentally delivered to the seafloor (Jeffreys *et al.* 2011). It is thus not clear how benthic megafauna is adapted to process the phytodetritus input arriving from the surface. In our case, the diversity of basal resources (estimated by the $\delta^{13}C$ range, dCR) varied non-significantly between depths and basins, with slightly increased values in the deep sites with respect to slope sites. This may reflect slightly different sources of organic matter from the surface, generating a wider spectrum of types of carbon reaching the deep seafloor.

Two distinct carbon pathways were observed especially in the Western basin (see Figure 5.1). The more carbon-light pathway consists mainly of benthopelagic species (i.e. the smooth-head *Alepocephalus rostratus*), exploiting suprabenthos and gelatinous plankton, especially the jellyfish *Pelagia noctiluca* (Carrassón & Matallanas 1998). The other pathway comprises demersal species and includes all macrourid fish and benthic crustaceans. This suggests a split of the deep benthic food-web at the suprabenthic level, with a benthic detritus-based chain and a more pelagic-linked one. This phenomenon has been observed also in the deep Pacific Ocean (Drazen *et al.* 2008). It seems that *A. rostratus* is not trophically constrained in the benthic food web but rather it is short-circuiting the bentho-pelagic coupling, feeding directly on plankton migrating from the water column. High quantities of gelatinous plankton that reach the lower slope (Sabatés *et al.* 2010) may be the possible factor explaining the dominance of *A. rostratus* in the demersal community between 1200 and 1350 m depth, in the western Mediterranean basin.

Also noteworthy are the high levels of carbon isotope enrichment with shifts to more positive values in the benthic megafauna with respect to basal components (i.e. surface zooplankton and mesopelagic macroplankton). The enrichment per trophic level stands on the higher end of the ranges usually considered in the literature, which range from 0 to 5.5 ‰ (Fry 1988). The single consistent explanation found in literature of this phenomenon is that as particulate organic matter (POM) sinks in the water column, pelagic components and bacteria perform a metabolic degradation that shifts the carbon isotopic ratio to heavier levels (Nadon & Himmelman 2006). If this is the case, then it would be justified by the height of the water column that POM would travel during its fall. This would also be accentuated by the high and constant water temperatures found in the Mediterranean Sea below 200 m depth (13-14 °C), which increase the levels of prokaryotic degradation of organic matter (Tyler 2003).

The isotopic bi-space did not show any particular pattern over depth and longitude, neither with fishes nor with crustaceans. These results confirm the complexity of the food webs of the deep benthonic and supra-benthonic communities. Results found in the deep western Pacific Ocean suggested that species specialism increased with depth and environmental stability, creating clusters of taxonomically-related species that share common environmental niches (Zintzen *et al.* 2011). This would be in accordance with other results in the western Mediterranean Sea (Carrassón & Cartes 2002), which showed cluster of species overlapping in diet composition, and suggest an important path for further studies. It is still a matter of speculation whether the changing trophic conditions over large geographic scales may modulate the response of the whole deep-sea benthic communities. In a study conducted at the Porcupine Abyssal Plain in the deep Atlantic ocean, it is explained that competition may be reduced by either increasing niche specialization or by vertically expanding the trophic structuring (Iken *et al.* 2001). In the case of the Mediterranean Sea, it remains to be proven whether the increase in generalist trophic habits of the benthos would help to reduce competition for resources.

Conclusions

Deep-sea ecosystem structure and functioning have been related to seasonality, mainly determined by intra-annual variations of surface primary productivity and climate-driven atmospheric events. In this chapter, we concluded that (i) no clear pattern of trophic niches can be observed over large spatial scales for the deep Mediterranean megabenthos, and (ii) primary and secondary production processes at the surface are the drivers of choice for possible detailed studies on this topic.

This is the first study to directly address the patterns of niche width in the deep-sea benthos over such a large spatial scale. It ultimately follows that trophic relations in such food-limited systems are far from being understood in detail, and that other ecosystem-wide approaches will surely be needed to address these topics in the near future.

Table 5.2. Values of δ^{13}C and δ^{15}N for all sampled benthic megafauna (Mean ± S.D.), along with the measured carbon/nitrogen ratio (C:N) and the number of analysed specimen.

Site	*Species*	δ^{13}C	δ^{15}N	C:N	(n)
WM-1200					
	Alepocephalus rostratus	-19,12 ± 1,64	9,86 ± 0,19	4,81	4
	Aristeus antennatus	-17,59 ± 0,43	9,61 ± 0,2	3,67	4
	Bathypterois mediterraneus	-18,05 ± 0,51	10,97 ± 0,29	3,88	7
	Galeus melastomus	-16,93 ± 0,13	10,04 ± 0,21	3,01	4
	Geryon longipes	-17,29 ± 0,53	9,22 ± 1,16	3,77	6
	Lepidion lepidion	-17,56 ± 0,26	11,2 ± 0,25	3,89	4
	Meganyctiphanes norvegica	-20,53 ± 0,34	5,19 ± 0,57	4,00	5
	Mora moro	-17,27 ± 0,13	11,78 ± 0,23	3,80	4
	Nettastoma melanurum	-18,45 ± 0,49	11,7 ± 0,46	5,30	2
	Nezumia sclerorhynchus	-16,54 ± 0,83	12,22 ± 0,31	3,64	4
	Opistoteuthis calypso	-18,38 ± 0,11	7,67 ± 0,53	4,05	2
	Trachyrincus scabrus	-16,86 ± 0,58	10,25 ± 0,41	3,63	4
	Suprabenthos	-20,16 ± 0,88	6,39 ± 1,67	4,34	3
WM-2000					
	Acantephyra eximia	-18,11 ± 0,35	8,35 ± 0,76	3,80	4
	Alepocephalus rostratus	-19,54 ± 1,87	10,59 ± 1,12	5,18	8
	Aristeus antennatus	-17,28 ± 0,4	10,35 ± 1,5	3,63	4
	Bathypterois mediterraneus	-17,3 ± 0,78	11,2 ± 0,2	3,83	4
	Bonellia viridis	-16,21 ± 2,21	9,18 ± 3,51	4,73	2
	Cataetyx laticeps	-18,3 ± 2,97	12,75 ± 0,16	9,66	5
	Centroscymnus coelolepis	-16,87 ± 0,25	11,35 ± 1,13	2,99	4
	Chaceon mediterraneus	-16,81	11,66	3,87	1
	Chauliodus sloani	-18,39	8,31	4,21	1
	Coryphaenoides guentheri	-16,87 ± 0,43	11,11 ± 0,47	3,76	5
	Coryphaenoides mediterraneus	-16,56 ± 0,41	10,97 ± 0,36	3,74	5
	Lampanyctus crocodilus	-20,07	7,59	4,46	1
	Lepidion lepidion	-16,61 ± 0,44	11,65 ± 0,71	3,63	5
	Nematocarcinus exilis	-17,11 ± 0,34	9,7 ± 0,42	3,83	4
	Sergestes arcticus	-20,71 ± 0,58	6,4 ± 0,75	3,89	2
	Sergia robusta	-19,85 ± 0,25	7,16 ± 0,48	3,71	2
	Stereomastis sculpta	-17,2 ± 0,33	10,94 ± 0,22	3,78	4
	Suprabenthos	-20,87	6,63	4,87	1
WM-3000					
	Acantephyra eximia	-17,98 ± 0,47	8,87 ± 1,12	3,68	4
	Acantephyra pelagica	-18,28 ± 0,16	7,33 ± 0,34	3,65	4
	Argyropelecus hemigymnus	-20,52	7,80	4,81	1
	Aristeus antennatus	-17,29	10,53	3,66	1
	Bathypterois mediterraneus	-17,99 ± 0,33	11,58 ± 0,17	3,73	4
	Centroscymnus coelolepis	-17,82 ± 0,2	11,69 ± 0,23	3,31	2

	Chaceon mediterraneus	-17,84 ± 0,17	10,21 ± 0,67	3,81	3
	Coryphaenoides guentheri	-17,21 ± 0,03	10,74 ± 0,66	3,86	2
	Coryphaenoides mediterraneus	-17,32 ± 0,35	11,27 ± 0,18	3,66	4
	Gennadas elegans	-20,89 ± 1,69	5,48 ± 0,28	5,95	4
	Lampanyctus crocodilus	-19,77 ± 0,76	8,46 ± 0,44	4,15	3
	Nematocarcinus exilis	-17,1 ± 0,29	9,14 ± 0,24	3,64	4
	Pelagia noctiluca	-19,14 ± 0,96	5,32 ± 0,47	3,46	4
	Stereomastis sculpta	-17,01 ± 0,38	10,47 ± 0,48	4,29	4
	Suprabenthos	-19,29	5,72	4,58	1
CM-1200					
	Acantephyra pelagica	-17,83 ± 0,19	6,01 ± 0,78	3,75	3
	Argyropelecus hemigymnus	-18,41	6,76	3,64	1
	Aristeus antennatus	-17,39 ± 0,26	8,34 ± 0,37	3,68	4
	Bathypterois mediterraneus	-17,9 ± 0,4	10,03 ± 0,62	3,80	4
	Chauliodus sloani	-17,75 ± 0,64	7,07 ± 0	3,91	2
	Chimaera monstrosa	-14,29	11,16	2,74	1
	Coelorincus mediterraneus	15,86	10,28	3,57	1
	Etmopterus spinax	-17,03 ± 0,29	9,29 ± 1,02	3,00	3
	Galeus melastomus	-15,92 ± 1,23	8,14 ± 1,16	2,88	2
	Hexanchus griseus	-14,52	10,58	2,98	1
	Lampanyctus crocodilus	-19,58 ± 1,31	8,09 ± 0,47	5,51	5
	Lepidion lepidion	-16,89 ± 0,03	10,49 ± 0,32	3,78	2
	Mora moro	-16,59 ± 0,28	9,89 ± 0,26	3,72	4
	Nettastoma melanurum	-17,54 ± 1,29	9,8 ± 0,66	4,57	3
	Nezumia sclerorhynchus	-16,38 ± 0,46	11,32 ± 0,52	3,68	7
	Notacanthus bonaparte	-17,57 ± 1,42	8,5 ± 0,55	5,53	4
	Phycis blennoides	-16,00	12,51	3,70	1
	Polycheles typhlops	-17,03 ± 0,12	8,87 ± 0,13	3,62	4
	Sergestes corniculum	-19,81 ± 0,32	3,79 ± 0,44	3,63	3
	Sergia robusta	-19,13 ± 0,47	5,96 ± 0,68	3,74	8
	Stomias boa	-17,23	8,51	3,70	1
CM-2000					
	Acantephyra eximia	-17,89 ± 0,19	6,71 ± 0,38	3,76	4
	Acantephyra pelagica	-19,25 ± 0,31	4,67 ± 0,9	4,16	2
	Aristeus antennatus	-17,79 ± 0,48	6,97 ± 0,84	3,81	6
	Bathypterois mediterraneus	-17,63 ± 0,32	10,23 ± 0,47	3,69	6
	Coryphaenoides guentheri	-15,97	10,62	3,73	1
	Coryphaenoides mediterraneus	-18,69 ± 1,61	18,6 ± 1,8	3,72	2
	Isopoda sp.	-18,46	6,46	4,43	1
	Lampanyctus crocodilus	-20,46	5,57	4,37	1
	Leptocephalus larvae	-21,6 ± 0,16	3,91 ± 0,93	5,04	2
	Lepidion lepidion	-18,34	6,97	3,62	1
	Nematocarcinus exilis	-17,62 ± 0,44	5,66 ± 0,28	4,01	4
	Suprabenthos	-20,52	4,61	5,32	1

CM-3000					
	Acantephyra eximia	-18,01 ± 0,18	7,38 ± 0,84	3,86	4
	Bathypterois mediterraneus	-18,15	10,87	3,95	1
	Cataetyx laticeps	-18,79 ± 1,28	10,85 ± 0,3	6,10	3
	Chaceon mediterraneus	-18,77 ± 1,74	10,15 ± 0,87	5,76	4
	Chauliodus sloani	-19,10	5,03	3,95	1
	Coryphaenoides guentheri	-15,98 ± 0,09	11,04 ± 0,46	3,78	2
	Coryphaenoides mediterraneus	-18,2 ± 1,68	8,5 ± 2,79	3,79	3
	Melanostigma atlanticum	-18,56	8,51	4,40	1
	Nematocarcinus exilis	-17,98 ± 0,55	6,16 ± 0,56	3,77	4
	Phronima sedentaria	-19,13	3,27	4,47	1
	Pyrosoma atlanticum	-21,75 ± 0,05	1,59 ± 0,06	6,14	3
EM-1200					
	Acantephyra eximia	-17,78 ± 0,31	4,89 ± 0,26	3,75	2
	Argyropelecus hemigymnus	-19,67	3,66	3,80	1
	Aristeus antennatus	-17,45 ± 0,16	6,28 ± 0,07	3,89	4
	Bathypterois mediterraneus	-17,48 ± 0,64	7,68 ± 0,3	3,68	6
	Dalatias licha	-16,79	7,81	2,97	1
	Galeus melastomus	-16,35	7,72	2,77	1
	Mora moro	-16,34	8,81	3,79	1
	Nettastoma melanurum	-16,71	7,72	4,15	1
	Nezumia sclerorhynchus	-16,93 ± 0,42	9,41 ± 0,9	3,80	7
	Plesionika acantonotus	-17,24 ± 0,2	5,06 ± 0,44	3,66	3
	Polycheles typhlops	-17,13 ± 0	7,3 ± 0,09	3,72	2
	Pyrosoma atlanticum	-21,39 ± 0,16	1,22 ± 0,7	7,26	2
	Sergia robusta	-19,28	3,39	3,62	1
	Suprabenthos	-19,41	5,01	4,39	1
EM-2000					
	Acantephyra eximia	-17,26 ± 0,74	5,84 ± 0,35	3,70	4
	Argyropelecus hemigymnus	-18,62	4,89	3,63	1
	Aristeus antennatus	-17,4 ± 0,11	7,06 ± 0,47	3,82	2
	Bathypterois mediterraneus	-17,85 ± 0,63	7,84 ± 2,1	3,76	5
	Cataetyx laticeps	-20,03 ± 0,29	8,86 ± 0,34	10,14	3
	Coryphaenoides mediterraneus	-16,50	7,27	3,66	1
	Leptocephalus larvae	-21,04	1,40	5,39	1
	Lepidion lepidion	-18,44 ± 1,06	6,92 ± 2,25	3,87	2
	Nematocarcinus exilis	-17,91 ± 0,48	4,36 ± 0,29	4,24	5
	Pasiphaea multidentata	-17,76	4,52	3,719243	1
	Polycheles typhlops	-16,8	6,17	3,568011	1
	Sergestes corniculum	-19,24 ± 0,31	2,43 ± 0,1	3,832676	3
EM-3000					
	Acantephyra eximia	-18,38 ± 0,31	5,58 ± 0,39	3,918422	4
	Coryphaenoides mediterraneus	-16,6	8,36	3,788555	1
	Nematocarcinus exilis	-17,88 ± 0,41	4,18 ± 0,61	4,06055	4

Food web structure and vulnerability

6

INTRODUCTION

The deep sea represents an optimal benchmark for exploring the functioning of marine systems. Deep-water ecosystems are known to host a large reserve of biodiversity, the vast majority of which remains unexplored (Danovaro *et al.* 2010, Ramírez-Llodra *et al.* 2010a). The extent of the relation between deep-sea biodiversity and the possible ecosystem services it supplies is yet to be clarified (Loreau 2008), but it is widely accepted that, under the precautionary principle, significant measures of conservation should be applied to avoid population collapses (Norse *et al.* 2012).

Fisheries have been expanding their footprint on marine ecosystems over the last five decades (Coll *et al.* 2008, Swartz *et al.* 2010). Commercial exploitation of benthic resources has been shifting in the last decades from the continental shelf deeper onto the continental slope (Morato *et al.* 2006). This shift has raised concerns on the degree of vulnerability of deep-sea fishing resources and associated species. It is generally accepted that many deep-water fish species present biological characteristics that make them especially vulnerable to fishing exploitation: K-type life-history traits, low fecundity, and aggregation behaviour in restricted topographic areas (Merrett & Haedrich 1997, Koslow *et al.* 2000). Due to their low productivity but their generally relative high levels of biomass, these species are subject to a consistent fishing interest while, at the same time, they are unable to support high levels of exploitation (Norse *et al.* 2012). Important deep-water fishing stocks, such as in the Atlantic Ocean (Large *et al.* 2003), are currently considered to be harvested beyond safe biological limits. No current method of fishing regulation has been able to act efficiently and prevent the overexploitation of deep-sea fisheries so often observed in recent decades (Roberts 2002).

The Mediterranean Sea presents particular conditions that increase concerns about management and conservation. These include high human densities at the coasts, a long history of exploitation of marine resources, and a bounded general water circulation that renders it particularly vulnerable to contamination and, more generally, to human impact (Coll *et al.* 2010, Ramírez-Llodra *et al.* 2011). Moreover, the Mediterranean Sea is the only enclosed sea for which a complete ban has been approved for commercial trawling below 1000 m depth (EC Regulation 1967/2006). This ban was originally approved in a period when no fishing activities were made below that depth; therefore no substantial enforcing solutions were put in place. There is thus concern that increasing pressure from the fishing industry will eventually lead to a *de facto* lifting of the ban, opening the lower continental slope to exploitation of the commercially important resources that can be found at those depths, especially the highly-valued Mediterranean red shrimp *Aristeus antennatus*.

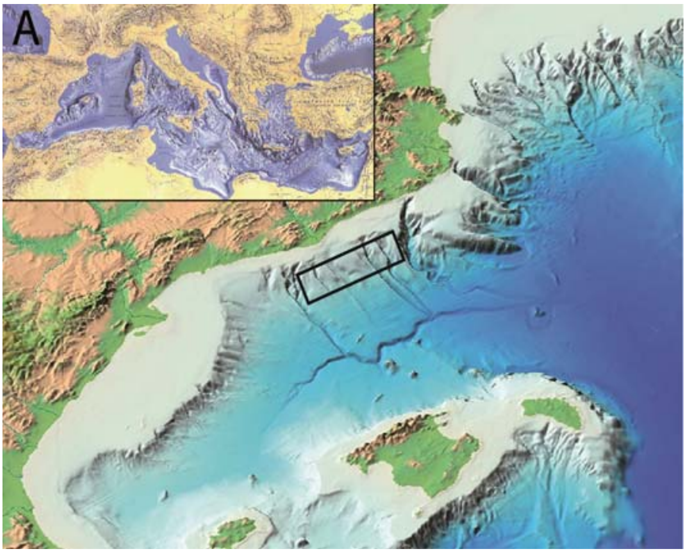
Yet, the absence of trawl fishing below 1000 m depth in the Mediterranean Sea offers a unique opportunity to study quasi-pristine environments (WWF/IUCN 2004). Ecosystem-wide study approaches in non-fished Mediterranean areas have recently been called for (Coll & Libralato 2012). Analyses of areas not subjected to fishing pressure permit focusing on the impact of species interactions and environmental factors, usually masked by fishing effects in coastal and shelf areas, and provide a solid baseline if in the future fishing activities expand to deeper habitats.

In the framework of ecosystem studies, a modelling approach can be pivotal in determining how various compartment interactions may change in response to environmental gradients and globally changing processes (Soetaert & Van Oevelen 2009). This is especially true for deep-sea areas, for which an endemic scarcity of data is present due to extreme difficulties in exploration and sampling. Among the various modelling platforms available, the *Ecopath with Ecosim* (EwE) approach and software has been intensively developed over the last three decades and it is now successfully applied throughout the world (Polovina 1984, Christensen & Walters 2004). One important advantage of EwE over other approaches is its capability of parameterisation with a relatively limited pool of data. In the Mediterranean Sea, the Ecopath with Ecosim modelling approach has been applied in numerous cases to address fisheries questions (e.g., Pinnegar & Polunin 2004, Coll *et al.* 2006, Pranovi & Link 2009, Tsagarakis *et al.* 2010), to evaluate environmental and other human-related impacts (e.g., Coll *et al.* 2009, Piroddi *et al.* 2011), and to study the general trophic structure of marine communities (e.g., Coll *et al.* 2007, Navarro *et al.* 2011).

The European Science Foundation-funded BIOFUN and the Spanish PROMETEO research projects conducted studies during 2009 of the deep open slope system of the Catalan continental margin, in the north-western Mediterranean. The sampled area spanned from 900 to 2700 m depth. A concentration of benthic biomass was found around the 1200 m depth boundary, and this represented a peak in biomass for the western Mediterranean continental margins. Possible explanations for this peak included ecological forcings such as depth-range ecotone-like effects, increased deposit of organic matter at those depths, and/or specific water mass circulation conditions (see Chapter 4). Therefore, the biomasses retrievable at around 1200 m depth may be of particular interest for fisheries in the area.

In this chapter, the field data collected under the present research work was used to model a snapshot of the biomasses and flows in a deep western Mediterranean ecosystem, with the objectives to (a) study the trophic structure of a deep continental slope system in the north-western Mediterranean Sea, (b) analyse the ecological role of the main species in the ecosystem, and (c) identify potential consequences in the event of a fishery being developed in the area to exploit deep-sea resources. This is, to my knowledge, the first modelling attempt for a deep Mediterranean Sea ecosystem.

Figure 6.1. Map of the Catalan-Balearic basin (north-western Mediterranean Sea) showing the modelled area (black rectangle)



MATERIALS AND METHODS

Study area

The Catalan continental slope, in the north-western Mediterranean Sea, is one of the most studied deep-sea areas in the world due to its relative closeness to land and thus accessibility, and the presence of a highly profitable trawl fishery (Fig. 6.1). This fishery developed in the 1940s mainly targets the Mediterranean red shrimp *Aristeus antenntatus*, and it has sparked the interest of fisheries scientists since its inception (Margalef 1985, Bas 2002). The red shrimp is the most economically important fishing resource in the western Mediterranean Sea, and it is collected by benthic trawling by a specialized fleet of high-powered vessels (between 400 and 2200 HP, even though the legal maximum power is of 500 HP). This fishery is active year round, has reached bottom depths of 800 m in the last years, and the data indicates an increasing depth trend (Carbonell *et al.* 1999, Sardà *et al.* 2009a).

The depths of 1200-1300 m have been identified as hosting the highest peak in biomass of the whole Catalan continental margin (Chapter 4, and Stefanescu *et al.* 1993). The benthic community is mainly composed of fishes and decapod crustaceans, while the Risso's smooth-head (*Alepocephalus rostratus*) and the common mora (*Mora moro*) dominate the fish community in terms of biomass (Stefanescu *et al.* 1993). The main inputs of organic matter in the area are derived by marine snowing from the upper strata and, on a scale yet to be fully clarified, by the advection of coastal nutrients, both from riverine input and from atmospheric events (Company *et al.* 2008, Sanchez-Vidal *et al.* 2012).

The benthic ecosystem of the northern Catalan continental slope was modelled, to represent an average situation for 2009 and for the depth range between 1000 m and 1400 m depth.

Although the majority of samples were collected in the Blanes open slope area (with a surface area of 100 km^2), it is possible to extrapolate the food web structure to a larger region, from the Blanes canyon to the Foix canyon (in front of the city of Barcelona), with a total covered area of approximately 850 km^2 (Figure 6.1). This wider area presents similar environmental and biological characteristics (Margalef 1985).

Modelling approach

The *Ecopath with Ecosim* (EwE) approach and software platform is widely applied to model aquatic ecosystems. It consists of a snapshot trophic mass-balanced linear model of the ecosystem (module Ecopath) and a time-dynamic module to address temporally changing factors (Ecosim, Walters *et al.* 2000) that can be further developed into a spatial model, named *Ecospace* (Christensen & Walters 2004, Christensen *et al.* 2008). The Ecopath model uses a matrix of linear equations to establish mass balance and estimate mass fluxes between a number of *a priori* established compartments or functional groups (i.e. ontogenetic fractions of a species, single species or group of species sharing common ecological traits and trophic habits). Each group is parameterised with its biomass (B, t km^{-2}), its production rate over biomass (P/B, year^{-1}), its consumption rate over biomass (Q/B, year^{-1}) and the interactions with its prey and predators in the form of a diet matrix (DC).

The Ecopath routine then solves a set of linear equations, based on the following two equations for each group defined in the model:

$$(Eq.1)\; B\left(\frac{P}{B}\right)_i = \sum_j B_j \left(\frac{Q}{B}\right)_j DC_{ij} + Y_i + E_i + BA_i + B_i\left(\frac{P}{B}\right)_i (1 - EE_i)$$

(Eq. 2) Consumption = Production + Unassimilated food + Respiration

Equation (1) expresses the production of the *i*th group as a function of the consumption ratio (Q/B) of its predators (j), of the fishing mortality (Y_i), net migration (E_i), biomass accumulation (BA_i), and the fraction of mortality not explained within the model (1 – EE_i) such as mortality due to disease or old age. The proportion of the production of each group that is used within the system, either exported out of the ecosystem (e.g., by fishing activity) or consumed by predators within it, is called ‘ecotrophic efficiency’ or EE, and, under the assumption of mass-balance; it cannot be > 1. Equation (2) ensures the energy balance of each group.

Parameterisation of the model

A total of 20 functional groups were modelled, including 18 consumer groups and two detritus groups (Table 6.1). Biomass estimates were compiled from the trawl surveys, conducted within this research work. Data came primarily from cruises conducted in May and June. Different sampling gears (Otter-trawl Maireta System, Agassiz dredge, mid-water trawl net) were used depending on the domain of each group: demersal, strictly benthic and benthopelagic, respectively. The P/B and Q/B ratios were estimated using empirical equations or obtained from literature (Appendix 1). The diet matrix was constructed using mainly literature data on stomach content analyses, giving preference to studies from the same area or similar areas (Table 6.2 and Appendix 1).

Steady-state was assumed for the initial Ecopath model parameterisation, with both biomass accumulation and net migration rates set to zero. We defined two detritus groups, one for benthic detrital matter and one for 'marine snow', representing the annual input of particulate organic matter (POC) from the surface strata. An estimate of the input provided by large carcasses was also provided as the annual import for benthic detritus (Appendix 1). For groups that are known to conduct wide vertical migrations or that spend a portion of their time outside the model area, we modelled their migrations by considering a portion of their diet as import for the model (see Appendix 1 for details), and directing a proportion of their detritus production to the 'marine snow' group instead of 'benthic detritus'. For the groups that performed migrations in and out of the modelled area, this was taken into account including a portion of the time out of the area as "import" in the diet matrix parameterisation.

The model was considered balanced when all EEs values were < 1 (i.e. realistic estimates of the missing parameters were calculated), the gross food conversion efficiencies ($g_i = \left(\frac{P}{B}\right) \Big/ \left(\frac{Q}{B}\right) = \frac{P}{Q}$) were in the interval of 0.1-0.3 for most consumer groups (may be lower for top predators), and no violations of energy balance (equation 2) were encountered, i.e. respiration rates were consistent with each group's activity levels (Christensen & Walters 2004). The balancing approach was 'top-down', starting with the top predator groups and moving down the food web to balance inconsistencies. When modifications of the data had to be performed, diet compositions were modified first, and then ratios of P/B and Q/B. Biomasses were considered as less uncertain, thus these were rarely modified during balancing. On the other hand, activity levels of deep-sea species, which determine P/B and Q/B, are still widely unknown (Merrett & Haedrich 1997). For this reason, we considered those parameters more uncertain, as can indeed be expected for output from empirical equations (described in Appendix 1).

Ecopath includes a routine to summarise the quality of the data entered, and estimates an overall Pedigree value between 0 and 1, 0 indicating that data is of low quality, and 1 indicating that data is of high quality (i.e. rooted in local studies and highly precise). For each group, we documented the origin and precision of the source data used to enter B, P/B, Q/B and the diets' matrices, and we used Ecopath default Pedigree parameters to estimate the model Pedigree index (Christensen *et al.* 2008).

Network analysis

Trophic network analysis was performed directly within the EwE platform, after mass-balance was achieved. The trophic level (TL) for each functional group (i) was calculated from its diet, as the weighted average of the trophic levels of its prey; i.e. according to the following equation:

$$(Eq.3)\; TL_i = 1 + \sum_{j=1}^{N} DC_{ij}\, TL_j$$

where DC_{ij} is the fraction of the prey item (j) in the diet of predator (i), and assuming a TL of 1 for detritus groups (marine snow and benthic detritus). The omnivory index (OI) of each consumer group was calculated as:

$$(Eq.4)\; OI_i = \sum_{j=1}^{N} \left(TL_j - (TL_i - 1)\right)^2 \cdot DC_{ij}$$

The OI represents the trophic specialisation of the predator, assuming values close to zero when the consumer is fully specialized, feeding on a single trophic level, and higher values when the predator feeds on several TLs (Pauly *et al.* 1993).

The Mixed Trophic Impact (MTI) routine was applied to evaluate the possible impact of the direct and indirect interactions in the food web. This analysis shows the theoretical impact that an infinitesimal change in biomass of one group would have on the biomasses of all the other groups in the system (Ulanowicz & Puccia 1990). Although this is a static analysis and does not account for temporal scale changes, the MTI can be used as a sensitivity analysis to explore possible impacts of biomass variations and to identify those groups, which may benefit from a refining of their model parameters.

The "keystoneness" index (KS) was calculated for each functional group, to identify which groups possess a high overall effect on the other groups compared to their biomass. Calculations were made according to both indices included in the approach, i.e. indices defined by Libralato et al. (2006) and Power et al. (1996), and plotted against the biomass of each group. The first index ranks higher those functional groups that have both high biomass and high trophic impact on the system, while the second index tends to consider as more important those groups with low biomasses, increasing the keystoneness of rare species (Coll *et al.* 2012).

Flows and biomasses in the system were aggregated by discrete trophic levels, creating a linear food chain in which flows are expressed as entering or exiting a single trophic level

(Lindeman 1942). The result, called "Lindeman spine plot", is useful to breakdown flows and identify which levels carry the predominant part of the biomass transfer. The Lindeman spine plot also includes the fraction of the biomass directed to detritus by each trophic level ('flow to detritus') and the transfer efficiency (TE) from one level to the upper next.

Ecosystem-wide statistics were calculated, including the sum of consumption, exports, respiratory flows, production, and all flows into detritus. The Total System Throughput (TST) was calculated as the sum of all fluxes occurring in the system. We also calculated the System Omnivory Index, defined as the average of the OIs of the individual groups, weighted by the logarithm of each consumer's intake (Pauly *et al.* 1993, Christensen & Walters 2004). The Finn's cycling index (FCI) was calculated as the percentage of all fluxes generated by cycling, and is considered an expression of ecosystem stability and resilience (Finn 1976, Christensen *et al.* 2008).

To put the results of this model within a comparative framework, results from ecosystem-wide statistics are compared with results from other available EwE models of different exploited ecosystems from shallower areas in the Mediterranean Sea: the southern Catalan Sea model (Coll *et al.* 2006) representing a 1994 situation of a neighbouring area of 4500 km^2 in the coastal and continental shelf of the Catalan margin from 50 to 400 m depth, the north-central Adriatic Sea (Coll *et al.* 2007) representing the 1990s of an area of 55.000 km^2 from 10 to 200 m depth, the northern Aegean Sea (Tsagarakis *et al.* 2010) representing an average of the year 2003 of an area of 8374 km^2 from 20 to 300 m depth, and the north-eastern Ionian Sea (Piroddi *et al.* 2010) representing the year 1964 of an area of 1020 km^2 from coastal to 200 m depth.

Time-dynamic simulation

The temporal module Ecosim re-calculates the initial Ecopath snapshot for each time-step, taking into account a series of variations in fishing effort, biomass accumulation, and other external/environmental factors. The Ecosim equation that models the biomass growth rate for each group (i) is:

$$(Eq.5)\ \frac{dB_i}{dt} = g_i \sum_j Q_{ji} - \sum_j Q_{ji} + I_i - (M_i + F_i + e_i)B_i$$

Where I_i and e_i are the immigration and emigration rates, respectively (which in our system were zero), M_i is the natural mortality and F_i is the fishing mortality (Walters *et al.* 1997, Christensen & Walters 2004).

The theoretical basis of Ecosim started with the typical Lotka-Volterra function that represents prey-predator interactions. However, Ecosim is based on the 'foraging arena' theory, by which only a fraction of the biomass of each functional group is available to predators at any given time (Walters & Martell 2004). This reduces the typical chaotic fluctuations that appear if the simulation is run based only on the Lotka-Volterra interactions and permits a fine-tuning of the model responses. The foraging arena concept is modelled within EwE by a key parameter, the 'vulnerability', representing the degree to which an increase in predator biomass will cause mortality for a prey. A low value of vulnerability (i.e. close to 1) will indicate a 'bottom-up' controlled interaction, while a high value of vulnerability will indicate that mortality of the prey is controlled by the predator biomass, as in a 'top-down' control (Christensen & Walters 2004). This parameter is linked to the carrying capacity for the predators.

The modelled ecosystem was considered to be at or close to carrying capacity due to the absence of direct fishing activities. Thus, the vulnerability parameters were set at 1.1 for the top predator groups (bluntnose sixgill shark *Hexanchus griseus*, demersal sharks (*Galeus melastomus, Etmopterus spinax, Dalatias licha*) and the monkfish *Lophius piscatorius*), and at 2.0 for all the other groups.

We modelled the current benthic trawler fishery operating in the shallower area of our case study by using data from the landing records of the Blanes and Palamós ports in 2009. The targeted groups were monkfish, greater forkbeard (*Phycis blennoides*), common mora, cephalopods, Mediterranean red shrimp, mesopelagic crustaceans, and other benthic invertebrates. These groups were assigned a landing ratio with very low values (0.0001 t km^{-2} year^{-1}) to avoid disturbing the initial starting snapshot. In addition, low values of discards for the Risso's smooth-head, benthic crustacean and other benthic invertebrates were assigned based on preliminary data on the shallower-operating trawling fleet (J.B. Company, unpublished data).

We generated a hypothetical expansion of the current red-shrimp fishery inside the modelled area. The target maximum fishing mortality rate was 1.5 year^{-1}, calculated as the mean value between males and females of published data from shallower depths of the same area (Demestre & Lleonart 1993). We simulated an increase of the fishing effort over 10 years, from zero in 2009 to a level so that the red shrimp would receive a fishing mortality of 1.5 year^{-1} in 2019. After reaching this maximum level, the fishing effort was kept stable for another 10 years. This simulation evaluates a situation where the current fishing effort targeting the highly commercial red shrimp at shallower depth would move deeper completely. We then analysed the temporal variations in biomass of the functional groups, the mean trophic level of the catch, and the ratios of fishes and invertebrates over the total living biomass.

RESULTS

Ecopath model

The balanced ecosystem model comprises four trophic levels (Table 6.1) with the topmost functional group, the bluntnose sixgill shark, presenting a TL of 4.34. The group of other "demersal sharks" also has a TL > 4. The other fish groups have a trophic level ranging from 2.76 (benthopelagic fish) to 3.95 (monkfish). Zooplankton groups were classified between 2.05 and 2.62. The flow diagram resulting from the model represents each group with a circle that is proportional to the logarithm of its biomass (Figure 6.2), along with all predator/prey interactions.

The ecotrophic efficiencies of top-consumers after balancing are low overall (compared with standard values), ranged between 0.36 and 0.73, not considering the bluntnose sixgill shark with an EE of 0 due to lack of predation on it. In the mid-web, the EEs are higher (e.g., 0.93 for other demersal fish; 0.94 for benthic invertebrates, crustaceans) underlining the importance of the mid-trophic web relations in the ecosystem. Meiobenthos EE is 0.94, suggesting high biomass turnover rates for this group and that they play an important role as prey for other organisms in the ecosystem.

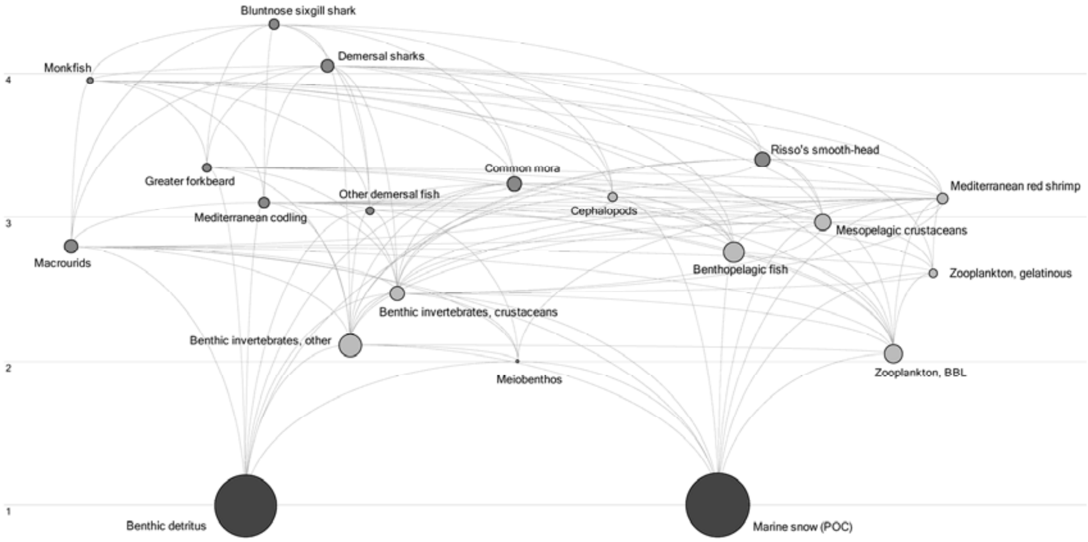


Figure 6.2. The ecosystem in the deep Catalan continental slope, north-western Mediterranean Sea: diagram of functional groups and flows. The circles represent 3-dimensional volumes, the area of which are proportional to the logarithm of group biomasses.

The Pedigree index calculated by the model is 0.542 (measure of fit = 2.58), at the mid-upper end of the range reviewed for recent models (Morissette 2007) and comparable with the Pedigrees of the other published models in the Mediterranean (Table 6.3). The model is thus based on a reasonable quality of source data, especially considering the usual scarcity and fragmentation of data available for deep-sea environments.

The ecological role of species

The omnivory of the modelled groups (Table 6.1), estimated with the Omnivory Index (OI), is low overall (0.05 – 0.51) except for the bluntnose sixgill shark (OI = 0.801). This indicates a general specialisation of the fauna, feeding on a narrow range of trophic levels.

The Mixed Trophic Impact analysis (Figure 6.3) highlights that the upper level consumers such as monkfish, greater forkbeard, Mediterranean codling (*Lepidion lepidion*), and common mora, are influenced by Elasmobranchii (the two shark groups 1 and 2). Demersal sharks exert widespread influence throughout the food web, due to the variety of predation flows they are involved with. Risso's smooth-head shows a strong link with gelatinous zooplankton, its main prey, and is impacted by demersal sharks and monkfish. Macrourids, a dominant fish family in the region, present a diffuse impact pattern, mainly influencing cephalopods and benthic invertebrates. Mesopelagic crustaceans impact mostly the Mediterranean red shrimp and themselves (due to both competition and cannibalism in their diet). Benthic invertebrates (seen as both crustacean and other) influence a wide variety of groups, especially meiobenthos and mid-web fish species. Marine snow (POC) positively impacts the zooplankton groups and non-crustacean benthic invertebrates, while benthic detritus mainly influences Mediterranean codling, macrourids, common mora, meiobenthos, and invertebrate megabenthos. This underlines the importance of the detritus-based interactions across the whole vertical axis of the food web.

The Keystoneness index using Libralato et al. (2006) is the highest for other benthic invertebrates (K = -0.112) and for demersal sharks (K = -0.168), which also present the maximum values of relative total impact (Figure 6.4a). Other groups with relatively high keystoneness and low biomass are the bluntnose sixgill shark and gelatinous zooplankton. Since the biomass of other benthic invertebrates is high, this group is likely a key structuring group in the ecosystem, rather than a keystone group.

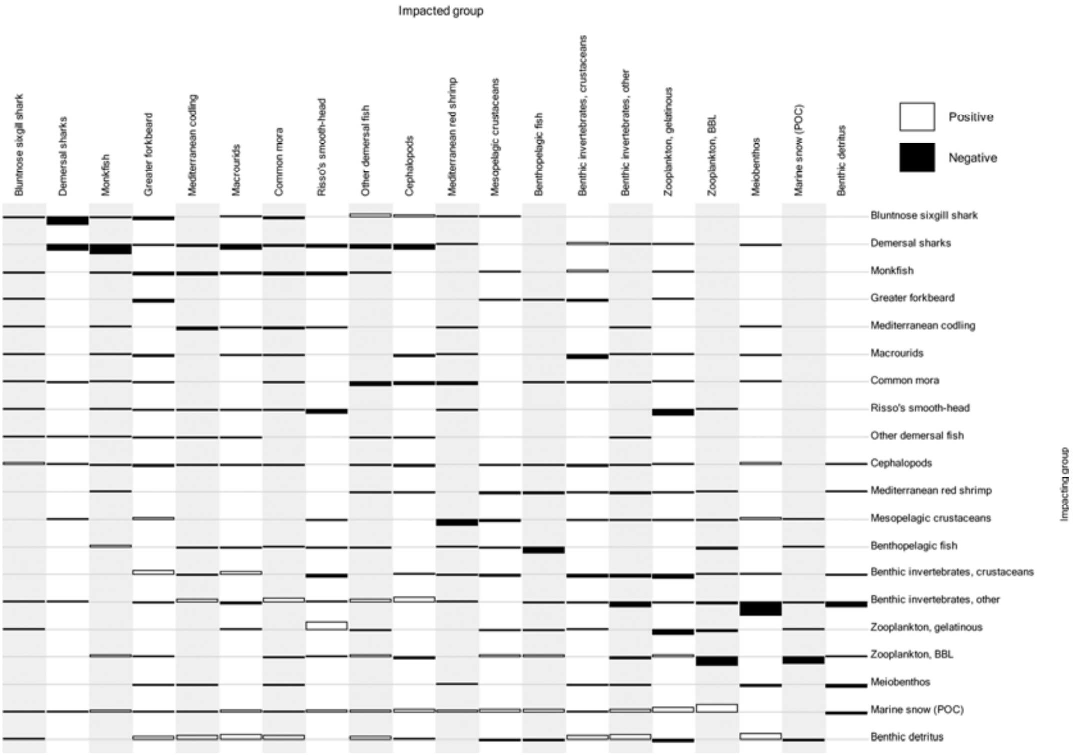


Figure 6.3. Mixed trophic impact of each functional group on the other groups in the model. A white rectangle above the baseline indicates positive impact, while a black rectangle below indicates negative impact. The dimensions of rectangles are relative and comparable between groups, and represent the impact that an infinitesimal increase of the impacting group will have on the impacted groups.

The group of crustacean benthic invertebrates also showed high keystoneness and high biomass, thus being identified as another possible key structuring group. This confirms the results of mixed trophic impact, indicating that the benthic components of the ecosystem play a strong role for the general functioning. The keystoneness index calculated according to Power et al. (1996) identifies the two elasmobranch groups (bluntnose sixgill shark, demersal sharks) and gelatinous zooplankton as the most keystone species, with high overall impact and relative low biomasses (Figure 6.4b). This is in line with results from the first index. Calculated keystoneness indexes for meiobenthos differed greatly between the two methods (-0.98 and 1.54), while its relative total impact is the lowest of the food web. This is an indication that while being neither a keystone nor a structuring group in the model, meiobenthos limits overall trophic flow because of its low biomass (see Figure 6.2).

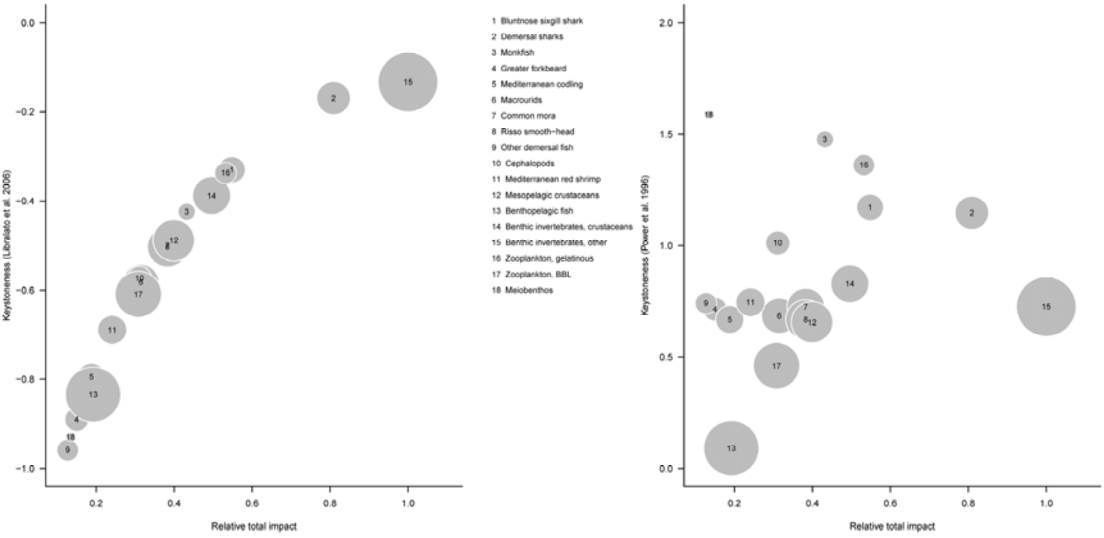


Figure 6.4. Keystoneness indexes for each functional group, plotted against their relative total impact, calculated (A) following Libralato *et al.* (2006) and (B) following Power *et al.* (1996). Area of circles is proportional to group biomass.

Ecosystem structure and functioning

The Lindeman spine plot of flows and biomasses indicates that the highest proportion of fluxes in the modelled system are concentrated at trophic level 2, which hosts over 24% of the total system throughput and 43% of the living biomass of the system (Fig. 6.5). TL 2 also produces the highest flow to detritus of the ecosystem. Since there are no primary producers within the system, the contribution of primary production to deep-water systems is through marine snow and the support of vertically migrating consumers only. Thus, the marine snow import is directly transferred to the consumer groups. In addition, respiration is maximised in flows originating from TL 2, underlining the importance of this TL in the general ecosystem functioning. The total transfer efficiency of the system is 15.7%, significantly higher than the average of 10% reviewed in aquatic ecosystems (Pauly & Christensen 1995) and of other Mediterranean Sea ecosystems (Table 6.3). Transfer efficiencies steadily decrease with TL, a typical pattern of non-exploited ecosystems (Coll *et al.* 2009).

The general ecosystem statistics and sums of total flows are reported on Table 6.3, along with values of the same statistics for published models of shallow-water environments in the Mediterranean Sea. The total system throughput (TST), representing the sum of all flows in the system, is comparatively low with respect to TSTs from other models set up in Mediterranean Sea coastal areas, which range from 1657 to 3844 t km^{-2} y^{-1}. Compared with the neighbouring modelled ecosystem at shallower waters of the Catalan margin, the deep-sea model shows lower TST, sum of all consumption, exports, respiratory flows, flows into detritus, total production and total biomass. This is also due to the higher oligotrophic nature of the deep-sea area compared with coastal waters.

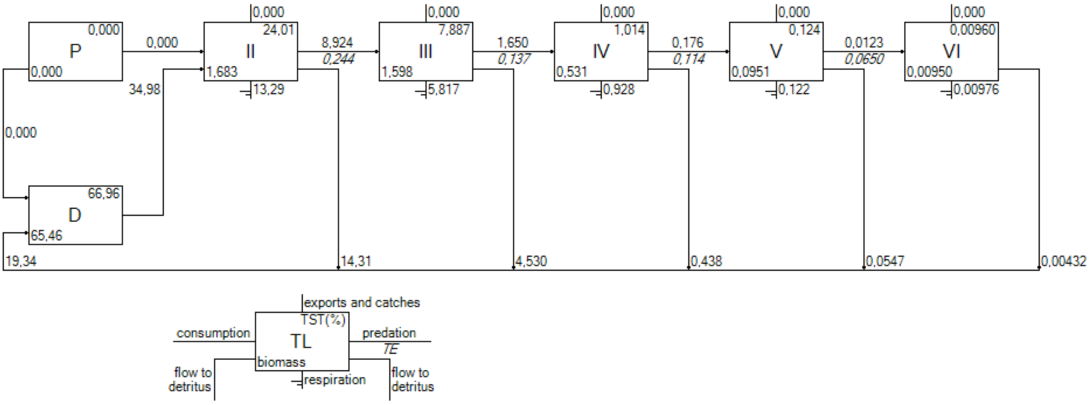


Figure 6.5. Lindeman spine plot of flows and biomasses, aggregated by discrete trophic levels

The System Omnivory index is low compared to expected values, but higher than the values obtained for the shallow-water Mediterranean models (Table 6.3) indicating that the modelled system is more web-like (as opposed to chain-like). The Finn's cycling index is exceedingly low compared to the above-cited shallow-water models in the Mediterranean Sea, which presented FCIs of 8.0-25.2% (Pinnegar & Polunin 2004, Coll *et al.* 2006, Pranovi & Link 2009). In our modelled system, less than 5% of all fluxes are generated by cycling.

Potential impact of fishing activities

Based on the simulation, Ecosim generated a steady increase in fishing effort over the first 10 years of simulation, followed by 10 years of stable fishing effort (Figure 6.6). A reduction in the mean trophic level of the catch, from 2.93 to 2.67, was observed at the end of the simulation in 2029. Total living benthic biomass would be reduced by 6.8% from the initial value while the target species, the red shrimp, would have been reduced to 38% of its initial biomass. The other more impacted groups were common mora, greater forkbeard, and monkfish, whose biomasses were reduced to 4.8%, 13% and 19% of the original level, respectively, after 20 years. Fishes – both Elasmobranchii and Actinopterygii – which accounted for 51% of the total biomass in 2009, would reduce their contribution down to 45.6%. On the contrary, the biomass of invertebrate groups (cephalopods, red shrimp, mesopelagic crustaceans, benthic invertebrates, and zooplankton) would transition from 36.9% of contribution over total biomass to 41,9% at the end of the simulation.

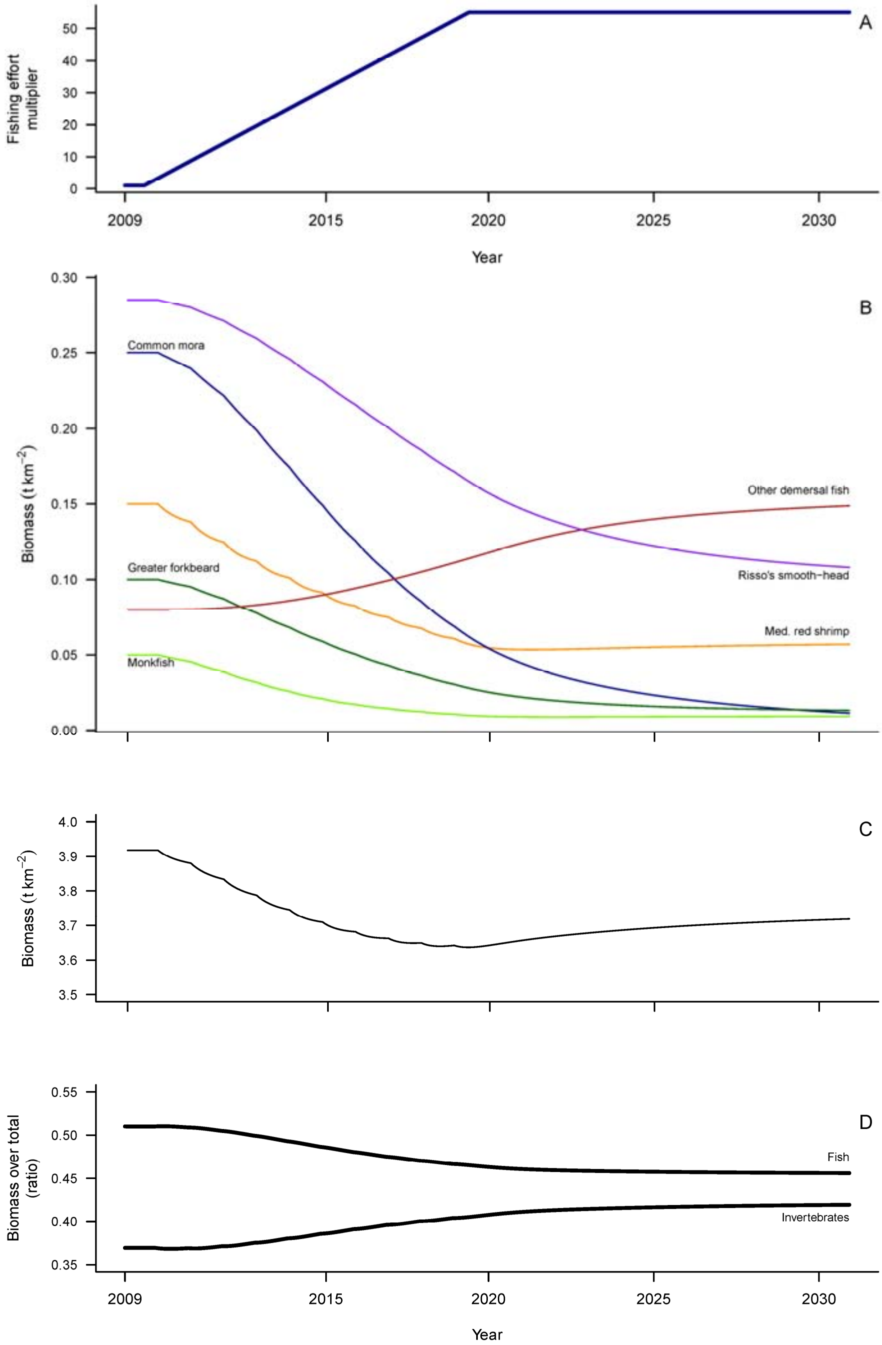


Figure 6.6. Time-dynamic simulation of fishery development in the modelled area. A: simulated increase in fishing effort. B: biomass trends of the groups, which showed a variation of more than 20% of their original biomass at the end of the simulated period. C: Total living biomass of the ecosystem (i.e. excluding detritus groups). D: ratio of fish biomass and invertebrate biomass over the total ecosystem living biomass.

DISCUSSION

Ecosystem functioning

The functioning of deep-sea ecosystems is extremely constrained by their heterotrophicity, i.e. their ultimate dependence on an allochthonous food supply. Ecosystems in the deep Mediterranean Sea have been closely linked to surface production, but also to the levels of sediment organic matter and its turnover (see Chapter 3). In this context, the Catalan continental slope is affected primarily by local-scale surface events such as fluctuations in river discharge and atmospheric events in the nearby Gulf of Lions, which can trigger periodic shelf-water cascading that brings enriched waters to the lower-slope depths (Company *et al.* 2008, Sanchez-Vidal *et al.* 2012). These factors contribute to the unusually high quantities of organic matter reaching the modelled area and to the highest concentration of biomass of the whole continental slope (Zuñiga *et al.* 2009).

The functional groups identified as keystone, and those that mostly impacted other groups in the system were similar. Demersal sharks act as a top predator group, feeding on a wide variety of prey. The other identified keystone group was "benthic invertebrates, other", which include both suprabenthos and megabenthos, both strictly in association with the substrate and also employing a wide variety of feeding strategies. A keystone group is identified as having a high structuring impact on the other groups in the system, in spite of having a relatively low biomass (Power *et al.* 1996). The same group "benthic invertebrates, other" also had an important biomass in the system, therefore they are likely to be key structuring groups (i.e. foundation stones), rather than keystone. Gelatinous zooplankton also plays a keystone role in the ecosystem. This group includes jellyfish, and its importance and keystone status is expected due to the high importance of this group in the organic matter transfer from the shallow layers to the deep sea, and to its contribution to the diet of a dominant fish species, the Risso's smooth head. Deep benthos survival is strictly linked to the flux of organic matter from the photic layer to the seafloor, or "marine snow" (Polunin *et al.* 2001). It has been shown, in recent years, that climate-driven changes modulate the surface primary production, which may, in turn, provoke massive changes in benthic communities and in general deep-sea processes (Yasuhara *et al.* 2008, Smith *et al.* 2009). In addition, plankton is also a main diet component of jellyfish (Pauly *et al.* 2009). Therefore, a reduction in the annual flux of marine snow could directly impact the benthic community, triggering a cascade change in the community. This adds to the vulnerability of the deep-sea system and is another aspect that should be addressed in subsequent studies.

Food-web analysis

The results from the network analysis indicate higher levels of flow connexions between ecosystem components as compared to shallow-water benthic environments in the

Mediterranean Sea. Moreover, the TL 2, and to a lower extent TL 3, are the trophic levels hosting the majority of ecosystem functioning. With the exception of the bluntnose sixgill shark, a top predator in the area, omnivory at group level was generally low, while at system level it was higher than in shallow water systems, but still near the lower end of the possible range (0 - 1). The majority of flow interactions take place at the level of primary consumers, which in the deep Mediterranean Sea are predominantly benthic deposit feeders (Fanelli *et al.* 2009). For the bluntnose sixgill shark, the high OI may reflect its feeding pattern, in that this species has been observed to spend considerable time slowly foraging for a wide variety of prey items (Ebert 1994). This is in accordance with results by Sardà *et al.* (2009b) who found general low values of omnivory in deep continental slopes of the Mediterranean Sea. Overall, these results suggest that the food-web topology at TL 2 is nearly saturated, confirming the importance of low trophic levels in the functioning of the modelled ecosystem. In addition, fauna seems to feed on a narrow range of trophic levels, an occurrence inverse to what was observed in the deep oceans (Gage & Tyler 1991). This may be due to the dominance of fishes in the deep Mediterranean waters, which tend to be more active predators than unselective deposit feeders (Merrett & Haedrich 1997). The calculated System Omnivory index is low if compared to expected values, but is nevertheless considered a robust indicator in network analysis, its fluctuations being not significantly influenced by the number of functional groups or the model focus (Pinnegar *et al.* 2005). Overall, the low system omnivory indicates that under the assumptions of Odum's theory (Odum 1985), the modelled system is more web-like (as opposed to chain-like) and is the result of a long-term ecological succession process.

Flow cycling (measured by the FCI) was extremely low in comparison with shallow-water benthic systems of the Mediterranean Sea. This indicates that most of the flows are direct, further confirming the trophic saturation mentioned above. Notwithstanding, high levels of consumer cycling may also reflect a response of the ecosystem to disturbance, as suggested by Pranovi and Link (2009), by which resources are mobilised from the upper trophic levels and enter recycling pathways in the mid-web. In our case, the levels of recycling were low compared to all other food-web models set up in the Mediterranean Sea; thus this could indicate low levels of disturbance. In addition, this result may imply that biomass is being conserved in standing stocks of high-level consumers, another indication that the ecosystem has reached climax stability.

In the ecosystem modelling approach, the food web flows are defined *a priori* primarily by means of diet composition data, then adjusted in due course to balance the model. An accurate definition of these flows is pivotal to obtain a model that is sufficiently representative of the ecosystem. However, no existing deep-sea ecosystem model takes into account the spurious trophic interactions that may take place in these extremely food-limited environments. For example, an atypical trophic behaviour in deep-sea megabenthos was recently observed in the deep Pacific Ocean, with scavenger gastropods switching their behaviour by actively feeding on a naturally fallen fish carcass (Aguzzi *et al.* 2012). This is a good example of a type of food-web flow that could not be easily captured by the currently available ecosystem models. Moreover, these types of feeding behaviour are difficult to quantify and thus, to relate to the general ecosystem functioning. For example, in the present model, we attempted to quantify the amount of organic matter inputted by whale carcasses (Smith & Baco 2003), but the obtained values have to be taken only as empirical. This leaves open questions about the food web dynamics in deep-sea systems. Are we really catching the majority of the trophic interactions or are these deep-sea systems opportunity-dominated? And, more importantly, how could we improve data collection for models to account for these atypical interactions?

Impact of potential fishing

Results from the Ecosim time-dynamic simulation show that increasing fishing effort in this area may produce a reduction of total live benthic biomass of up to 5% in 20 years, and that this reduction would be concentrated on the more mobile species of the community. In addition, common mora, monkfish, and greater forkbeard would suffer a collapse. Even if this reduction may be considered a modest change, one should consider that slight biomass changes, together with biodiversity loss, may provoke significant reduction in the general benthic ecosystem functioning (Danovaro *et al.* 2008a). Nektobenthic species, which have high movement capability, participate significantly in the dynamics of the benthic boundary layer through, e.g., trophic interactions, seasonal migrations, and dispersion processes (Mees & Jones 1997). As the exact consequences of this biomass reduction are yet to be clarified, it is logical to proceed according to the precautionary principle when advising on possible future fishery exploitation of the deep Catalan continental slope.

Ecosim simulations can greatly benefit from a calibration of the vulnerability parameters. Normally, this calibration is carried out by fitting the model response to biomass time-series. In our case, time-series of catch data were not available as the area is unexploited; however, results from the network analysis showed that the system may indeed have reached ecological stability, thus supporting the choice of applying low values for the vulnerability parameter.

Given the recent commentary about the applicability of the EwE approach to deep-sea ecosystems (Heymans *et al.* 2011), it remains to be verified if the results presented here are supported by a sufficient amount of empirical knowledge and acceptably large datasets. Heymans *et al.* (2011) identified biomass estimates as a critical parameter. In the present case, biomass was the least uncertain parameter since it was obtained by integrating results from a multi-year, seasonal, and multi-gear sampling plan. The critical parameters for this model were the P/B and Q/B ratios, which were primarily obtained from empirical relationships that have not yet been demonstrated fully valid for deep-sea fish and other organisms. Exhaustive studies should be conducted on production levels of deep-sea fish if a more detailed parameterisation is to be carried out.

Caution has to be expressed in that our results from the dynamic simulation may be optimistic. The reduction of biomass of the red shrimp by 62% over 20 years does not take into account the decadal fluctuations of landings of this species, typical of the northern Catalan region. These fluctuations are caused, among other factors, by the periodic dense shelf-water cascading events, that ‘flush’ the populations of red shrimp downslope, away from fishing grounds (Company *et al.* 2008). While such processes may be incorporated in the modelling, this has not been considered for the present study, since additional biological data is needed. In fact, it has been hypothesised that the occurrence of cascading events may enhance the recruitment of red shrimp in subsequent years (Sardà & Company 2012), and that this process may be the reason for the decadal stable trend in landings, even if present data suggests the resource is being overexploited. For this reason, variations in the periodicity of these events could increase the vulnerability of this species to fishing pressure, thus producing even higher drops in biomass than those reflected in the present model. The risk of observing the typical “boom and bust” fishery pattern, seen in other deep-sea fisheries around the world (Norse *et al.* 2012), is thus further enhanced in this modelled ecosystem.

Conclusions

Results from this ecosystem model indicate that the benthic system in the Catalan continental margin presents markedly different characteristics with respect to the shallower habitats in the Mediterranean Sea. This work will hopefully help define the specific biotope of the Mediterranean deep benthic habitats. Specifically, it was found that (i) detritus-based interactions are highly important along the whole food web, (ii) primary consumers and particularly benthic deposit feeders are structural groups in the ecosystem, while more motile species play keystone roles, and (iii) the expansion of the current red shrimp trawl fishery to deeper areas may provoke changes in the relative proportion of species, with possible collapses of fish species.

In conclusion, we presented here the first ecosystem model of a deep-water environment in the Mediterranean Sea, an enclosed basin with peculiar characteristics. The absence of direct human exploitation below 1000 m depth facilitated the parameterisation and provided a unique opportunity of observing *quasi-pristine* environments in an otherwise highly exploited Large Marine Ecosystem (Coll *et al.* 2010). I conclude by stressing the importance of deep-sea primary consumers in benthic-pelagic coupling processes, and reiterating the concern of vulnerability of these ecosystems to potential future exploitation.

Table 6.1. Input and main output parameters for the deep Catalan Sea ecosystem model. Numbers in **bold** indicate parameters estimated by the model. U/Q: fraction of unassimilated consumption, inputted directly into detritus.

		Biomass (t km^{-2})	P/B (y^{-1})	Q/B (y^{-1})	EE	U/Q	Trophic level	OI	Net efficiency	Flow to detritus (t km^{-2} y^{-1})	Relative total impact
1	**Bluntnose sixgill shark**	0.128	0.148	1.14	**0.00**	0.20	4.34	0.81	0.16	0.05	0.52
2	**Demersal sharks**	0.200	0.250	1.90	**0.61**	0.20	4.05	0.16	0.16	0.10	0.78
3	**Monkfish**	0.050	0.430	2.70	**0.40**	0.20	3.95	0.13	0.20	0.04	0.41
4	**Greater forkbeard**	0.100	0.508	2.75	**0.36**	0.20	3.35	0.41	0.23	0.09	0.16
5	**Mediterranean codling**	0.140	0.736	2.91	**0.45**	0.20	3.11	0.48	0.32	0.14	0.18
6	**Macrourids**	0.225	0.516	3.35	**0.52**	0.20	2.80	0.51	0.19	0.21	0.32
7	**Common mora**	0.250	0.283	2.45	**0.67**	0.20	3.23	0.45	0.14	0.15	0.37
8	**Risso's smooth-head**	0.285	0.265	2.03	**0.73**	0.20	3.41	0.28	0.16	0.14	0.39
9	**Other demersal fish**	0.080	1.050	3.60	**0.93**	0.20	3.05	0.24	0.36	0.06	0.12
10	**Cephalopods**	0.105	2.190	7.30	**0.59**	0.20	3.14	0.36	0.38	0.25	0.31
11	**Mediterranean red shrimp**	0.150	3.000	8.59	**0.65**	0.20	3.13	0.39	0.44	0.42	0.20
12	**Mesopelagic crustaceans**	0.305	3.115	8.90	**0.68**	0.20	2.96	0.48	0.44	0.85	0.41
13	**Benthopelagic fish**	0.540	1.300	7.40	**0.61**	0.20	2.76	0.49	0.22	1.07	0.17
14	**Benthic invertebrates, crustaceans**	0.255	2.768	10.40	**0.94**	0.20	2.47	0.45	0.33	0.57	0.49
15	**Benthic invertebrates, other**	**0.652**	3.190	16.20	0.99	0.30	2.11	0.10	0.28	3.19	1.00
16	**Zooplankton, gelatinous**	0.080	22.000	56.00	**0.46**	0.40	2.62	0.38	0.65	2.75	0.50
17	**Zooplankton, BBL**	**0.369**	18.000	50.00	0.95	0.40	2.05	0.05	0.60	7.71	0.30
18	**Meiobenthos**	0.012	60.000	240.00	**0.94**	0.35	2.00		0.38	1.05	0.11
19	**Marine snow (POC)**	35.80			**0.70**		1.00			11.16	
20	**Benthic detritus**	29.66			**0.30**		1.00				

Table 6.2. Diet compositions for the deep Catalan continental slope model after balancing. Diets sum to 1.

	Prey \ predator	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Bluntnose sixgill shark																		
2	Demersal sharks	0.130	0.030																
3	Monkfish	0.020	0.015																
4	Greater forkbeard	0.040	0.015	0.030	0.010														
5	Mediterranean codling	0.060	0.050	0.080		0.020													
6	Macrourids	0.080	0.090	0.080		0.010													
7	Common mora	0.090	0.050	0.085		0.010													
8	Risso's smooth-head	0.080	0.070	0.095		0.010													
9	Other demersal fish	0.050	0.100	0.090		0.005		0.030											
10	Cephalopods	0.150	0.200	0.080			0.010	0.030											
11	Mediterranean red shrimp		0.050	0.100	0.030	0.060	0.000	0.085	0.040		0.020		0.040		0.010				
12	Mesopelagic crustaceans		0.140	0.080	0.200	0.090	0.050	0.085	0.070	0.080	0.100	0.075	0.030		0.030				
13	Benthopelagic fish		0.050	0.180	0.100	0.005	0.000	0.080			0.050	0.055		0.050					
14	Benthic invertebrates, crustaceans		0.030		0.350	0.070	0.250	0.110	0.010	0.100	0.160	0.050	0.020						
15	Benthic invertebrates, other		0.030		0.100	0.380	0.100	0.410	0.180	0.420	0.420	0.250	0.100		0.150				
16	Zooplankton, gelatinous					0.040	0.040		0.600			0.050	0.030		0.100				
17	Zooplankton, BBL			0.100	0.060	0.080	0.070			0.215		0.150	0.350	0.400	0.050	0.060	0.380	0.050	
18	Meiobenthos						0.060			0.050		0.050			0.010	0.050			
19	Marine snow (POC)						0.150				0.150	0.100	0.130	0.150	0.200	0.430	0.270	0.950	0.300
20	Benthic detritus				0.150	0.220	0.270	0.170	0.100	0.135					0.450	0.460			0.700
	Import	0.300	0.080								0.100	0.220	0.300	0.400			0.350		

Table 6.3. General ecosystem statistics calculated for the present model, along with statistics reported for other shallow-water models in the Mediterranean Sea: the southern Catalan Sea (Coll *et al.* 2006), the Adriatic Sea (Coll *et al.* 2007), the northern Aegean Sea (Tsagarakis *et al.* 2010) and the north-eastern Ionian Sea (Piroddi *et al.* 2010).

	Catalan margin					
	Deep open slope (2009)	**Southern Catalan Sea (1994)**	**North-central Adriatic Sea (1990s)**	**Northern Aegean Sea (2003)**	**North-eastern Ionian Sea (1964)**	
Sum of all consumption	51.36	852.11	1305.04	868.83	851.90	t km^{-2} y^{-1}
Sum of all exports	20.09	61.27	730.15	274.81	162.10	t km^{-2} y^{-1}
Sum of all respiratory flows	20.19	327.16	421.09	269.48	503.30	t km^{-2} y^{-1}
Sum of all flows into detritus	65.84	416.91	1387.46	562.53	748.80	t km^{-2} y^{-1}
Total system throughput (TST)	157.48	1657.00	3844.00	1976.00	2266.10	t km^{-2} y^{-1}
Sum of all production	14.83	658.00	1566.00	791.00	813.60	t km^{-2} y^{-1}
Calculated total net primary production	0.00	386.68	1149.85	535.48	664.90	t km^{-2} y^{-1}
Total primary production/total respiration	0.00	1.18	2.73	1.99	1.30	
Net system production	-20.19	59.52	728.76	265.99	161.60	t km^{-2} y^{-1}
Total transfer efficiency	15.7	12.6	10.0	17.4		%
Total primary production/total biomass	0.00	6.55	8.82	16.21	15.00	
Total biomass/total throughput	0.02	0.04	0.03	0.02	0.02	
Total biomass (excluding detritus)	3.93	58.99	130.30	33.04	44.30	t km^{-2}
System Omnivory index	0.29	0.19	0.19	0.18	0.10	
Finn's cycling index (FCI)	4.20	25.19	14.70	14.60	14.43	% of TST
Ecopath pedigree index	0.54	0.67	0.66	0.61	0.50	

APPENDIX 1 – Groups parameterisation

Unless otherwise noted, biomasses for each group were calculated by standardizing catches for the trawled area (both for the OTMS and Agassiz gears). The empirical equations applied to calculate P/B and Q/B of some groups are as follows (Pauly 1980, Palomares & Pauly 1998):

$$P/B = K^{0.65} \cdot L_{\infty}{}^{-0.279} \cdot T_c{}^{0.463}$$

$$\log(Q/B) = 7.964 - 0.204\,logW_{\infty} - 1.965\,T' + 0.083\,A + 0.532\,h + 0.398\,d$$

In the first equation, K is the growth parameter from the Von Bertalanffy Growth Function (year^{-1}), L_∞ is the asymptotic total length (cm) and T_c is the system water temperature (°C). In the second equation, W_∞ is the asymptotic body weight (g), A is the aspect ratio of the caudal fin, *h* is a dummy variable with a value of 1 for herbivores and 0 for carnivores and detritivores, and *d* is another dummy variable with a value of 1 for detritivores and 0 for herbivores and carnivores. The term T' is an expression of the system water temperature, calculated as $T' = \frac{1000}{T_c + 273.15}$.

1. Bluntnose sixgill shark

This sixgill shark (*Hexanchus griseus*) has rarely been seen in catches and trawl survey data, and in the Mediterranean Sea it has been recorded in the western and central basins (Compagno *et al.* 2005). Its diet composition was adapted from Ebert (1994), including a 30% of import outside the model, to take into account the time spent foraging off the bottom or moving out of the study area. Production and consumption parameters were estimated with empirical equations using data from FishBase and the equations in this Appendix, above, while biomass was estimated considering the absences of catches.

2. Demersal sharks

This group includes the black-mouthed dogfish *Galeus melastomus,* the velvet-belly *Etmopterus spinax* and the kitefin shark *Dalatias licha.* Diets were weighted by the relative biomass contribution for each species, considering both local and global literature data (Cortés 1999, Valls *et al.* 2011), and were partially modified to balance the model. P/B and Q/B values were calculated with empirical relationships based on parameters from FishBase.

3. Monkfish

Diet of monkfish (*Lophius piscatorius*) was taken from reviewed data (Stergiou & Karpouzi 2002), while P/B and Q/B parameters were calculated using empirical relationships.

4. Greater forkbeard

The greater forkbeard (*Phycis blennoides*) is present in the modelled area as the deepest part of its depth range distribution. Estimated biomass ranged from 0.05 to 0.30 t km^{-2}, and a value of 0.10 t km^{-2} was chosen to balance the model. Diet was taken from detailed studies on stomach contents (Macpherson 1978), while the P/B and Q/B values were obtained by empirical relationships using parameters from FishBase.

5. Mediterranean codling

The Mediterranean codling (*Lepidion lepidion*) is a dominant species at the lower slope of the western Mediterranean waters and its diet has been studied in detail (Carrassón *et al.* 1997). P/B and Q/B parameters were obtained from empirical relationships.

6. Macrourids

The species of grenadiers dwelling in the Catalan Sea lower continental slope are the smooth rattail *Nezumia aequalis, Coelorinchus mediterraneus,* the Gunther's grenadier *Coryphaenoides guentheri*, and the roughsnout grenadier *Trachyrincus scabrus*. These species were all grouped due to their high dietary and habitat overlap (Macpherson 1979). Their diet was weighted by the relative biomass contribution of each species and consisted mainly of benthic invertebrates, various planktonic sources, and detritus (Macpherson 1979, Carrassón & Matallanas 2002a). The resulting diet was then partially changed to balance the model. Due to lack of data about production and consumption rates of these species, the P/B and Q/B values were estimated by empirical equations using only data from *T. scabrus*, obtained from FishBase.

7. Common mora

This morid (*Mora moro*) is a co-dominant fish species in the modelled depth range along with the next species, both in terms of abundance and biomass. Diet was obtained from (Carrassón *et al.* 1997). Values of P/B and Q/B were calculated with empirical relationships using parameters from FishBase.

8. Risso's smooth-head

The Risso's smooth-head (*Alepocephalus rostratus*) is another co-dominant species between 1100 and 1400 m depth in the Catalan sea slope (Stefanescu *et al.* 1993). Its diet was integrated starting from detailed studies on stomach contents (Carrassón & Matallanas 1998, Stergiou & Karpouzi 2002). P/B and Q/B were obtained by empirical relationships starting from data by Morales-Nin *et al.* (1996).

9. Other demersal fish

This group includes *Cataetyx alleni*, the spiderfish *Bathypterois mediterraneus,* the shortfin spiny eel *Notacanthus bonaparte* and the blackfin sorcerer *Nettastoma melanurum*, considered benthic scavengers and sit-and-wait predators. Diets were retrieved from literature and weighted by the relative biomass contribution for each species (Carrassón & Matallanas 2001, 2002b, Stergiou & Karpouzi 2002). Similarly, P/B and Q/B were obtained with data from FishBase, weighting the results of the empirical equations by the relative biomass.

10. Cephalopods

This group included the bathyal octopods *Bathypolipus sponsalis* and *Opistoteuthis calypso*, with a small fraction considered for occasional immigrations by the benthopelagic squid *Todarodes sagittatus* (6% of the group biomass). The value of Q/B was adapted from a shallower model of the Catalan Sea (Coll *et al.* 2006), and a gross food conversion efficiency of 0.30 was applied to calculate the P/B ratio. Diet is known only for *B. sponsalis*, thus its diet was used in the model (Quetglas *et al.* 2001).

11.Mediterranean red shrimp

The red shrimp (*Aristeus antennatus*) is the most valuable commercial species fished in the continental slope of the Mediterranean basin, down to depths of 800 m. The published diet was considered as semi-quantitative (Cartes 1994) and was partially modified to balance the model. Q/B was calculated with empirical relationships starting from data by García-Rodriguez and Esteban (1999) while P/B was a guesstimate based on previous knowledge of this species' metabolism (Company *et al.* 2003 and J.B. Company, pers. comm.).

12. Mesopelagic crustaceans

This group includes the decapod shrimps *Acantephyra eximia, A. pelagica, Pasiphaea multidentata, P. sivado*, *Sergestes arcticus* and *Sergia robusta.* All these species are known to perform wide benthopelagic movements in and out the benthic boundary layer. Their biomass was estimated by using pelagic mid-water trawls. Consumption/biomass was calculated by empirical equations, taking *A. eximia* as the reference species, and P/B was then calculated assuming a production/consumption ratio of 0.35. Detritus fate for this group was considered as 70% directed to marine snow and 30% directed to sediment detritus.

13. Benthopelagic fish

This group comprises small-sized planktonic fish species that perform vertical migrations between the mesopelagic domain and the benthic-boundary layer. It consisted mainly of the spottail anglermouth *Cyclothone braueri*, the short-silver hatchet-fish *Argyropelecus hemigymnus*, the jewel lanternfish *Lampanyctus crocodilus* and other species of Myctophidae. Biomass was obtained by mid-water trawl surveys, conducted in the framework of the same sampling plans

(Gonzalez-Barrios, unpublished data). Diet of this group was considered only as qualitative, based on data on *L. crocodilus* (Stefanescu & Cartes 1992). P/B and Q/B were calculated with empirical relationship using data from FishBase. Detritus fate for this group was directed as 70% to marine snow and 30% to sediment detritus.

14. Benthic invertebrates, crustaceans

This group includes the reptantian decapod crustaceans: *Geryon longipes*, *Munida tenuimana*, *Pagurus alatus*, *Paromola cuvieri* and *Polycheles typhlops*. P/B and Q/B ratios were calculated using empirical relationships for marine invertebrates (Brey 2001). Diet compositions were retrieved from detailed literature studies (Cartes & Abelló 1992, Cartes 1993a, Cartes 1993b), weighted by the relative contribution in biomass of each species, and were partially changed to balance the model.

15. Benthic invertebrates, other

This group includes the non-crustacean megabenthos and macrobenthos invertebrates. Values of P/B and Q/B were taken from the open Atlantic ocean model (Vasconcellos & Watson 2004) and corrected for temperature differences according to Opitz (1996). Diet composition for this group came from general knowledge about the related species, and was partially modified to balance the model. We let the model estimate the biomass of the group, assuming that the model fully explains mortalities, i.e. with an ecotrophic efficiency of 0.99.

16. Zooplankton, gelatinous

Trawl surveys in the area identified a large dominance of the jellyfish *Pelagia noctiluca* in this group. Also included in this group were *Nausithoe* sp., *Salpa* sp. and *Pyrosoma atlanticum*. Values of P/B and Q/B were taken from another model set up in the coastal area of the southern Catalan Sea (Coll *et al.* 2006, Pauly *et al.* 2009). Quantitative dietary data is available for *P. noctiluca*, in the same region (Sabatés *et al.* 2010).

17. Zooplankton, BBL

This is primarily mesozooplankton from the benthic-boundary layer (sensu Dauvin & Vallet 2006). We let Ecopath estimate the biomass of this group by assuming an ecotrophic efficiency of 0.95. Values of P/B and Q/B were taken from a seamount model in the Northern Atlantic Ocean (Morato & Pitcher 2005) and corrected for temperature differences. Diet was assumed as 10% zooplankton (cannibalism) and 90% marine snow.

18. Meiobenthos

Biomass for this group was estimated from literature (Gambi *et al.* 2010), using a conversion factor of 12.4% between organic carbon content and wet weight for nematodes (Jensen 1985). P/B and Q/B were obtained from Heip *et al.* (1990) and corrected for temperature, following published guidelines from another deep-sea model in the northern Atlantic Ocean (Van Oevelen *et al.* 2011). Diet was assumed as 35% marine snow and 65% detritus. The proportion of unassimilated food for this group was estimated at 0.35, based on recommendations by Heip *et al.* (1990).

19-20. Detritus compartments

Dead organic matter is modelled in Ecopath as *detritus*, a functional group that receives inputs from the other living groups based on their 'other mortality' and their proportion of 'unassimilated food'. Two different detritus groups were set up to model the carbon input from allochthonous food sources in the deep ecosystems. A *marine snow* group represents the annual POC input falling from the upper strata, which has been estimated to 35.80 t km^{-2} year^{-1} based on sediment traps data in the model area (Danovaro *et al.* 1999). A *benthic detritus* group represents the non-living particulate organic matter deposited on the sediment; biomass of sediment detritus was estimated at 29.66 t km^{-2}.

Large carcasses of marine mammals have been considered separately: the two most abundant whales in the area are the common finback whale *Balaenoptera physalus* and the sperm whale *Physeter macrocephalus*. We estimated the amount of wet weight reaching the deep seafloor by multiplying density data by the mean adult weight and by the calculated mortality rate, for both species (Forcada *et al.* 1992, Gannier *et al.* 2002, Smith & Baco 2003). The obtained value of 0.0613 t km^{-2} year^{-1} was added as import for the benthic detritus group. This will allow for rapid updating of the parameter should new data were to become available.

Conclusions

7

This study was the first attempt to sample the deep megafauna over the entire longitudinal axis of the Mediterranean Sea, simultaneously investigating their biodiversity, community composition, trophic habits, and the possible environmental drivers for the observed patterns. The data on deep Mediterranean megafauna are still limited, but the results obtained in this study have presented an initial basin-wide picture of these deep ecosystems, providing a baseline from which to build a sound understanding of the biodiversity and ecosystem functioning in the Mediterranean basin. This knowledge will be essential also for predicting changes in the deep communities in the face of potential anthropogenic impact and, especially, climate change, and for developing sustainable management and conservation options. On a small spatial scale and specifically in the Catalan continental margin, this work provided a first description of the seasonal fluctuations of benthic community structure and helped define the essential habitat at lower-slope depths, laying the basis from which detailed explanations will be searched for in the next future.

At the end of this study, we can discard the null hypothesis established at the beginning, and conclude that the structure and functioning of marine ecosystems in the deep Mediterranean Sea are indeed related with geographic position and depth. However, the processes regulating this interaction are more complex than previously thought and surely require further exploration. Regarding the submarine canyon environment, we can also discard the initial null hypothesis, as the Blanes submarine canyon proved to host higher biodiversity than that of the outer continental slope, and a different community composition.

The following conclusions may then be summarized:

1. The otter-trawl Maireta system and the Agassiz dredge are complementary samplers in the study of deep-sea megafauna, providing an integrated image of the benthic compartment and of the overlying fractions of nektobenthic and benthopelagic species. Their combined use permits the retrieval of a wider collection of species, especially improving studies on biodiversity.
2. Depth, strictly speaking, cannot be considered a univocal descriptor of species distribution over large bathymetric gradients. Depth co-varies with a number of other physical and biotic variables, thus de-linearizing their interactions with biota.
3. The biogeography of the deep Mediterranean benthos is the product of local environmental selections from a wider regional species pool. The relatively young age of this pool, in a geological time scale, and its separation from the one in the Atlantic Ocean, will have to be accurately considered when studying macro-ecological processes in the Mediterranean Sea.

4. The dynamics of the surface layers, and the consequent modulation of food availability at the seafloor, are key processes in determining the variations of community composition and biomass, at the bathyal and abyssal bottoms of the deep Mediterranean Sea over large spatial scales. Sediment grain size and its particulate organic carbon content were also identified as modulating drivers, further strengthening the linkage between pelagic, in-faunal, and benthic processes.
5. Bathymetric patterns of benthic biomass present a typical absolute peak at depths of 1000-1400 m with respect to the whole continental margin of the western Mediterranean Sea. The actual process generating this peak is not yet understood, being the current explanations only mechanistic.
6. Even if traditionally considered as stable and temporally invariable, the communities in the Catalan continental margin evidence significant seasonal fluctuations, with a dynamic zone above 1000 m depth where populations of highly mobile species perform year-round migrations throughout the slope, and a more static region below 1000 m (i.e. the twilight zone end) with substantially less inter-annual variations. These fluctuations seem to be driven by movements of deep-water masses, and particularly by the arrival of new Western Mediterranean Deep Water from the deep basin to the lower slope during the spring season, which may produce a biomass accumulation above 1000 m depth.
7. The Blanes submarine canyon in the north-western Mediterranean hosts a more diverse community than the one in the outer continental slope, but with comparable levels of abundance and biomass. The canyon habitat may act as a refuge area for species impacted by fishing activities in the outside regions. Results strengthen the concept of submarine canyons as hotspots of biodiversity and underline the importance of their future conservation as diversity repositories.
8. The stable isotopes analysis has been proven a useful technique to address hypotheses in trophic ecology, when the collection of standard dietary data is marred by sampling difficulties, such as in the deep sea. The width of trophic niches in the deep Mediterranean habitats is correlated with water-column processes such as primary and secondary productivity at the surface, further strengthening the linkage between surface, mesopelagic, and benthic environments.
9. From an ecological point of view, the ecosystem of the deep Mediterranean continental slope, according to what was observed in the ecotrophic model, is now at a level of ecological stability, possesses a trophic web with a bottom-up driven structure, and general characteristics of vulnerability from possible future human exploitation.

10. The interactions between human fishing activities, climate-driven processes, and the natural fluctuations of deep benthic communities deserve special attention, by both scientists and policy-makers. Governance of these areas is particularly complex. This is especially true in the Catalan margin region, over which large parts of the human populations carry out activities related to the sea. The possible future effects, which would include collapses of fish species and thus provoke extended social impacts, are aspects that cannot be overlooked any more.

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