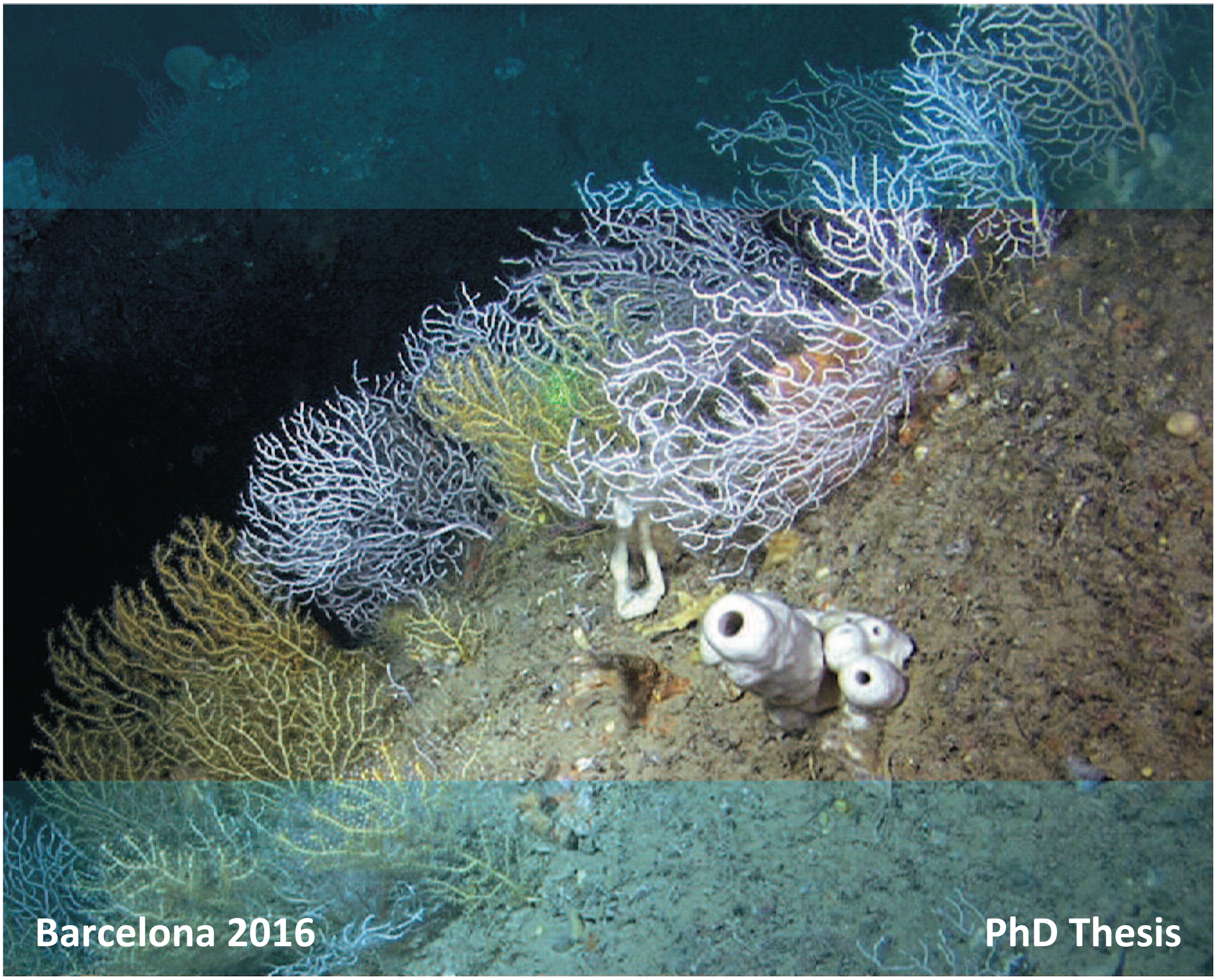
Ecological study of benthic communities in the continental shelf and upper slope in the Menorca Channel (North Western Mediterranean)

JORDI GRINYÓ



Barcelona 2016

PhD Thesis

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Ecological study of benthic communities in the continental shelf and upper slope in the Menorca Channel (North Western Mediterranean Sea)

Estudi ecològic de les comunitats bentòniques a la plataforma i talús continental somer al Canal de Menorca (Mediterrani Nord Occidental)

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Tesi presentada per l'obtenció del títol de Doctor
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"The sea, once it casts its spell, holds one in its net of wonder forever."

Jacques Yves Cousteau

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Summary

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Technological development and increased availability of remotely operated vehicles (ROVs) and manned submersibles have significantly increased accessibility to deep environments revealing the presence of rich and diverse macrobenthic assemblages dominated by suspension feeders. These assemblages have been largely exposed to the impacts of fishing activities (especially bottom trawling) in the Mediterranean Sea. Consequently, it is currently urgent to increase knowledge on their distribution, as well as on the ecology and biology of the main species that constitute these macrobenthic assemblages in order to implement effective management and conservation measures. This thesis addressed the characterization of macrobenthic assemblages in the continental shelf and upper slope (40–360 m depth) of the Menorca Channel through a multidisciplinary approach at different ecological levels.

Six macrobenthic assemblages mainly segregated by substrate and depth. Hard substrates hosted sponge grounds and coral gardens, whereas crinoid and brachiopod beds occurred on soft sediments. Highest diversity values were found in the shelf edge, probably as a consequence of the bottom heterogeneity and the constant hydrodynamic conditions. Gorgonians were one of the most important and diverse bioengineering organisms in the Menorca Channel, forming dense assemblages that extended over vast areas. Gorgonian assemblages on the continental shelf and upper slope were mostly monospecific, whereas shelf edge assemblages were highly multispecific. Small colonies were dominant throughout the studied bathymetric range, but in deeper environments intermediate and large colonies were more abundant. The good preservation state of most of the observed benthic assemblages and gorgonian populations is probably related to the low pressure of bottom trawling, which is mostly concentrated in deeper areas of the continental and the exceptional fishing practices exerted by the local artisanal fisherman. *Paramuricea macrospina* is the most abundant gorgonian species in the Menorca Channel. Throughout its distribution, three different chromatic forms were observed. Two forms occurred on the continental shelf, and a third occurred on the shelf edge. Colony shape, sclerite size and shape, and the genetic variability of mitochondrial markers were compared to elucidate if these chromatic forms represented different taxonomic units. Colony morphology barely changed

among the three forms resulting as a high conservative character. Conversely, sclerite size and shape significantly differed amongst the three forms, possibly conditioned by genetic and environmental factors. However, no significant differences were observed in the studied mitochondrial markers. The reproductive cycle, energy storage and metabolic requirements of *P. macrospina* showed significant differences compared to Mediterranean coastal gorgonians. Its reproductive cycle was delayed 2–3 months respect to shallow species, possibly following the late summer increase in seawater temperature occurring on the Mediterranean continental shelf. Moreover, internal brooding in *P. macrospina* contrasts with surface brooding in the congeneric *Paramuricea clavata*. Lipid content was lower and more constant in *P. macrospina* than in shallow species, and $\partial^{13}C$ and $\partial^{15}N$ composition showed almost no seasonal variation, suggesting that food availability in the continental shelf is lower but more constant than in shallower environments. The high oligotrophic conditions of the Balearic Sea apparently contrasts with the high abundance and diversity of active and passive suspension feeders observed in the Menorca Channel. Indeed, the downward particle fluxes quantified during two consecutive years in the Menorca Canyon were comparatively low within the Mediterranean context, reflecting the oligotrophic nature of the study area and the lack of continental inputs of particulate matter. Hydrodynamic settings and physical processes that cause sediment resuspension appeared to be more important than surface primary production in the control of the magnitude and composition of the total mass flux in the study area, likely playing a major role in determining the distribution of the macrobenthic assemblages.

Resum

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Les millores tecnològiques i la major disponibilitat de vehicles operats remotament (ROVs) i submarins tripulats han incrementat l'accessibilitat a entorns profunds, revelant la presència associacions d'organismes bentòniques riques i diverses, dominades per suspensívors. Aquestes agregacions han estat altament impactades per la pesca (en especial el ròssec) al Mediterrani. És urgent incrementar el coneixement, sobre la distribució d'aquestes associacions i aspectes claus de la biologia i ecologia de les principals espècies que les constitueixen per tal d'establir mesures de conservació efectives. En aquesta tesi es caracteritzen les associacions bentòniques de la plataforma i talús continentals del Canal de Menorca a través d'una aproximació multidisciplinària a diferents nivells ecològics.

S'han identificat sis associacions bentòniques diferents, que es diferencien principalment pel tipus de substrat i el rang batimètric. En els substrats durs s'hi ha trobat fons d'esponges i coralls, en els fons tous s'hi han trobat camps de crinoïdeus i braquiòpodes. Al marge continental s'hi concentren la diversitat més elevada, probablement resultant de l'heterogeneïtat ambiental i l'hidrodinamisme d'aquest entorn. Al Canal de Menorca les gorgònies són un dels organismes bioenginyers més importants i diversos, formant denses agregacions sobre amplies zones. Les agregacions de gorgònies a la plataforma i part superior del talús eren principalment monoespecífiques, mentre que les del marge continental eren multiespecífiques. Les colònies petites eren les més abundants al llarg de tot el rang batimètric. Tot i així, en entorns profunds les colònies mitjanes i grans incrementaven la seva abundància. El bon estat de les associacions bentòniques i les poblacions de gorgònies probablement resulta de la baixa pressió del ròssec, que es concentra a major fondària, i a les pràctiques excepcionals dels pescadors artesanals. *Paramuricea macrospina* és la gorgònia més abundant del Canal de Menorca. Al llarg de la seva distribució batimètrica es varen observar tres variants cromàtiques diferents. Dues es varen trobar a la plataforma i una tercera es trobà al marge continental. Per tal d'esbrinar si les diferents variants eren unitats taxonòmiques diferents s'han estudiat la forma colonial, la variabilitat de tamany i formes dels esclerits així com la variabilitat en marcadors mitocondrials. Es va detectar molt poca variabilitat en la morfologia colonial de les tres variants, suggerint que és un caràcter conservatiu. El

tamany i la morfologia dels esclerits diferia significativament entre les tres variants, suggerint que estan influenciats per factors ambientals i genètics. No es varen trobar diferències entre els tres marcadors mitocondrials. S'ha avaluat el cicle reproductor, l'acúmul energètic i les necessitats metabòlics de *P. macrospina*. El cicle reproductiu d'aquesta espècia està retardat 2–3 mesos respecte les espècies de gorgònia mediterrànies somes. Aquesta situació podria sorgir de l'augment tardà de la temperatura de l'aigua a la plataforma continental mediterrània. El fet que *P. macrospina* sigui una *internal brooder* contrasta amb que *Paramuricea clavata* sigui una *surface brooder*. El contingut lipídic de *P. macrospina* és més baix però més constant que el d'espècies somes mediterrànies. D'altra banda la composició de la $\partial^{13}C$ i la $\partial^{15}N$ gariebé no presentaven fluctuacions estacionals. Suggerint que la disponibilitat d'aliment a la plataforma és més baixa però més constant que en entorns soms. Al marge sud del Canal de Menorca hi trobem el Canyó de Menorca. Tot i l'elevada oligotròfia del mar Balear, les associacions d'organismes bentònics a la capçalera del canyó i proximitats són d'una elevada riquesa. Per aquest motiu s'ha avaluat el flux de partícules durant dos anys consecutius al canyó. Els fluxos recollits eren comparativament inferiors que els registrats en d'altres canyons mediterranis, reflectint la manca d'aportacions continentals i l'oligotròfia de l'àrea d'estudi. Els processos físics que causaven resuspensió i diferents paràmetres hidrodinàmics, semblen ser més importants en el control, la magnitud i la composició del flux de partícules que no pas la producció primària superficial. Aquest resultats suggereixen que els fluxos de partícules en canyons insulars Mediterranis, sense sistemes fluvials importants, són semblants als observats en el mar profund, però segurament juguen un paper important en la distribució d'associacions d'organismes macrobentònics.

Introduction

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Benthic assemblages in shallow environments have received plenty of attention due to the wide diffusion of SCUBA diving. The recent technological development and increased availability of video-equipped towed gears, remotely operated vehicles (ROVs), manned submersibles and autonomous under water vehicles (AUVs) have remarkably increased accessibility to deep environments, allowing the direct observation and quantitative study of benthic assemblages (e.g. Miller et al., 2012; White et al., 2012; Pineda et al., 2016). Several surveys using ROVs and manned submersible vehicles have been carried out in the deep sea, below a depth of 150 m (Sink et al., 2006). Conversely, continental shelf and upper slopes have received relatively less attention (Cau et al., 2015).

Highly diverse benthic assemblages dominated by passive (e.g. gorgonians, corals, black corals) and active (e.g. sponges, brachiopods) benthic suspension feeders have been recently found in continental shelf, slopes and seamounts worldwide (e.g. Clark et al., 2010; Kenchington et al., 2013; Buhl-Mortensen et al., 2016). In these deep environments, hydrodynamic processes provide high and stable food supply allowing the development of dense aggregations of suspension feeders, such as crinoid beds, sponge grounds, coral gardens and cold-water coral reefs (Lavaleye et al., 2002; Rice, 1990; Thiem et al., 2006). Benthic suspension feeders induce a significant flow of matter and energy from the pelagic to the benthic system by capturing plankton and particulate organic matter, playing a remarkable role in the benthopelagic coupling and biogeochemical cycles (e.g. Bell, 2008; Cathalot et al., 2015). From a structural point of view, they play an important ecological role by forming complex three-dimensional structures that may provide shelter, enhance food availability and act as nursery grounds for numerous associated species (Bo et al., 2015; Buhl-Mortensen and Mortensen, 2005; Henry et al., 2013). Consequently, high diversity and biomass of associated fauna is promoted in these benthic assemblages (Beazley et al., 2013). Amongst the associated fauna there are numerous decapod and fish species of commercial interest, which are targeted by the fishing industry (Clark and O'Driscoll, 2003; Colloca et al., 2004; Shester and Ayers, 2005). Consequently, many sponge grounds, coral gardens and cold-water coral reefs have been widely degraded due to the repeated exposure to destructive fishing activities, such us bottom trawling and long line fishing (Althaus et al., 2009; Taylor et al., 2013). Due to the slow growth and high longevity of most of the macrobenthic species that constitute these assemblages, their recovery may last centuries to millennia (Clark et al., 2016).

In the Mediterranean Sea, the first studies concerning benthic assemblages on the continental shelf and upper slope date back to the 1950s, when the Swiss engineer August Piccard performed several dives in areas of the Western Mediterranean Sea (Martin, 1960). However, it was not until the 1960s and 1970s when the first bionomical studies with detailed characterizations of deep Mediterranean benthic assemblages were performed (e.g. Pérès and Picard, 1964; Pérès, 1967; Vacelet, 1969; Fredj, 1972). These studies were based on the observations made on board of the manned submersible Scoupe Plongeante, developed by the French marine pioneer Jacks Costeau (Laban et al., 1963). After 1970s, research concerning deep Mediterranean benthic environments abruptly decreased, mainly due to the technical difficulties and high economic costs that imply working in deep-waters. During this period, most research derived from by-cached or withdrawn by blind destructive techniques (Relini et al., 1986; Gili et al., 1987). It was not until the 2000s when the increased affordability of ROVs and manned submersibles, and the increasing interest for cold-water corals, reinforced research in deep Mediterranean benthic environments (Taviani et al., 2005; Rossi et al., 2008; Orejas et al., 2009). In recent years, ROVs and manned submersibles have been used to characterize benthic assemblages on the continental shelf and slopes, reporting the presence of rich and dense assemblages such as coral gardens, sponge grounds or bryozoan beds (e.g. Bo et al., 2013, 2011; Fabri et al., 2014; Michez et al., 2014; Cau et al., 2015). However, in many cases these assemblages also presented clear signs of fishing derived damages (Orejas et al., 2009; Bo et al., 2014; Fabri et al., 2014; Angiolillo et al., 2015) (Fig. 2).

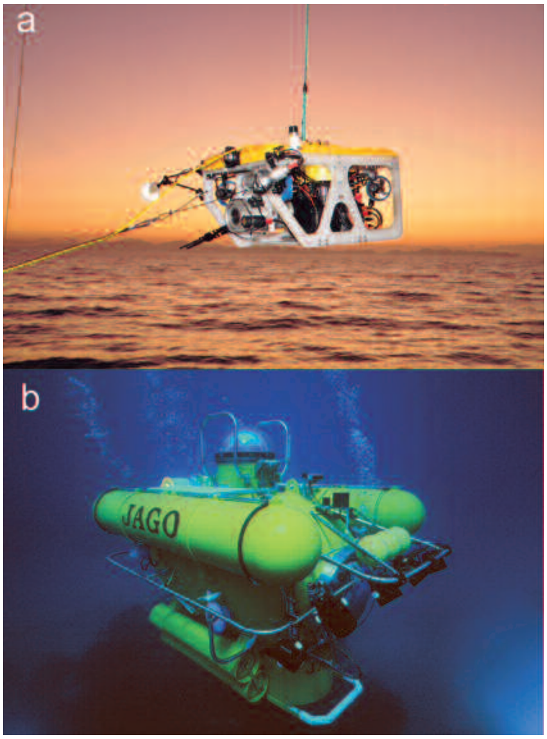

Fig. 1. a) the remotely operated vehicle NEMO (G. Newman) b) the manned submersible JAGO (IFM-GEOMAR) used during the field work of this thesis.

The increasing demand for fishing resources in Mediterranean countries, has lead to an intensification of fishing activities, resulting in overexploitation of fish stocks and the severe damage of benthic habitats, turning the Mediterranean Sea in one of the most impacted seas in the world (Lotze et al., 2011; Coll et al., 2012). Bottom trawling fishing has gradually increased in intensity since the first half of the 20th century, progressively expanding to greater depth (Sacchi, 2008). Consequently, decades of chronic trawling have widely impacted large areas of the Mediterranean continental shelf and slope (e.g. Fabri et al., 2014), probably constraining the presence of macrobenthic assemblages to remote (Díaz et al., 2015) or in-

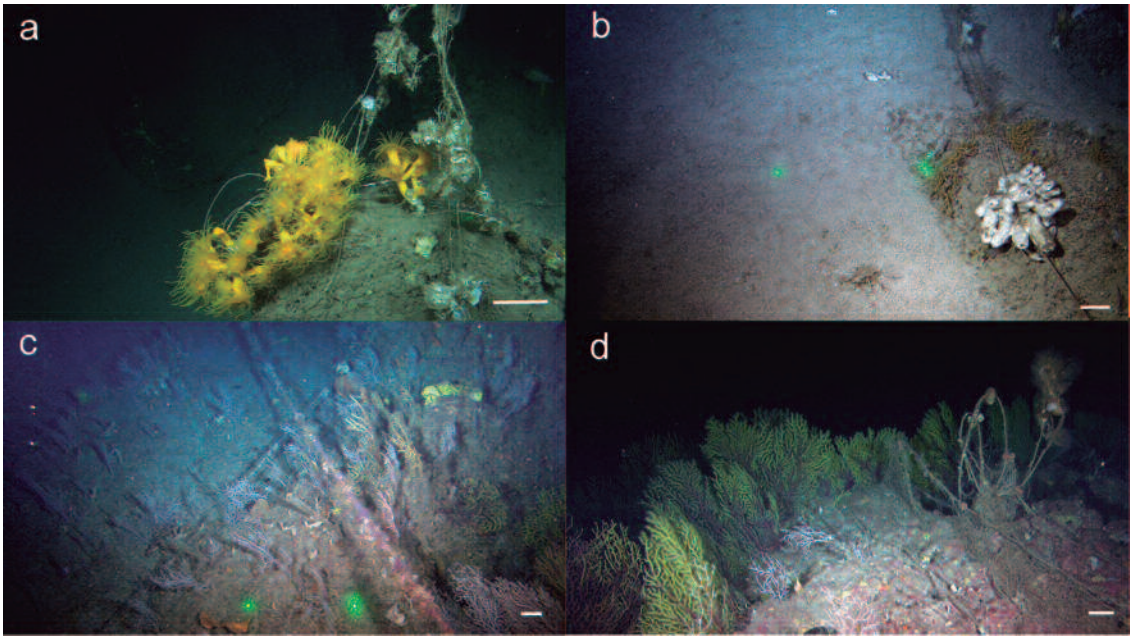

Fig. 2. a) Colony of the cold-water coral *Dendrophylia cornigera* entangled in a long line b) A large sponge *Haliclona magna* cut by a long line c) Long lines in a coral garden d) Trammel net entangled in the gorgonian *Paramuricea clavata*. Scale bar = 10 cm.

accessible areas (Bo et al., 2015). The vulnerability and low resilience of these assemblages (Althaus et al., 2009) have motivated the implementation of management measures, such as the restriction in the use of bottom trawling and other bottom contact gears (Pipitone et al., 2000) and the establishment of large marine protected areas on continental shelf and slope (Barberá et al., 2012). The European Union Natura 2000 initiative has developed a strong policy framework to deal with the multiple challenges that shallow and deep marine environments are facing, and ensure a sustainable-based approach for their marine resources (http://ec.europa.eu/environment/nature/natura2000/marine/index_en.htm9). From 2009 to 2014, the LIFE+ INDEMARES project has been studying deep benthic environments on Spain's territorial waters in order to contribute to the protection and sustainable use of 10 different sites of community interest (Fig. 3) that eventually will become special areas of conservation. These areas represent an increment of more than 7 million hectares to the Spanish Natura 2000 Network, covering more than 8% of Spain territorial waters (http://www.indemares.es/en/project/description). Ultimately, the LIFE+ INDEMARES project laid the foundation for future management plans for Natura 2000 marine areas. Moreover the project also contributes to fulfill the objective established by the Convention on Biological Diversity from the United Nations, to reach the goal of protecting 10% of coastal and marine areas by 2020 (https://www.cbd.int/2011-2020/goals). Amongst the 10 areas studied in the LIFE+ INDEMARES project, the Menorca Channel covers a vast extension of the continental shelf and upper slope between the Mallorca and Menorca Islands (Balearic Archipelago) from 40 to 360 m depth.

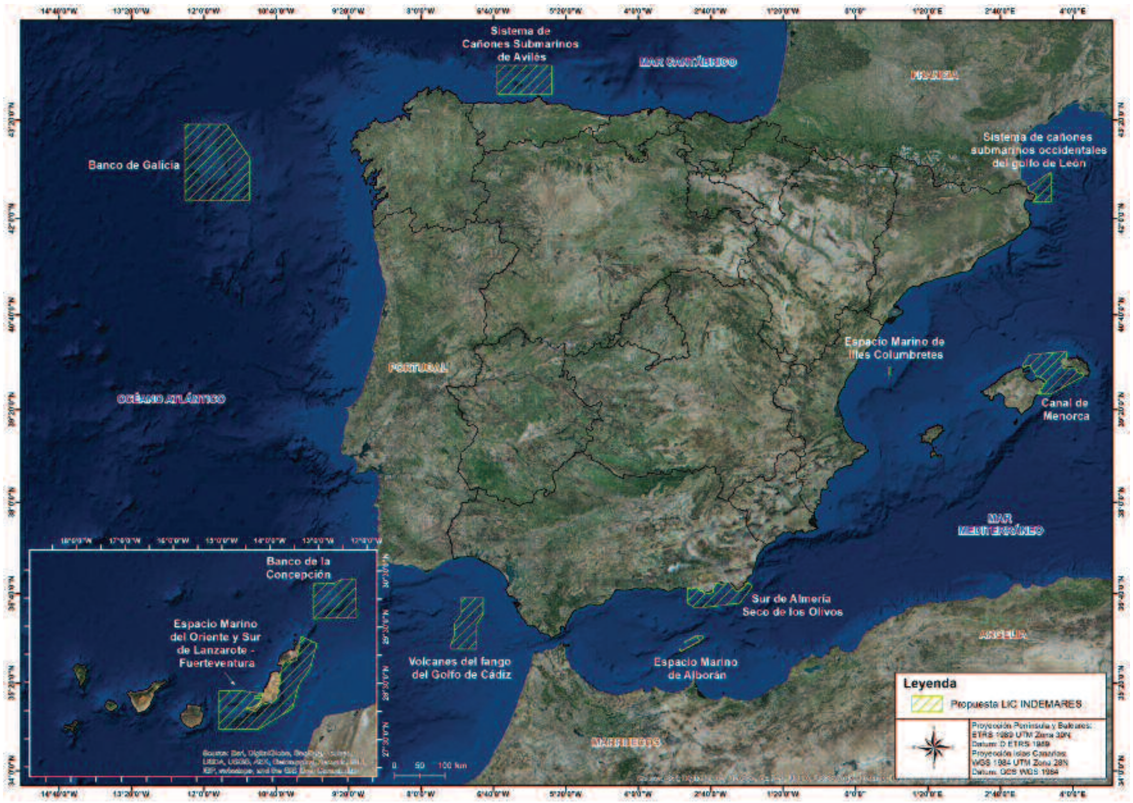

Fig. 3. Project LIFE+ INDEMARES study areas (http://www.indemares.es/areas-marinas).

GENERAL OBJECTIVES

The present thesis addressed the characterization of the benthic assemblages in the continental shelf and upper slope of the Menorca Channel by means of five different studies:

Chapter 1 - Macrobenthic assemblages on the Mediterranean continental shelf edge and upper slope (Menorca Channel, Western Mediterranean Sea).

This study focuses on the exploration of the deep continental shelf and upper slope of the Menorca Channel in order (1) to characterize the composition of the macrobenthic assemblages, (2) to asses their geographical and bathymetric distribution, (3) to quantify their biodiversity variation with depth, and finally (4) to discuss the possible relationship between macrobenthic assemblages distribution and the pressure of bottom trawling fishing in the area.

Chapter 2 - Diversity, distribution and population size structure of deep Mediterranean gorgonian assemblages (Menorca Channel, Western Mediterranean Sea).

Gorgonians resulted to be the most relevant macrobenthic species forming highly diverse and dense assemblages in the Menorca Channel. This study aimed at (1) characterize the diversity and quantify the abundance of gorgonians species on a large area of the continental shelf and upper slope at 40–360 m depth, (2) assess their geographical and vertical distribution, (3) explore their population size structure, and (4) to gain insight into the possible factors affecting their abundance and distribution.

Chapter 3 - Morphological and molecular variability of the gorgonian *Paramuricea macrospina* on the Mediterranean continental shelf (Menorca Channel, Western Mediterranean Sea).

Paramuricea macrospina resulted to be the most abundant gorgonian species in the Menorca Channel. Throughout its range of distribution, three different chromatic forms were observed. Two forms occurred on the continental shelf, and a third one occurred deeper on the shelf edge. This study aims to elucidate if the different *P. macrospina* chromatic forms represent distinct taxonomic units, by exploring differences in their (1) colony shape, (2) sclerite size and shape, and (3) genetic variability in mitochondrial markers (msh1, Igr1 and COI).

Chapter 4 - Reproductive cycle, energy storage and metabolic requirements of the gorgonian *Paramuricea macrospina* from the Mediterranean continental shelf (Menorca Channel, Western Mediterranean Sea).

The aim of this chapter was to explore, for the first time, the reproductive ecology and the dynamic of energy storage and metabolic demands of a Mediterranean gorgonian on the continental shelf. For this purpose, the annual development of sexual products, lipid content, free fatty acid content and composition, and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) composition were assessed over an annual cycle in the gorgonian *P. macrospina* from 70 m depth, to address the following questions: (1) Are there differences in the reproductive timing and gonadal output compared to shallow species? (2) Are there differences in the annual dynamic of energy storage and metabolic requirements compared to shallow species? (3) How are the gorgonian reproductive cycle, energy storage and metabolic demands related in the Mediterranean continental shelf environment?

Chapter 5 - Composition and temporal variability of particle fluxes in an insular canyon of the northwestern Mediterranean Sea.

The Baleric Archipelago is one of the most oligotrophic environments in the Western Mediterranean Sea. The only submarine canyon of the Archipelago is the Menorca Canyon. Despite the high oligotrophy of the Balearic Sea, benthic assemblages around the Menorca Canyon are highly diverse, with dense gorgonian and black coral assemblages. This study explored the environmental characteristics and flux of organic matter in which these communities thrive by characterizing (1) the temporal variability of downward particle fluxes, (2) assessing their geochemical and macroscopic composition, (3) identifying the main processes that modulate particle fluxes in the Menorca Canyon, and (4) compare the studied canyon with previous research developed in the Mediterranean Sea.

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Chapter 1

Macrobenthic assemblages on the Mediterranean continental shelf edge and upper slope (Menorca Channel, Western Mediterranean Sea)

ABSTRACT

Highly diverse macrobenthic assemblages dominated by passive and active suspension feeders have been recently reported in shelf edge environments of the Mediterranean Sea. Due to the frequent association with species of commercial interest, these assemblages have been heavily impacted by fishing practices. The vulnerability and low resilience of these assemblages, composed mainly by long-living and slow-growing species, have motivated the implementation of management measures such as the restriction of bottom trawling fishing, and the establishment of large protected areas including deep environments. The Menorca Channel is one of these large areas recently included in the protection frame of the European Union Natura 2000 network. Quantitative analysis of video transects recorded at 95–360 m depth by manned submersible and remotely operated vehicles were used to characterize macrobenthic assemblages, and assess their geographical and bathymetric distribution. Six different assemblages were identified, mainly segregated by substrate and depth. Hard substrates hosted coral gardens and sponge grounds, whereas soft sediments were mainly characterized by vast extensions of the crinoid *Leptometra phalangium* and the brachiopod *Gryphus vitreus*. The good preservation of most of the observed assemblages is probably related to the low pressure of bottom trawling fishing in this area, mainly concentrated deeper, on the continental slope. Because of their biological and ecological value, management and conservation measures need to be established to preserve these benthic assemblages.

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ADDITIONAL RESOURCES

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1. INTRODUCTION

Knowledge about macrobenthic assemblages in coastal areas has greatly increased during the past decades thanks to the wide diffusion of SCUBA diving; conversely, continental shelf and slope have received relative less attention due to the difficulties related to working in deep environments (Cau et al., 2015). However, the most recent technological developments and increased availability of remotely operated vehicles (ROVs), manned submersibles and video-equipped towed gears have significantly increased accessibility to these deep environments, allowing the direct observation and quantitative study of macrobenthic assemblages (e.g. Etiope et al., 2010; Buhl-Mortensen et al., 2016). Rich and high diverse macrobenthic assemblages mostly dominated by passive (e.g. gorgonians, corals and black corals) and active (e.g. sponges) suspension feeders (e.g. Emig, 1997; Bo et al., 2013; Bertolino et al., 2013), have been recently reported on the continental shelf, slope and seamounts in several locations of the Mediterranean Sea (Bo et al., 2009; 2012; 2015; Deidun et al., 2014). These assemblages have shown to provide habitat (Mastrototaro et al., 2010; Porteiro et al., 2013) and act as nurseries (Colloca et al., 2004; Bo et al., 2015) for a wide variety of associated species, many of which are of commercial interest (Abella et al., 2005; Maynou and Cartes, 2012).

Due to their distribution deeper than 50 m, these assemblages have been largely exposed to the impacts of bottom trawling (Maynou and Cartes, 2012; Fabri et al., 2014) and, to a lesser extent, long-line and trammel net fishing (Orejas et al., 2009; Sampaio et al., 2012; Mytilineou et al., 2014). These fishing practices have dramatic effects on macrobenthic species, which are often removed or severely damaged (Fosså et al., 2002; Mytilineou et al., 2014), resulting in a decline in the biodiversity and abundance of the associated fauna (Althaus et al., 2009; Clark et al., 2016). In the Mediterranean Sea, bottom trawling has progressively increased in intensity since the first half of the 20th century, progressively expanding to greater depth (Sacchi, 2008). Consequently, decades of chronic trawling have widely impacted large areas of the Mediterranean continental shelf and slope (e.g. Fabri et al., 2014), reducing the presence of relatively well preserved macrobenthic assemblages to remote (Díaz et al., 2015) or inaccessible areas (Bo et al., 2015). The ecological effects of bottom trawling are extremely long lasting, as no signs of recovery of macrobenthic assemblages have been observed in areas closed to trawling after more then ten years (Althaus et al., 2009; Williams et al., 2010). Due to the slow-growth and high longevity of most of the macrobenthic species that constitute these assemblages, it has been suggested that their possible recovery may take centuries to millennia (Clark et al., 2016).

The vulnerability and low resilience of these communities (Althaus et al., 2009) have motivated the recent implementation of management measures, such as the restriction in the use of bottom trawling and other bottom contact gears (Pipitone et al., 2000; Armstrong et al., 2014), and the establishment of managed and protected areas on continental shelf, continental slope (Spalding et al., 2013; Bennecke and Metaxas, 2016) and seamounts (Sheppard et al., 2012; Huvene et al., 2016) worldwide. Deep-water protected areas are often much more extensive than coastal ones (De Santo, 2013; Spalding et al., 2013; Bennecke and Metaxas, 2016). Large marine protected areas extend over wide bathymetrical and geographical range, covering several habitat types (Fernandes et al., 2005; Sheppard et al., 2012) and offering refuge to a wide variety of mobile and sessile species with high larval dispersal (Roberts et al., 2003). In the Mediterranean Sea, marine protected areas are mostly restricted to littoral environments (e.g. Francour et al., 2001). However, the establishment of large marine protected areas (>100 km^2), covering large areas of continental shelf and slope, has been recently proposed as a tool for the ecosystem based management of marine resources and environments (European Union, Natura 2000 network, http://www.eea.europa.eu/data-and-maps/data/natura-2). The Menorca Channel (Fig. 1) is one of these large area recently included in the protection frame of the European Union Natura 2000 network. Previous studies have investigated and characterized benthic assemblages in the coastal (e.g. Coma et al., 2006) and shallow continental shelf (<100 m) (e.g. Joher et al., 2012; Barberá et al., 2012) of the Menorca Channel. Conversely, composition and distribution of benthic assemblages on the deep continental shelf and upper slope still remain widely unknown. This information is basic for the establishment of effective management and conservation measures, as well as to monitor their effectiveness.

To this purpose, the aims of this study were: (1) to characterize the composition of macrobenthic assemblages on the deep continental shelf and upper slope of the Menorca Channel, (2) to asses their geographical and bathymetric distribution, (3) to quantify their biodiversity variation with depth, and finally (4) to identify a potential relationship between macrobenthic assemblages distribution and the pressure of bottom trawling fishing in the area.

2. MATERIALS AND METHODS

2.1 Study area

The Menorca Channel is located in the Western Mediterranean Sea between Mallorca and Menorca Islands (39° 53′ 0.73″ N, 3° 29′ 51.16″ E) (Fig. 1a), as part of the Balearic Promontory (Acosta et al., 2002). The study area covered the deep continental shelf (90–110 m), the shelf edge (110–180 m) and the upper slope (180–350 m) of the channel. The continental shelf is characterized by smooth reliefs covered by maërl beds alternated with coralligenous outcropping rocks and detritic coarse sediments (Barberá et al., 2012). Fine sands cover vast areas of the northern continental shelf at 100–110 m depth. Smooth reliefs and large extensions of detritic sediments with few isolated patches of outcropping rocks characterize most of the shelf edge and continental slope. Only near Cap Formentor (Fig. 1b) and in the Menorca Canyon (Fig. 1c) sharply edged rock outcroppings and rocky vertical walls are the dominant substrates.

The northern shelf edge and continental slope of the study area is mainly influenced by the Balearic Current (Balbín et al., 2012) and its associated front (Ruiz et al., 2009), which originates from a branch of the Liguro-Provençal-Catalan current flowing northward over the continental slope of the Balearic archipelago at ~200 m depth (Ruiz et al., 2009). Secondary currents flow northward from the Algerian sub-basin entering the Balearic sub-basin and feed the Balearic current (Pinot et al., 2002; Amores et al., 2013). Conversely, the southern shelf edge and upper slope are not influenced by a steady current (Amores and Montserrat, 2014), but by the sporadic arrival of mesoscale structures detached from the Algerian Current and the Almería-Oran front (Millot, 1987; García et al., 2005).

2.2 Sampling procedure

A total of 45 video transects (Fig. 1) were recorded during four surveys conducted on board of the R/V “García del Cid” (September 2010, April 2011, October 2011, June 2012). During the first two surveys, 17 video transects were recorded with the manned submersible JAGO (IFM–GEOMAR), whereas 28 video transects were recorded with the ROV NEMO (Gavin Newman) during the last two surveys. Both instruments were equipped with a 1080 horizontal line resolution camera, a grabber and two parallel laser beams which provided a scale to define a fixed width within the transects (0.5 m) during the subsequent video analysis. Transects were recorded in a close-zoom (~0.5–1.5 m width) and in a digital format. Positioning of JAGO and NEMO was achieved with underwater acoustic positioning system (LinkQuest TrackLink 1500 HA). Both JAGO and NEMO moved at a constant speed of ~0.3 knots, and transect lengths ranged between 309 and 2375 m, over depths ranging from 90 to 347 m. Transects were haphazardly located in order to cover the entire study area, but areas showing morphological features possibly related to the presence of rocky bottoms were explored more intensively (Fig. 1). Overall, a total of 37.7 km were video recorded along the seabed.

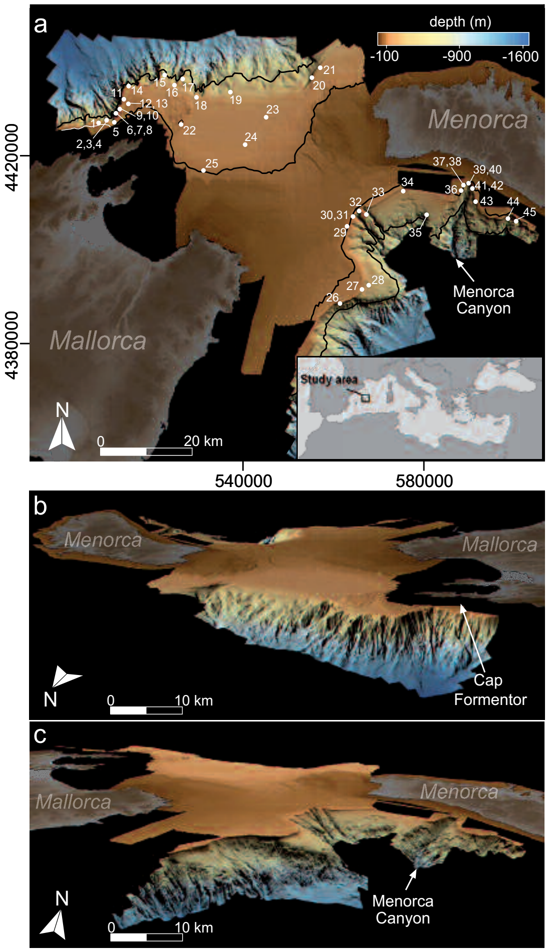


Fig. 1. The study area. (a) Three-dimensional bathymetry of the Menorca Channel: the map shows the location of the video transects (1 to 45) and the location of the study area in the western Mediterranean. Detail of the (b) northern and (c) southern subareas

In order to confirm the taxonomic identification of the macrobenthic organisms observed in the video transects, voucher organisms were collected by means of the JAGO and NEMO grabbers. Sampled organisms were fixed and preserved in 10% formalin as well as in absolute ethanol for posterior taxonomical and genetic analyses. Organisms were identified to the lowest possible taxon, which corresponded to species or genus.

2.3 Video analysis

Quantitative video analysis was performed according to the methodology described in Gori et al. (2011) using Apple's Final Cut Pro software. In order to correctly estimate the length of each transect, all the pauses in the movement of the JAGO or NEMO were removed from the footage. Sequences with poor image quality or too far away from the seafloor were considered unsuitable for analysis. The remaining useful sequences comprised 93.3% of the total recorded material and corresponded to a total distance of 35 km. All macrobenthic organisms observed within a width of 0.5 m (based on the laser beams) along each video transect were identified and assigned a time reference derived from the time elapsed since the beginning of the video transect to the crossing of the laser beams with the organism (Gori et al., 2011). This time reference was posteriorly converted into position along the transect according to the known velocity from the positioning system $P = t \cdot v$, where t is the time reference expressed in seconds, and v is the velocity expressed in meters per second). A similar procedure was used to characterize seabed substrate types and slope along every transect, within the same width of 0.5 m. Seabed substrate type was classified (based on the Wentworth scale) into five categories: fine sands, medium sands to gravels, cobbles and pebbles, maërl, and rock. Seabed slope was classified into three categories: horizontal (0°–30°), sloping (30°–80°) and vertical (80°–90°); slope was estimated from the video by looking at the two parallel laser beams and the depth sensor (Gori et al., 2011; Ambroso et al., 2013).

2.4 Data treatment

2.4.1 Sampling unit characterization

Each transect was divided into a string of sampling units. Different size of sampling units (2, 5, 10, 15, 20, 30, 40 and 50 m^2) were used in the canonical correspondence analysis (see below) in order to explore how the size of the sampling units may affect the identification of the benthic assemblages, and their associated main environmental features. Each sampling unit was characterized by the number of macrobenthic organisms of each identified species, as well as by the average depth and coverage percentage for each substrate and slope category.

2.4.2 Canonical correspondence analysis (CCA)

Relationship between benthic macrofauna abundance and depth, substrate type and slope were explored by means of canonical correspondence analysis (CCA). CCA is a multivariate constrained ordination technique used to elucidate the relationships between species abundances (response variables) and environmental variables (explanatory variables) (Greenacre and Primiceiro, 2013). Taxa that appeared with less than three individuals were discarded from the analysis in order to avoid distortions. Depth of sampling units was coded into three fuzzy categories that conserve all the information in the variables while reducing it to a categorical scale (Aschan et al., 2013). This fuzzy-coding allows taking into account possible nonlinear relationships between fauna abundance and depth (Greenacre and Primiceiro, 2013). Environmental variables were standardized by standard deviation, and CCA were performed with the R-language function cca in the ca library (Nenadic and Greenacre, 2007) of the R software platform (R Core Team 2014). Additionally, environmental factors were tested to determine which was the best predictor by means of the R-language function anova (Chambers and Hastie, 1992). CCA was performed with several sampling unit sizes (2, 5, 10, 15, 20, 30, 40 and 50 m^2) in order to explore how the identification of the benthic assemblages, and their associated main environmental features, change with the size of the sampling units.

2.4.3 Geographic distribution of macrobenthic assemblages

Geographical distribution of each identified macrobenthic assemblage in the study area was reported by mapping its occurrence in the video transects on a geographically referenced map using GIS (ESRI ArcGIS ArcInfo v10).

2.4.4 Variation of macrobenthic diversity with depth

Sampling units were grouped in 5 m depth intervals, and the exponential of Shannon's diversity index was calculated based on the observed macrobenthic abundance. This diversity index was chosen as it weights all species by their frequency without favoring rare or most common ones (Jost, 2006). Analysis was per-

Table 1. Results of the CCA analysis performed at different sampling unit

Sampling Unit Size	Species Nº	Sampling Units Nº	Total Inertia	Restricted Inertia	Unrestricted Inertia	% of Inertia explained by env. fact.	Macrobenthic assemblage Nº
2	69	8639	28.36	3.03	25.32	10.69	6
5	69	3372	20.96	3.28	17.68	15.6	6
10	69	1537	18.5	3.39	15.11	18.3	5
15	69	985	16.15	2.46	13.69	15.2	5
20	69	713	15.17	3.44	11.73	22.6	5
30	69	443	13.8	3.45	10.37	24.97	5
40	67	316	13.25	3.57	9.67	13.25	5
50	67	248	12.59	3.61	8.98	28.71	4

formed with the diversity function of the vegan library (Oksanen et al., 2015) of the R software platform.

2.4.5 Fishing activity in the study area

Fishing activity and location of fishing grounds (i.e. areas consistently fished through time) in the study area were assessed by means of the Vessel Monitoring System (VMS) through the analysis of the number of fishing events per surface units. VMS data were obtained in the framework of the LIFE+ INDEMARES project from the General Directorate of Fisheries Management of the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA). Available VMS records for the period January 2007 to July 2012 were included after examination to exclude erroneous vessel identity, position or speed. To select the records referring to fishing activity, a set of common criteria (Lee et al., 2010) was followed: duplicated records and records close to ports were removed, and the interval between records was calculated in order to only retain vessels moving at 2–5 knots. Distribution of fishing activity in the study area was assessed based on counts of fishing events per cell unit based on a point summation method (Hintzen et al., 2010). A 250 m^2 cell size was selected as the most suitable considering geographical factors and dataset (for further details, see Piet and Quirijns, 2009). Only one record per vessel, day and cell was retained to avoid overestimation of fishing activity (Hintzen et al., 2010). The centroids of each cell were extracted and weighted by the sum of fishing events for this cell. These weighted centroids were used to identify statistically significant hotspots of fishing activity in the study area using the Gi* statistic (Getis and Ord, 1996).

3. RESULTS

3.1 Canonical Correspondence Analysis (CCA)

CCA analysis performed with different sampling unit sizes revealed a general increase of the amount of inertia explained by environmental factors as sampling unit size increased

Table 2. Macrobenthic taxa occupancy and abundance in the study are
the set of sampling units); abundance (number of organisms). (subc

Phylum	Class	Nº Species	Occupancy		Abundance	
			Number	(%)	Number	(%)
Porifera	Desmospongia	21	936	27.76	11805	30.96
Cnidaria	Octocorallia (subc.)	19	858	25.44	6861	17.99
	Hexacorallia (subc.)	8	248	7.35	1170	3.07
	Hydrozoa	3	14	0.42	16	0.04
Briozoa	Gymnolaemata	1	31	0.92	47	0.12
Brachiopoda	Rhynchonellata	1	467	13.85	3219	8.20
Annellida	Polychaeta	4	491	14.56	2334	6.12
	Echiura	1	49	1.45	55	0.14
Echinodermata	Crinoidea	1	549	16.28	12126	31.8
	Ophiuroidea	1	64	1.90	416	1.09
	Echinoidea	4	127	3.77	144	0.38
	Holothuridae	2	15	0.44	15	0.04
	Astroidea	1	11	0.33	12	0.03
Chordata	Ascidiacea	2	6	0.18	6	0.02

(Table 1). However, as sampling unit size increased, the resolution of the CCA output decreased (Table 1). This was due to (1) a reduction in the total number of sampling units, (2) an increase in the number of sampling units discarded from the analysis affected by unsuitable sequences, and (3) an increase in the variability of each sampling unit composition (due to the presence of several bottom types in large sampling units) (Table 1). A 5 m^2 sampling unit size was chosen as the best balance between the inertia explained by environmental factors, and the number of macrobenthic species assemblages identified (Table 1).

Using 5 m^2 sampling units, a total of 38,136 organisms belonging to 69 macrobenthic species were considered in the analysis. Crinoidea, only represented by *Leptometra phalangium*, was the most abundant and the third most frequent taxon accounting for 31.8% of all observed organisms, occurring in 16.3% of all sampling units (Table 2). Desmospongia, represented by 21 species, was the second most abundant and the first most frequent taxon, accounting for 30.9% of all observed organisms, in 27.7% of all sampling units (Table 2). The subclass octocorallia, represented by 19 species, was the third most abundant and the second most frequent taxon, accounting for 17.9% of observed organisms, in 25.4% of all sampling units (Table 2). The remaining taxa were represented by 1–4 species, accounting for 0.02–8.2% of observed organisms, in 0.2–14.5% of all sampling units (Table 2).

In the CCA, sampling units and species were displayed constrained according to environmental factors. Environmental factors explained 15.6% of the variation in species abundance. The first axis (CCA1) explained 26.8% of the variance, and the second axis (CCA2) explained 22.7% of the variance. Substrate was the best predictor (inertia = 2.1935) followed by depth (inertia = 1.3017), and slope (inertia = 0.6974). Six different macrobenthic assemblages (Fig. 2a), characterized by environmental factors (Fig. 2b) and most contributing species (Fig. 2c) were identified in the CCA analysis:

Assemblage A: occurring on shallow maërl beds (Fig. 2b), is mostly characterized by the sponges *Haliclona* cf. *elegans* and *Aplysina cavernicola* (Figs. 2c, 3a and 3b).

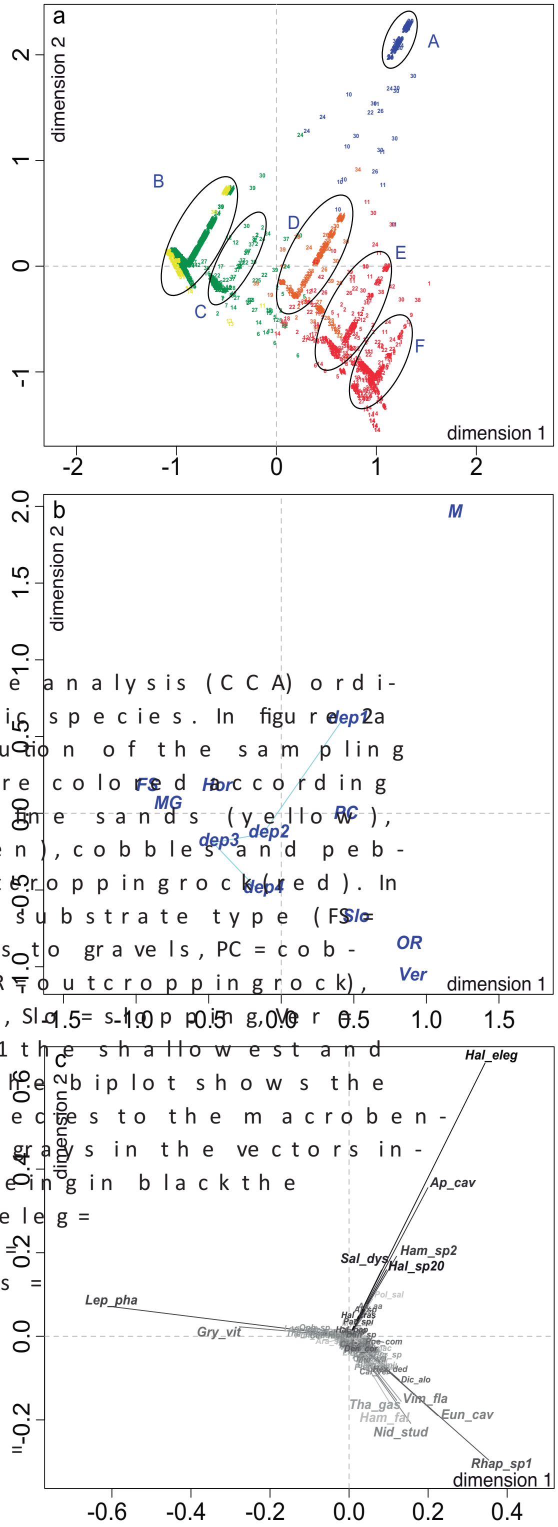


Fig. 2. Canonical correspondence analysis (CCA) ordination biplots of macrobenthic species. In figure 2a the biplot shows the distribution of the sampling units (n = 3372), sampling units are colored according to the dominant substrate: fine sands (yellow), medium sands to gravels (green), cobbles and pebbles (orange), maërl (blue), outcropping rock (red). In figure 2b the biplot shows the substrate type (FS = fine sands, MG = medium sands to gravels, PC = cobbles and pebbles, M = maërl, OR = outcropping rock), seabed slope (Hor = horizontal, Slo = sloping, Ver = vertical) and depth, being dep1 the shallowest and dep4 the deepest. In figure 2c the biplot shows the contribution of the different species to the macrobenthic assemblages, the scale of grays in the vectors indicate the degree of correlation, being in black the highest correlation (Hal_eleg = *Haliclona* cf. *elegans*, Ap_cav = *Aplysina cavernicola*, Ham_sp2 = *Hamacantha* sp., Hal_sp20 = *Haliclona* sp., Sal_dys = *Salmacina dysteri*, Lep_pha = *Leptometra phalangium*, Gry_vit = *Gryphus vitreus*, Tha_gas = *Thalamophyllia gasti*, Ham_fal = *Hamacantha falcula*, Vim_fla = *Viminella flagellum*, Eun_cav = *Eunicella cavolinii*, Nid_stu = *Nidalia studeri*, Rhab_sp2 = *Rhabderemia* sp.

Assemblage B: occurring on horizontal grounds covered by fine and coarse sands throughout most of the explored depth range (Fig. 2b), is mostly characterized by the presence of the crinoid *Leptometra phalangium* (Figs. 2c and 3c).

Assemblage C: occurring on horizontal grounds covered by medium sands to gravels in deep environments (Fig. 2b), is characterized by the presence of the brachiopod *Gryphus vitreus* (Figs. 2c and 3d).

Assemblage D: mostly found at intermediate depth on mixed cobbles and pebbles bottoms (Fig. 2b), is characterized by the polychaete *Salmacina dysteri* and two unidentified sponge species belonging to the genus *Hamacantha* and *Haliclona* (Figs. 2c, 3e and 3f).

Assemblage E: occurring in slopping rocky outcrops at shallow and intermediate depths (Fig. 2b), is characterized by the presence of the gorgonians *Eunicella cavolinii*, *Viminella flagellum*, the soft coral *Nidalia studeri*, the solitary coral *Thalamophyllia gasti* and the incrusting sponge *Hamacantha falcula* (Figs. 2c, 3g, 3h, 3i, 3j and 3k).

Assemblage F: occurring on vertical rock outcropping mostly at intermediate and deep environments (Fig. 2c), is characterized by an incrusting sponge of the genus *Rhabderemia* (Figs. 2c and 3l).

3.2 Geographic distribution of macrobenthic assemblages

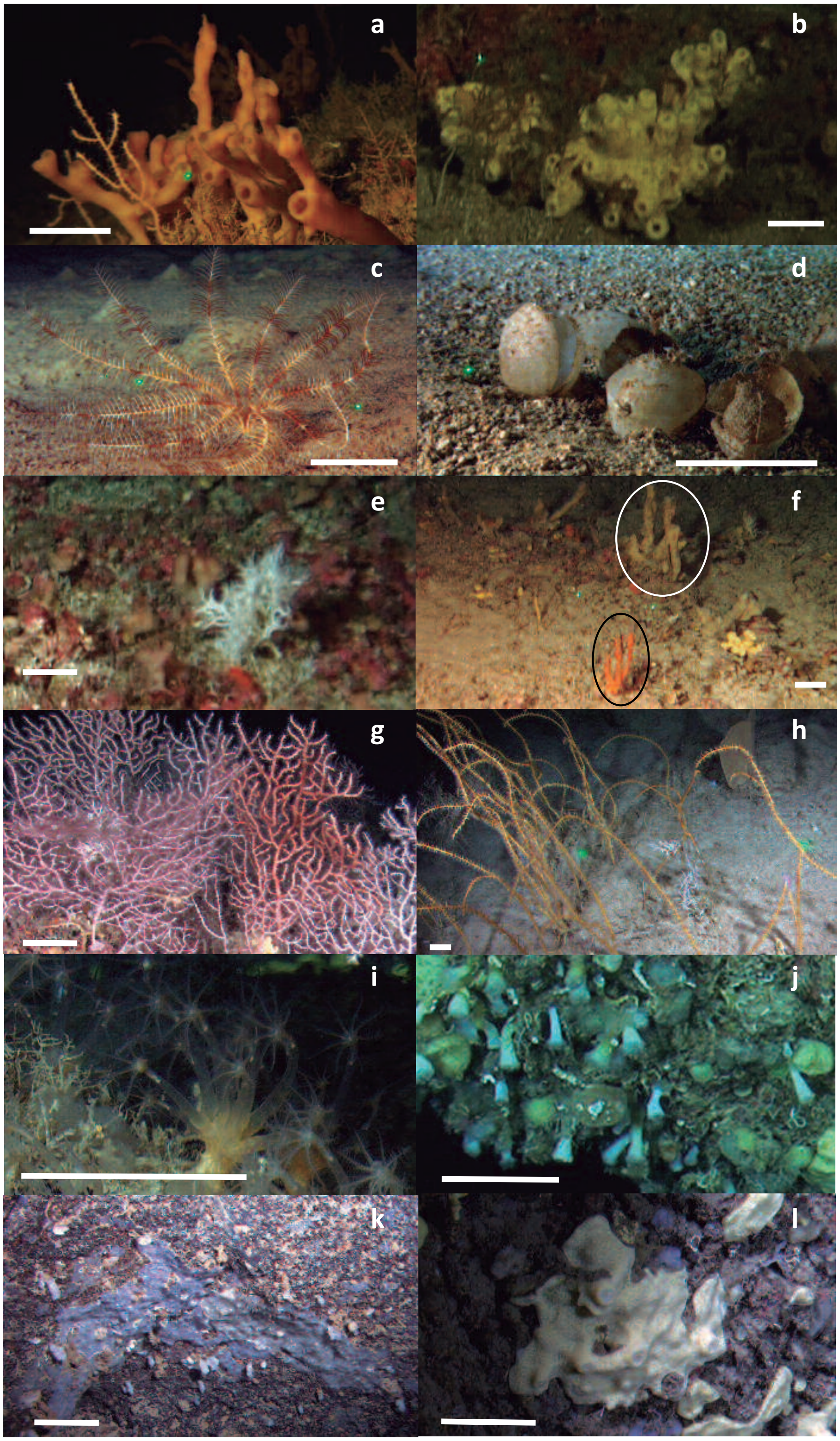
Assemblage A was only observed in one single transect in the northeastern side of the Menorca Channel (Fig. 4). Assemblages B and C were widely distributed in both the northern and southern areas of the channel (Fig. 4). Both assemblages presented a certain degree of overlapping, but assemblage C was restricted to deeper environments. Assemblage D was observed in only four locations in the channel, three in the northern slope and one in the head of the Menorca Canyon (Fig. 4), and partially coincided with assemblages A, E and F. Finally, assemblages E and F mostly co-occurred in the transects located in the proximity of Cap Formentor and the Menorca Canyon's head (Fig. 4).

3.3 Variation of macrobenthic diversity with depth

Highest sampling effort was conducted at 90–240 m depth, with sampling effort progressively decreasing below this depth. The exponential of Shannon's index presented the highest values (3.5–4.0) in the shelf edge depth range (110–160 m depth) (Fig. 5). Intermediate values of the Shannon's index (2.2–3.0) occurred at 160–250 m depth, whereas values decreased (<2.5) below 250 m depth, reaching minimum values at 330–350 m depth (Fig. 5).

3.4 Fishing activity in the study area

A total of 34 fishing vessels, from eleven ports were observed to regularly trawl in the Menorca Channel. The vessels from the ports of Cala Ratjada, Alcúdia and Ciutadella (Fig. 6) accounted for 77.5% of the localizations. One large (E) and two small (D and C) hot spot areas of bottom trawling were identified on the continental shelf of the channel at 50–75 m depth (Fig. 6). Four additional large hotspots were identified deeper on the continental slope in the north (A) and south area (F, G and H) of the channel at 500–600 m depth (Fig. 6). Finally, a small hot spot area was also observed near Cap Formentor (B) at ~50 m depth (Fig. 6). Three hotspots (A, E and G) concentrated 78.8% of the total bottom trawling fishing effort in the area.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4. DISCUSSION

Six different macrobenthic assemblages distributed throughout the continental shelf and upper slope were identified in the Menorca Channel. These assemblages were mainly segregated by the substrate type and depth range.

Sponge grounds composed by large desmosponge species (>15 cm in height), mostly characterized by the branched *Haliclona* cf. *elegans* and the tubular sponge *Aplysina cavernicola* (Assemblage A), occurred on a shoal located near the shelf edge at 95–110 m depth (Fig. 4). This shoal presented a highly irregular topography that alternated vertical walls with flat areas covered by maërl and flat coralligenous outcrops. The close proximity of the shoal to the shelf edge (Fig. 4) and its highly irregular topography, agrees with sponge ground distribution in other areas of the world (e.g. Klitgaard and Tendal, 2004). It has been suggested that the irregular topography of these environments may cause the acceleration of local currents (Rice et al., 1990) potentially favoring the presence of sponges (Beazley et al., 2015). *A. cavernicola* and *H.* cf. *elegans* accounted for 73% of all observed organisms, followed by other desmosponge species such us *Poecillastra compressa, Aaptos aaptos*, *Haliclona poecillastroides* and *Syphoncahlina* sp., representing 21% of the observed sponges. Species composition resembled that of other sponge grounds from rocky outcrops of the Tyrrhenian Sea continental shelf (Bertolino et al., 2013). Overall, sponge densities (9.2 ± 8.4 individuals m^{-2}, (mean ± SD), max = 43 individuals m^{-2}) were similar to those registered in other other Mediterranean sponge grounds (Bo et al., 2011a), and exceeded those observed in deep multispecific sponge grounds in the North Atlantic (Kutti et al., 2013; Howell et al., 2016). The lack of gorgonians (only represented by a few colonies of *Paramuricea macrospina*) and antipatharians basically distinguished this assemblage from Assemblage E, in which almost all the same sponge species (except *A. cavernicola*) co-occurred together with large gorgonians and antipatharians (see below). It has been suggested that under high oligotrophic conditions, such as those observed in the Balearic Archipelago, sponge and other active suspension feeders may become the dominant group in coralligenous community replacing gorgonians (Ballesteros, 2006). However, since gorgonians dominate vast areas of the continental shelf and upper slope of the Menorca Channel (Grinyó et al., 2016), the existence of the observed sponge ground might have been driven by other environmental or historical settings. Sponge grounds increase the structural complexity of habitats and the biodiversity of the associated macrofaunal community (Klitgaard, 1995; Beazley et al., 2013). Moreover, the high water processing capacity of sponges (Morganti, 2016) indicates that sponge grounds play a major role in the benthic-pelagic coupling (Ribes et al., 2005) through their influence on the microbial loop (Yahel et al., 2007) and the cycling of carbon and other elements (Kutti et al., 2013; Cathalot et al., 2015; Maldonado et al., 2015).

Leptometra phalangium (Fig. 3c) was widely distributed in both coarse and fine sands (Assemblage B), reaching high densities of more

⇦ **Fig. 3**. Most contributing species: a) *Haliclona* cf. *elegans*, b) *Aplysina cavernicola*, c) *Leptometra phalangium*, d) *Gryphus vitreus*, e) *Salmacina dysteri*, f) *Hamacantha* sp. 2 (white circles) and *Haliclona* sp. 1 (black circle), g) *Eunicella cavolinii*, h) *Viminella flagellum* i) *Nidalia studeri*, j) *Thalamophyllia gasti*, k) *Hamacantha falcula*, l) *Rhabderemia* sp. Scale Bar: 10 cm.

than 30 individuals m^{-2} (5.3 ± 3.2 individuals m^{-2} (mean ± SD)), as previously observed on the base of seamounts in the Tyrrhenian Sea (Bo et al., 2010). In the Menorca Channel, this almost monospecific assemblage (*L. phalangium* represented 95% of all observed organisms) mostly occurred on the shelf edge (96% of occupied sampling units) (Fig. 4), agreeing with studies that characterized it as a typical shelf edge assemblage (e.g. Fredj, 1964; Kallianiotis et al., 2000; Mangano et al., 2010). It has been suggested that *Leptometra* beds are mainly constrained to shelf edge environments as they depend upon the exposition to bottom currents that regularly carry high concentrations of suspended organic particles (Lavaleye et al., 2002; Colloca et al., 2003). Absence of trawling activity is fundamental for the persistence of high-density *L. phalangium* populations, since the fragility of this organism makes it extremely vulnerable to mechanical damage (Smith et al., 2000). *L. phalangium* beds have been associated with elevated densities of benthopelagic fish, with high juvenile abundances, suggesting that this assemblage may play a crucial role in the life cycle of numerous fish species, some of which are of high commercial interest (e.g., *Merlucius merlucius* and *Mullus barbatus*) (Colloca et al., 2004). The sea pen *Funiculina quadrangularis*, the polychaete *Lanice conchilega* and the anemones *Cerianthus membranaceus* and *Arachnanthus oligopodus* accompanied *L. phalangium* on coarse sands, together with the sea pen *Virgularia mirabilis*, the soft coral *Alcyonium palmatum*, and the sponge *Thenea muricata* on fine sands. The presence of *T. muricata* on the shelf edge (~130 m depth) was rather surprising, as it has mostly been reported in deeper areas of the continental slope (e.g. Pérès, 1967; Michez et al., 2014).

The brachiopod *Gryphus vitreus* (Fig. 3d) constituted 81% of all observed organisms in the Assemblage C. As previously described in other areas of the Mediterranean, this species sparsely covered (2 ± 3 individuals m^{-2} (mean ± SD)) sandy grounds on and beyond the shelf

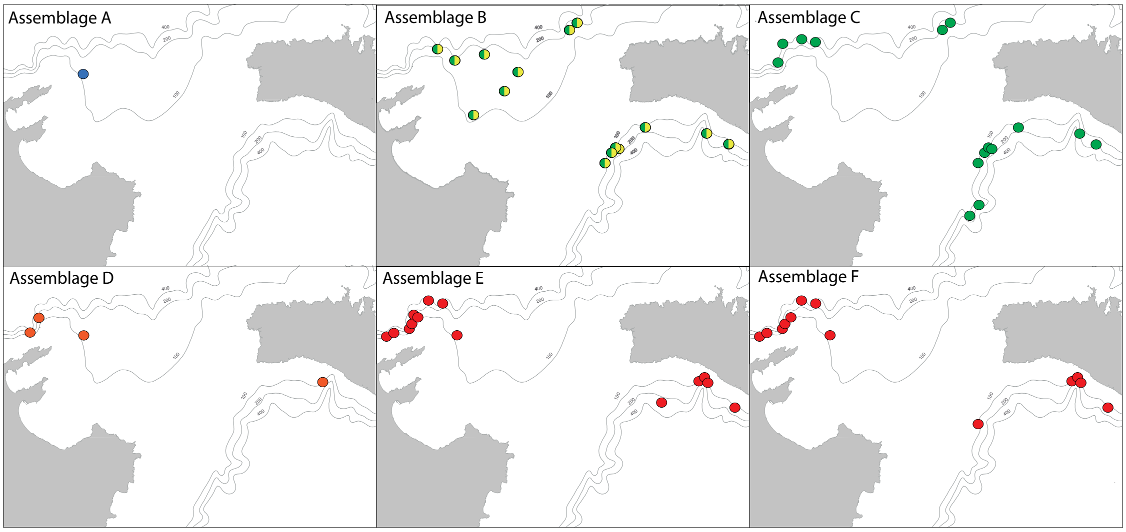

Fig. 4. Macrobenthic assemblages distribution. Assemblages were repr
transects where they occur, and following the colors as in figure 2: fin
(green), cobbles and pebbles (orange), maërl (blue), outcropping rock

edge, at 110–330 m depth (e.g. Emig, 1987; 1989). Assemblage C presented similar distribution of Assemblage B, occasionally co-occurring for tens of meters. However, while assemblage B was observed on both fine and coarse sands, Assemblage C was basically restricted to coarse sands (Figs. 2 and 4). This substrate segregation responds to the necessity of *G. vitreus* to fix to small pebbles or shells on the surface of the sediment (Emig, 1987). In the upper slope, at 180–200 m depth, *G. vitreus* formed dens aggregations with more than 25 individuals m^{-2}. However, these densities observed in the study area are rather low if compared to those reported from continental slopes exposed to intense bottom currents (600 individuals m^{-2}) (Emig, 1987). In this regard, *G. vitreus* and other brachiopod occurrence and abundance appear to be directly related to hydrodynamic conditions (Eshleman and Wilkens, 1979; Emig, 1987). The composition of accompanying species in coarse sands of assemblage B and C was very similar, with the sea pen *F. quadrangularis*, the polychaete *L. conchilega* and the anemones *C. membranaceus* and *A. oligopodus* amongst the most abundant. *A. oligopodus* and *L. conchilega*, have commonly been reported in soft sediments of the shelf edge and continental slope of the Mediterranean Sea (e.g. Pérès and Picard, 1964; Emig, 1997; Michez et al., 2014), occasionally formed high density (9 individuals m^{-2}) aggregation in the Menorca Channel.

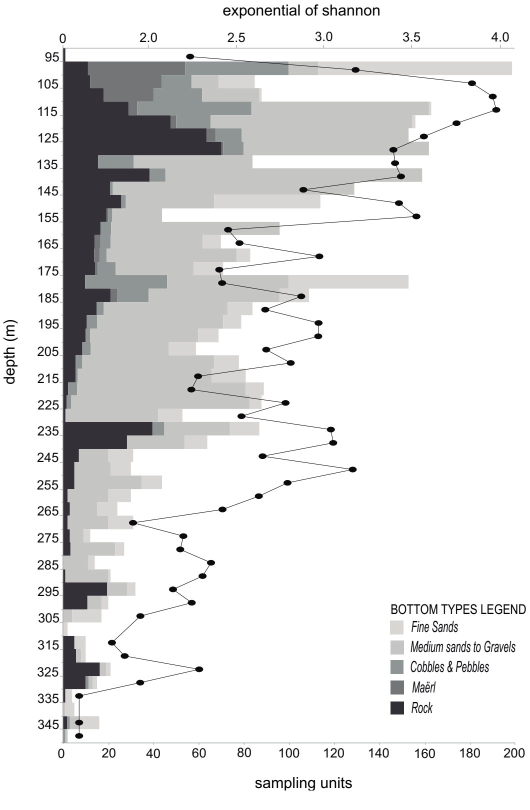

Fig. 5. Vertical distribution of the Shannon's exponential entropy index along the studied bathymetric range. Depth was subdivided at 5m intervals. Gray scale histograms represent the total number of sampling units for each substrate type (see legend).

The polychaete *Salmacina distery* (Fig. 3e) and two unidentified sponges belonging to the genus *Hamacantha* and *Haliclona* (Fig. 3f) characterized the Assemblage D, occurring on coarse sand scattered with cobbles and pebbles, at 95–140 m depth. These three species represented 63% of all observed organisms, and rarely exceeded densities of 1.5 individuals m^{2}. Despite the mixed substrate where this assemblage was observed, the vast majority of the organisms were associated to hard substrates (e.g. the sponges *P. compressa, A. aaptos* and *Pachastrella monilifera* (26% of observed organisms), and the gorgonians *E. cavolinii* and *Swiftia pallida* (10% of observed organisms). Conversely, coarse sand patches were mainly occupied by echinoids, holothurians and polychaetes (1% of observed organisms). Isolated *L. phalangium* were also occasionally observed. The occurrence of Assemblage D in the same transects than Assemblages A and E (Fig. 4) (mainly in the immediate proximities of outcropping rocks and vertical walls on the shelf edge, and in areas of dead maërl rodolyths in the proximities of shelf shoals) suggests that Assemblage D is a transition between assemblages occurring on hard substrates and those located in coarse sand ones.

The gorgonians *Eunicella cavolinii* and *Viminella flagellum*, the soft coral *Nidalia studeri*, the small solitary coral *Thalamophyllia gasti* and the incrusting sponge *Hamacantha falcula* (Figs. 3g, 3h, 3i, 3j and 3k) characterized (43% of all observed organisms) the sloping rocky substrates (Assemblage E) from the continental shelf to the upper slope (95–340 m depth). Despite this wide bathymetric distribution, this coral garden assemblage (*sensu* Stone, 2006; Buhl-Mortensen and Buhl-Mortensen, 2013) mostly occurred on the shelf edge at 110–190 m depth (72% of observed sampling units), on rocky outcrops and vertical walls near Cap Formentor and the Menorca canyon head (Fig. 4). These environments are most likely exposed to regular currents that may enhance food availability for the large sized passive suspension feeders that characterize this assemblage (Shepard et al., 1974; Balbín et al., 2012). Both the gorgonians *E. cavolinii* and *V. flagellum* had previously been reported as dominant species of rocky assemblages on the Mediterranean shelf edge (Bo et al., 2011b; Angiolillo et al., 2014). In the Menorca Channel, both species mostly formed mixed assemblages along with other species (Grinyó et al., 2016), but they can punctually form dense monospecific patches of up to 15 colonies m^{-2}, as also observed in other areas of the Mediterranean (Pedel and Fabri, 2011; Angiolillo et al., 2014). *N. studeri* and *T. gasti* formed dense monospecific assemblages (30 colonies m^{-2}). *N. stud-*

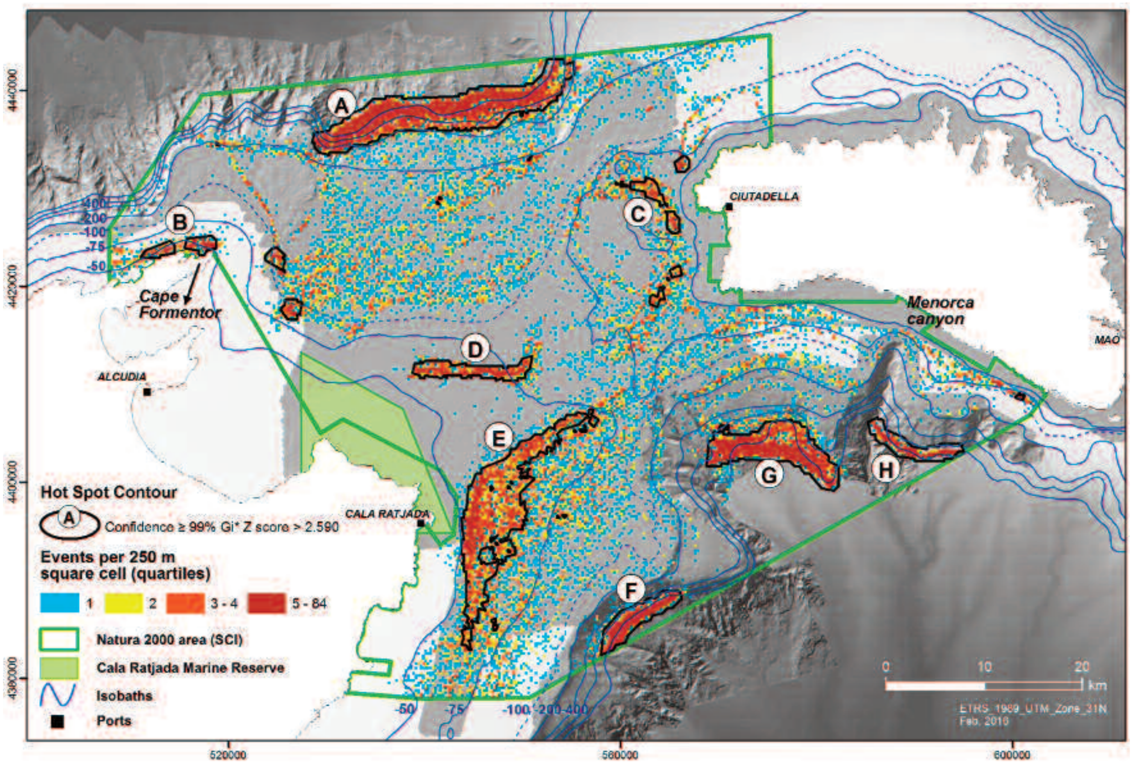

Fig. 6. Bottom trawling fishing effort in the Menorca Channel.

eri mostly occurred on gently sloping rocky outcrops, whereas *T. gasti* on steeply sloping rocky outcrops. The incrusting sponge *H. falcula* showed low maximum densities of 5 individuals m^{-2}, but large dimensions (>20 cm diameter), covering large areas of the rocky bottom, and perhaps excluding other species (Fig. 3k). Assemblage E was the most diverse of the six macrobenthic assemblages identified in the study area. Eighteen desmosponges were observed as accompanying species (25% of all observed organisms) mostly as scattered individuals spread throughout the transects (2.4 ± 4 individuals m^2 (mean ± SD)), and only punctually reaching high densities (15 individuals m^{-2}). Some of these sponges reached large dimensions (>20 cm height and width) such as the massive *Haliclona magna* or the fan shaped *Phakellia robusta*, *P. monilifera* and *P. compressa*. Anthozoans accounted for 57% of all observed organisms, with 22 different species. Besides *E. singularis* and *V. flagellum,* eight gorgonian species were also observed in this assemblage: *Acanthogorgia hirsuta*, *Bebryce mollis*, *Callogorgia verticillata*, *Corallium rubrum*, *Muriceides lepida*, *Paramuricea clavata*, *P. macrospina* and *S. pallida*. Gorgonians formed multispecific high-density patches (~20 colonies m^{-2}) (Grinyó et al., 2016), which contrast with the lower gorgonian diversity in coastal and bathyal Mediterranean zones (e.g. Gori et al., 2011; Cartes et al., 2013). The antipatharians *Antipathella subpinnata*, *Antipathes dichotoma*, and *Leiopathes glaberrima* occurred at low densities (1–4 colonies m^{-2}) in mixed assemblages with gorgonians, as also observed at similar depth in other areas of the Mediterranean Sea (e.g. Bo et al., 2009, 2012; Deidun et al., 2014). Finally, the recently described soft coral *Chironephthya mediterranea* (López-González et al., 2015), and *Paralcyonium spinulosum* were also largely represented in these rocky bottoms. Coral gardens are known to provide habitat to numerous organisms (Auster et al., 2013; De Clippele et al., 2015) and act as nursery grounds for several fish species (Bo et al., 2015; Etnoyer and Warrenchuk, 2007). Due to the complex three-dimensional structure they form, and the slow growth of gorgonian and antipatharian species (Sherwood and Edinger, 2009), coral gardens are extremely vulnerable to fishing damages (Angiolillo et al., 2015) and pollution (Silva et al., 2015), showing extremely slow recovery from anthropogenic impacts (Althaus et al., 2009).

Finally, vertical rocky substrates at 95–210 m depth were dominated by incrusting sponges (Assemblage F), with an unidentified incrusting sponge of the genus *Rhabderemia* (Fig. 3l) accounting for 64% of all observed organisms (max = 23 individuals m^{-2}), and other incrusting sponges representing 10% of all observed organisms. Incrusting sponges seem to be the only organisms adapted to colonize this environment, alternating with the conversely highly diverse coral gardens (Assemblage E), depending on the substrate inclination.

Unlike areas of the Gulf of Lions (Pérès and Picard, 1964; Orejas et al., 2009; Gori et al., 2013; Fabri et al., 2014) and the Catalan margin (Lastras et al., 2016), only a few colonies of the cold-water coral *Madrepora oculata* were observed on vertical rocky walls at 320 m depth in the Menorca Channel. This could suggest that this species might be restricted to deeper environments in the study area (> 350 m depth), as observed in other areas of the Mediterranean (e.g. Etiope et al., 2010; Taviani et al., 2015).

Macrofauna biodiversity was higher on the shelf edge, and progressively decline with depth (Fig. 5). This diversity pattern probably results from the synergy caused by habitat heterogeneity and hydrodynamic conditions in the shelf edge. Indeed, rocky outcrops and vertical walls alternate with different grain sized soft sediments grounds in the shelf edge. Moreover, both passive and active suspension feeders may benefit from the stable environmental conditions and hydrodynamic processes that enhance particle suspension in the near-bottom water layers on the shelf edge (Thiem et al., 2006). It has also been suggested that the observed increment in macrofauna diversity at intermediate depth may result from the merging of species with shallower and deeper distributions, causing a mid domain effect (Colwell and Less, 2000) as previously observed for deep coral communities in the north Pacific (Stone, 2006; Matsumoto et al., 2007). Benthic macrofauna in general, erected and massive sponges (Klitgaard and Tendal, 2004), gorgonians (Maynou and Cartes, 2012), antipatharians (Koslow et al., 2001) and crinoids (Smith et al., 2000) in particular, are extremely sensitive to bottom trawling fishing. Consequently, the low trawling pressure occurring at 100–500 m depth in the study area (Fig. 6) may be one of the main reasons for the preservation of the observed high-density and high-diverse macrofauna assemblages on the continental shelf edge. Decades of chronic bottom trawling on the continental shelf and edge (Watling and Norse, 1998) have probably heavily impacted and fragmented macrobenthic assemblages (Hall-Spencer et al., 2002), confining them to remote an inaccessible environments (Díaz et al., 2015; Bo et al., 2015). In this sense, the observed complexity and high-diversity of benthic macrofauna, could suggest how benthic community had been in several areas of the Mediterranean continental shelf and shelf edge, before the cumulative impact of decades of bottom trawling fishing. Management and conservation measures based on the knowledge of deep benthic communities composition and distribution should aim at limiting towed bottom fishing gears over the most sensitive areas, and establish large protection zones to protect and ensure conservation of these increasingly rare macrobenthic assemblages. Although the establishment of these large protection zones may pose potentially challenging situations amongst stakeholders, large protection zones may have economic benefits including the recovery of degraded fisheries (Pipitone et al., 2000) and the maintenance of ecosystem services (Balmford et al., 2002).

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Chapter 2

Diversity, distribution and population size structure of deep Mediterranean gorgonian assemblages (Menorca Channel, Western Mediterranean Sea)

ABSTRACT

Gorgonians are a key group of organisms in benthic marine communities with a wide bathymetric and geographical distribution. Although their presence on continental shelves and slopes has been known for more than 100 years, knowledge concerning the ecology of deep gorgonian species is still in a very preliminary stage. To overcome this situation, gorgonian assemblages located at 40–360 m depth were studied over a large geographical area on the continental shelf and upper slope of the Menorca Channel (Western Mediterranean Sea). A quantitative analysis of video transects recorded by a manned submersible and a remotely operated vehicle, were used to examine the diversity, distribution and demography of gorgonian species. Results showed high gorgonian diversity within this depth range (a total of nine species were observed) compared to Mediterranean coastal areas. Gorgonian assemblages on the continental shelf and upper slope were mostly monospecific (respectively 73% and 76% of occupied sampling units contained one single species), whereas shelf edge assemblages were highly multispecific (92% of occupied sampling units contained several species). This contrasts with the monospecificity of Mediterranean coastal gorgonian assemblages. Gorgonian populations on the continental shelf were mostly dominated by small colonies (88% of measured colonies) with few intermediate and large colonies (12% of measured colonies). In deeper areas small colonies were still dominant (60% of measured colonies), but intermediate and large colonies were much more abundant (40% of measured colonies). This suggests high recruitment rates on the continental shelf, but perturbations (trammel nets, long lines and strong storms) may limit the presence of intermediate and large colonies. Conversely, on the shelf edge and upper slope a more stable environment may allow colonies to reach larger dimensions. The identification and ecological characterization of these deep assemblages further extends the current knowledge about Mediterranean gorgonians, and is fundamental in improving the management and conservation of deep benthic ecosystems.

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1. INTRODUCTION

Gorgonian assemblages play an important structural and functional role in several marine benthic ecosystems all over the world, promoting a high diversity and biomass of associated fauna (Gili and Coma, 1998). From a structural point of view, gorgonians act as ecosystem engineers (*sensu* Jones et al., 1994) and foundation species (*sensu* Dayton, 1972) forming complex three-dimensional structures that provide a suitable habitat and refuge for numerous species (Buhl-Mortensen and Mortensen, 2005; Roberts et al., 2009; De Clippele et al., 2015). Current flow, food availability, and sediment re-suspension, vary widely within the complex structures formed by the gorgonian colonies, and this heterogeneity increases the abundance and functional diversity of the associated fauna (Witherell and Coon, 2001; Stone, 2006; Cerrano et al., 2010). From a functional point of view, gorgonians promote a significant flow of matter and energy from the pelagic to the benthic system by capturing plankton and suspended particulate organic matter (Lewis, 1982; Ribes et al., 1999; Sherwood et al., 2008), playing a paramount role in benthic-pelagic coupling processes and biogeochemical cycles (Gili and Coma, 1998; Coma et al., 2001; Hill et al., 2014).

Knowledge about diversity, distribution, ecology and state of conservation of gorgonian assemblages in coastal areas has significantly increased in recent decades based on the research carried out within the depth range of traditional SCUBA diving (~40 m depth) (e.g. Grigg, 1977; Yoshioka and Yoshioka, 1989; Linares et al., 2008). Far less is known about deep gorgonian assemblages located on the continental shelf and slope (e.g. Mortensen and Buhl-Mortensen, 2004; Matsumoto et al., 2007; Salomidi et al., 2009; Stone et al., 2014), where gorgonian presence was mostly known due to specimens by-cached by fishermen or withdrawn by blind destructive techniques (Relini et al., 1986) for taxonomical studies (Carpine and Grasshoff, 1975; Grasshoff, 1992). The most recent technological development and increased availability of remotely operated vehicles (ROVs), manned submersibles, and video-equipped towed gears have significantly increased accessibility to deeper areas, allowing for controlled sampling and quantitative study of deep rocky bottoms communities (e.g. Reed et al., 2006; Mortensen et al., 2008; Orejas et al., 2009). Abiotic features change considerably with depth (Bell and Barnes, 2000; Garrabou et al., 2002; Bak et al., 2005), and the overall variability in environmental conditions is dampened at greater depths (below ~100 m depth) because temperature, currents, and other water column features are more constant than in shallower areas (e.g. Puig et al., 2000; De Mole et al., 2002; Fernández de Puelles et al., 2007). Such stability in the main environmental conditions could allow deep gorgonians to grow larger and develop populations dominated by medium and large-sized colonies (Grigg, 1975; Watanabe et al., 2009; Gori et al., 2011b). Toppling and detachment by strong currents or wave action is considered one of the main causes of mortality of large gorgonian colonies (Grigg, 1977; Weinbauer and Velimirov, 1996; Weinberg and Weinberg, 1979). Indeed, both the environmental suitability and stability of a habitat may be reflected in the size structure of gorgonian and coral populations, because the size structure reflects the factors affecting recruitment, growth, and mortality rates in a particular habitat for a period of time matching the longevity of the population (Grigg, 1975; Gilmour, 2004; Alvarado-Chacón and Acosta, 2009).

Earlier ecological studies on deep gorgonian assemblages in the Mediterranean Sea revealed that they can reach density values as high as those observed in littoral environments (e.g. Bo et al., 2009; Angiolillo et al., 2014). However, both the continental shelf and upper slope are areas where fishing is more intense (Watling and Norse, 1998; Hall-Spencer, 2002). Gorgonians are among the most common species of the by-catch of bottom trawling, trammel nets, and long line fishing (Van Dolah et al., 1987; Krieger, 2001; Mytilineou et al., 2014). Since gorgonians are usually long-lived and slow growing (Linares et al., 2007; Risk et al., 2002; Sherwood and Edinger, 2009), fishing impacts can have far-reaching and long-lasting effects (Althaus et al., 2009). Consequently, deep gorgonian assemblages located on the continental shelf and upper slope are nowadays highly threatened by fishing activity (Hall-Spencer et al., 2002), and knowledge about their diversity, distribution and state of conservation is urgently needed to implement effective management and conservation measures of deep benthic ecosystems.

To this purpose, the aims of this study were: (1) to characterize the diversity and quantify the abundance of gorgonians forming Mediterranean gorgonian assemblages on a large area of the continental shelf and upper slope at 40–360 m depth (2) to assess the geographical and vertical distribution patterns of the observed species; (3) to appraise their population size structure, and (4) to gain insight into the possible factors affecting their abundance and distribution.

2. MATERIAL AND METHODS

2.1 Study area

The Menorca Channel is located in the Western Mediterranean Sea between Mallorca and Menorca Islands (39° 53′ 0.73″ N, 3° 29′ 51.16″ E) (Fig. 1a), as part of the Balearic Promontory (Acosta et al., 2002). The continental shelf in this area extends between 40 and ~110 m depth, and is largely covered by maërl alternating with patches of coastal detritic sediments and coralligenous outcrops (Barberá et al., 2012). Conversely, smooth reliefs and large extensions of detritic sediments with few isolated patches of outcropping rocks characterize most of the shelf edge and continental slope. Only near Cap Formentor (Fig. 1b) and in the Menorca Canyon (Fig. 1c) sharply edged rock outcroppings and rocky vertical walls are the dominant substrates.

The northern shelf edge and continental slope of the study area is mainly influenced by the Balearic Current (Balbín et al., 2012) and its associated front (Ruiz et al., 2009), which originates from a branch of the Liguro-Provençal-Catalan current, flowing northward over the continental slope of the Balearic archipelago at ~200 m depth (López García et al., 1994; Ruiz et al., 2009). Secondary currents flow northward from the Algerian subbasin entering the Balearic subbasin and feed the Balearic current (Alemany et al., 2006; Amores et al., 2013). Conversely, the southern shelf edge and upper slope is not influenced by a steady current (Amores and Montserrat, 2014), but by the sporadic arrival of mesoscale structures detached from the Algerian Current and the Almería-Oran front (Millot, 1987; García et al., 2005).

2.2 Sampling procedure

A total of 73 video transects (Fig. 1a) were recorded during five surveys conducted on board of the R/V "García del Cid" (September 2010, April 2011, October 2011, June 2012) and the R/V "Miguel Oliver" (August 2011). During the first two surveys, 20 video transects were recorded with the manned submersible JAGO (IFM-GEOMAR), whereas 53 video transects were recorded with the ROV NEMO (Gavin

Newman) during the last three campaigns. Both instruments were equipped with a 1080 horizontal line resolution camera, a grabber and two parallel laser beams which provided a scale to define a fixed width of the transects (0.3 m) during the subsequent video analysis. Transects were recorded in a close-zoom (~0.5-1.5 m width of view) and in a digital format. Positioning of JAGO and NEMO was achieved with underwater acoustic positioning system (LinkQuest TrackLink 1500 HA). Both JAGO and NEMO moved at a constant speed of ~0.3 knots, and transect lengths ranged between 309 and 2375 m, over depths ranging from 52 to 347 m. Transects were haphazardly located in order to cover the whole study area, but areas showing morphological features possibly related to the presence of rocky bottoms were explored more intensively (Fig. 1). Overall, a total of 57.8 km was video recorded along the seabed.

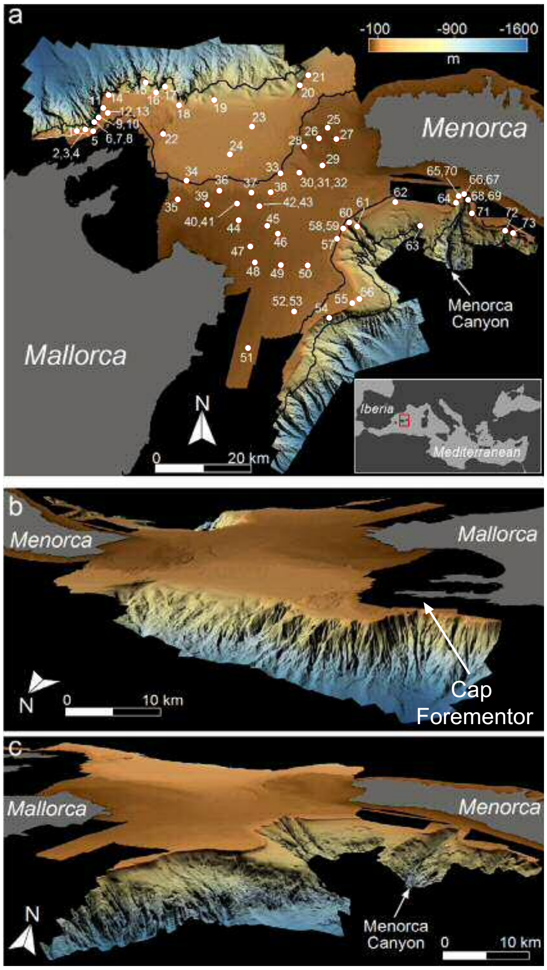


Fig. 1. The study area. (a) Three-dimensional bathymetry of the Menorca Channel: the map shows the location of the video transects (1 to 73) and the location of the study area in the western Mediterranean. Detail of the (b) northern and (c) southern subareas.

In order to confirm the taxonomic identification of the species observed in the video-transects, voucher colonies of gorgonian species observed along the transects were also collected. Colonies were fixed and preserved in 10% formalin until analyzed in the laboratory.

2.3 Video analysis

Quantitative video analysis was performed according to the methodology described in Gori et al. (2011a) using Apple's Final Cut Pro software. All the pauses in the movement of the JAGO or NEMO were removed from the footage, in order to correctly estimate the length in each transect, and those sequences with poor image quality or too far away from the seafloor were considered unsuitable for analysis. The remaining useful sequences comprised 94.6% of the total recorded material and corresponded to a total distance of 54.7 km. Every gorgonian observed within a width of 0.3 m (based on the laser beams) along each video transect was identified with a time reference derived from the time elapsed since the beginning of the video transect to the crossing of the laser beams with the base of the colony (Gori et al., 2011a). This time reference was posteriorly converted into position along the transect according to known velocity $P = t \cdot v$, where t is the time reference expressed in seconds, and v is the velocity expressed in meters per second). A similar procedure was used to characterize seabed substrate types and slope along every transect, within the same width of 0.3 m. Seabed substrate type was classified (based on the Wentworth scale) into four categories: sand to gravel, cobbles and pebbles, maërl, and rock. Seabed slope was classified into three categories: horizontal (0º–30º), sloping (30º–80º) and vertical (80º–90º); slope was estimated from the video by looking at the two parallel laser beams and the depth sensor (Gori et al., 2011a; Ambroso et al., 2013).

To study population size structure, the maximum height of each observed gorgonian colony was measured using the Macnification 2.0.1 software (Schols and Lorson, 2008) on still images extracted from recorded footage. The distance from the two lasers beams was used to calibrate the images. Measurements were done on still images in which the colony base was in the same plane as the laser beams, while the colony lied perpendicular to the video (Gori et al., 2011b). This methodological constraint entails that only a subsample of observed gorgonians could be measured for the study of population size structure (81% of the total observed colonies, 63%–100% of the colonies in each transect).

2.4 Species identification

Identification of the observed species was based on the existing taxonomic works on Atlanto-Mediterranean gorgonians (Carpine and Grasshoff, 1975; Grasshoff, 1992), and confirmed by the examination of the voucher specimen collected (see section 2.2), looking at the colonial branching pattern, the distribution and shape of calyces and polyps along branches, as well as the shape and size of sclerites. For this purpose, small fractions of the sampled colonies were placed in a sodium hypochlorite solution until organic matter was dissolved and sclerites disaggregated. Sclerites were rinsed with distilled water, and observed under a stereoscope (Olympus SZ-60). Three *Paramuricea macropsina* (Koch, 1882) chromatic morphotypes were differentiated in the video, two chromatic morphotypes on the continental shelf (M1, M2) and a third one on the shelf edge (M3) (more details in Grinyó et al. (submitted) (Figs. 2c, d and e).

2.5 Data treatment

2.5.1 Gorgonian occupancy and abundance

To quantify gorgonian occupancy (frequency of occurrence in the set of sampling units) and abundance (number of colonies per sampling unit), and examine the species composition of gorgonian assemblages within the study area, each transect track was divided into a string of 2 m^2 (0.3 m width and 6.66 m long) sampling units, using ArcMap 10.1 software (ESRI ArcGIS ArcInfo v10). Such sampling unit size was chosen as representative of Mediterranean octocorals on rocky substrate (based on Weinberg, 1978), as well as to allow a comparison with previous studies on shallow gorgonian and coral species (Gori et al., 2011a; Ambroso et al., 2013). A total of 8221 sampling units were obtained from the 73 transects, corresponding to a total area of 0.016 km^2. Each sampling unit was characterized by the number of colonies of each gorgonian species, as well as by its depth and coverage percentage for each substrate and slope type. For each gorgonian species, occupancy and abundance were quantified. Gorgonian assemblages were assessed based on species composition using a non-metric multidimensional scaling ordination (nMDS) of the sampling units, with gorgonian abundances square root transformed, and ordination by a Bray-Curtis similarity matrix. Adonis permutation multivariate analysis of variance and subsequent pairwise tests were used to test for significance of differences in gorgonian assemblages based on depth: continental shelf (40–100 m depth), shelf edge (100–180 m depth), upper slope (180–360 m depth), as well as substrate type (sand to gravel, cobbles and pebbles, maërl and rock), and slope (horizontal, sloping and vertical). Dominant substrate and slope type was assigned to each sampling unit for this analysis. Ordination and permutation multivariate analysis of variance and subsequent pairwise tests were performed using the r-language functions metaMDS and adonis, respectively, which are available in the vegan library (Oksanen et al., 2015) of the R software platform (R Core Team 2014). The adonis test was performed considering depth, substrate and slope simultaneously. Additionally, a similarity percentage procedure analysis (SIMPER, Clarke and Warwick, 1994) was performed to identify which gorgonian species contributed the most to the different assemblages considering the previously mentioned environmental features using the software PRIMER v6 (Clarke and Warwick, 2001).

2.5.2 Geographical and vertical distribution

Geographical distribution of each species in the study area was studied by mapping the observed density on a geographically referenced map using GIS (ESRI ArcGIS ArcInfo v10). Vertical distribution of each species was studied grouping sampling units in 20 m depth intervals (based on their depth), and estimating the median (first and third quartile, and the range between minimum and maximum values) of gorgonian density in each depth interval.

2.5.3 Population size structure

Size structure of gorgonian populations was analyzed in terms of descriptive statistics using distribution parameters such as skewness and kurtosis. Skewness is a measure of the symmetry of a distribution using its mean, reflecting the proportion of small versus large colonies in a gorgonian population. If skewness is significant ($p<0.05$) population size structure is asymmetrical. Positive skewness denotes the prevalence of small size colonies, while negative skewness denotes the dominance of large size colonies in the population. Kurtosis is a measure of the peakedness of a distribution near its central mode. A significant kurtosis value ($p<0.05$) indicates longer tails than would be expected for a normal distribution, and therefore a particular colony size prevails in the population. Skewness and kurtosis were calculated by means of the r-language functions agostino.test (Komsta and Novomestky, 2012) and anscombe.test (Anscombe and Glynn, 1983), which are available in the moments library of the R software platform. Only populations with more than 40 colonies were studied for their population size structure in order to perform meaningful skewness and kurtosis estimates.

2.5.4 Relationship with environmental features

Relationships between gorgonian abundances and depth, substrate type and slope were explored by means of canonical correspondence analysis (CCA), using the ordination software Canoco 4.5 (ter Braak and Smilauer, 2002). For this analysis, each sampling unit was characterized by its depth and the percentage coverage of each substrate and slope type. Gorgonian's abundance was log transformed, and the statistical significance of the species-environment relationship was evaluated using Monte Carlo permutation test using 999 unrestricted permutations.

2.5.5 Spatial structure

Finally, spatial structure in the distribution of one gorgonian species respect to the others was analyzed along 3 of the video transects, chosen in order to be representative of the assemblages identified in the study area. Abundance of each gorgonian species along the transects was displayed in density plots, obtained by transforming each transect into a string of contiguous quadrats (0.2 x 0.3 m) and counting the number of colonies of each species inside each quadrat. Spatial covariance between pairs of species was analyzed with the Three-Term Local Quadrat Covariance (3TLQC) function, using the Passage 2.0 software (Rosenberg, 2008). In the 3TLQC, the variance as a function of block size for species A (Var A), specie B (Var B), and the combined number (Var A+B) (Cov=Var A+B-Var A-Var B) is examined (Dale, 1999). To test the null hypothesis of an independent distribution of one species with respect to the other along each transect, randomized transects were generated for comparison by randomly permuting the "labels" (species A or B) of all the observed colonies along each transect. For statistical significance (95% confidence interval) the values at the limit of 2.5% tails of 999 of these ran-

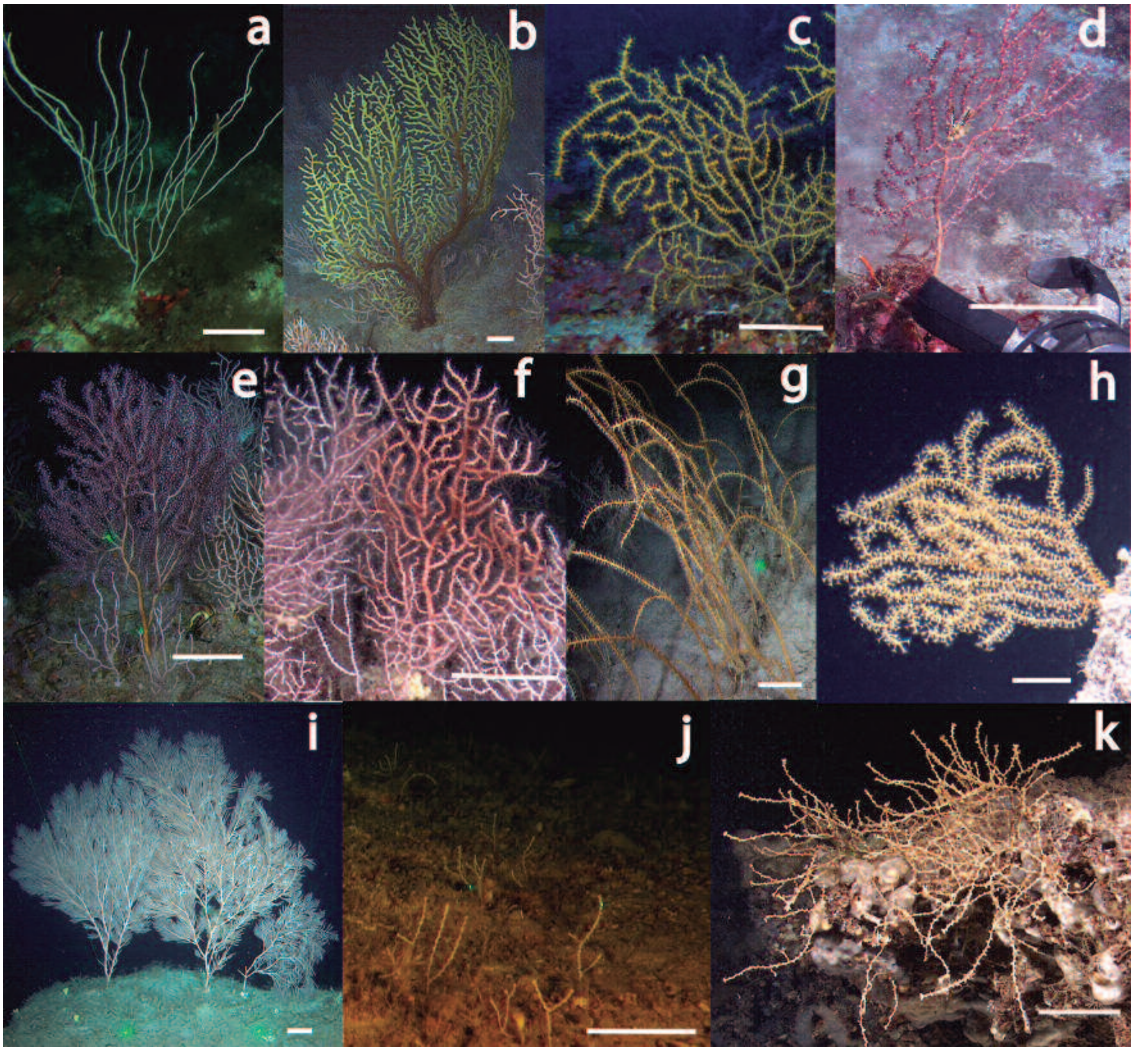


Fig. 2. Studied species. (a) *Eunicella singularis*, (b) *Paramuricea clavata*, (c) *Paramuricea macrospina* morphotypes (M 1), (d) M 2, (e) M 3, (f) *Eunicella cavolini*, (g) *Viminella flagellum*, (h) *Acanthogorgia hirsuta*, (i) *Callogorgia verticillata*, (j) *Swiftia pallida*, (k) *Bebryce mollis*. Scale Bar: 10 cm.

domizations was used. In the produced plots of covariance as a function of block size, position of a significantly positive deviated sample statistic from the bounds of the confidence interval (peaks) indicates the scale of any positive association (attraction) between species, whereas significant negative deviations (valleys) indicate the scale of any negative association (repulsion) (Dale, 1999).

3. RESULTS

3.1 Gorgonian occupancy and abundance

A total of 7802 colonies of nine gorgonian species (Fig. 2) were observed along all transects (Table 1), occurring in 18.9% of the 8220 sampling units. Overall, *P. macrospina* was the most abundant and frequent species, with its three morphotypes representing 44% of observed colonies present in 6.8% of the sampling units. *Eunicella cavolinii* (Koch, 1887) was the second most abundant and the third most frequent species, whereas *Eunicella singularis* (Esper, 1791) was the third most abundant and the second most frequent species. *Swiftia pallida* Madsen, 1970 and *Viminella flagellum* (Johnson, 1863) were the fourth and fifth most abundant and frequent species. The other species accounted for less than 2% of the observed colonies, occurring in less than 1% of the sampling units.

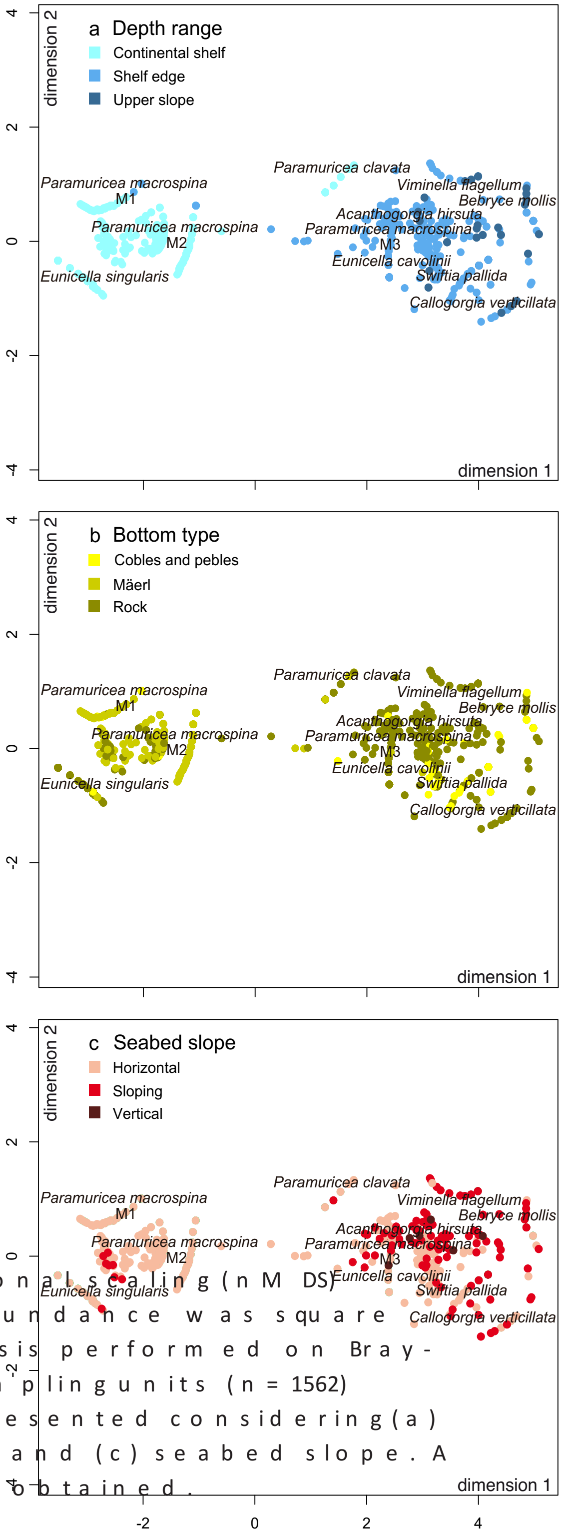


Fig. 3. Non-metric multidimensional scaling (nMDS) ordination plot. Gorgonian abundance was square root transformed and analysis performed on Bray-Curtis dissimilarity matrix. Sampling units (n = 1562) containing gorgonians are represented considering (a) depth range, (b) bottom type and (c) seabed slope. A stress estimate of 0.025 was obtained.

Table 1. Gorgonian occupancy and abundance in the study area. Occup
sampling units) is given for each bathymetric range and species; a
maximum density of each species is given per each bathymetric ra

Depth-range	Sampling Units			Species	Occupancy		Abundance		Mean density ± SD	Max density
	Nº	with gorgonians	(%)		Nº	(%)	Nº	(%)	(colonies · m^{-2})	(colonies · m^{-2})
Continental shelf (40 - 100 m)	2723	735	(27)	*E. singularis*	357	(48.5)	966	(23.0)	1.2 ± 1	7.5
				P. clavata	22	(3.1)	48	(1.1)	1.1 ± 0.8	3
				P. macrospina M1	292	(39.7)	1899	(44.0)	3.2 ± 5	33
				P. macrospina M2	208	(29.0)	1370	(32.0)	3.6 ± 3.9	18.5
Shelf edge (100 - 180 m)	3338	751	(22.5)	*P. clavata*	10	(1.3)	16	(0.5)	1.2 ± 1	3.0
				P. macrospina M1	3	(0.4)	4	(0.1)	0.7 ± 0.3	1.0
				P. macrospina M2	7	(0.9)	11	(0.3)	0.8 ± 0.7	2.5
				P. macrospina M3	51	(6.8)	149	(4.6)	1.5 ± 1.7	9.0
				E. cavolinii	308	(41.0)	1768	(55.1)	2.9 ± 2.7	24
				V. flagellum	65	(8.7)	425	(13.2)	3.2 ± 5.4	27.5
				A. hirsuta	17	(2.3)	34	(1.1)	1.0 ± 1.0	4.5
				C. verticillata	102	(13.6)	102	(3.2)	1.0 ± 0.9	5
				S. pallida	141	(18.8)	606	(18.9)	2.1 ± 2.6	13
				B. mollis	47	(6.3)	93	(2.9)	1.0 ± 1.0	5.5
Upper slope (180 - 360 m)	2159	75	(3.5)	*E. cavolinii*	8	(10.6)	16	(5.1)	1.0 ± 0.9	3
				V. flagellum	19	(25.3)	118	(37.9)	3.1 ± 4.5	17
				C. verticillata	23	(30.7)	32	(10.3)	0.7 ± 0.3	1.5
				S. pallida	23	(30.7)	79	(25.4)	1.6 ± 2.8	14
				B. mollis	26	(34.2)	66	(21.2)	1.2 ± 0.8	3

Two major groups could be identified in the nMDS, one represented by sampling units from the continental shelf covered by rock and maërl and presenting an horizontal inclination, and a second one formed by sampling units on the shelf edge and upper slope mostly covered by rock and presenting a flat or sloping inclination (Fig. 3).

Permutation multivariate analysis of variance and subsequent pairwise test revealed that for all environmental categorizations gorgonian assemblages were significantly different ($p<0.001$) from one another. The SIMPER analysis showed an average similarity in species composition that ranged from 17.89% to 31.68% (Table 2). The number of species contributing up to 90% of the similarity varied between two to four (Table 2). *E. singularis* dominated assemblages located on the continental shelf as well as those located on rocky and horizontal sampling units (Table 2). *E.*

cavolinii was especially relevant on assemblages located on the shelf edge as well as on sloping and vertical rocky outcrops (Table 2). *Bebryce mollis* Phillipi, 1842 dominated assemblages located in the upper slope, whereas *P. macrospina* M1 on those located on maërl beds, and *V. flagellum* dominate in vertical rocky bottoms (Table 2).

3.2 Geographic and vertical distribution

Only three species were observed on the continental shelf of the study area, *P. macrospina* (M1, M2), *E. singularis* and *Paramuricea clavata* (Risso, 1826) (Fig. 4, Table 1). *P. macrospina* (M1, M2) *and E. singulairs* were the most frequent species scattered over the continental shelf (Fig. 4) at 65–100 m and 52–88 m depth, respectively (Fig. 5). Both morphotypes of *P. macrospina* were much more abundant in terms of colony number than *E. singularis* (Table 1). Conversely, *P. clavata* was only found on the continental shelf in two low-density patches on coralligenous banks at 67–92 m depth, and few colonies on the shelf edge at 109–120 m depth (Figs. 4 and 5). The shelf edge and upper slope were much more diverse than the continental shelf in terms of species richness. In this depth range, gorgonian assemblages were highly localized in the areas dominated by rocky outcrops and vertical walls near Cap Formentor and in the Menorca Canyon (Fig. 4). *E. cavolinii* was the most frequent and abundant species of the shelf edge, with the highest abundances concentrated at 100–160 m depth, together with *P. macrospina* M3 (Figs. 4 and 5). *V. flagellum*, *S. pallida*, *C. verticillata* were also abundant on the shelf edge, but extended their distribution beyond 240 m depth, with one colony of *S. pallida* observed at 324 m (Figs. 4 and 5). *Acanthogorgia hirsuta* Gray, 1857 was the species showing the most restricted distribution, occurring in few locations of the shelf edge at 149–176 m depth (Fig. 5). *B. mollis* extended its distribution from the shelf edge to the upper slope, where it was the most frequent species, followed by *C. verticillata* and *V. flagellum* (Table 1). *V. flagellum* was the most abundant species in the upper slope, followed by *S. pallida*, *B. mollis* and *C. verticillata* (Table 1). Finally, some colonies (~20) of the precious coral, *Corallium ruburm*, were observed at 80–120 m depth, whereas only few isolated colonies were seen below 150 m depth.

3.3 Population size structure

Overall, the maximum height of 81% of all the observed colonies was measured (63–100% of the colonies in each transect). All the analyzed populations, indistinctively of the species, were unimodal (Figs. 6a and b). Most populations of *P. macrospina* M1, *V. flagellum* and the only analyzed population of *C. verticillata* were positively skewed, indicating the dominance of small colonies (Figs. 6a, b and Table 3). Conversely, most population of *P. macrospina* M2, *E. cavolinii*, *S. pallida* and all the *E. singularis* populations were not skewed, being dominated by medium sized colonies (Figs. 6a, b and Table 3). One of the two-studied populations of *P. macrospina* M3 was positively skewed (Fig. 6b, Table 3). All the *P. macrospina* M1 populations showed significant kurtosis (Table 3), indicating that they were slightly more peaked or over-centralized than normal distributions. Conversely, only a few populations showed significant kurtosis in the other species (Table 3). The shrub-like morphology, and the densely intertwined branches of *B. mollis* did not allow the study of population size structure in this species.

3.4 Relationship with environmental features

Depth, substrate and slope explained ~20.8% of the total inertia (i.e. explained variation of the data) in the CCA, with the first two axis accumulating 17.1% of the species variances, and 82.3% of the species-environment relation variance (Fig. 7). According to the Monte Carlo

Table 2. SIMPER analysis. Species that belong to the similarity group of 90
Percentages in bold letters are the average similarity values for each

Depth range	Species	Sim/SD	Contribution%	Cum. Contribution%
Continental shelf: 22.43%	*E. singularis*	0.48	60.33	60.33
	P. macrospina M1	0.34	24.59	84.92
	P. macrospina M2	0.25	14.86	99.79
Shelf edge: 21.45%	*E. cavolinii*	0.65	78.43	78.43
	S. pallida	0.25	14.70	93.14
Upper slope: 17.89%	*B. mollis*	0.33	38.55	38.55
	C. verticillata	0.26	24.83	63.38
	S. pallida	0.27	23.30	86.68
	V. flagellum	0.21	12.13	98.81
Bottom type	**Species**	**Sim/SD**	**Contribution%**	**Cum. Contribution%**
Rock: 18.63%	*E. singularis*	0.37	54.89	54.89
	E. cavolinii	0.33	32.61	87.50
	S. pallida	0.12	3.68	91.19
Cobbles and pebbles: 17.67%	*S. pallida*	0.42	60.77	60.77
	E. cavolinii	0.21	14.08	74.85
	P. macrospina M1	0.16	10.86	85.70
	B. mollis	0.15	9.93	95.64
Maërl: 28.68%	*P. macrospina* M1	0.62	60.20	60.20
	P. macrospina M2	0.42	33.55	93.75
Seabed Slope	**Species**	**Sim/SD**	**Contribution%**	**Cum. Contribution%**
Horizontal: 18.11%	*E. singularis*	0.35	49.09	49.09
	P. macrospina M1	0.28	25.11	74.19
	P. macrospina M2	0.29	14.93	89.13
	E. cavolinii	0.15	7.12	96.25
Slopping: 19.66%	*E. cavolinii*	0.50	66.28	66.28
	B. mollis	0.17	9.94	76.22
	S. pallida	0.17	9.26	85.48
	V. flagellum	0.17	7.03	92.51
Vertical: 31.68%	*E. cavolinii*	0.56	53.09	53.09
	V. flagellum	0.51	44.75	97.84

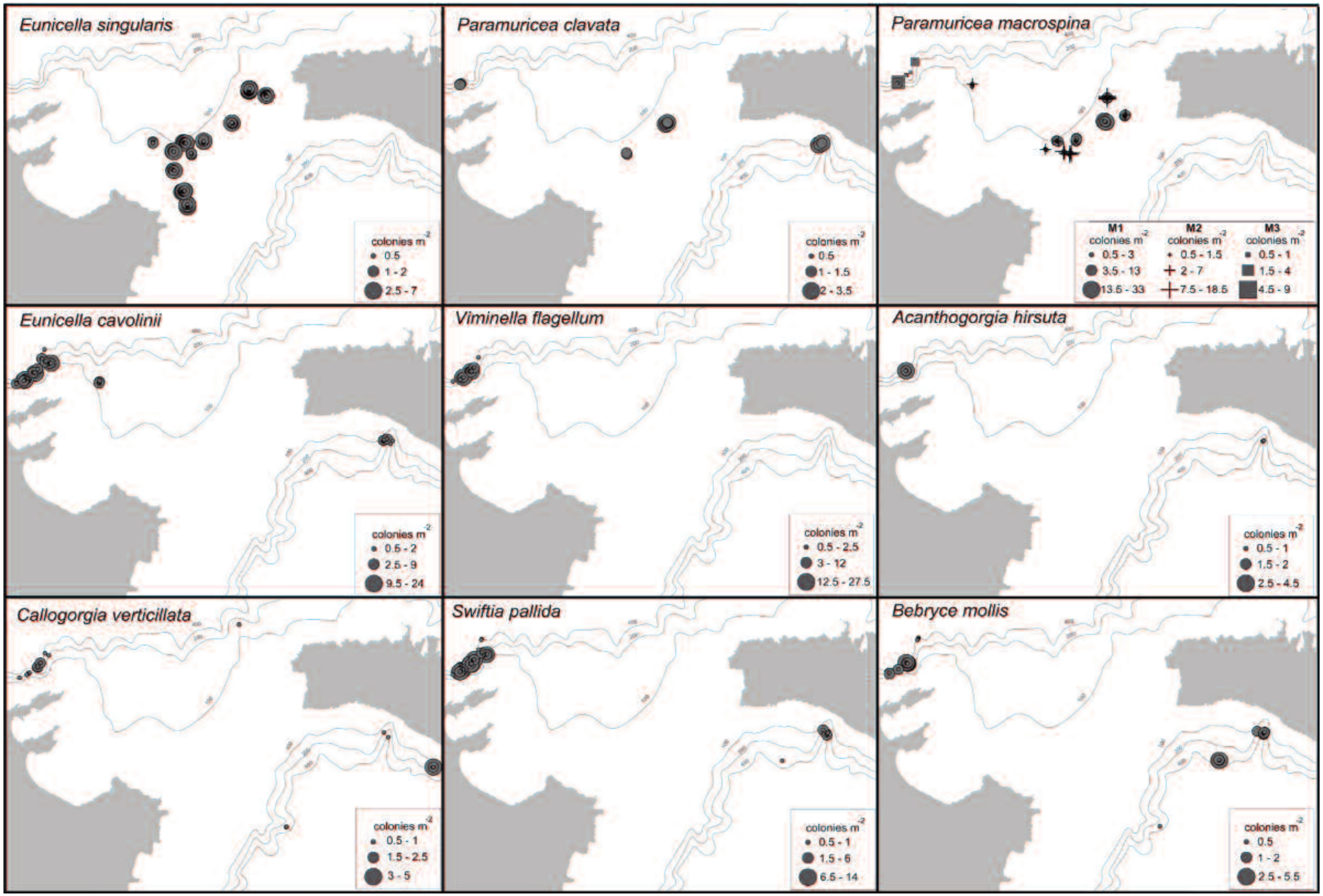


Fig. 4. Geographical distribution. *E. singularis*, *P. clavata*, *P. macrospina* (M1, M2, M3), *E. cavolinii*, *V. flagellum*, *A. hirsuta*, *C. verticillata*, *S. pallida*, and *B. mollis* distribution is represented on the study area based on sampling unit density. Species have been displayed from shallowest to deepest distribution.

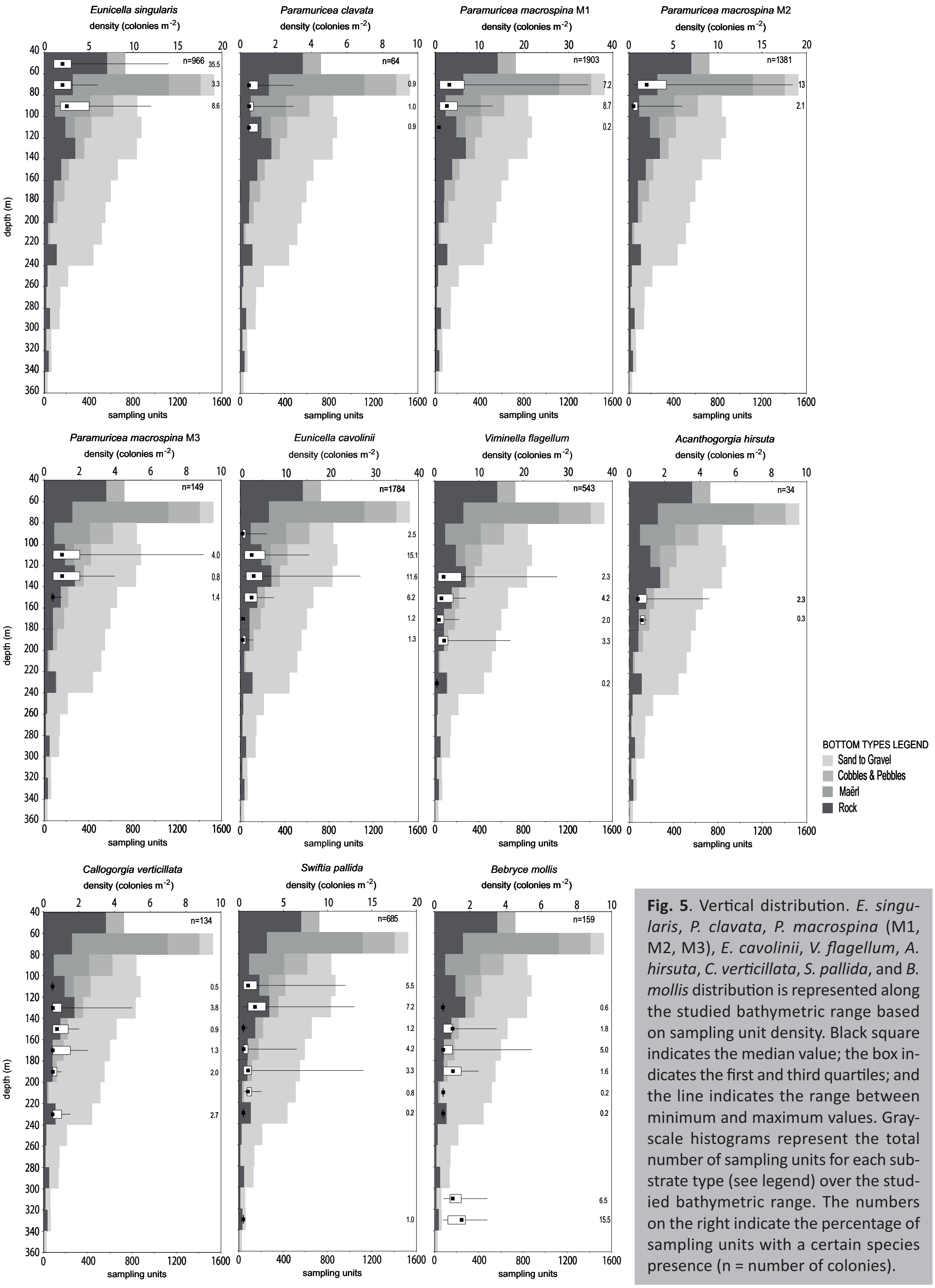


Fig. 5. Vertical distribution. *E. singularis*, *P. clavata*, *P. macrospina* (M1, M2, M3), *E. cavolinii*, *V. flagellum*, *A. hirsuta*, *C. verticillata*, *S. pallida*, and *B. mollis* distribution is represented along the studied bathymetric range based on sampling unit density. Black square indicates the median value; the box indicates the first and third quartiles; and the line indicates the range between minimum and maximum values. Grayscale histograms represent the total number of sampling units for each substrate type (see legend) over the studied bathymetric range. The numbers on the right indicate the percentage of sampling units with a certain species presence (n = number of colonies).

Table 3. Size structure characteristics of the studied morphotypes and species: colony height, skewness and kurtosis. Significant skewness or kurtosis are indicated with one (p-value <0.05) two (p-value < 0.01), or three asterisks (p-value < 0.001).

			Height (cm)			Skewness			Kurtosis		
Species	**Transect**	**Colonies**	**Mean**	**SD**	**Max.**	**Skew.**	**p-value**	**Sig.**	**Kurt.**	**p-value**	**Sig.**
E. singularis	37	169	25.30	10.35	55.1	0.541	0.062		2.708	0.497	
	47	45	8.86	4.58	23	0.066	0.209		3.216	0.449	
P. macrospina (M1)	28	98	6.26	5.83	29.4	1.775	<0.001	***	5.870	<0.001	***
	29	81	5.90	3.36	18.4	1.377	0.004	**	4.939	0.008	**
	30	1099	8.72	6.9	33.1	1.056	<0.001	***	3.335	0.037	*
	37	65	10.82	6.10	33.8	0.829	0.075		4.550	0.027	*
	38	276	8.24	6.17	41.9	2.013	<0.001	***	7.596	<0.001	***
	43	251	12.10	6.24	29.4	0.185	0.421		2.247	<0.001	***
P. macrospina (M2)	28	461	6.82	5.13	30.1	1.302	<0.001	***	4.415	<0.001	***
	42	166	15.05	5.75	31.35	0.346	0.222		2.570	0.222	
	43	57	14.52	5.61	25.1	-0.242	0.590		2.366	0.286	
P. macrospina (M3)	2	70	17.22	9.07	41.31	0.784	0.086		2.831	0.967	
	3	52	17.95	11.43	55.65	13.566	0.018	*	4.037	0.272	
E. cavolinii	2	206	10.55	7.43	35.63	0.985	<0.001	***	3.205	0.417	
	3	384	15.16	10.06	50.45	0.739	<0.001	***	2.912	0.843	
	4	78	16.03	7.98	43.51	0.081	0.059		3.621	0.177	
	6	156	14.59	8.77	47.15	0.579	0.056		2.909	0.990	
	7	82	13.03	6.65	31.94	0.694	0.092		3.136	0.542	
	10	63	19.07	9.83	47.65	0.276	0.524		2.775	0.950	
	11	207	9.08	7.30	38.55	1.492	<0.001	***	4.940	<0.001	***
	13	110	11.93	6.93	29.75	0.442	0.202		2.468	0.198	
	22	71	9.81	4.92	22.42	0.686	0.116		2.703	0.785	
V. flagellum	2	56	42.35	22.20	96.41	0.308	0.500		2.558	0.606	
	4	230	38.19	25.14	148.3	1.045	<0.001	***	4.721	<0.001	***
	7	130	46.00	30.86	136.6	0.740	0.0300	*	3.045	0.689	
C. verticillata	73	54	26.43	21.52	115.31	2.513	<0.001	***	9.467	<0.001	***
S. pallida	3	186	5.35	1.98	13.54	0.932	<0.001	***	4.452	<0.001	***
	4	88	7.12	2.12	12.17	0.224	0.548		2.655	0.615	
	7	83	6.83	2.71	13.96	0.753	0.069		3.190	0.480	
	9	90	6.66	2.81	17.46	1.061	0.013	*	5.329	0.002	**
	13	91	4.62	1.75	10.47	0.552	0.151		3.641	0.156	
	69	40	5.32	1.99	10.39	0.439	0.408		2.571	0.759	

permutation test, the three factors contributed significantly ($p<0.001$) to the ordination. Depth clearly segregates gorgonians found on the continental shelf (*P. macrospina* M1 and M2, *E. singularis*, *P. clavata*) from those from the shelf edge and continental slope (*P. macrospina* M3, *V. flagellum, E. cavolinii, S. pallida, B. mollis, C. verticillata*, and *A. hirsuta*). On the continental shelf, *P. macrospina* M1 and M2 were mainly associated with horizontal maërl beds, whereas *E. singularis* and *P. clavata* with rocky bottoms (Fig. 7). On the shelf edge and continental slope, *E. cavolinii* and *P. macrospina* M3 were mainly associated with sloping rocky bottoms. At increasing depth *V. flagellum*, *S. pallida*, *C. verticillata,* and *A. hirsuta* were also mainly associated with sloping bottoms (Fig. 7). Finally, *B. mollis* was the species most strongly associated with highest depths (Fig. 7).

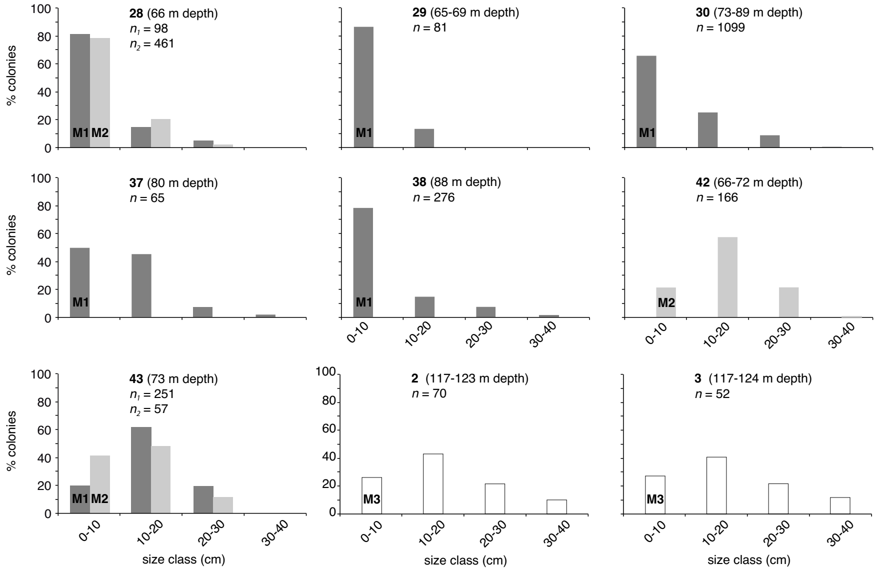
3.5 Spatial structure

Spatial structure of gorgonian distribution was analyzed along three of the video transects (Fig. 8), representative of the continental shelf (transect 30) and slope (transects 4 and 6). On the continental shelf, *E. singularis* was positively associated with *P. macrospina* M1 at large spatial scales (more than 30 m), whereas they were independently distributed at smaller scales (Fig. 8, Table 4). *E. singularis* was negatively associated to *P. clavata* at small scales (less than 5 m) and tended to be independently distributed at large and intermediate scale. *P. clavata* was independently distributed with respect to *P. macrospina* at all scales (Fig. 8 and Table 4). On the continental slope, in transect 4, all species were independently distributed at small and medium scales (few to tens of meters) (Fig. 8 and Table 4). At larger spatial scales (more than 30 m), *C. verticillata*, *S. pallida* and *E. cavolinii* were positively associated, whereas *V. flagellum* occurred in exclusion to the other species (Fig. 8, Table 4). In transect 6, *E. cavolinii, P. macrospina* M3, *V. flagellum* and *B. mollis* were positively associated at large spatial scales (more than 30 m) (Fig. 8 and Table 4). *A. hirsuta* tended to be independently distributed at small (less than 30 m) and large spatial scale (more than 30 m) (Fig. 8 and Table 4). *E. cavolinii* presented no association with *B. mollis* and *V. flagellum*, and the same situation was found for *P. macros*pina M3 when compared to *A. hirsuta*.

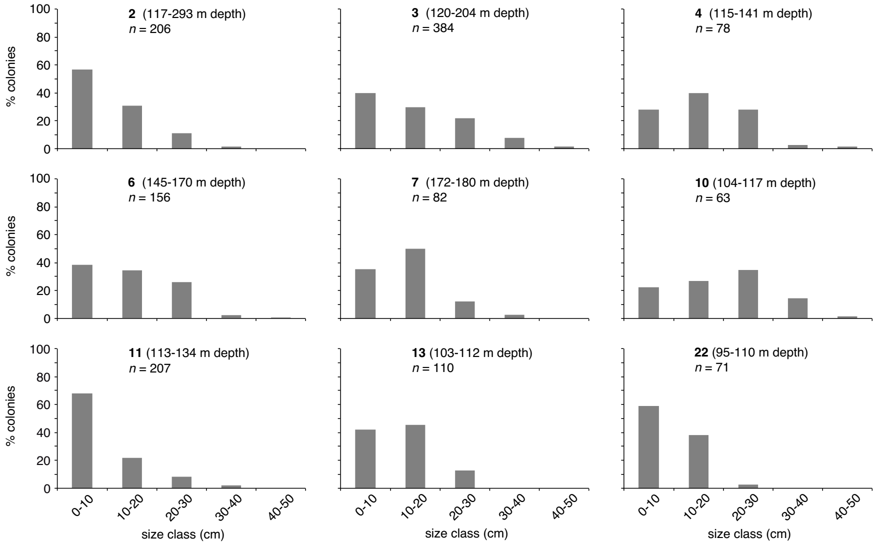
4. DISCUSSION

The diversity of the studied deep gorgonian assemblages was much higher than in shallow Mediterranean coastal areas (Weinberg, 1978; Linares et al., 2008; Gori et al., 2011a). Nine species were observed to dwell between 40 and 360 m depth within the study area, while only 4–5 species are commonly present in littoral areas (Velimirov, 1973; Gori et al., 2011a; Garrabou et al., 2001). Gorgonian diversity resembled that reported of Atlantic coastal areas (Cúrdia et al., 2013), subtropical areas (Opresko, 1973) and similar depth ranges in the Aleutian Islands (Stone, 2006). Conversely, gorgonian density observed in these deep multispecific assemblages reached high values (~20 colonies m^{-2}) comparable to those reported for Mediterranean coastal species (Weinbauer and Velimirov, 1996; Linares et al., 2008; Gori et al., 2011a). These high density values are similar to those found in temperate (Grigg, 1975; 1977; Cúrdia et al., 2013) and tropical (Lasker and Coffroth, 1983; Yoshioka and Yoshioka, 1989) coastal gorgonian assemblages, and clearly exceed those found at similar depths (180–500 m) in the North Atlantic (Mortensen and Buhl-Mortensen, 2004; Buhl-Mortensen et al., 2014), and polar continental shelves and slopes (Orejas et al., 2002; Miller et al., 2012).

Paramuricea macrospina



Eunicella cavolinii



Eunicella singularis

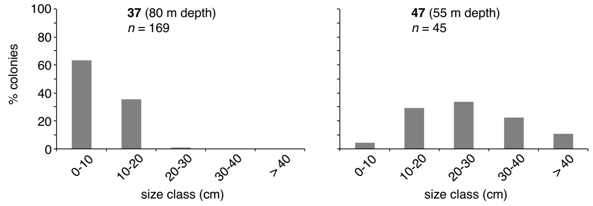


Fig. 6a. *P. macrospina* (M1, M2, M3), *E. cavolinii* and *E. singularis* population size frequency distribution (transect number as in Fig. 1 and Table 3, depth range, n = number of colonies).

However, the observed high gorgonian diversity was mainly concentrated on the shelf edge (100–180 m depth). A clear vertical zonation can be established regarding species distribution: (1) coastal species extending their distribution to deep coralligenous banks (*E. singularis* and *P. clavata*); (2) dominance of one single species on the continental shelf (*P. macrospina*); (3) concentration of several gorgonian species on the shelf edge (*E. cavolinii*, *P. macrospina*, *S. pallida*, *A. hirsuta*, *V. flagellum, B. mollis* and *C. verticillata*); and (4)

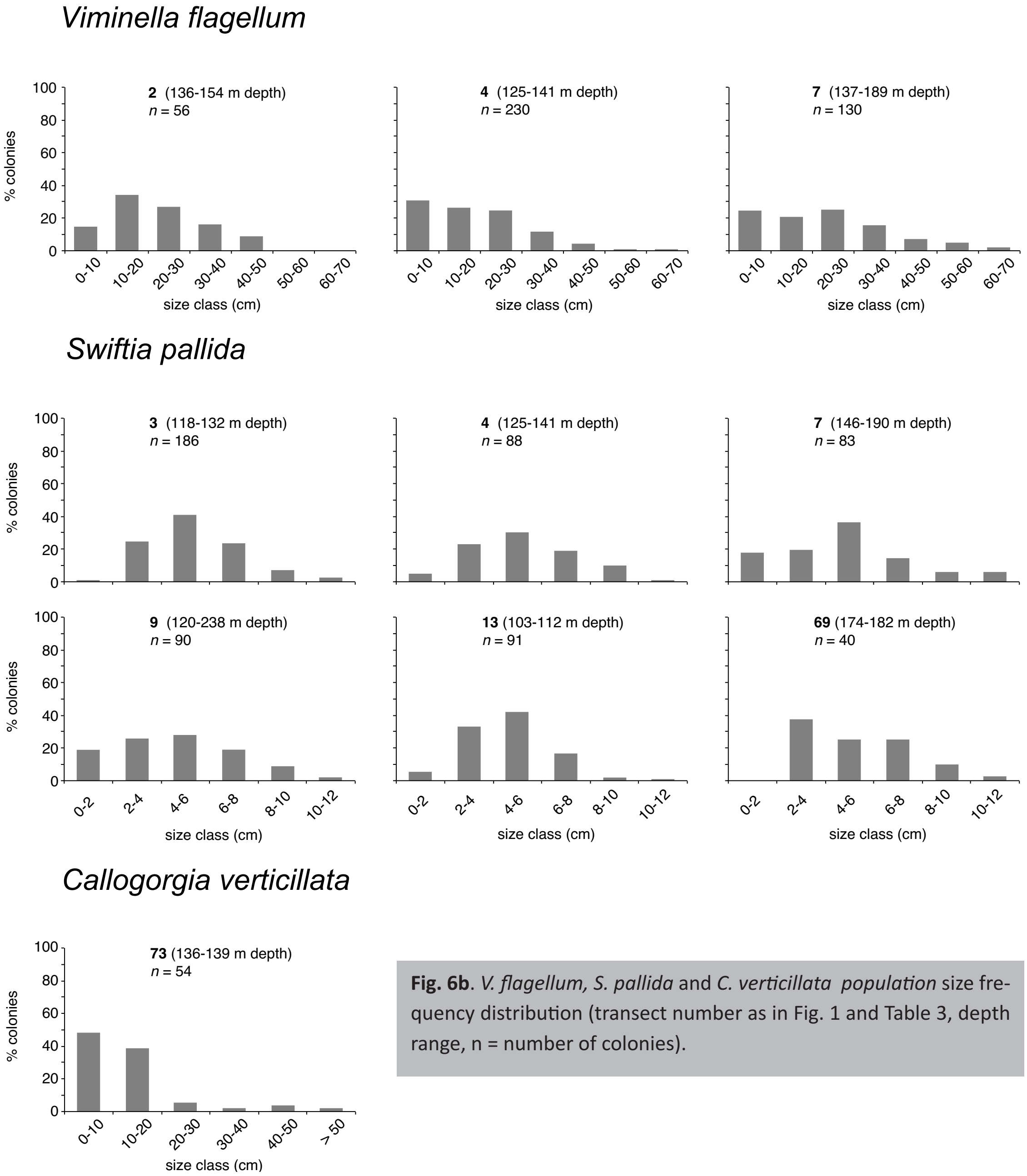


Fig. 6b. *V. flagellum, S. pallida* and *C. verticillata population* size frequency distribution (transect number as in Fig. 1 and Table 3, depth range, n = number of colonies).

Table 4. Summary of the three-term local quadrat covariance (3TLQ). Significant spatial covariance between pairs of species along the three analyzed transects.

Transect Number	Compaired species	Negative	Random	Positive
30	*P. macrospina M1 vs. E. singularis*		0-30 // 55-75	30-55 // >75
	P. macrospina M1 vs. P. clavata			All transect
	P. clavata vs. E. singularis	2-5	>5	
4	*C. verticillata vs. E. cavolinii*		0-50	>50
	C. verticillata vs. S. pallida		0-5	>5
	C. verticillata vs. V. flagellum	>55	0-55	
	E. cavolinii vs. S. pallida		0-50	>50
	E. cavolinii vs. V. flagellum	>34	0-34	
	S. pallida vs. V. flagellum	>48	0-48	
6	*A. hirsuta vs. B. mollis*	30-60	0-30 // >60	
	A. hirsuta vs. E. cavolinii	11-20	0-11 // 20-40	>40
	A. hirsuta vs. P. macrospina M3		All transect	
	A. hirsuta vs. V. flagellum	30-65	0-30 // >65	
	B. mollis vs. E. cavolinii		All transect	
	B. mollis vs. P. macrospina M3		20-30	0-20 // >30
	B. mollis vs. V. flagellum	11-20	0-11 // 20-32	>32
	E. cavolinii . P. macrospina M3		0-35	>35
	E. cavolinii vs. V. flagellum		All transect	
	P. macrospina M3 vs. V. flagellum	12-22	0-12 // 22-60	> 60

species extending their distribution deeper into the upper slope (*B. mollis*, *C. verticillata*, *V. flagellum* and *S. pallida*).

The common Mediterranean coastal species *E. singularis* and *P. clavata* were found on deep coralligenous banks on the continental shelf of the studied area (45–100 m depth). The high water transparency allows a very deep distribution of coralligenous banks (Ballesteros and Zabala, 1993), which in turns could explain the deep distribution of *E. singularis* within the study area (down to 100 m depth). In coastal environments characterized by high water turbidity, this species is limited to 70 m depth (Gori et al., 2011a). In the study area continental shelf both species' density was much lower than that reported in shallow coastal environments (Linares et al., 2008; Gori et al., 2011a). This could indicate that these species' deep bathymetric limit lies around this depth, according to our data. Interestingly, the observed colonies of *E. singularis* presented the same morphology as the deep asymbiotic morphotype reported in coastal environments below 40 m depth (Théodor, 1969; Gori et al., 2012). These populations were dominated by medium sized colonies, corresponding to what has been observed in deep coastal environments (60 m depth) (Gori et al., 2011b). Conversely, in shallow coastal environments (20 m depth) these species populations are mainly composed of small colonies (Linares et al., 2008). Populations of *P. clavata* extending beyond 40 m have also been reported in several coastal areas (Gori et al., 2011a; Angiolillo et al., 2015), as well as on top of seamounts (Bo et al., 2011) and on rocky bottoms of the continental shelf and slope (Bo et al., 2012, 2014). In these deep environments, *P. clavata* colonies generally achieve larger sizes (50 ± 15 cm (mean ± SD), min = 25 cm, max = 90 cm) than in shallower environments (Linares et al., 2008), as a possible consequence of the higher environmental stability of deeper areas (Grigg, 1975). Deep populations of *E. singularis* and *P. clavata* are probably protected from high hydrodynamic processes (Teixidó et al., 2013) as well as from unusually high-temperature conditions associated to mass mortality events in shallow benthic communities (Garrabou et al., 2009).

Maërl beds on the continental shelf of the studied area are covered by very high abundances of *P. macrospina* (M1 and M2). In this environment this species presents high frequency of occurrence over large areas (Figs. 4 and 8) and can reach densities of up to 33 colonies m^{-2}, which is at odds with previous data on this species, having been only reported on rocky substrates and never as a dominant species (Bo et al., 2011; Topçu and Öztürk, 2015). Preservation of these *P. macrospina* populations may be a consequence of low trawling pressure over large areas of the continental shelf (Moranta et al., 2014), as well as the habit of local artisanal fishermen to clean their nets *in situ* (Díaz et al., 2015). This habit consists of releasing by-catch over the same fishing grounds. This practice might result in partial damage of entangled colonies of *P. macrospina*, but as long as their holdfast remains attached to the maërl rodholite, their survival might be favored (Díaz et al., 2015). Population size structures of *P. macrospina* were mostly asymmetrical and positively skewed (Table 4), suggesting high recruitment rates (Linares et al., 2008) and implying that maërl beds are particularly suitable habitats for this species. However, unlike *E. singularis* and *P. clavata* that occur on coralligenous banks, the structural instability of maërl may limit the presence of large colonies of *P. macrospina* in the continental shelf (Tunnicliffe and James, 1983). Furthermore, fishing activities might be particularly detrimental to large colonies, which are probably more susceptible than smaller colonies to get entangled and broken by nets used by artisanal fishermen in the area. Indeed, populations dominated by small colonies have been reported in intensely fished areas (Althaus et al., 2009).

Highest gorgonian diversity was concentrated on the rocky bottoms of the shelf edge (at 100–180 m depth). In this environment, gorgonian assemblages were mostly multispecific (92% of occupied sampling units contained several species) and included seven of the nine observed species (Figs. 4 and 5). This diversity is probably influenced by the joint effect of habitat heterogeneity and hydrodynamic sta-

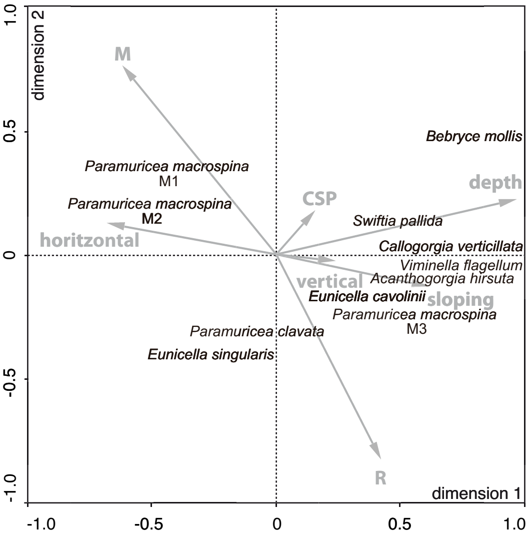


Fig. 7. anonical correspondence analysis (A): biplot showing the ordination of gorgonian species and the roles of the significant environmental variables. M: maërl, SP: cobbles and pebbles and R: rocky substrates.

bility. In the study area's shelf edge, boulders, slaps and vertical walls alternate over short distances. Shelf edge environments are mainly influenced by steady flow currents and stable hydrodynamic conditions (e.g. Puig et al., 2000), such as the Balearic current associated with the Balearic front, which flows from south to north along the western shelf edge of the Balearic Promontory (Ruiz et al., 2009). Stable hydrodynamic conditions, together with the hydrodynamic processes that increase particle suspension in the near-bottom water layers, may also suppose enhanced food availability for gorgonians on the shelf edge (Thiem et al., 2006). Finally, the observed increase in gorgonian diversity on the shelf edge could also result from the merging of species with shallower and deeper distributions, causing a mid-domain effect (Colwell and Lees, 2000), as previously suggested for deep coral diversity in other locations (Stone, 2006; Matsumoto et al., 2007). Most gorgonian populations located on the shelf edge had a bell-shaped, unimodal size structure dominated by medium sized colonies (Figs. 6a and b). *P. macrospina* (M3) populations on the shelf edge were bell-shaped, in contrast to its populations on the continental shelf (M1 and M2) that were mostly dominated by small size colonies. When compared to maërl, rocky substrates provide higher structural stability allowing

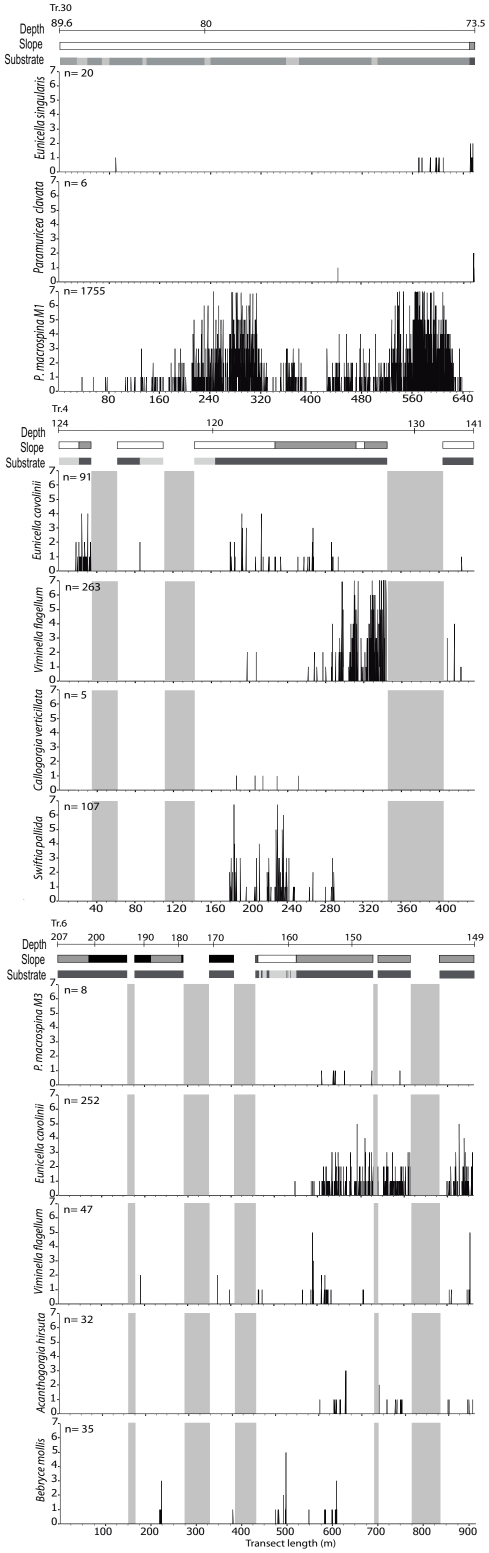


Fig. 8. Density plots. Species densities are plotted along representative transects from the continental shelf (transect 30) continental shelf edge and slope (transect 4 and 6, respectively). Bottom type and seabed slope are indicated (see legend). Vertical gray rectangles indicate invalid sequences for the analysis (Tr = transect, n = number of colonies).

BOTTOM TYPES LEGEND
░ Sand to Gravel
▒ Cobbles & Pebbles
▓ Maërl
█ Rock

SLOPE TYPES LEGEND
■ Vertical
▨ Slopping
□ Horizontal

colonies to achieve larger sizes. In these multispecific gorgonian assemblages of the shelf edge, the fine scale spatial covariance of species appears to be related to the combined effect of density, occupancy, and colony size. When a large species such as *V. flagellum* achieves high-densities over extended areas, it tends to exclude any other species (Fig. 8). Conversely, coexistence is observed at low and intermediate densities among large (*C. verticillata*), medium (*E. cavolinii*) and small sized species (*S. pallida*). This could indicate that differences in size may reduce interspecific competition for space and food (Schoener, 1974). Species with similar size probably feed on the food particles that are transported by the same water layer, with a potential competition for food (Kim and Lasker, 1997). Conversely, species with different size are probably feeding on different water layer, thus avoiding potential shading processes. Coexistence at medium spatial scales (~30 m) may also occur at low and intermediate densities among species of similar size and morphology (*E. cavolinii* and *A. hirsuta*). In this case, however, spatial exclusion occurred at smaller scales (~10 m), suggesting that these species share the same habitat but partially compete for space.

Overall, the species dwelling on the shelf edge within the study area, have been recently found with increasing frequency on rocky substrates at similar depths in other areas of the Mediterranean Sea (Bo et al., 2009; 2011; 2012; 2014), suggesting the general validity of the observed pattern of high gorgonian diversity.

Below the shelf edge, gorgonian abundance strongly decreases, and only *B. mollis*, *S. pallida, V. flagellum* and *C. verticillata* were observed to extend their distribution under 200 m depth (Fig. 4). Gorgonian assemblages are here mainly monospecific, (76% of occupied sampling units contained one single species) and with low densities (~0.75 ± 0.3 colonies m^2 (mean ± SD)). These species are typically found along the continental slope in other Mediterranean locations (Mytilineou et al., 2014; Deidun et al., 2014; Bo et al., 2015) where they mostly concentrate along the edges, maximizing their exposure to currents (Genin et al., 1986). On the scarce rocky substrates occurring in these deeper environments, gorgonian abundance tends to decrease with increasing depth, and antipatharians become the dominant arborescent species (Deidun et al., 2014; Bo et al., 2015). Finally, the soft sediments of these deeper environments can be colonized by the bamboo coral *Isidella elongata* (Maynou and Cartes, 2012; Bo et al., 2015), which can form extended assemblages in bathyal muds (Pérès, 1967).

Due to their arborescent morphology, gorgonians are especially vulnerable to fishing activities (Mytilineou et al., 2014; Bo et al., 2014), and their slow growth rate (Coma et al., 1998; Sherwood and Edinger, 2009) makes their recovery from related fishing damages very slow (Althaus et al., 2009). The high diversity and abundance of deep gorgonian assemblages in the rocky areas of the shelf edge, and the vast area covered by high densities of *P. macrospina* on the continental shelf are probably related to the low trawling pressure and the exceptional fishing practices exerted by local artisanal fishermen. This represents an example of the possibility of conserving deep gorgonian assemblages and their associated high-diverse fauna (e.g. Buhl-Mortensen and Mortensen, 2005) through better management of fishing activities. These high-density deep gorgonian assemblages may provide an approximate idea of how Mediterranean continental shelves and upper slopes stood before decades of bottom trawling.

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Chapter 3

Morphological and genetic features of the gorgonian *Paramuricea macrospina* on the Mediterranean continental shelf (Menorca Channel, Western Mediterranean Sea)

ABSTRACT

Morphological variability in gorgonians is frequent and commonly associated to habitat variability, often resulting in segregated morphotypes. *Paramuricea macrospina* is an endemic Mediterranean gorgonian species found on rocky bottoms between 40–100 m depth. It has recently been reported as one of the most abundant species in continental shelves and shelf edges. Three different chromatic forms of *P. macrospina* were observed in the Menorca Channel: a yellow and a light purple forms occurring on maërl beds of the continental shelf, and a dark purple form occurring on rocky substrates of the shelf edge. The objective of the present work is to verify if these *P. macrospina* forms may represent distinct taxonomic units by analyzing colony shape, sclerite size and shape, and mitochondrial markers of the three chromatic forms of *P. macrospina*. No significant differences were found in colony shape, suggesting that environmental variability between the continental shelf and the shelf edge is not influential enough to significantly alter colony morphology. Significant differences in sclerite size and shape were found amongst all forms, suggesting that sclerites may be more likely influenced by environmental conditions. However, the coexistence of yellow and light purple forms on the continental shelf suggests that both environmental factors and genetic differentiation are conditioning sclerite size and shape. Molecular analysis revealed no differences amongst the three forms but proportioned new insight on the phylogenetic relationship of this gorgonian genus.

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1. INTRODUCTION

Modularity is a successful strategy widely spread amongst sessile organisms as it facilitates adaptation to different environmental conditions (Huges, 1989). In marine environments, morphological variability of sessile modular organisms is very common, especially among coral and gorgonian species (Bruno and Edmunds, 1997; Sánchez et al., 2007; Vermeij et al., 2007). Indeed, these organisms can present a wide plasticity in their growth under different environmental conditions (Todd, 2008). Variability can affect colony color and morphology (Sánchez et al., 2007; Prada et al., 2008), as well as sclerite size and shape (Gutiérrez-Rodríguez et al., 2009) and the presence of specific secondary compounds (Puyana et al., 2004). This can suppose an additional challenge for taxonomical, evolutionary and ecological studies (Vermeij et al., 2007).

Coral and gorgonian morphological variability has commonly been related to habitat differences (Bruno and Edmunds, 1997; Helmuth et al., 1997), often resulting in segregated morphotypes adapted to different hydrodynamic or light conditions (Sebens, 1987; Sánchez et al., 2007; Gori et al., 2012). Colony transplant experiments have shown that the same genotype can generate different morphological phenotypes confirming the importance of phenotypic plasticity amongst gorgonians (West et al., 1993; Joseph et al., 2015).

In the last 20 years, several studies have addressed morphological variability in shallow gorgonian species (<40 m depth) mainly in relation with the

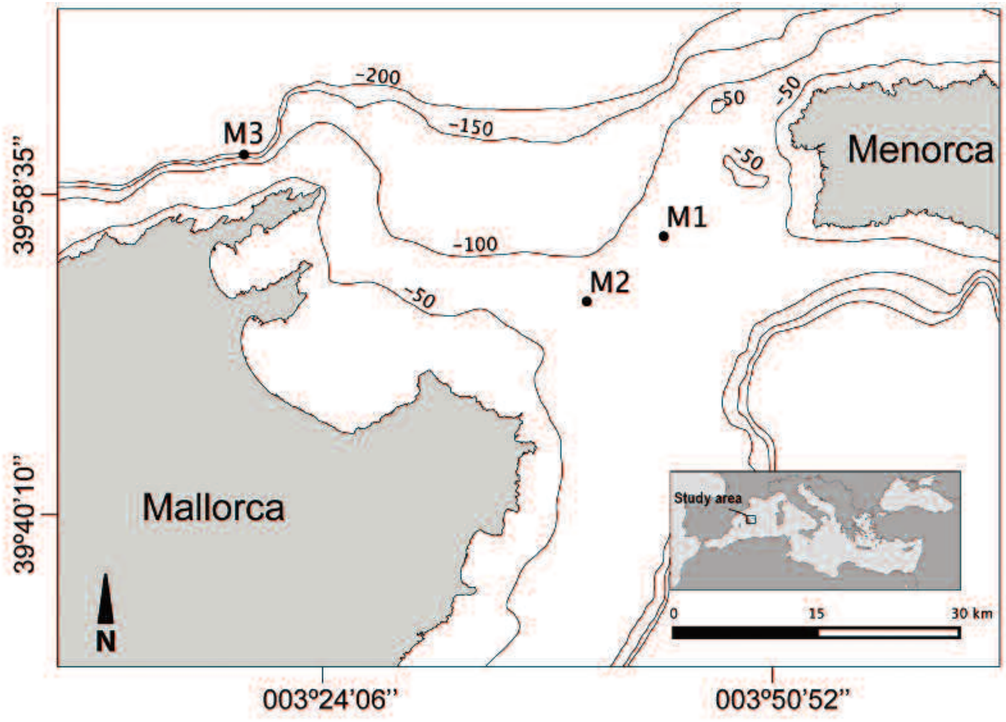

Fig. 1. Map of the Menorca Channel. Black dots indicate the sampling locations of each chromatic form.

dominant hydrodynamic conditions (e.g. Weinbauer and Velimirov, 1995; Skoufas, 2006; Sánchez et al., 2007). On the contrary, studies concerning deep gorgoninan morphological variability are still very scarce (e.g. Mortensen and Buhl-Mortensen, 2005; Quattrini et al., 2013; Doughty et al., 2013). However the increased accessibility to deep environments proportioned by remotely operated vehicles (ROVs) and manned submersibles has revealed that deep gorgonians species may occur over a wide bathymetric range and through different environmental settings (Stone, 2006; Buhl-Mortensen et al., 2014). The increasing observation of deep gorgonian assemblages is showing that, as their shallow counterpart, deep gorgonian species may also present a certain degree of morphological variability (Mortensen and Buhl-Mortensen, 2005). *Paramuricea macrospina* (Koch, 1882) is an endemic Mediterranean gorgonian mostly found on rocky bottoms at 40–100 m depth (Carpine and Grasshoff, 1975; Bo et al., 2012). This species has been recently found to be amongst the most abundant gorgonian on the Mediterranean continental shelf (Bo et al., 2012; Grinyó et al., 2016). In the Menorca Channel (Western Mediterranean Sea) (Fig. 1), *P. macrospina* is the dominant gorgonian species at 65–90 m depth on the continental shelf, but is also commonly found on the shelf-edge associated to other gorgonians at 110–160 m depth (Grinyó et al., 2016). Two different chromatic forms of *P. macrospina* were observed on the continental shelf, a yellow one (M1) and a light purple one (M2) (Figs. 2a and 2b, respectively). Both forms occur on maërl beds, covering vast areas and punctually reaching very high densities (Grinyó et al., 2016). On the shelf edge, a third dark purple form (M3) was observed on rocky substrates (Fig. 2c).

In the last few years several studies started to explore the biology and ecology of deep Mediterranean gorgonian species (e.g. Bo et al., 2012; Topçu and Öztürk, 2016). In this context, this study aims to elucidate if the different *P. macrospina* forms observed in the Menorca Channel represent distinct taxonomic units, by exploring differences in their (1) colony shape, (2) sclerite size and shape, and (3) genetic variability in mitochondrial markers (msh1, Igr1 and COI).

MATERIAL AND METHODS

Colony shape

P. macrospina's colony shape was analyzed on pictures obtained from videos recorded on the

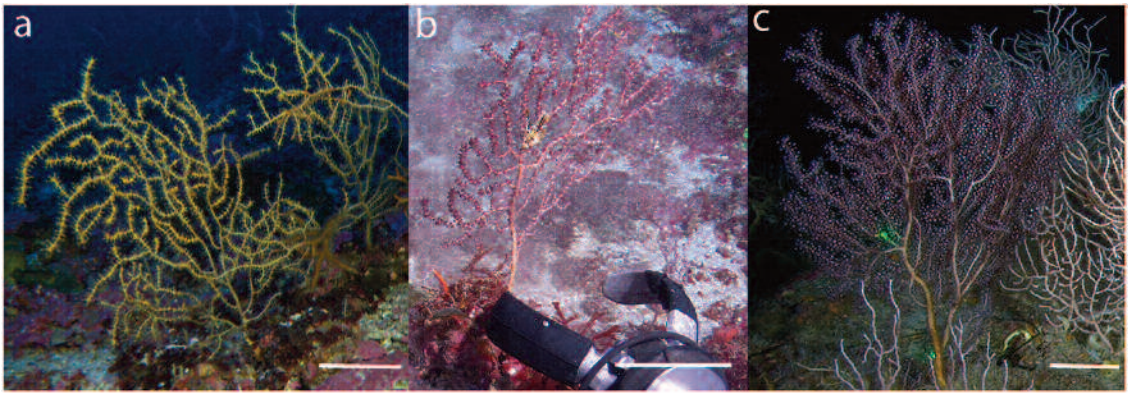

Fig. 2. Images of colonies of different *Paramuricea macrospina*'s chromatic forms (a) M1, (b) M2 and (c) M3. Scale Bar: 10 cm.

continental shelf (60–80 m depth) and the shelf-edge (110–160 m depth) of the Menorca Channel (39° 53′ 0.73″ N, 3° 29′ 51.16″ E) in September 2010 and April 2011. Videos were recorded with the manned submersible JAGO (IFM-GEOMAR) equipped with a 1080 horizontal line color video camera, and two parallel laser beams that provide a scale for the images (50 cm). In each picture, colonies were perpendicular to the camera, and lasers were in the same plane as the colony. Twenty pictures of colonies of each form (M1 and M2 from the continental shelf, and M3 from the shelf-edge) were selected for the subsequent analysis. Analyses were performed with the Macnification 2.0.1 (Orbicule Enhanced Labs) software and laser beams were used as reference to calibrate each picture.

From each picture, the maximum height and width of the gorgonian colony was measured; mean width was calculated as the mean of three measurements taken at equidistant positions and perpendicular to the height (Gori et al., 2012). Fan surface area was estimated by measuring the area defined by a continuous line that linked the tips of all the branches located on the external perimeter of the colony (Weinbauer and Velimorov, 1995). The ramification pattern was determined by establishing a branch ordination pattern following Brazeau and Lasker (1988). Most distal branches were defined as first order branches, second order branches occurred when two first order branches joined. Higher order branches only appear when two branches of equal lower order join. Branches were also classified as "tributary" or "source", where source branches are those that join same order branches whereas tributary branches are those that join a higher order branch (Brazeau and Lasker, 1988; Gori et al., 2012). Finally, the length of all primary branches was measured.

For each colony, the following shape features were calculated: height to width ratio, height to mean width ratio, ramification density (number of ramifications/fan surface area), primary branches density (number of primary branches/fan surface area), order of the colony's base, the primary branches maximum and mean length, the bifurcation ratio ($R_b = \frac{1}{m} \sum_{i=1}^{m} \frac{n_i}{1 + n_i}$ where n is the number of branches of order i, and m is the total number of orders) and the tributary to source ratio of primary and secondary order branches.

Sclerite size and shape

Colonies of *P. macrospina* were collected from the continental shelf (~75 m depth) and shelf-edge (~130 m depth) of the Menorca Channel in April 2011 and July 2012 (Fig. 1). Sampling was performed by means of the manned submersible JAGO (IFM-GEOMAR) and the ROV NEMO (Gavin Newman), both equipped with a grabber. Fifteen colonies of each of the two continental-shelf forms (M1 and M2) were sampled, whereas six colonies were sampled for the shelf-edge form (M3). A fragment of each colony was immediately preserved in absolute ethanol for genetic analyses, whereas the rest of the colonies were fixed in 4% buffered formalin in seawater, and finally preserved in 70% ethanol.

In order to compare sclerites from polyps with a similar size, several primary branches were placed under a binocular stereomicroscope (Olympus SZ-60) and photographed in different angles with a Moticam 2300 camera. Polyp length was measured with the software Macnification 2.0.1 (Orbicule Enhanced Labs) as the distance from the base of the calix to the tips of the most distal calix sclerites. Measurements were done on fully contracted polyps placed perpendicular to the camera. A total of 200 polyps were measured for the M1 and M2 forms, and 55 polyps could be measured for the M3 form. Polyps length ranged from 0.3 mm to 1.8 mm. Polyps of 1–1.5 mm were the most abundant, accounting for

62%, 55% and 60% of polyps in M1, M2 and M3 forms, respectively. Consequently, for each sampled colony, a total of 10 polyps 1–1.5 mm in size from the middle section (2 cm below the tip) of primary branches were dissected under a binocular stereomicroscope (Olympus SZ-60). Collected polyps were immersed in a sodium hypochlorite solution until all organic matter was dissolved and sclerites were disaggregated. Sclerites were rinsed several times with distilled water. A minimum of 20 sclerites were haphazardly selected from each colony, placed on a double stick-tape and posteriorly sputter-coated with Au-Pd. Sclerite observations and images were obtained with a Scanning Electron Microscope (SEM) HITACHI S-3500 N at 5.0 KV, images were obtained at X110. Images were pre-processed with Photoshop C5 (Adobe System) creating a black background, while sclerites were kept grey shaded alongside the scale caption. This procedure facilitated the subsequent measurement by means of the software Macnification 2.0.1 (Orbicule Enhanced Labs). The following measures were taken for each sclerite: total length, perimeter length, area, maximum width, and bottom, middle and top widths (Kim et al., 2004; Prada et al., 2008; Gutiérrez-Rodríguez et al., 2009; Gori et al., 2012). The number of ramifications at the base of large sclerites were counted and referred to as root number. Mean width was calculated as the mean among bottom, middle and top width. Sclerite shape was also evaluated by the following ratios: length/maximum width and area/perimeter (Bramanti et al., 2013).

Genetic markers

Total genomic DNA was extracted from ethanol-preserved samples of the three forms using the E.Z.N.A. DNA kit (OmegaBiotech) following the manufacturer's instructions. The mtMutS (msh1), and Igr1+COI mitochondrial regions were sequenced as an octocoral barcode proposed by McFadden et al. (2011) for comparative purposes. The start of the mtMutS (msh1) region was amplified using the primers ND42599F and MUT3458R (France and Hoover, 2002; Sánchez et al., 2003). Igr1+COI region was amplified using the primers COII8068F and COIOCTR (McFadden et al., 2004; France and Hoover, 2002). Each PCR used 1 U of DNA Stream Polymerase (BIORON), 0.2 mM of dNTPs, 0.3 µM of each primer, approximately 25 ng of genomic DNA, and was brought to a final volume of 25 µL with H_2O. MtMutS PCR was carried out using the following cycle profile: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55–58°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. The Igr1+COI PCR used the same cycle profile, but 58ºC as annealing temperature and 40 s for extension duration on each of the 35 cycles. PCR products were purified using the NucleoSpin® Extract II DNA Purification Kit following the manufacturer's instructions. Purified products were electrophoresed on an ABI PRISM® 3730xl Genetic Analyzer and sequence traces were edited using Sequencher™ 4.0. The obtained sequences were compared with homologous sequences from the EMBL-Bank of Plexauridae species.

The alignments of the sequences were carried out using MUSCLE, implemented in MEGA5 (Tamura et al., 2011). After alignment, the best nucleotide substitution model was selected using Modeltest implemented in MEGA 5, according to Akaike Information Criterion (AIC) and hierarchical likelihood ratio test (hLRT) values.

The mtMutS comparison involved 41 nucleotide sequences, while the COI, mtMutS+COI and mtMutS+Igr+COI comparisons only involved 11, 11 and 10 nucleotide sequences, respectively. All positions containing missing data were eliminated. Positions containing internal gaps were maintained but not considered by the analysis. The aligned mtMutS sequences had 660 positions, with a total of 121 variable and 69 parsimony-informative sites. The aligned COI sequences had 752 positions, with a total of 63 variable and 32 parsimony-informative sites. The aligned mtMutS+COI concatenated sequences had 1412 positions, with a total of 145 variable and 65 parsimony-informative sites. The aligned mtMutS+Igr1+COI concatenated sequences had 1524 positions, with a total of 139 variable and 69 parsimony-informative sites. The genus *Swiftia* (*Swiftia pallida*, HM106337 and FJ264905) was selected as outgroup, this genus is currently included in Plexauridae, but molecular analyses often aligned it close to the Gorgoniidae genera (see Wirshing and Baker, 2015; Vargas et al., 2014). The phylogenetic reconstruction was obtained applying Maximum Likelihood (ML) and Bayesian inference methods. Maximum likelihood method (ML) was developed in MEGA5, and based on the T92+G (Tamura, 1992) model for all set of sequences, using the NNI heuristic method (Nearest Neighbor Interchange) and 1000 bootstraps replications (Felsenstein, 1985). The Bayesian Inference was carried out online in Bioportal (Kumar et al., 2009) in MrBayes 3.1.2 program (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), using the model GTR+G (lset nst=6 rates=gamma), 10^7 generations and discarding 25% initial trees.

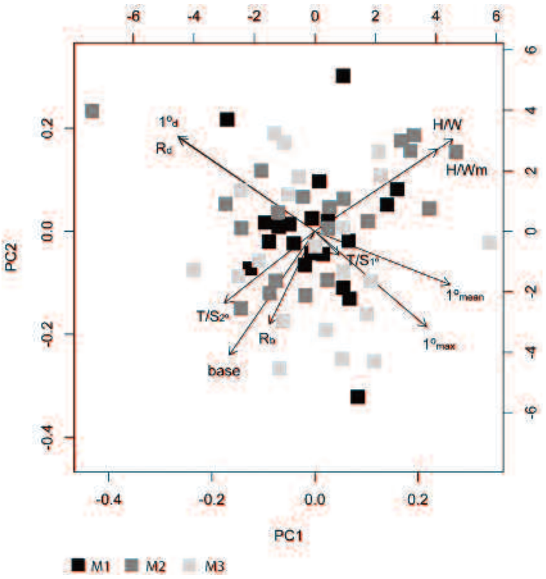

Fig. 3. Principal component analysis (PCA) biplot showing the ordination of the studied colonies (n = 60) regarding their colony shape, and the role of the analyzed features; H/W = height to width ratio; H/Wm = height to mean width ratio; Rd = ramification density; 1ºd = primary branch density, base = order of the colony base; 1ºmax = maximum length of primary branches; 1ºmean = mean length of primary branches; Rb = bifurcation ratio; T/S1º = tributary to source ratio of primary branches; T/S2º = tributary to source ratio of secondary branches.

Statistical analyses

Distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was employed to test the null hypothesis of no significant differences among forms in colony shape, and sclerite size and shape. Each term of the analysis was tested using 9,999 permutations. Data were standardized respect to their mean absolute deviation ($\mathrm{MAD} = \frac{1}{n} \sum_{j=1}^{n} [\, x_{ij} - \bar{x}_i \,]$, where x_i is the value of the i variable observed in the n colonies) (García Pérez, 2005). Significant relations relevant to the hypothesis were investigated using a posterior pair-wise post-hoc test. Finally, an ordination of the analyzed colony shape and sclerite size and shape was obtained with a principal component analysis (PCA). PERMANOVA and pair-wise post-hoc tests were performed with the PaST software (Hammer et al., 2001). PCA was performed with the R-language function princomp, which is available in the Vegan library (Oksanen et al., 2005) of the R software platform (R Core Team, 2014).

RESULTS

Colony shape

No significant differences in colony shape (PERMANOVA, Pseudo-F=0.983, p=0.517) were found amongst the three forms. The first two principal components of the PCA explained 55.4% of the data variance, the first component accounted for 21.2% of the variance. A strong correlation occurred between primary branch density and ramification density, as well as between height to maximum width and mean width ratios (Fig. 3). M1 was characterized by high primary branch and ramification densities (Figs. 3 and 4), whereas M2 by high values of height to maximum width ratio and height to mean width ratio (Figs. 3 and 4). M3 colonies were characterized by long primary branches, high base orders and tributary to source ratio of secondary branches (Figs. 3 and 4).

Sclerite size and shape

Two different types of sclerites were observed to form the *P. macrospina* polyp calyxes: thorns

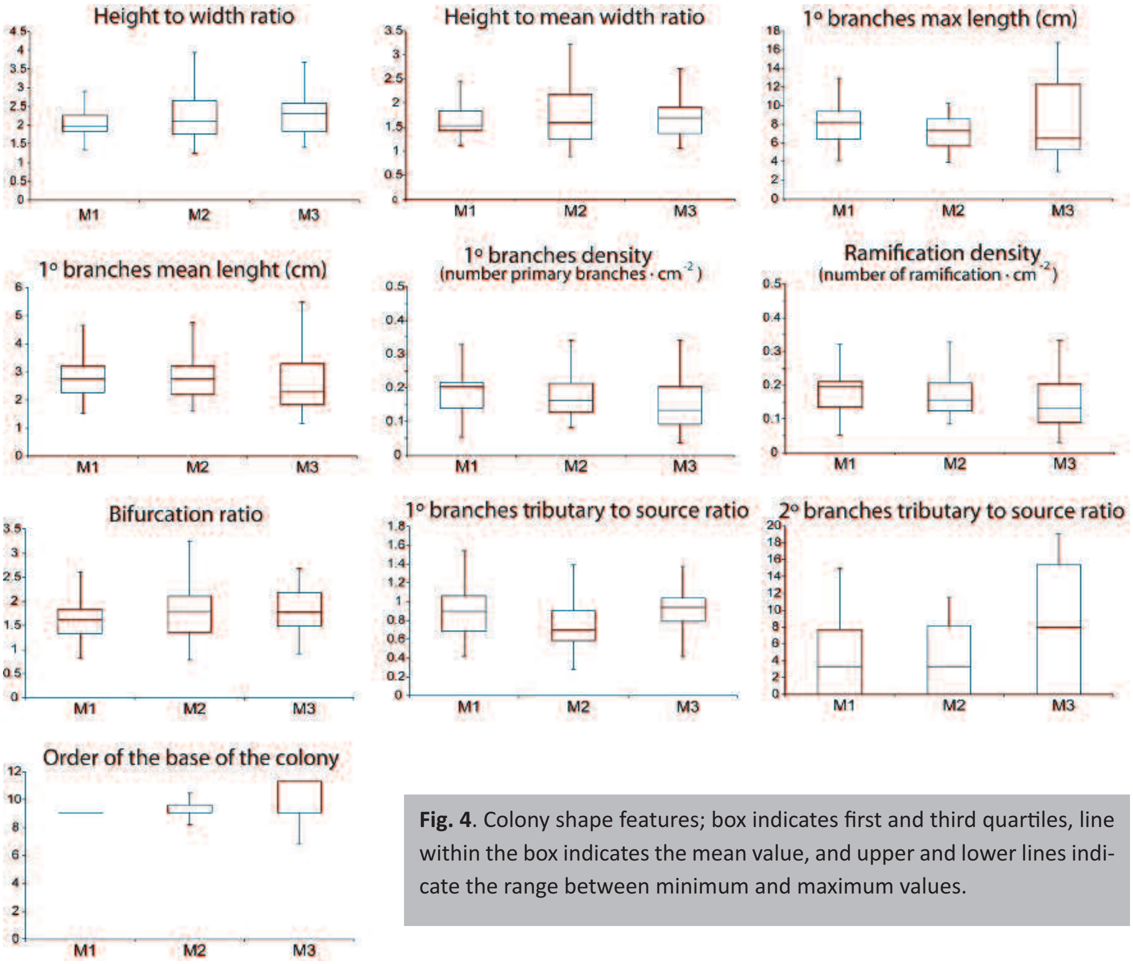

Fig. 4. Colony shape features; box indicates first and third quartiles, line within the box indicates the mean value, and upper and lower lines indicate the range between minimum and maximum values.

Fig. 5. Thorns (a–h) and spindles (i–n) from the calyx of *Paramuricea macrospina* M1.

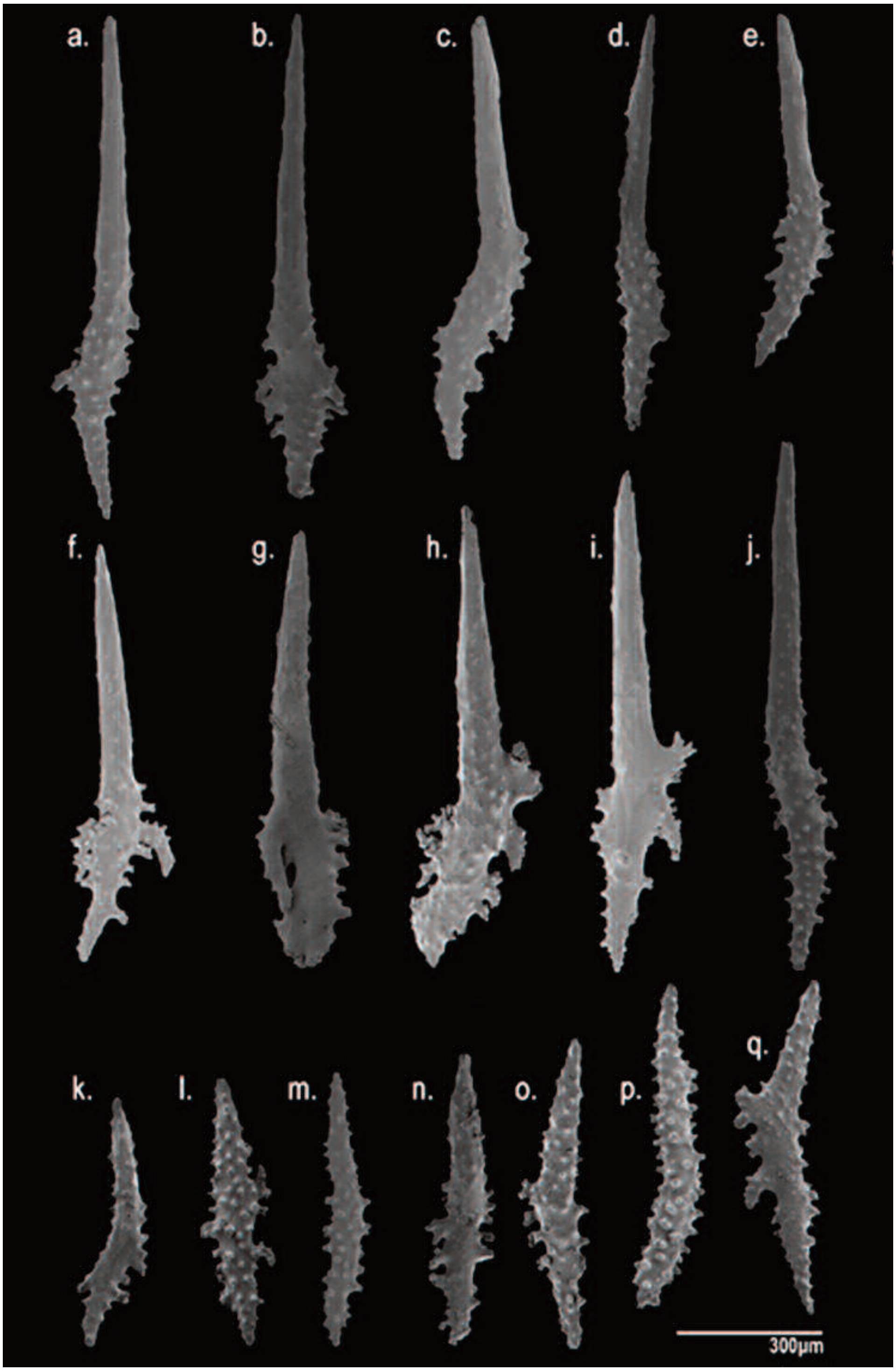

Fig. 6. Thorns (a–j) and spindles (k–q) from the calyx of *Paramuricea macrospina* M2.

Fig. 7. Thorns (a–i) and spindles (j–q) from the calyx of *Paramuricea macrospina* M3.

and spindles (Figs. 5, 6, and 7). Significant differences amongst the three forms were observed in the thorns (PERMANOVA, Pseudo-F = 6.812, p<0.001) as well as in the spindles (PERMANOVA, Pseudo-F = 6.586, p<0.001) (Table 1). The first two principal components of the PCA explained 84% of the data variance for thorns, and 88% for spindles; first components accounted for 65% and 64% of the variance, respectively. Thorn size and shape changed gradually, being larger in M1, intermediate in M2, and smaller in M3 (Fig. 8). Spindle size and shape presented less variability amongst forms (Fig. 9). M1 colonies were characterized by thorn and spindle with high mean and maximum width, as well as by high number of thorn roots (Figs. 8, 9, 10 and 11). M2 colonies presented intermediate values (Figs. 8, 9, 10 and 11), whereas M3 colonies were mainly characterized by thorn and spindle with high length and high length to width ratios (Figs. 8, 9, 10 and 11).

Table 1. Pairwise test for thorns and spindles forms. Significance is indicated with one (p-value <0.05), two (p-value <0.01), or three asterisks (p-value <0.001).

Morfotypes	Thorns		Spindles	
	p-value	Significance	p-value	Significance
M1 vs. M2	0.0158	*	0.0237	*
M1 vs. M3	0.0002	***	0.0009	***
M2 vs. M3	0.0423	*	0.0366	*

Phylogenetic analyses

Uncorrected *p*-distances based on mtMutS pointed out that all *Paramuricea macrospina* genotyped specimens shared the same sequence. The set of sequences obtained in GenBank for *P. biscaya, P. multispina* and *P.*

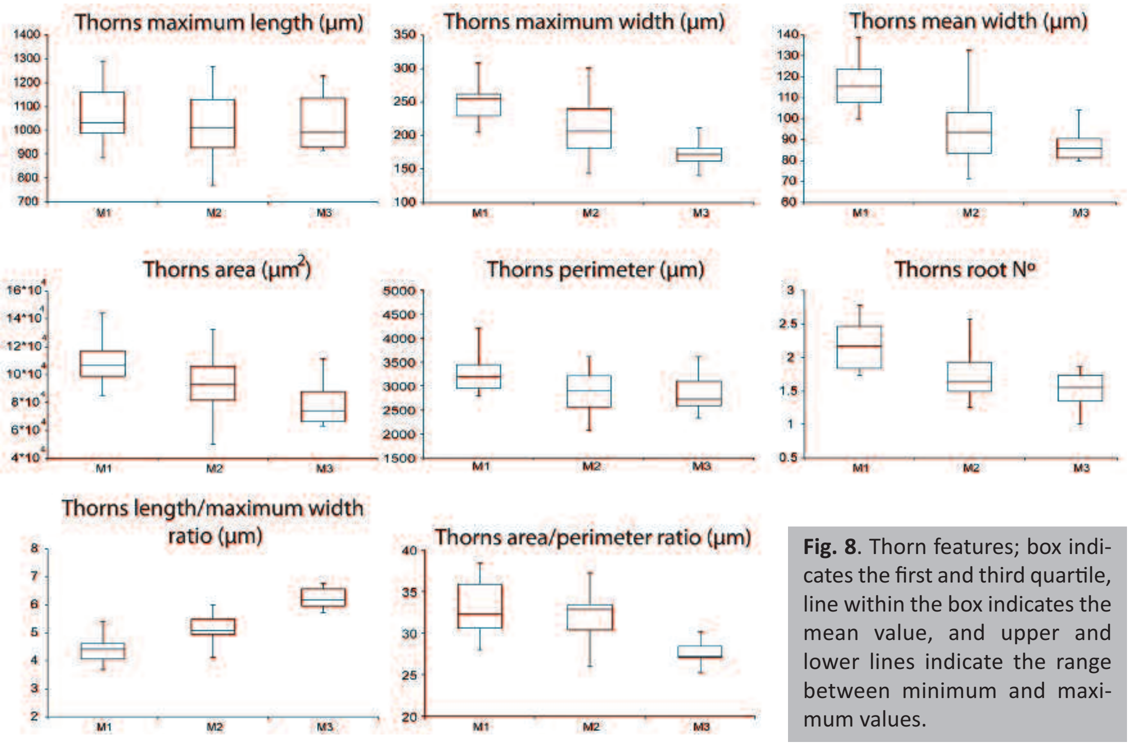

Fig. 8. Thorn features; box indicates the first and third quartile, line within the box indicates the mean value, and upper and lower lines indicate the range between minimum and maximum values.

placomus show these species to be polyphyletic (Fig. 12). The species used in this comparison of the genera *Paramuricea* and *Placogorgia* are reunited in a well-supported clade, but they are nested in the analysis, showing a possible future generic synonymy when a higher number of species and better diagnostic specific characters will be defined. Internal relationships within the *Paramuricea-Placogorgia* clade are poorly supported, just a set of *P. biscaya* (I, J, and K), the different *Paramuricea macrospina* forms here considered, a group of *P. placomus*, *Placogorgia* spp., and two additional sets of sequences [*P. biscaya* (L)-*Placogorgia sp.* (A), and *P. multispina* (B) - *P. placomus* (A)] are highly supported (see Fig. 12). The two Mediterranean *Paramuricea* species, *P. clavata* and *P. macrospina* are distinctly divergent (p-distance 2.1%). *Paramuricea macrospina* is closely related (p-distance 0.2%) to the set of sequences of *P. biscaya* A to H, *P. multispina*, *P. tenuis*, and *P. terceira*. The "intra-generic" p-distances considering all species in the *Paramuricea-Placogorgia* clade ranged from 0 to 3.9%, being 0–2.3% when excluding *Placogorgia* sp. (C), which is separated from the rest of *Paramuricea-Placogorgia* species by 2.3–3.9% in p-distance. The analysed species here included in the *Paramuricea-Placogorgia* clade are widely separated from other Plexauridae genera such as *Villogorgia*, *Bebryce*, *Alaskagorgia* and *Swiftia* by distances between 5.6% and 7.30%.

Analyses based on COI, and concatenated mtMutS+COI and mtMutS+Igr1+COI sets of sequences (Fig. 13), with a reduced number of species represented by the poor knowledge of COI sequences in *Paramuricea-Placogorgia*, have similar results as for mtMutS. *Paramuricea biscaya* is also showed as a polyphyletic taxon, the two Mediterranean *Paramuricea* species are also clearly differentiate by COI, and the *Paramuricea-Placogorgia* clade is well supported,

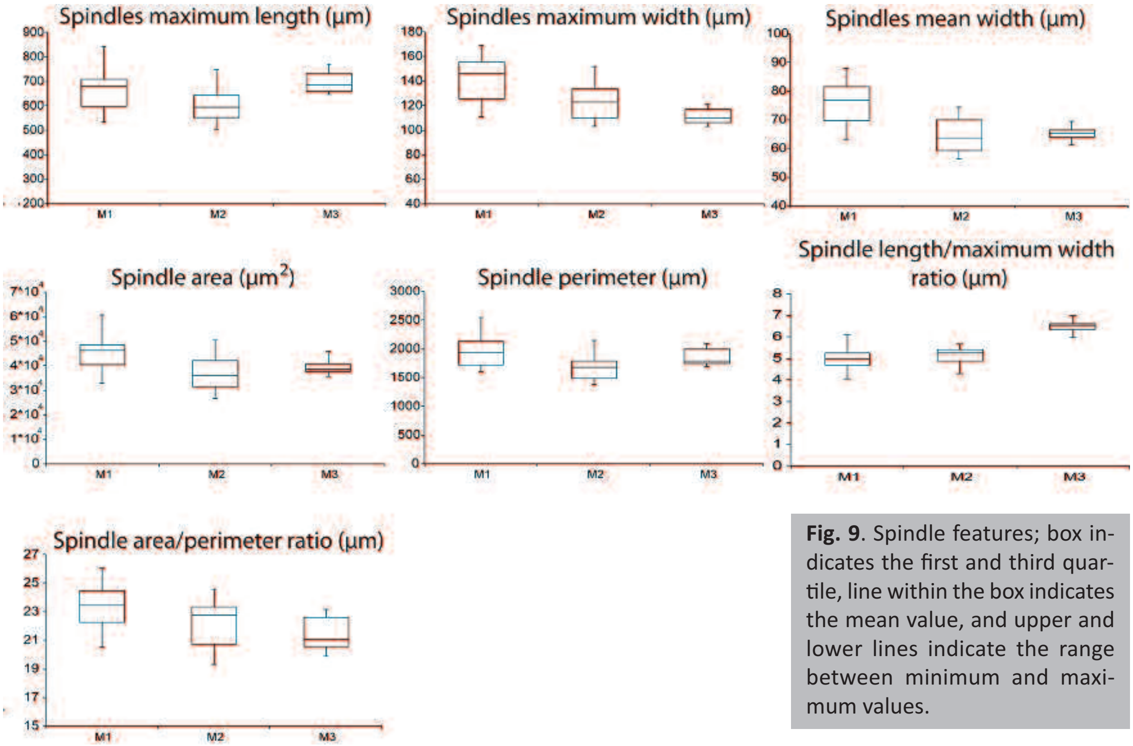

Fig. 9. Spindle features; box indicates the first and third quartile, line within the box indicates the mean value, and upper and lower lines indicate the range between minimum and maximum values.

although internal relationships remain in general poorly resolved. Comparing between the two Mediterranean *Paramuricea* species, and despite the reduced number of bases in the available sequence mtMutS of *Paramuricea clavata*, this species retains 11 mutations respect to its homologue sequence in *P. macrospina*, expressed in 7 amino acid differences. The available COI sequence of *P. clavata* is 205 bases shorter than the homologous in *P. macrospina* (M3), differing in 13 silent mutations that do not produce any amino acid difference.

DISCUSSION

The results of this study show that the three chromatic forms of the gorgonian *Paramuricea macrospina* observed on the continental shelf and slope of the Menorca Channel, significantly differs in terms of sclerite size and shape, but not in terms of molecular markers and colony shape.

Most colonial morphological features barely changed among the three forms (Fig. 4). Therefore, *P. macrospina*'s colonial morphology is a conservative character that experiences little variation across different environments, as similarly observed in other gorgonian species (Mitchell et al., 1993). This invariability contrasts with other studies, where depth-related changes in environmental conditions result in significant morphological variation in coral species (e.g. Helmuth and Sebens, 1993; Prada et al., 2008; Nir et al., 2011; Gori et al., 2012). However, most research has been conducted in shallow littoral environments, where environmental conditions considerably change over a narrow depth range (Garrabou et al., 2002). Conversely, the increased stability of environmental conditions at greater depth (>50 m) (e.g. Puig et al., 2000; Fernández de Puelles et al., 2007), may explain the lack of variability in the colonial morphology of the three chromatic forms of *P. macrospina*. The shallow forms (M1 and M2) occurring on maërl beds on the continental shelf may only suffer the effects of sporadic intense hydrodynamic events (Puig et al., 2001; Teixidó et al., 2013) that have been shown to cause damage to sessile organism at similar depths (Woodley et al., 1981; Bongaerts et al., 2013). Conversely, the structural stability of shelf-break rocky bottoms sheltered from strong hydrodynamic events may allow deep M3 colonies to grow larger, as previously observed in other gorgonian species (West et al., 1993; Gori et al., 2012). This may explain why M1 and M2 colonies are significantly (PERMANOVA $p<0.05$) smaller (23 ± 8.7 SD and 22 ± 9.1 S cm, respectively) than deeper M3 ones (34 ±

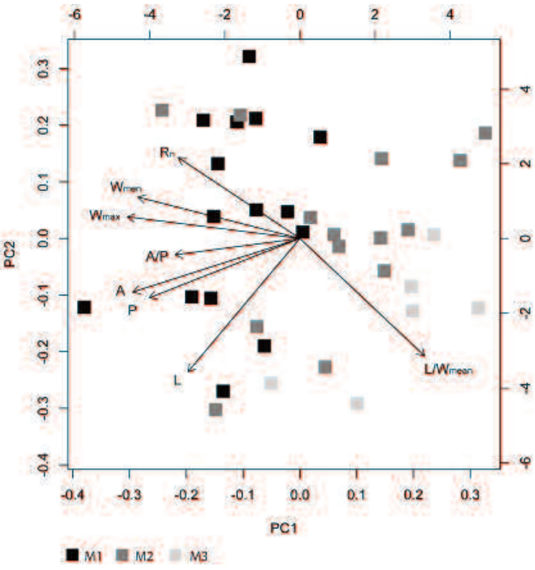

Fig. 10. Principal component analysis (PCA) biplot showing the ordination of the studied colonies (n= 36) regarding their thorn size and shape, and the role of the analyzed features; L = thorn length; P = thorn perimeter; A = thorn area; W_{max} = thorn maximum width; W_{mean} = thorn mean width; Rn = number of thorn roots; L/W_{mean} = thorn length to mean width ratio; A/P thorn area to perimeter ratio.

15 SD cm). Moreover, it has been proposed that in deep environments exposed to unidirectional currents, gorgonians may benefit from developing larger colonies as they maximize surface exposed to perpendicular flow and increase prey capture efficiency (Sebens and Johnson, 1991). However, these differences in the stability of the substrate and possible exposure to intense hydrodynamic events of *P. macrospina* on the continental shelf are evidently not influential enough to cause any significant changes in its colonial morphology.

Unlike colony shape, thorn and spindle size and shape significantly differed among the three forms. Differences in *P. macrospina* sclerite shape mostly derived from width rather than length, differently from what has been more frequently observed in other gorgonian species (e.g. West, 1997; Gori et al., 2012). Both thorns and spindles had the same length but were much wider and heavily rooted in M1 and M2 than in M3 colonies. Other Mediterranean coastal gorgonian species had also larger sclerites in shallow environments than in deeper ones (Velimirov, 1976; Skoufas, 2006; Gori et al., 2012). It has been suggested that larger sclerites would proportion higher flexion capacity to gorgonian colonies in shallow or exposed habitats (Skoufas, 2006). However, deep colonies of Caribbean gorgonians had longer sclerites than shallow ones (West, 1993; Kim et al., 2004; Prada et al., 2008) as a possible adaptation to reduced water motion, since large sclerites are more susceptible to breakage than smaller ones (West, 1998). This would agree with studies reporting a negative correlation between sclerite size and water motion (West et al., 1993; Kim et al., 2004). In this regard, the less robust morphology of M3 sclerites could be an adaptation to less intense hydrodynamic conditions. In addition to their structural role, sclerites can also act as defense against predators (Van Alstyne et al., 1992; Puglisi et al., 2002), with larger sclerites being more effective than smaller ones in deterring predators (West, 1998). Therefore larger sclerites in M1 and M2 could also be an adaptation to higher predation pressure. This would agree with previous studies that generally reported higher predation pressure in shallow than in deeper areas (West et al., 1993). However predation processes on deep-water gorgonians are still highly unknown.

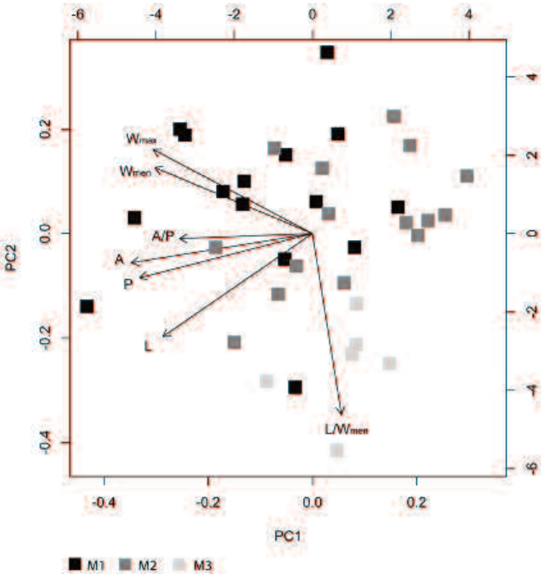

Fig. 11. Principal component analysis (PCA) biplot showing the ordination of the studied colonies (n=36) regarding their spindle size and shape, and the role of the analyzed features; L = spindle length; P = spindle perimeter; A = spindle area; W_{max} = spindle maximum width; W_{mean} = spindle mean width; L/W_{mean} = spindle length to mean width ratio; A/P spindle area to perimeter ratio.

Overall, it could be supposed that differences in environmental conditions on the continental shelf and shelf-edge are not enough to induce any change in the *P. macrospina* colony shape, but may be influential enough to affect sclerite size and shape. However, this is not supported by the significant differences observed among the two continental shelf forms (M1 and M2), which occurs side by side on the same mäerl beds (Grinyó et al., 2016). In this sense, differences between M1 and M2 sclerite could indeed suggest a genetically controlled processes rather than an environmental driven one. M2 sclerites present intermediate values between M1 and M3 forms (Figs. 8 and 9). However, differences between M2 and M3 sclerites were less pronounced than between M2 and M1 (Table. 1). Considering the similar coloration in M2 and M3 colonies and the less marked differences in sclerite size and shape, it could alternatively be suggested that M2 and M3 are a shallow and a deep forms of a single morphotype occurring over a wide depth range comprising both the continental shelf and slope, whereas M1 is a different morphotype only occurring on the continental shelf.

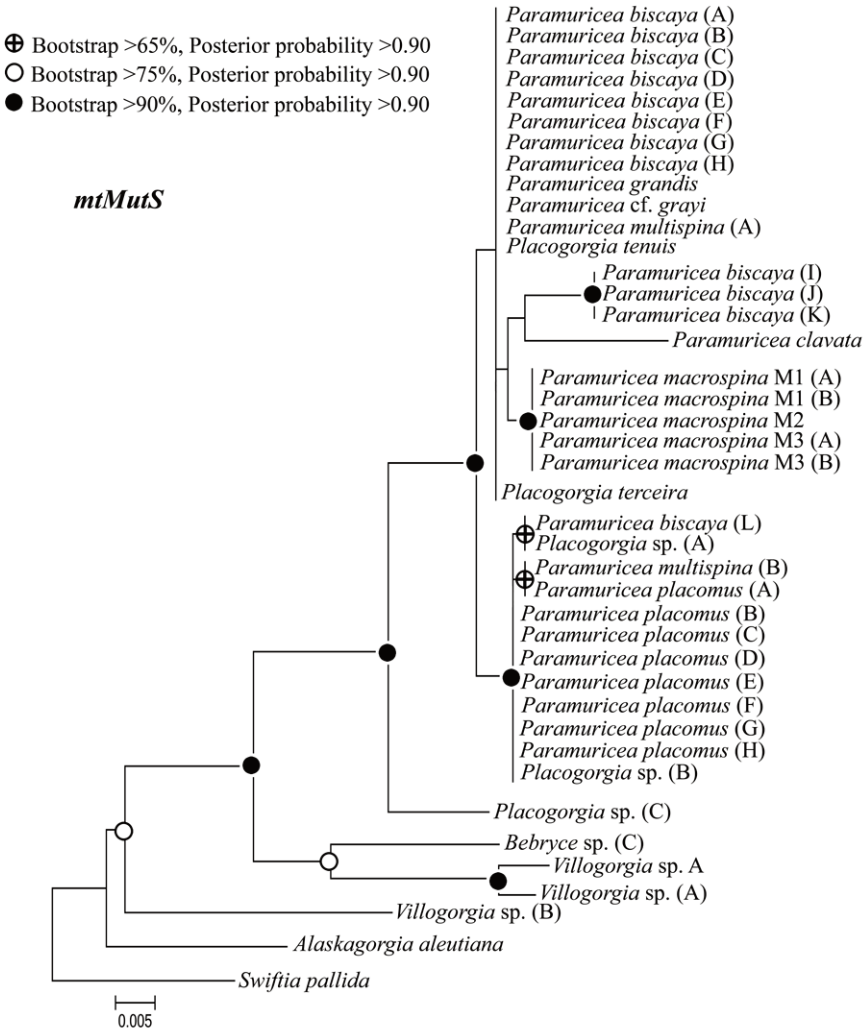

Fig. 12. Molecular analysis by ML method. Relationship of Paramuricea-Placogorgia species and related plexauriid genera using *Swiftia pallida* (HM106337) as outgroup; the analysis is based in mtMutS. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

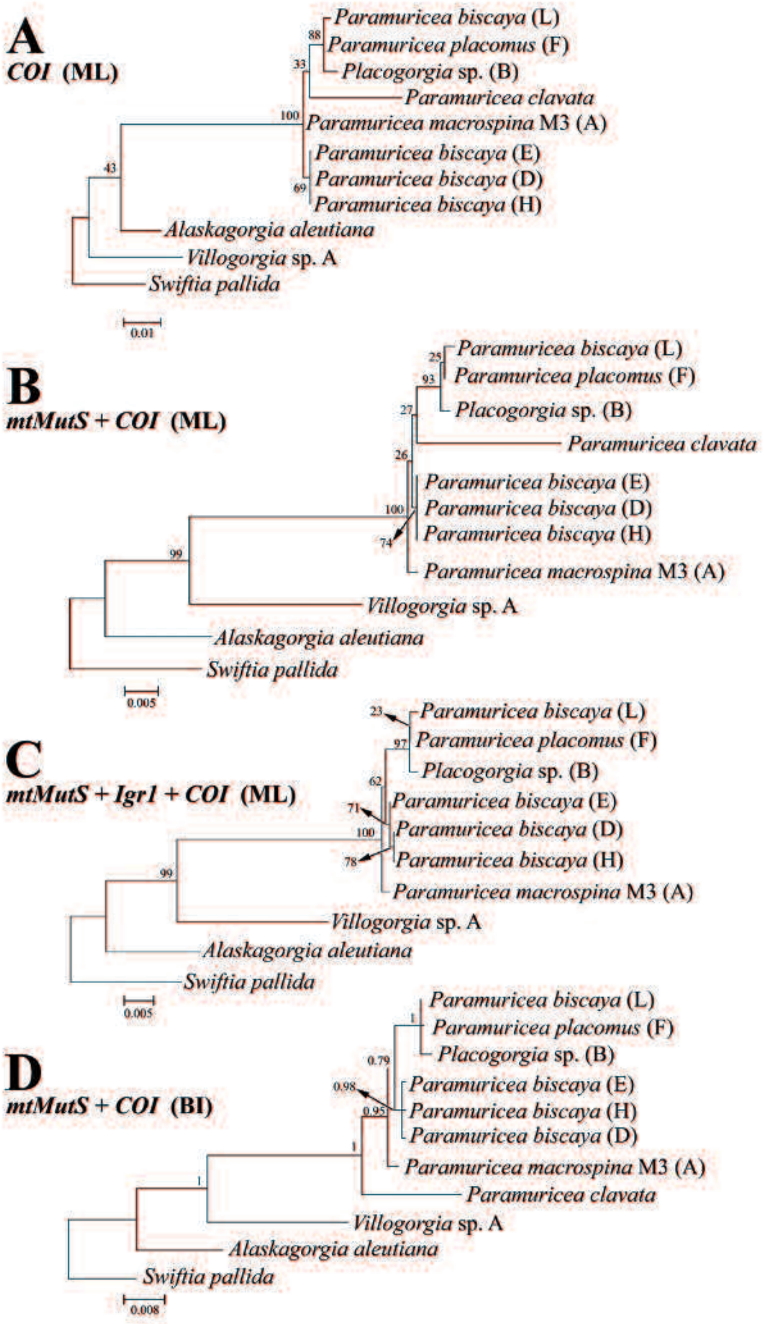

Fig. 13. Additional molecular analysis. Relationship of Paramuricea-Placogorgia species and related plexauriid genera using *Swiftia pallida* (HM106337 for mtMutS and FJ264905 for Igr1+COI) as outgroup. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Abbreviations: ML, maximum likelihood; BI, Bayesian inference.

However, mtMutS sequences obtained in this study for the three forms of *Paramuricea macrospina* (M1, M2, and M3) were identical. Among the mitochondrial genes, the mtMutS (msh1) is the most rapidly evolving protein-coding region, being genetic distances (uncorrected *p*-distances) usually 2–3 times those for other segments as COI (McFadden et al., 2011). Unfortunately, only a single COI sequence of *P. macrospina* (form M3) could be successfully obtained in this study, being sequences of this marker poorly available in the *Paramuricea-Placogorgia* clade in general. Both mtMutS and COI have proven to be good discriminators sequences among octocoral species (e.g. Quattrini et al., 2013; 2014). However, different octocoral species may also share identical COI or mtMutS sequence (e.g. McFadden et al., 2011; Gori et al., 2012; Doughty et al., 2013). The lack of resolution in mtMutS is not necessary indicative of species identity (see also Moore et al., 2016 for *Primnoisis* species). In this sense, a recent speciation process that separated M1 versus M2+M3 forms of *P. macrospina* cannot be discarded. Indeed, the molecular information currently available neither resolves the relationships between the two Mediterranean endemic *P. macrospina* and *P. clavata*, since basal nodes of the clades including these species are not well supported in the mtMutS nor in the mtMutS+COI analyses (Figs. 12 and 13). The nested structure of the *Paramuricea-Placogorgia* clade is similar to that observed for other pairs of octocoral genera, such *Siphonogrogia-Chironephthya* (see López-González et al., 2015) or *Lepidisis-Keratoisis* (see Dueñas et al., 2014), where apparently clear morphological features distinguishing genera do not found molecular support. The polyphyletic appearance of *P. biscaya, P. multispina* and *P. placomus* observed in the mtMutS phylogenetic hypothesis in this study (and at least *P. biscaya* when including COI and Igr1+COI) could be explained as the result of: 1) cryptic speciation, or 2) current instability (or understood variability) of the morphological diagnostic characters that should be used in the identification of these species. In the first case, cryptic species (morphologically identical or extremely similar, but genetically distinct) have already been detected in octocoral genera such *Cornularia* (McFadden and van Ofwegen, 2012), *Incrustatus* (McFadden and van Ofwegen, 2013), and the soft-coral genera *Sarcophyton-Lobophytum* (McFadden et al., 2006). In the second case, supposed well-understood morphology is diluted when a large sampling effort intra- and interspecies is carried out (e.g. Gori et al., 2012 for Mediterranean *Eunicella* spp.). Additional mistakes in the assignation of species names could also be the result of the identification by direct comparison to sequences in accessible public databases whose reliable identifications had not been fully assessed, or simply by errors in the interpretation of the diagnostic characters proposed. In this sense, the deposit of voucher specimens of the published sequences will surely facilitate further consults, reinterpretation of morphological features, and corrections of the identifications initially proposed, when necessary.

Overall, colonial morphology of the three forms of *P. macrospina* observed in the Menorca Channel did not show any clear relation with possible changes in environmental conditions with depth in the continental shelf versus shelf-edge. However, it would be interesting to extend the morphological comparisons to also include shallow coastal areas, where the species has been recently reported (20–40 m depth, Topçu and Öztürk, 2015), and where hydrodynamic conditions are probably much more contrasted. Conversely, the observed differences in sclerite size and shape probably suggest that both environmental and genetic factors are conditioning sclerite size and shape. Transplant experiments between depths would be desirable to further explore the possible role of phenotypic plasticity and genetic divergence on the morphological variability of this very abundant Mediterranean deep gorgonian species (Grinyó et al., 2016). Gorgonians are capable to adapt to different environmental settings that can derive in morphological variability (Weinbauer and Velimirov, 1995; Gori et al., 2012). In this regard, *P. macrospina*'s broad bathymetric distribution and occurrence in very different environments reflects its wide adaptability (Bo et al., 2012; Topçu and Öztürk, 2015; Grinyó et al., 2016). While it is clear that morphological variability can be driven by environmental features, we hypothesis that morphological variability could also be reflecting a more complex speciation process occurring in the studied species. In this regard, new genetic markers are needed to elucidate the fine line between species and morphological variation among Mediterranean gorgonians.

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Chapter 4

Reproductive cycle, energy storage and metabolic requirements of the gorgonian *Paramuricea macrospina* from the Mediterranean continental shelf (Menorca Channel, Western Mediterranean Sea)

ABSTRACT

Paramuricea macrospina is an endemic Mediterranean gorgonian species found on hard substrates at 40–160 m depth. This study examined the sexual reproductive cycle, energy storage, and metabolic requirements of *P. macrospina* in a continental shelf environment (~75 m depth). *P. macrsopina* resulted to be a gonochoric, internal brooding species with a 1:1 population sex ratio. Oogenesis lasted ~12–14 months, whereas spermatogenesis was significantly shorter only lasting 6 months. Fertilization occurred during late summer (August) and larval release occurred during early autumn (September - October). The organic matter, total lipid content and stable isotopic composition showed very little seasonal variability, reflecting a general stability in gorgonian food sources on the continental shelf. Conversely, the free fatty acid composition varied seasonally reflecting changes in *P. macrospina* energetic demands probably related to the gametogenesis and the larval brooding. *P. macrospina* reproductive ecology and biochemical composition differed from most shallow Mediterranean gorgonian species, most likely reflecting the higher environmental stability of deeper environments.

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1. INTRODUCTION

In the past few decades there has been a substantial increase in the ecological characterization of coral-dominated communities located at 40–150 m depth. In tropical areas, mesophotic coral reefs represent a direct extension of shallow-water reef ecosystems reaching depths of over 150 m (Hinderstein et al., 2010; Kahng et al., 2010). Mesophotic coral ecosystems have revealed extensive, productive and rich communities, which differ significantly from their shallow-water counterparts (Bongaerts et al., 2010; Kahng et al., 2010; 2014; Loya et al., 2016). High-density gorgonian and black coral assemblages have been also recently reported on rocky bottoms in deep coastal areas, continental shelf and upper slope at 40–200 m depth in the Mediterranean Sea (Bo et al., 2011, Gori et al., 2011; Grinyó et al., 2016). These assemblages are composed of shallow species that extend their distribution to deeper environments (Bo et al., 2011; Gori et al., 2011), as well as by depth specialist species with distribution restricted to the continental shelf and upper slope (Bo et al., 2012; Grinyó et al., 2016). Despite the recent increase in knowledge about species composition and abundance of Mediterranean coral and gorgonian assemblages at intermediate depths (40–200 m depth) (e.g. Cau et al., 2015; Grinyó et al., 2016), very few studies have addressed basic aspects of their biology, such as the reproductive ecology and the dynamic of energy storage and metabolic demands (Gori et al., 2012).

Reproductive success and posterior larval settlement are crucial processes in the maintenance of Mediterranean shallow gorgonian populations (Coma et al., 1995; Ribes et al., 2007) as well as for their recovery after perturbations (Cerrano et al., 2005). In general, oogenesis has a longer duration than spermatogenesis (e.g. Coelho and Lasker, 2014). In broadcast spawning species, sperms and oocytes are released and fertilized in the water column; whereas in surface brooder species oocytes are retained by mucous material and fertilized on the surface of female colonies, and in internal brooder species oocytes are fertilized inside female polyps, where larvae develop (Kahng et al., 2011). Gorgonian larvae are lecithotrophic, and thus their survival success solely depends on the energetic reserves transferred from the maternal colony during the oogenesis (Thorson, 1950; Pechenik, 1990). However, the quantity of energy transferred by maternal colonies is limited since the energetic reserves are finite and need to be partitioned into respiration, growth, defense, and reproduction (Stearns, 1992). These energetic requirements are primarily supported by lipids (Oku et al., 2002; Grottoli et al., 2004; Brodte et al., 2008), which are also the main structural constituents of cellular membranes (Tchernov et al., 2004; Dalsgaard et al., 2003). Thus, lipid content reflects the nutritional condition of corals and gorgonians, which results from the balance between food inputs and respiration output, tissue replenishment and reproductive investment (Arai et al., 1993; Ward, 1995). When used as energetic source, lipid reserves are oxidized to provide energy in form of free fatty acids (FFA) that produce high ATP per molecule (Gurr et al., 2002). The FFA content can increase under stress situations, such as starvation and thermal anomalies, and thus their content can be used as a measure of metabolic demands (Sargent et al., 1999). On the other hand, FFA composition may reflect the nature of these metabolic demands (i.e., energetic requirements) (Imbs, 2013; Viladrich et al., 2016). For example, Poly Unsaturated Fatty Acids (PUFA) are highly energetic fatty acids (FA), essential for overcoming stress conditions, since they can be converted into many other FA (Müller–Navarra et al., 2000; Wacker and Von Elert, 2001), whereas Mono Unsaturated Fatty Acids (MUFA) and Saturated Fatty Acids (SFA) are mainly used to cover basic metabolic energy consumption (Sargent et al., 1999; Dalsgaard et al., 2003).

Environmental conditions affecting food availability, gorgonian metabolism, and the quantity and quality of the ingested food will determine lipid storage, as well as their consumption (FFA content), and thus the nutritional condition of gorgonian colonies (Ward and Harrison, 2000). In a temperate sea such as the Mediterranean, shallow gorgonians exhibit a marked seasonality of activity and secondary production, as a consequence of the strong seasonal environmental variability (Coma et al., 2000). Food capture, growth, and lipid storage are enhanced during winter-spring, in correspondence with phyto- and zooplankton blooms (Ribes et al., 1999; Rossi et al., 2006; Coma and Ribes, 2003). Conversely, gorgonian activity is significantly reduced during summer in shallow waters, when the stratification of the water column results in severe depletion of food sources (Coma et al., 2000; Coma and Ribes, 2003), and gorgonian mainly rely on their lipid reserves (Rossi et al., 2006). Environmental variability is dampened with depth in Mediterranean coastal bottoms (Garrabou et al., 2002), because temperature and currents are more constant below the summer thermocline (Riedl, 1971; Ballesteros, 2006). This major environmental stability is reflected in the lower but constant lipid content in gorgonian tissue at 60 m depth, as well as in their lower reproductive output compared to shallow gorgonians at 20 m depth (Gori et al., 2012). Continental shelf environments are more stable than littoral ones, showing very little variation in seawater temperature and being sheltered from strong hydrodynamic forces (Puig et al., 2001; Fernández de Puelles et al., 2007). Food availability on Mediterranean

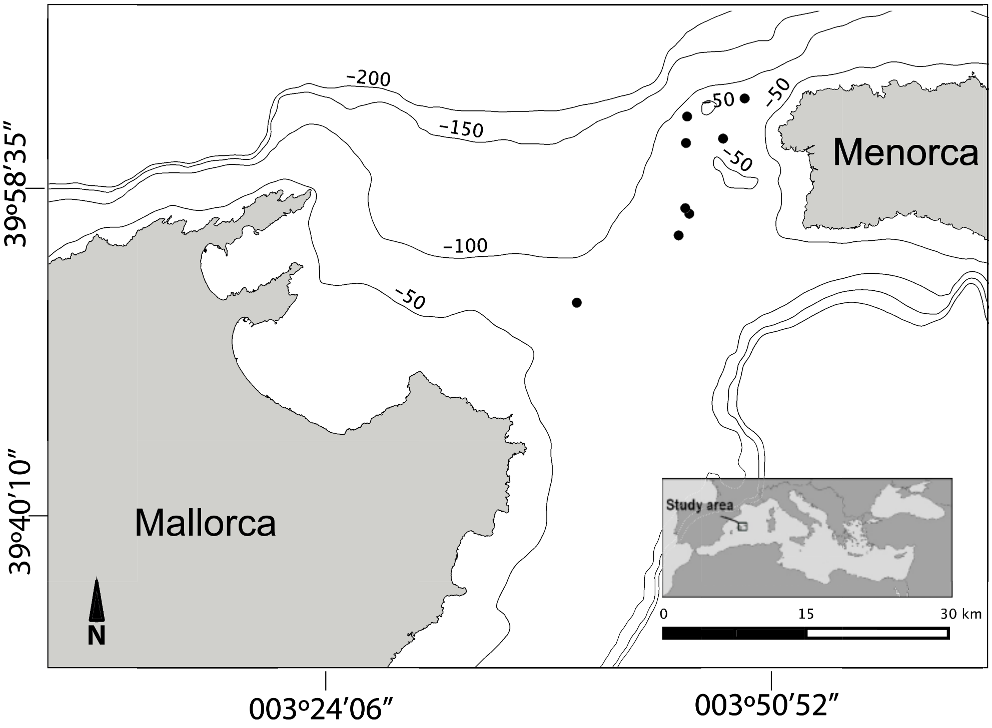

Fig. 1. Map of the study area, black dots indicate the location of the sampling stations.

continental shelves also follows a seasonal trend with highest inputs during winter and spring although, it is generally more constant during the year than in shallower environments (Danovaro et al., 2000; Rossi et al., 2003). Consequently, gorgonians are exposed to overall more stable environmental conditions on the continental shelf than in coastal areas, which can directly affect their annual reproductive cycle, energetic storage dynamic and metabolic requirements. Recently, *Paramuricea macrospina* has been reported as one of the most frequent and abundant species in Mediterranean gorgonian assemblages at intermediate depths. This species is the dominant gorgonian in maërl beds on the continental shelf at 65–100 m depth, and can also occur in dense multispecific assemblages on rocky shoals on the continental shelf and shelf edge at 90–160 m depth (Bo et al., 2012; Grinyó et al., 2016).

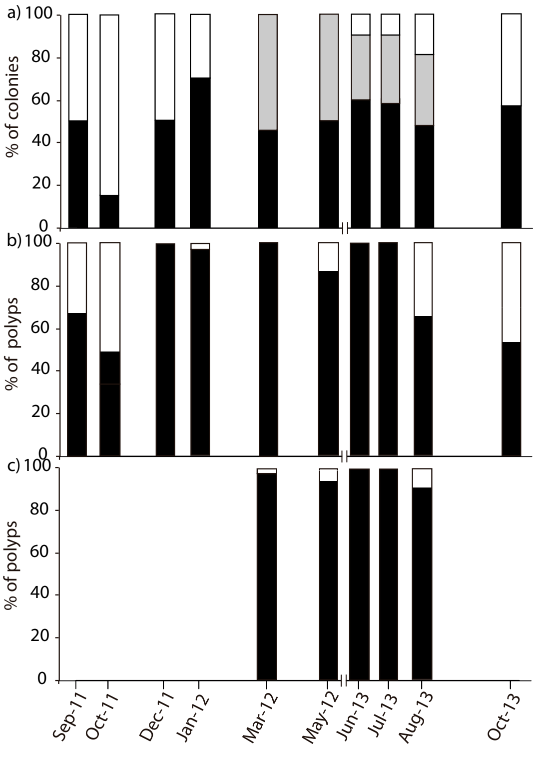

Fig. 2. Reproductive state of *Paramuricea macrospina* colonies (a) (black = female colonies, gray = male colonies, white = indeterminate colonies), and percentage of female (b) and male (c) fertile polyps (black = fertile polyps, white = empty polyps) (N polyps = 453; N examined colonies = 115).

The aim of this study was to explore, for the first time, the reproductive ecology and the dynamic of energy storage and metabolic demands of a Mediterranean gorgonian on the continental shelf. For this purpose, the annual development of sexual products, lipid content, FFA content and composition, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) composition were assessed over an annual cycle in the gorgonian *P. macrospina* from 70 m depth, to address the following questions: (1) Are there differences in the reproductive timing and gonadal output compared to shallow species? (2) Are there differences in the annual dynamic of energy storage and metabolic requirements compared to shallow species? (3) How are the gorgonian reproductive cycle, energy storage and metabolic demands related in the Mediterranean continental shelf environment?

2 MATERIALS AND METHODS

2.1 Sampling procedure

P. macrospina colonies were monthly sampled on the continental shelf of the Menorca Channel at 60–75 m depth (Fig. 1), from September 2011 to May 2012 as bycatch from trammel net experimental fisheries (project LANBAL). Since no colonies were caught in the experimental fisheries during the summer 2012, additional colonies were subsequently monthly sampled by SCUBA diving from June 2013 to October 2013. In November 2011, February and April 2012, and September 2013 no sample could be collected due to bad weather conditions. All sampled colonies were higher than 10 ± 0.5 cm (height distance from the base to the farthest point). Two primary branch fragments (~2 cm) were collected from each colony: one branch was fixed in 10% formalin in order to study the reproduction and population sex ratio; the other one was frozen at -20°C and freeze-dried during 12 h at -110°C and at 100 mbar pressure (Telstar Lyo Alfa 6 lyophilizer) for biochemical analyses.

2.2 Gametogenesis

Sex identification was performed under optical microscope and according to the color and appearance of sexual products (Coma et al., 1995; Ribes et al., 2007; Gori et al., 2007). Spermaries are pale, while oocytes present darker tonalities, harder consistency and are covered by a spotted membrane. Five female and five male colonies were examined for each sampling event, except for September 2011 when only nine colonies were sampled. For each colony, six polyps on the central portion of the branch were haphazardly selected and dissected under a binocular stereomicroscope (Olympus SZ-60). All sexual products were photographed with a Moticam 2300 photo camera, and pictures were analyzed with the image-processing software Macnification (Version 2.0.1 Orbicule Enhanced Labs). This software automatically counts the number of sexual products, and measures area and circularity (the proximity of the shape of an

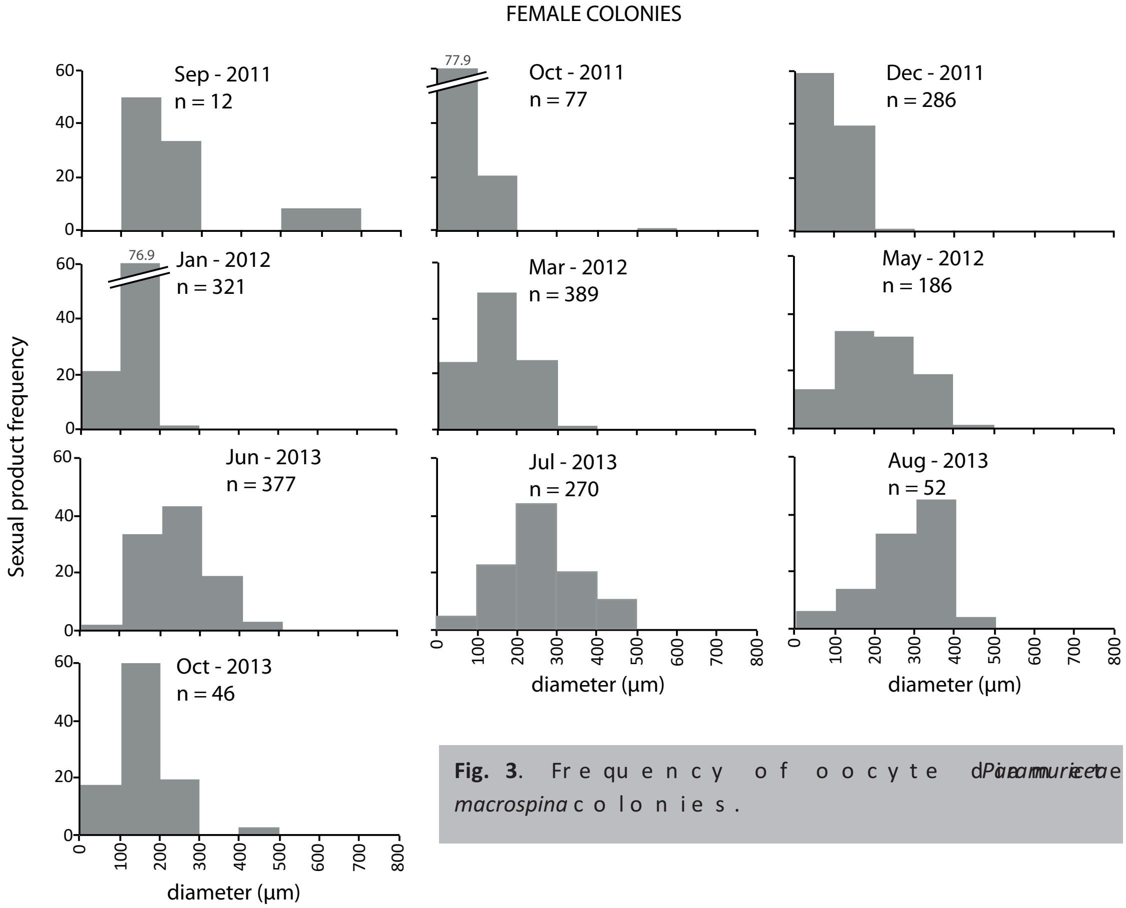

Fig. 3. Frequency of oocyte diameter (µm) in *Paramuricea macrospina* colonies.

object to that of a circle) of each one. Since circularity was always higher than 0.8, all sexual products were considered as spherical, and their measured areas (*a*) were converted to diameters ($d = 2\ (\sqrt{a/\pi})$). Diameters (*d*) were then transformed to volume ($v = \frac{4}{3}\pi\ (\frac{d}{2})^3$) in order to quantify the produced volume of sexual products per polyp. When observed inside female polyps, larvae were also quantified. A total of 594 polyps were dissected, and 3631 sexual products measured.

2.3 Population sex ratio

Colonies collected in June, July and August (when polyps are full of sexual products, see later) were used to quantify the population sex ratio. Based on Gori et al. (2007) branches in which no sexual products were found inside 10 polyps were not considered. A total of 90 colonies were examined.

2.4 Biochemical analyses

2.4.1 Organic matter

Organic matter (OM) in the coenenchyme was monthly quantified in four colonies. Approximately 10 mg (± 0.1 mg) of coenenchyme dry weight from each sample was reduced to ash during 4 h at 500 °C in a muffle (Relp 2H-M9), and the OM was calculated as the difference between the coenenchyme dry weight and ash weight (Slattery and McClintock, 1995;

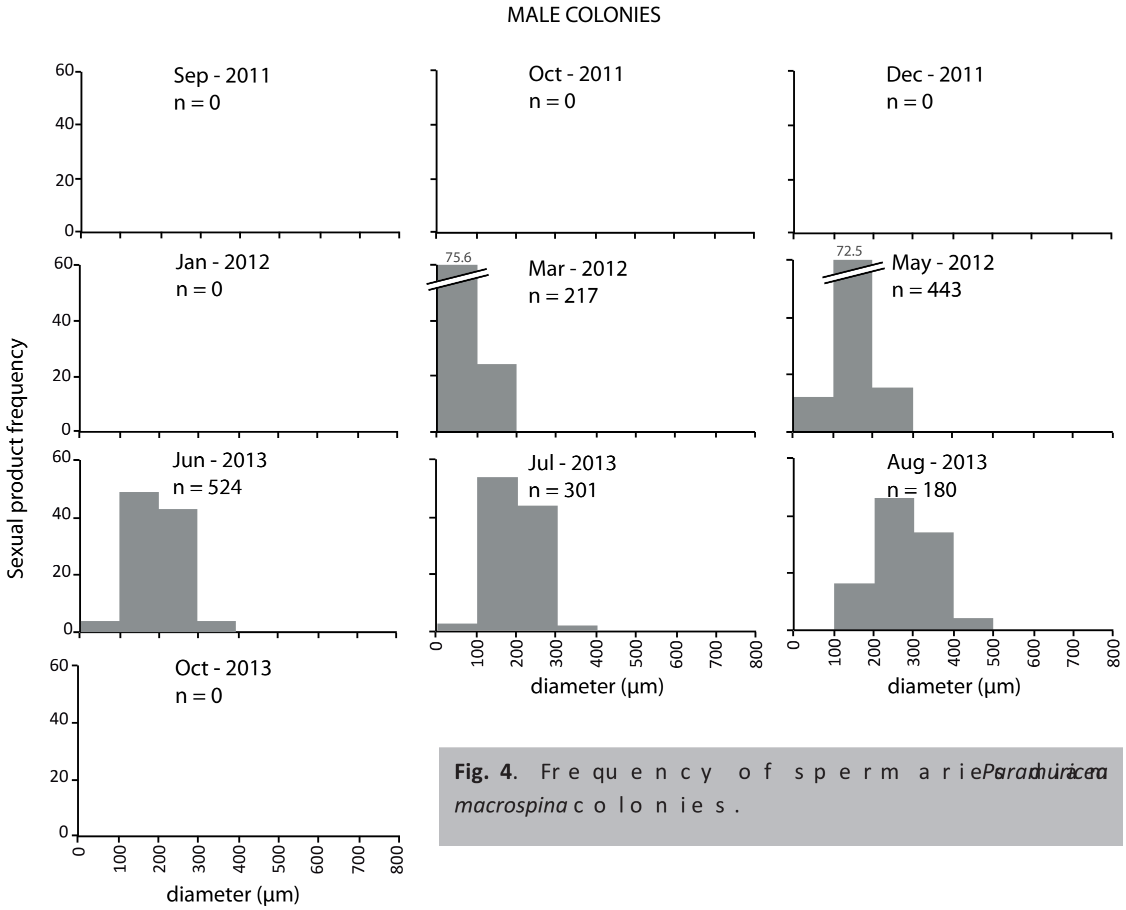

Fig. 4. Frequency of spermaries diameter (µm) *Paramuricea macrospina* colonies.

Rossi et al., 2006). Results are expressed in percentage.

2.4.2 Lipid content

Total lipid content in the tissue was quantified in five colonies per sampling event. Approximately 10 mg (± 0.1 mg) of coenenchyme dry weight from each sample were homogenized in 3 ml of chloroform:methanol (2:1), and total lipids were quantified colorimetrically (Barnes and Blackstock, 1973) with cholesterol as a standard. Results are expressed in µg of lipid mg^{-1} of OM.

2.4.3 Free Fatty acids and stable isotope composition

Five colonies for each sampling event were used to determine the FFA content and composition, following the methodology previously used by Viladrich et al. (2016). Approximately 11 mg (± 0.1 mg) of coenenchyme dry weight from each sample were dissolved in 3:1 DCM:MeOH (dichloromethane-methanol), and fatty acids were quantified with gas chromatography technique (Viladrich et al., 2016). Results are expressed in µg FA mg^{-1} of OM, and in percentage of SFA, MUFA and PUFA mg^{-1} of OM. The stable isotopes (SI) ($\delta^{13}C$ and $\delta^{15}N$) composition of the gorgonian tissue was assessed from monthly samples of three colonies. Approximately 2 mg (± 0.001 mg) of coenenchyme dry weight from each sample was acidified with HCl 1 M during 48 h to eliminate carbonates, and the $\delta^{13}C$ composition was determined with Thermo Finnigan EA1108 analyzer and a Thermo Finnigan MAT253 spectrometer. Finally, approximately 2 mg (± 0.001 mg) of coenenchyme dry weight from each sample was directly analyzed with the Thermo Flash EA112 analyzer and the Thermo Delta V advantage spectrometer to determine the $\delta^{15}N$ composition.

2.5 Statistical analyses

The population sex ratio was tested by means of a chi-square test using the R-language function chisq.test (Patefield, 1981) of the R software platform (R Core Team, 2014).

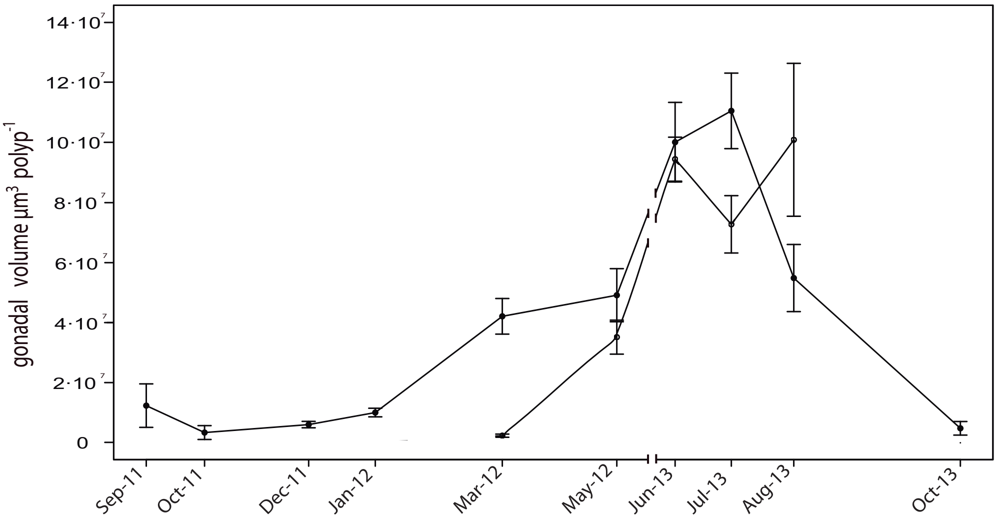

Fig. 5. Mean gonadal volume per polyp (mean ± SE) of *Paramuricea* (blac circ *macrospina* colonies (µm^3 polyp -1) (N female polyps = 312, N male polyps = 132).

Significant differences amongst seasons in OM, lipid content and SI composition were tested by means of a repeated measure ANOVA with the R-language function aov (Chambers et al., 1992) of the R software platform (R Core Team, 2014). Seasons were defined as follow: Autumn (September 2011, October 2012 and 2013), winter (December 2011 and January 2012), spring (March 2012, May 2012 and June 2013), summer (July and August 2013).

Colonies analyzed for FFA composition (n=50) were ordinated by means of a principal component analysis (PCA) performed on transformed data ($p' = \arcsin(\sqrt{p})$) with the R-language function princomp, which is available in the Vegan library (Oksanen et al., 2005) of the R software platform (R Core Team, 2014).

3. RESULTS

3.1 Population sex ratio

The recorded ratio of male to female colonies was 1.41 (36/51) and did not significantly deviate from 1:1 ($X^2 = 2.586$, df = 1, p-value = 0.108).

3.2 Gametogenesis

Colonies with female sexual products were observed during all sampling events (Fig. 2a). During late summer and autumn (August, September and October) fertile polyps were 45–66% of all the dissected polyps, whereas during the rest of the year almost all polyps (>80%) were fertile (Fig. 2b). Colonies with male sexual products were found from early spring to late summer (March to August) (Fig. 2a), with almost 100% of fertile polyps (Fig. 2c). Oocyte development took ~12–14 months to complete, beginning in early summer and ending in the next late summer (Fig. 3). Oocyte mean diameter progressively increased from early autumn to late summer (Table 1; Fig. 3) and oocyte number increased from early autumn to early summer (Table 1; Fig. 3). Smallest oocytes (<300 mm) were present in all sampling events, reaching its highest abundance during early autumn and winter (~98% and 100% observed oocytes, respectively) (Fig. 3). Large oocytes (>300 mm) were most abundant during late summer (80% of observed oocytes in August), whereas in early autumn their presence was residual (1–2% of observed oocytes in October) and they were completely absent in winter (Fig. 3). Spermaries development was considerably shorter, beginning in early spring (March) and ending in late summer (August) (Fig. 4). Spermaries mean diameter progressively increased from early spring to late summer (Table 1; Fig. 4). Mean number of spermaries per polyp increased from early spring (May) to early summer (June), and decreased during summer (Table 1).

Female gonadal volume per polyp progressively increased from early winter (October) to mid summer (July) when it reached its maximum volume (Fig. 5). From this point onward female gonadal volume decreased reaching its lowest values in mid autumn (October) (Fig. 5). Male gonadal volume per polyp increased from early spring to late summer when it reached its maximum (Fig. 5). A slight decrease in male volume was observed between early and mid summer (July) (Fig. 5).

One to five planulae larvae (1.3 ± 0.13 (mean ± SE)) were found inside 15.5% of female polyps in September and October.

3.4 Biochemical analyses

OM represented 27.2 ± 7.1% (mean ± SD) of the coenenchyma dry weight, with seasonal fluctuation ranging from 20.7 ± 2.5% in autumn to 34.1 ± 3.4% in summer (Fig. 6a). Summer

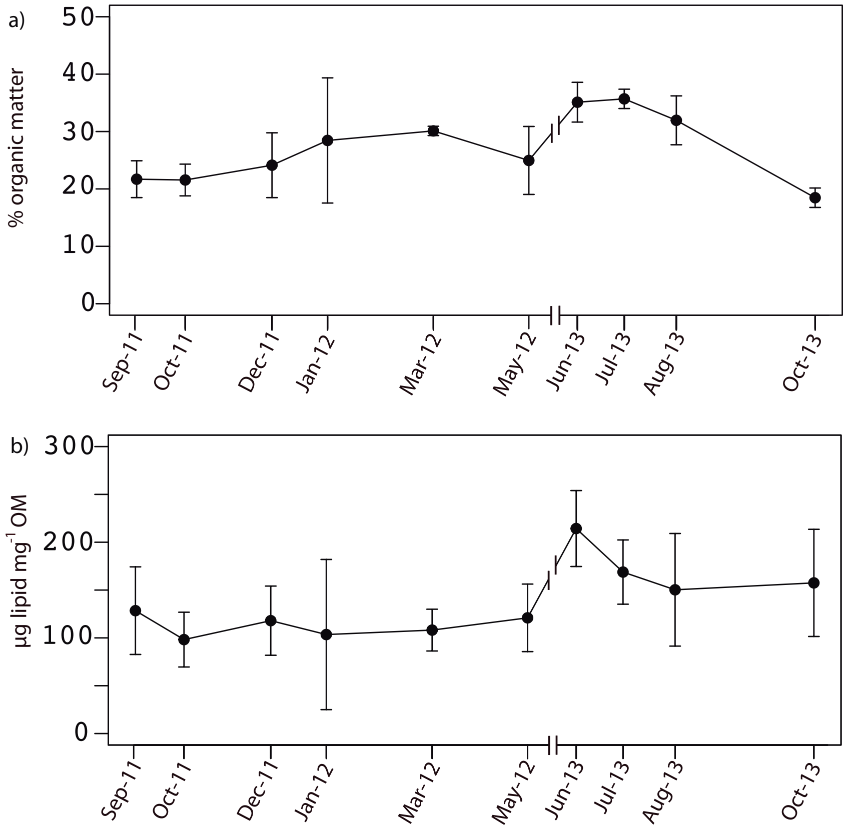

Fig. 6. Organic matter (a) in the *Paramuricea macrospina* colonies (N = 3), and lipid con (µg mg^{-1} OM) in the organic matter of *Paramuricea macrospina* colonies (N = 49).

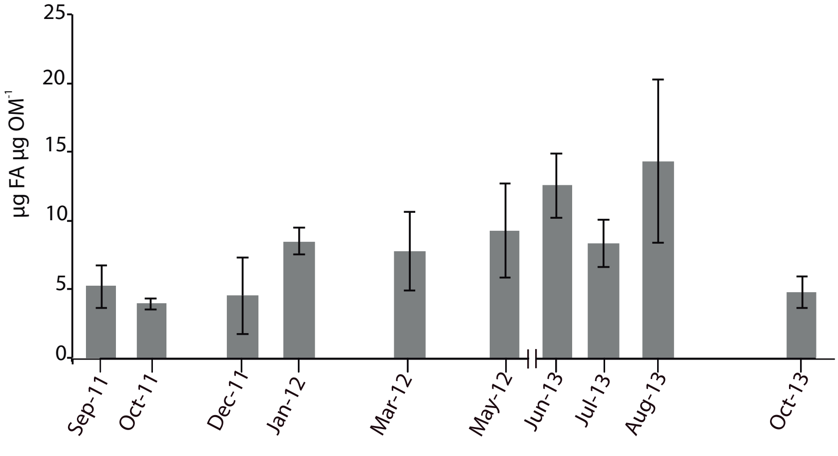

Fig. 7. Total free fatty acid (FFA) content (µg mg^{-1} OM) in the organic matter of *Paramuricea macrospina* colonies (N = 46).

OM content was significantly higher than in autumn and winter (ANOVA, F = 11, p<0.001), and spring OM content was significantly higher than in autumn (ANOVA, F = 11.01, p<0.001).

Average total lipid content was 137 ± 53.5 µg lipid mg^{-1} OM (mean ± SD), with significantly higher values (ANOVA, F = 5.8, p = 0.002) in summer (1778 ± 504 µg lipid mg^{-1} OM) (Fig. 6b).

The overall FFA concentration progressively increased from early autumn (October) to mid summer (August) (Fig. 7). PUFFAs and SFFAs were the most abundant fractions of the total FFA content, whereas MUFFAs only represented <15% of total FA (Fig. 8). A total of 36 fatty acids were identified (Table 2), with FFA composition showing seasonal changes characterized by SFFA markers during winter, and by PUFFA markers during spring and summer. The first component of the PCA accounted for 57.4%, and the second component accounted for 14.6% of the data variance, for a total 72% of explained variance. The PCA biplot revealed a seasonal gradient along the first component (Fig. 9), with autumn samples (orange squares) mainly characterized by 18:3 and 24:0, most winter samples (blue squares) characterized by 13:0, 14:0, 15:0 and 17:0 (all SFFA), spring samples (green squares) and most summer samples (red squares) characterized by 22:6, 20:4(n-3) and 18:4(n-3). SI composition presented very little seasonal variation (Fig. 10). The $\delta^{13}C$ ranged between -21.6 ± 0.3% (mean ± SD) in winter to -21.9 ± 0.1% in summer. The $\delta^{15}N$ ranged between 5.2 ± 0.5% (mean ± SD) in winter to 4.7 ± 0.4% (mean ± SD) in summer. No significant differences amongst seasons were found in the $\delta^{13}C$ and $\delta^{15}N$.

4 DISCUSSION

The results of this study showed the gorgonian *P. macrospina* from the Mediterranean continental shelf to sexually reproduce annually,

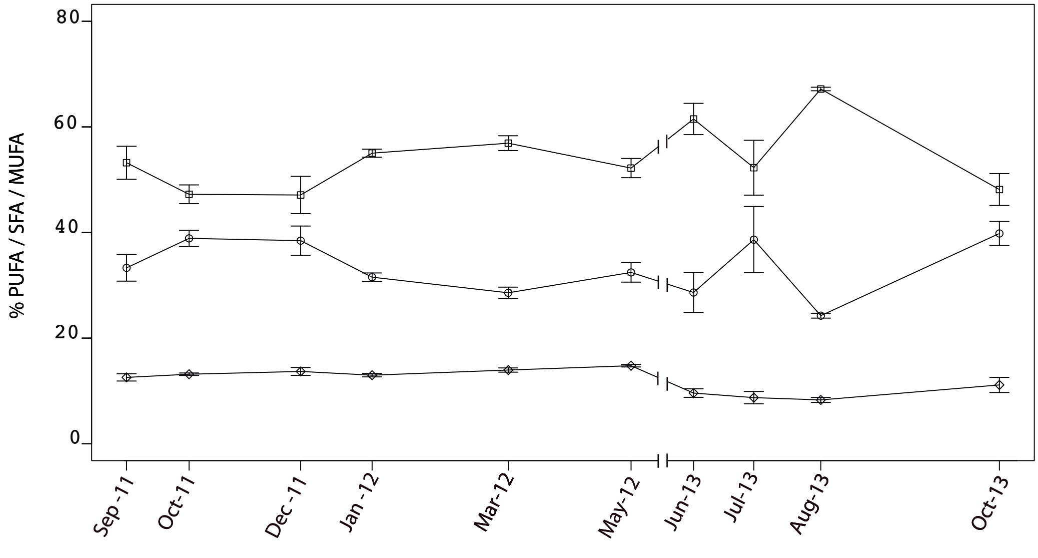

Fig. 8. Percentage of saturated (SFAs), monounsaturated (MUFAs) and pol
FFA of *Paramuricea macrospina* colonies (N = 46) (SFAs = circles, MUFAs = diamonds, PUFAs

Table 1. Changes in the diameter and number of *Paramuricea macrospina* sexual products (mean ± SE) (N

Sampling Event	Diameter (µm)				Number			
	Female		Male		Female		Male	
	Mean ± S	Max.	Mean ±	Max.	Mean ±	Max.	Mean ±	Max.
September 2011	248 ± 149	607			0.7 ± 1.1	4		
October 2011	88 ± 57	510			2.6 ± 3.4	11		
December 2011	94 ± 35	216			9.6 ± 6.2	21		
January 2012	128 ± 32	227			7.6 ± 5.9	28		
March 2012	158 ± 66	330	85.8 ± 21	150	13.0 ± 5.1	20	6.0 ± 6.4	24
May 2012	214 ± 91	403	153 ± 45	291	6.2 ± 4.7	15	14.8 ± 9.6	35
June 2013	236 ± 82	494	195 ± 56	335	10.5 ± 5.9	23	29.1 ± 11.8	56
July 2013	259 ± 99	509	195 ± 50	347	6,5 ± 3.1	14	13.9 ± 8.1	38
August 2013	373 ± 91	562	276 ± 81	491	1.7 ± 1.8	6	7.5 ± 8.2	30
October 2013	168 ± 101	502			1.1 ± 1.7	6		

with larval development taking place inside the female polyps, and larval release occurring in late summer-early fall. Gonochorism of *P. macrospina* colonies, and 1:1 population sex ratio, match the general pattern previously observed in shallow Mediterranean gorgonian species (Coma et al., 1995; Rossi et al., 2003; Ribes et al., 2007), and in the majority of octocorals in general (Kahng et al., 2011). In the same way, oogenesis (12–14 months) (Fig. 3) and spermatogenesis (~6 months) (Fig. 4) duration in *P. macrospina* was within the range observed in other shallow Mediterranean (Coma et al., 1995; Ribes et al., 2007) and temperate gorgonian species (e.g. Excoffon et al., 2004; Seo et al., 2008) with annual sexual reproduction (Kahng et al., 2011; Table 3). This long oogenesis duration results in the presence of a cohort of mature large oocytes during summer, together with a second cohort of immature small oocytes (<200 mm) that will slowly increase in size and number to mature during the following summer (Fig. 3). Conversely, spermaries maturation is much faster, starting in early spring (March) and ending with its release during late summer (August).

Spawning of male gametes and larval fertilization in *P. macrospina* is delayed 2–3 months respect to shallow Mediterranean gorgonian species, which generally spawn during late spring - early summer coinciding with the spring increase in shallow seawater temperature (Coma et al., 1995; Tsounis et al., 2006; Ribes et al., 2007). Gonadal development has been suggested to be conditioned by seawater temperature (Grigg, 1977; de Putron and Ryland, 2009), since gorgonian colonies occurring or maintained in colder environments showed a delay in gonadal development (Gori et al., 2013) and spawning respect to populations located in warmer environments (Gori et al., 2007; Pakes and Woollacott, 2008). Seawater temperature in the Balearic continental shelf (75 m) slightly increases a few degrees during late summer and early autumn (Fernández de Puelles et al., 2007) coinciding with the *P. macrospina* spawning. This might support that timing in *P. macrosina* reproductive cycle is conditioned by the late increase in seawater temperature occurring on the Mediterranean continental shelf. It is also interesting that larval release in September and October also coincides with the beginning of the fall phytoplankton bloom in the study area (Grinyó et al., submitted), which could suppose fruitful food availability for the primary polyps resulting from the larvae metamorphosis.

When compared to other internal brooding species, *P. macrospina* showed much smaller oocytes (Table 3), only exceeding those observed in species of the genus *Acabaria* (Ben-Yosef and Benayahu, 1999; Fine et al., 2005). The small size of *P. macrospina* oocytes is however compensated by high fertility compared to other internal brooding species (Table 3), which generally tend to develop few but large oocytes (e.g. Tsounis et al., 2006; Ribes et al., 2007; Orejas et al., 2007). In this sense, both oocyte size and fertility of *P. macrospina* are within the range observed in the congeneric Mediterranean *Paramuricea clavata* (Table 3; Coma et al., 1995; Gori et al., 2007), which mainly inhabit vertical rocky walls in coastal areas (Linares et al., 2008; Gori et al., 2011; Kipson et al., 2014). However, the two species clearly differ in their reproductive strategy, being *P. macrospina* an internal brooder, and *P. clavata* a surface brooder (Coma et al., 1995). Differences among congeneric species in the reproductive strategy have previously been reported in allopatric species of the genus *Corallium* (Waller and Baco, 2007; Nonaka et al., 2015; Priori et al., 2013) as well as in sympatric species of the genus *Anthillogorgia* (Coelho and Lasker, 2014), although the causes of this variability remain unknown. A possible hypothesis could be that fertilization in surface brooding species is mainly restricted to the few days when oocytes remain attach to the surface of the mother colony (Coma et al., 1995; Fiorillo et al., 2013). Thus, fertilization success is highly conditioned by the currents and proximity of male and female colonies. Conversely, in internal brooding species fertilization may probably occurs over a longer period, and thus colony proximity would possibly be less important for fertilization success. In this sense, surface brooding could be highly effective in a large-sized species with high population density (33 ± 14 colonies m^{-2}) such as *P. clavata* (Linares et al., 2008; Kipson et al., 2014). Conversely, internal brooding could be more effective for *P. macrospina*, which has smaller colonies mainly distributed in low to medium densities (3.2 ± 5 colonies m^{-2}) over vast ar-

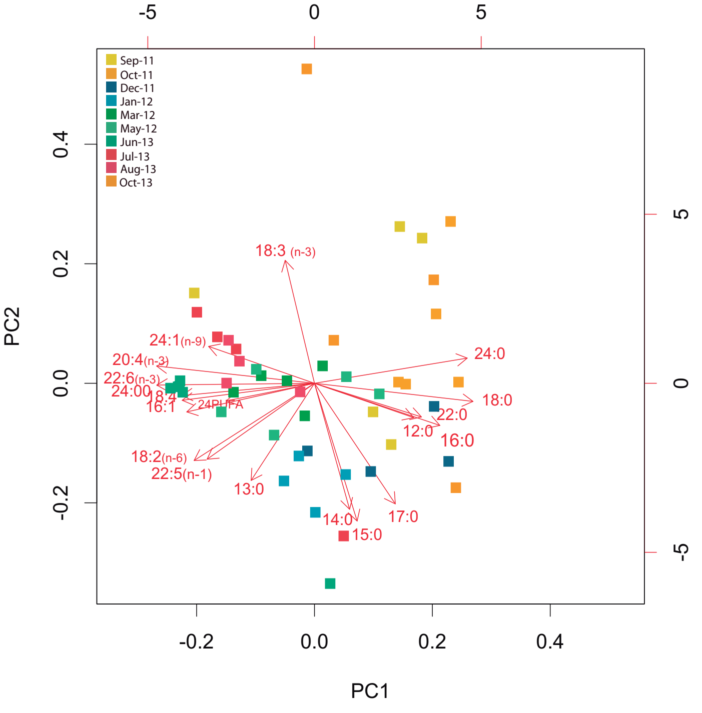

Fig. 9. Principal component analysis (PCA) biplot showing the ordination of studied colonies of *Paramuricea macrospina* regarding their composition in fatty acids

eas of the continental shelf (Grinyó et al., 2016). Moreover, from an energetic point of view, surface brooding probably requires a higher energetic investment than internal brooding, due to the production and secretion of large quantities of mucus (Viladrich et al., 2016). In this regard, *P. clavata* can probably rely upon high spring food availability in littoral environments (Rossi et al., 2003, 2006; Viladrich et al., 2016) exposed to intense turbulent flows (Riedl, 1971), whereas, food availability might be lower in continental shelf environments, where *P. macrospina* is exposed to mild unidirectional currents (Palanques et al., 2002).

Organic matter and total lipid content in *P. macrospina* showed little seasonal variation, with higher values during summer coinciding with the progressive augment of gonadal volume (Figs. 5 and 7). This fact, together with their lower content after larval release, can suggest a direct transfer of lipid from the parental colonies to the sexual products (Arai et al., 1993; Richmond, 1987). Remarkably, total lipid content was much lower and more constant in *P. macrospina* all year round than previously observed in shallow (25–30 m depth) colonies of *P. clavata* (Rossi et al., 2006; Viladrich et al., 2016). This lower and more constant lipid content in deep than shallow colonies has previously been observed in the Mediterranean gorgonian *Eunicella singularis* (Gori et al., 2012). Thus, these differences between depths may be due to lower but more constant food availability on the Mediterranean continental shelf than in littoral environments (Gori et al., 2012). A general stability in food availability for gorgonians on the Mediterranean continental shelf is also supported by the lack of seasonality in the $\partial^{13}C$ and $\partial^{15}N$ composition of *P. macrospina* tissue (Fig. 10). Both observed $\partial^{13}C$ and $\partial^{15}N$ values are in the range of those from heterotrophic Mediterranean coastal gorgonians (Fig. 11) (Gori et al., 2012; Cocito et al., 2013), and suspension feeders feeding on microzooplankton and particulate organic matter in general (Carlier et al., 2007). The $\partial^{13}C$ signature of *P. macrospina* also resembled that observed in other gorgonian species on the Pacific continental shelf (Sherwood et al., 2005).

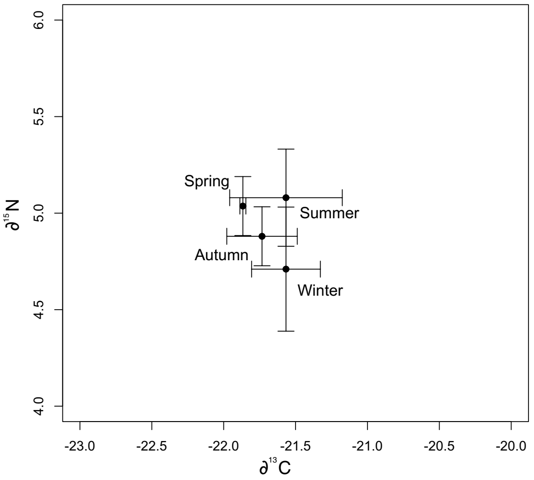

Fig. 10. Stable isotope (δ13C and δ1 N) com
of *Paramuricea macrospina* colonies (N = 12).

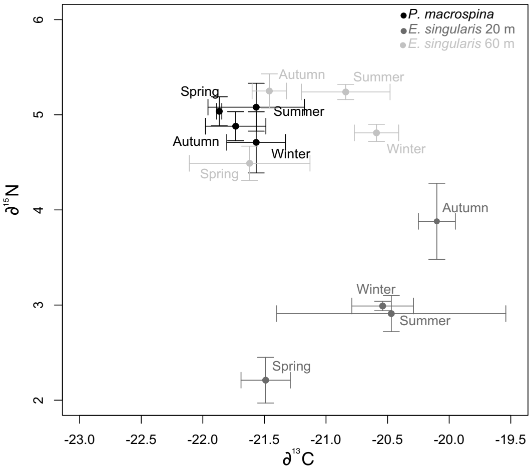

Fig. 11. Stable isotope (δ13C and δ1 N) com
of *Paramuricea macrospina* colonies (N = 12) and
Eunicella singularis from 20 m depth and 60 m d
(From Gori et al., 2012).

Table 2. Fatty acid composition (% of total fatty acids) of *Paramuricea macrospina* colonies (N=46) (mean ± SD).

Fatty Acids	Sep 11	Oct 11	Dec 11	Jan 12	Mar 12	May 12	Jun 13	Jul 13	Aug 13	Oct 13
	(n = 5)	(n = 5)	(n = 4)	(n = 4)	(n = 5)	(n = 5)	(n = 5)	(n = 5)	(n = 4)	(n = 4)
C12:0	0.003 ± 0.002	0.002 ± 0.001	0.003 ± 0.002	0.002 ± 0.000	0.001 ± 0.000	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.001	0.001 ± 0.000	0.003 ± 0.001
C13:0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000
C14:1	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.004 ± 0.009	0.000 ± 0.000	0.000 ± 0.000
C14:0	0.014 ± 0.006	0.02 ± 0.003	0.023 ± 0.03	0.030 ± 0.005	0.016 ± 0.003	0.019 ± 0.003	0.026 ± 0.015	0.031 ± 0.011	0.016 ± 0.04	0.017 ± 0.013
C15:0	0.007 ± 0.003	0.009 ± 0.000	0.010 ± 0.001	0.012 ± 0.002	0.007 ± 0.001	0.009 ± 0.001	0.010 ± 0.004	0.013 ± 0.004	0.006 ± 0.001	0.007 ± 0.005
C16:1	0.014 ± 0.003	0.012 ± 0.001	0.019 ± 0.003	0.023 ± 0.002	0.018 ± 0.004	0.022 ± 0.003	0.031 ± 0.008	0.027 ± 0.014	0.022 ± 0.005	0.017 ± 0.004
C16:0	0.0173 ± 0.04	0.192 ± 0.02	0.211 ± 0.056	0.160 ± 0.013	0.155 ± 0.023	0.181 ± 0.032	0.0167 ± 0.072	0.220 ± 0.101	0.138 ± 0.016	0.222 ± 0.049
C17:0	0.015 ± 0.005	0.017 ± 0.002	0.015 ± 0.002	0.015 ± 0.001	0.013 ± 0.002	0.014 ± 0.002	0.012 ± 0.004	0.016 ± 0.007	0.011 ± 0.001	0.012 ± 0.010
C18:3(n-6)	0.01 ± 0.01	0.000 ± 0.000	0.001 ± 0.002	0.005 ± 0.003	0.006 ± 0.004	0.006 ± 0.004	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.001	0.007 ± 0.002
C18:4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.001	0.001 ± 0.002	0.001 ± 0.002	0.004 ± 0.000	0.004 ± 0.002	0.003 ± 0.001	0.000 ± 0.000
C18:2(n-6)	0.02 ± 0.02	0.000 ± 0.000	0.013 ± 0.003	0.023 ± 0.002	0.026 ± 0.002	0.030 ± 0.003	0.026 ± 0.008	0.026 ± 0.008	0.021 ± 0.007	0.012 ± 0.015
C18:3(n-3)	0.02 ± 0.03	0.010 ± 0.001	0.001 ± 0.001	0.004 ± 0.001	0.006 ± 0.001	0.006 ± 0.000	0.013 ± 0.003	0.021 ± 0.021	0.009 ± 0.005	0.026 ± 0.039
C18:1(n-9)	0.09 ± 0.02	0.100 ± 0.009	0.098 ± 0.020	0.086 ± 0.010	0.093 ± 0.011	0.097 ± 0.007	0.038 ± 0.011	0.035 ± 0.022	0.042 ± 0.013	0.070 ± 0.048
C18:2(n-4)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.003	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.007	0.011 ± 0.018	0.000 ± 0.000	0.000 ± 0.000
C18:0	0.09 ± 0.02	0.116 ± 0.027	0.106 ± 0.025	0.084 ± 0.008	0.080 ± 0.010	0.088 ± 0.022	0.062 ± 0.019	0.096 ± 0.074	0.062 ± 0.04	0.102 ± 0.018
C19:0	0.002 ± 0.002	0.003 ± 0.002	0.004 ± 0.001	0.003 ± 0.000	0.003 ± 0.000	0.008 ± 0.000	0.003 ± 0.001	0.001 ± 0.002	0.002 ± 0.000	0.002 ± 0.001
C20:5(n-3)	0.3 ± 0.2	0.297 ± 0.158	0.356 ± 0.862	0.370 ± 0.021	0.348 ± 0.033	0.294 ± 0.021	0.343 ± 0.052	0.253 ± 0.110	0.436 ± 0.047	0.247 ± 0.151
C20:4(n-6)	0.07 ± 0.1	0.088 ± 0.154	0.021 ± 0.004	0.030 ± 0.004	0.031 ± 0.004	0.038 ± 0.009	0.047 ± 0.003	0.061 ± 0.042	0.058 ± 0.023	0.087 ± 0.110
C20:3(n-6)	0.01 ± 0.01	0.014 ± 0.003	0.02 ± 0.005	0.006 ± 0.004	0.010 ± 0.002	0.013 ± 0.004	0.011 ± 0.002	0.012 ± 0.002	0.009 ± 0.004	0.019 ± 0.013
C20:4(n-3)	0.003 ± 0.007	0.000 ± 0.001	0.000 ± 0.001	0.002 ± 0.002	0.005 ± 0.003	0.004 ± 0.005	0.009 ± 0.004	0.007 ± 0.006	0.006 ± 0.007	0.002 ± 0.003
C20:2(n-6)	0.008 ± 0.007	0.006 ± 0.001	0.006 ± 0.002	0.004 ± 0.000	0.006 ± 0.001	0.007 ± 0.001	0.008 ± 0.002	0.008 ± 0.002	0.006 ± 0.003	0.007 ± 0.002
C20:1(n-9)	0.007 ± 0.007	0.007 ± 0.001	0.007 ± 0.002	0.006 ± 0.002	0.007 ± 0.001	0.008 ± 0.002	0.011 ± 0.004	0.009 ± 0.004	0.007 ± 0.005	0.007 ± 0.003
C20:0	0.002 ± 0.002	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.002 ± 0.001
C21:0	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	0.002 ± 0.000	0.003 ± 0.000	0.003 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.004 ± 0.002
C22:5	0.002 ± 0.002	0.000 ± 0.001	0.001 ± 0.002	0.004 ± 0.000	0.005 ± 0.001	0.004 ± 0.002	0.005 ± 0.001	0.001 ± 0.001	0.004 ± 0.002	0.001 ± 0.001
C22:6(n-3)	0.03 ± 0.01	0.026 ± 0.005	0.036 ± 0.009	0.006 ± 0.019	0.076 ± 0.022	0.078 ± 0.027	0.086 ± 0.021	0.076 ± 0.039	0.078 ± 0.018	0.044 ± 0.010
C22:4	0.003 ± 0.002	0.000 ± 0.001	0.001 ± 0.001	0.003 ± 0.000	0.002 ± 0.000	0.002 ± 0.001	0.002 ± 0.001	0.000 ± 0.000	0.002 ± 0.001	0.001 ± 0.002
C22:5	0.001 ± 0.002	0.000 ± 0.000	0.000 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.001 ± 0.001	0.004 ± 0.002	0.001 ± 0.000	0.002 ± 0.002	0.001 ± 0.001
C22:1(n-9)	0.008 ± 0.006	0.006 ± 0.007	0.005 ± 0.004	0.009 ± 0.002	0.008 ± 0.004	0.007 ± 0.003	0.004 ± 0.001	0.003 ± 0.002	0.004 ± 0.001	0.009 ± 0.003
C22:2(n-6)	0.007 ± 0.004	0.013 ± 0.008	0.008 ± 0.003	0.008 ± 0.002	0.007 ± 0.002	0.007 ± 0.003	0.005 ± 0.001	0.004 ± 0.002	0.005 ± 0.001	0.007 ± 0.003
C22:0	0.003 ± 0.003	0.03 ± 0.001	0.003 ± 0.000	0.002 ± 0.000	0.003 ± 0.001	0.003 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.008 ± 0.011
C23:0	0.014 ± 0.02	0.021 ± 0.026	0.003 ± 0.001	0.002 ± 0.00	0.002 ± 0.000	0.003 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.018 ± 0.025
C24:PUFA	0.03 ± 0.02	0.012 ± 0.011	0.019 ± 0.009	0.017 ± 0.003	0.024 ± 0.004	0.019 ± 0.004	0.028 ± 0.007	0.021 ± 0.013	0.019 ± 0.013	0.015 ± 0.004
C24:PUFA	0.012 ± 0.015	0.004 ± 0.001	0.004 ± 0.004	0.011 ± 0.003	0.013 ± 0.004	0.013 ± 0.006	0.020 ± 0.006	0.017 ± 0.012	0.011 ± 0.009	0.005 ± 0.004
C24:1(n-9)	0.009 ± 0.001	0.007 ± 0.002	0.008 ± 0.002	0.006 ± 0.001	0.014 ± 0.002	0.014 ± 0.002	0.012 ± 0.003	0.009 ± 0.004	0.009 ± 0.003	0.008 ± 0.002
C24:0	0.009 ± 0.002	0.007 ± 0.001	0.007 ± 0.003	0.004 ± 0.001	0.005 ± 0.001	0.006 ± 0.002	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.009 ± 0.005

Table 3. Reproductive patterns in different gorgonian species. Med = Mediterranean, Isl. = island, S = sexuality, G = gonochoric, H = hermaphroditic, RS = reproductive strategy, IB = internal brooder, SB = surface brooder, BS = broadcast spawner, Spermat. = spermatogenesis.

Environment	Location	*Species*	S	RS	Oogenesis Duration	Oocyte Diameter (µm)	Fertility	Spermat. Duration	Spermary Diameter (µm)	Fertility	Reference
Littoral temperate	Med.	*Paramuricea macrospina*	G	IB	~12	87.9 ±6.5 – 330.04±15.6	2±0.3 – 10.4±0.2	5	85.8±1.5 – 276.7 ±4.3	8.36±0.4 – 29.11±0.5	This study
	Med.	*Paramuricea clavata*	G	SB	13 – 18	72±45 – 425±76	13±2.2	6 – 7	77 ±34 – 326±108	4.3±0.8 – 35±6.1	Coma et al., 1995
	Med.	*Eunicella singularis*	G	IB	13 – 17	141±46 – 829±250	0.69±0.16	4 – 6	123 ± 49 – 387±106	-	Ribes et al., 2007
	Med.	*Corallium ruburum*	G	IB	>12	170 – 520	~0.5 – ~2.4	8	50 – 480	~0.5 – ~2.7	Tsounis et al., 2006
	Med.	*Leptogorgia sarmentosa*	G	BS	>12	500	3 – 4	6 – 7	550	4 – 5	Rossi et al., 2009
	Med.	*Spinimuricea klavereni*	G	BS	-	<150 – 538	43±22 – 87±27	-	<150 – 680	29.7±12.9 – 65±17.5	Topçu and Öztürk, 2016
	Med.	*Acabaria erythraea*	H	IB	-	100	10 – 25	-	200	-	Fine et al., 2005
	S Atlantic	*Tripalea clavaria*	G	IB	11 – 12	40 – 700	7.2 ±3.7 – 14.3 ±5.2	6 – 7	900	10.1 ±3.8 – 3.8±2.1	Excoffon et al., 2004
	Jeju Isl.	*Anthoplexaura dimorpha*	G	BS	12	43±8 – 359±62	-	6	56±10 – 315±36	-	Seo et al., 2008
	California	*Muricea fruticosa*	G	IB	9 – 12	>750	-	-	~450	-	Grigg, 1970
	California	*Muricea californica*	G	IB	9 – 12	~800	-	-	~600	-	Grigg, 1970
Littoral tropical	S Taiwan	*Elisella robusta*	G	BS	-	360	3.2	-	-	-	Chang, 2007
	S Taiwan	*Subergorgia suberosa*	G	BS	-	322	1.4	-	-	-	Chang, 2007
	S Taiwan	*Subergorgia mollis*	G	BS	-	461	1.1	-	-	-	Chang, 2007
	S Taiwan	*Bebryce indica*	G	-	-	312	2.0	-	-	-	Chang, 2007
	Caribbean	*Briareum asbestinum*	G	SB	9 – 12	900	2.25 – 4.4±2.76	5	-	0.5±1.5 – 4.55±3.87	Brazeau and Lasker, 1990

Table 3 (cont.). Reproductive patterns in different gorgonian species. Med = Mediterranean, Isl. = island, S = sexuality, G = gonochoric, H = hermaphroditic, RS = reproductive strategy, IB = internal brooder, SB = surface brooder, BS = broadcast spawner, Spermat. = spermatogenesis.

Environment	Location	*Species*	S	RS	Oogenesis Duration	Oocyte Diameter (µm)	Fertility	Spermat. Duration	Spermary Diameter (µm)	Fertility	Reference
Littoral tropical	Red Sea	*Briaerum hambrum*	G	SB	12	180 – 750	14 – 16	8 – 10	250 – 550	-	Benayahu, 1989
	Caribbean	*Plexaura flexuosa*	G	BS	-	597±27	0.17±0.24 – 1.09±0.73	-	~450	-	Beiring and Lasker, 2000
	Caribbean	*Plexaura sp.*	G	BS	~9	200 – 600	-	-	-	-	Brazeau and Lasker, 1989
	Caribbean	*Plexaura homomalla*	G	BS	18	>100 – 640	1.97±0.26	6 – 8	-	-	Martin et al., 1982
	Caribbean	*Antillogorgia hystrix*	G	IB	9	101 – >700	~0.75 – ~3.5	4	101 – >601	-	Coelho and Lasker, 2014
	Caribbean	*Pseudopterogorgia elisabethae*	G	SB	~10	66±3.1 – 379.6±9.2	-	~2	70±4.5 – 296.6±11.8	-	Gutiérrez-Rodríguez and Lasker, 2004
	Red Sea	*Acabaria biserialis*	G	IB	10	<40 – 240	-	~10	<40 – 160	-	Ben-Yosef and Benayahu, 1999
Continental shelf and slope	N Atlantic	*Acanella arbuscula*	G	-	-	20.8±6.6 – 543±71.9	21.0±17.5	-	28.8±14 – 309.7±21	13.9±13.5	Beazley and Kenchington, 2012
	S Pacific	*Primnoa notialis*	G	-	-	100 – 690	18±4.51	-	-	-	Feehan and Waller, 2015
	N Pacific	*Swiftia beringi*	G	-	-	726.63	13.6±2.85	-	-	-	Feehan and Waller, 2015
	N Pacific	*Swiftia kofoidi*	G	-	-	561.81	3±1.53	-	-	-	Feehan and Waller, 2015
	N Pacific	*Swiftia pacifica*	G	-	-	150 – 664.81	4.6±2.06	-	-	-	Feehan and Waller, 2015
	N Pacific	*Swiftia simplex*	G	-	-	269 – 698.53	42.53±9.82	-	-	-	Feehan and Waller, 2015
	N Pacific	*Swiftia torreyi*	G	-	-	241 – 645.07	8±1.15	-	-	-	Feehan and Waller, 2015

Table 3 (cont.). Reproductive patterns in different gorgonian species. Med = Mediterranean, Isl. = island, S = sexuality, G = gonochoric, H = hermaphroditic, RS = reproductive strategy, IB = internal brooder, SB = surface brooder, BS = broadcast spawner, Spermat. = spermatogenesis.

Environment	Area	*Species*	S	RS	Oogenesis Duration	Oocyte Diameter (µm)	Fertility	Spermat. Duration	Spermary Diameter (µm)	Fertility	Reference
Continental shelf and slope	N Pacific	*Primnoa pacifica*	G	SB	~12	50 – 802	86±23	~12	500 – 1000	-	Waller et al., 2014
	N Atlantic	*Primnoa resedaeformis*	G	-	-	<100 – 1000	84.3±3.1	-	-	-	Mercier and Hamel, 2011
	N Atlantic	*Keratoisis ornata*	G	-	-	70 – 700	-	-	-	-	Mercier and Hamel, 2011
	N Pacific	*Paracorallium japonicum*	G	BS	~9	102.3 – 227.7	1 – 3	-	162.3 – 261.7	1 – 6	Nonaka et al., 2015
	N Pacific	*Corallaium elatius*	G	BS	~11	112.7 – 229.3	1 – 7	-	36.1 – 250.3	1 – 6	Nonaka et al., 2015
	N Pacific	*Corallium konjoi*	G	BS	-	76.3 – 168.8	1 – 7	-	50.6 – 287.2	1 – 8	Nonaka et al., 2015
	N Pacific	*Corallium lauuense*	G	BS	-	~650	-	-	-	-	Waller and Baco, 2007
	N Pacific	*Corallium secundum*	G	BS	-	~600	-	-	-	-	Waller and Baco, 2007
	Antarctica	*Dasystenella acanthina*	G	-	>12	50 – 1200	1.2±0.08	-	20 – ~790	2.6±0.19	Orejas et al., 2007
	Antarctica	*Thouarella sp.*	G	IB	>12	>100 – 550	1.1±0.1	-	>75 – 325	3.0±0.2	Orejas et al., 2007
	Antarctica	*Thouraella variabilis*	G	IB	>12	>50 – >800	-	-	>50 – >950	-	Brito et al., 1997
	Antarctica	*Fannyella rossii*	G	IB	>12	>100 – >350	1.5±0.06	-	75 – >150	5.0±0.21	Orejas et al., 2007
	Antarctica	*Fannyella spinosa*	G	IB	>12	>80 – >300	1.4±0.8	-	>80 – >300	2.6±0.21	Orejas et al., 2007

Seasonality in the energetic requirements of *P. macrospina* is highlighted by the seasonal changes observed in the FFA content and composition. Indeed, the slight increase of FFA content from early autumn to mid summer (Figs. 7 and 8), in coincidence with progressive augment of gonadal volume (Fig. 5), suggests that gonadal development supposes a high energetic demand that *P. macrospina* meets by mobilizing FFA as a source of immediate energy (Sargent et al., 1988; Viladrich et al., 2016). On the contrary, larval development inside the maternal polyp does not seem to require any high metabolic investment, as FFA content was minimum in September and October when larvae were found inside the polyps. This seasonality was also reflected in the FFA composition of *P. macrospina* tissue, which is mainly characterized by SFFA markers during winter, and by PUFFA markers during spring and summer (i.e., when gonadal volume progressively increases) (Fig. 9). Besides, the predominance of 18:4(n-3), 20:4(n-3) and 22:6(n-3) during gonadal development might have been directly related to increased fecundity, fertility and egg quality (Pernet et al., 2002; Viladrich et al., 2016). On the other hand, 18:3(n-3) and 24:0 predominate during late summer and early fall, when larvae are present inside the female polyps. The 18:3(n-3) is an essential FA that can be converted into the high energy and biologically active FFA 20:5(n-3) and 22:6(n-3) (Kelly and Scheibling, 2012). In this sense, larvae could be directly using 18:3(n-3) to fulfil their metabolic demands (Sargent et al., 1999).

Overall, dampening of environmental variability with depth (Puig et al., 2001; Garrabou et al., 2002; Fernández-Puelles et al., 2007) is reflected in the constant lipid content and SI composition of *P. macrospina* from the Mediterranean continental shelf, contrasting with the strong seasonality observed in shallow species (Coma et al., 2000; Rossi et al. 2006; Gori et al., 2012). The slight temperature increase occurring on the continental shelf in late summer (Fernández de Puelles et al., 2007) is probably driving the reproductive cycle of *P. macrospina*, with gametogenesis likely affecting its metabolic requirements (with a mobilization of high-energy PUFFA in spring and early summer). Internal brooding in *P. macrospina* contrasts with surface brooding of the congeneric *P. clavata*. The differences in the habitats where the two species occur, arises the question about the possible adaptive advantage of their reproductive strategies. Nevertheless, the observed differences in the reproductive features of *P. macrospina* compared to the shallower Mediterranean species, contributes to highlight the ecological diversity of gorgonians as a possible explanation of their wide spatial and bathymetric distribution in the Mediterranean Sea (Carpine and Grasshoff, 1975; Bo et al., 2012; Grinyó et al., 2016).

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Chapter 5

Composition and temporal variability of particle fluxes in an insular canyon of the northwestern Mediterranean Sea

ABSTRACT

Particle fluxes have widely been studied in canyons located in continental margins conversely particle fluxes in canyons incising small island margins have received very little attention and remain poorly understood. The Menorca Canyon is the only canyons system in the Balearic Archipelago. Moreover, the Menorca Canyon and surrounding areas host highly diverse communities dominated by benthic suspension feeders. Understanding the magnitude and variability of environmental factors influencing these communities thus remains crucial. In order to characterize the temporal variability of particle fluxes, analyze its geochemical and macroscopic composition and identify the main processes that modulate particle fluxes in the Menorca Canyon one instrumented line was deployed at 430 m from September 2010 to October 2012. Particle fluxes ranged between 0.2 – 2.3 $g\ m^{2}\ d^{-1}$ being one of the lowest ever registered in a Mediterranean submarine canyon's head. Asides opal, geochemical constituents were temporally highly correlated with particle fluxes. $CaCO_3$ fraction was the major constituent contrasting with the general trend observed in other Mediterranean canyons. Macroscopic constituents (fecal pellets, *Posidonia oceanica* detritus and pelagic and benthic foraminifera) constituents presented a wide variability throughout the sampling period and were poorly correlated with the total mass flux. The low magnitude of the registered fluxes and the lack of correlation with the observed environmental variables (e.g., currents, winds, wave height, chlorophyll-a biomass) suggest that there is no evident controlling mechanism. However, we could infer that resuspension processes and the presence of different hydrodynamic features (e.g. water masses) condition the magnitude and composition of particle fluxes.

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1. INTRODUCTION

Continental and island margins receive particulate matter derived from fluvial discharges (Syvitski and Morehead, 1999; Kineke et al., 2000), which stimulate primary production (González et al., 2010) that can result in higher sediment accumulation (Sanchez-Cabeza et al., 1999). Continental and insular margins are irrupted by submarine canyons (Harris and Whiteway, 2011) that act as conduits of sediments and organic matter from the continental shelf to deeper environments (Mullenbach et al., 2004; Lopez-Fernandez et al., 2013a). Particle fluxes within submarine canyons are characterized by strong seasonal fluctuations (e.g. Fabres et al., 2008) and can be orders of magnitude larger than those over the adjacent slopes (Martín et al., 2006; Zúñiga et al., 2009; Pasqual et al., 2013). The interaction of along slope currents and canyon topography may result in alterations of local currents that can promote cross shelf edge water exchange (Ahumada-Sempoal et al., 2015) leading to downwelling (Jordi et al., 2005) and upwelling processes within the canyon (Sobarzo and Djurfledt, 2004). Moreover, several hydrodynamic and atmospheric driven mechanisms can punctually increase particle fluxes in submarine canyons through resuspension and gravity-driven processes over short periods of time (Puig et al., 2014). Storms can cause sediment resuspension and gravity flows enhancing particle fluxes (Puig et al., 2004; Ross et al., 2009). Dense shelf water cascading can also cause dramatic increases in downward particle fluxes over few days span (Heussner et al., 2006) and can represent a major source of organic matter for deep environments (Tesi et al., 2010). Downward particle fluxes and the main mechanisms controlling them have been widely studied in submarine canyons associated to continental landmasses (e.g. Shepard et al., 1974; Xu et al., 2002; Khripounoff et al., 2003) and large islands with high mountain ranges (≥ 2000 m) and permanent rivers systems (e.g. Kineke et al., 2000; Liu et al., 2002). However, downward particle fluxes in canyons incising small island margins remain widely unknown.

In the Mediterranean, this situation is rather surprising due to the elevated number of islands found in this basin (~200), where many of them are incised by submarine canyons (e.g. Lo Iacono et al., 2011). Despite the relative abundance of Mediterranean insular canyons (Harris and Whiteway, 2011), most studies concerning particles have been conducted in less than 25 canyons located in the European margin. Moreover, many of them are located in river-dominated shelves (e.g. Bonnin et al., 2008) with high productivity relative to the Mediterranean average (Bosc et al., 2004). The Baleric Archipelago is one of the most oligotrophic environments in the Western Mediterranean Sea (Bosc et al., 2004). In this archipelago there is only one large canyon system, the Menorca Canyon, located in the southern slope of Menorca Island (Fig. 1). Benthic communities associated with the head of the submarine canyons and surrounding shelves are characterized by high biodiversity and biomass (De Leo et al., 2010; Fabri et al., 2014). Despite the high oligotrophy of the Balearic Sea, benthic communities in the proximities of the Menorca Canyon are highly diverse (Barberá et al., 2012), giving an interesting opportunity to assess the environmental characteristics, including the flux of organic matter, where these communities thrive.

In this sense the aims of this study are to characterize (1) the temporal variability of downward particle fluxes analysing its geochemical and (2) macroscopic composition and (3) identify the main processes that modulate particle fluxes in the Menorca Canyon, and (4) set the present results within previous research mainly developed in the Mediterranean.

1.2. Regional setting

The Menorca Canyon is located in the south-western slope of Menorca Island (Fig. 1) and is the largest submarine canyon system in the Balearic Archipelago (Acosta et al., 2002). The canyon's head is at approximately 80 m water depth and is less than 5 km off Menorca's coastline (Acosta et al., 2002). The axis of the canyon has a NNE-SSW orientation and reaches approximately 1000 m water depth (Acosta et al., 2002). The flanks are steep and characterized by vertical walls and escarpments up to 20 m height (Lo Iacono et al., 2014). The continental shelf surrounding the canyon is relatively narrow and it only extends few kilometres (3 to 6 km) (Alonso et al., 1988). Biogenic sands with > 65% of carbonate content constitute the sediments in the canyon and surrounding shelves (Alonso et al., 1988).

The Balearic archipelago separates the Balearic-subbasin in the north from the Algerian-subbasin in the south (Amores and Monserrat, 2014) in such a way that different hydrodynamic processes and water masses influence the northern and southern slopes of the archipelago (Balbín et al., 2014). The northern slope is influenced by the Balearic current (Balbín et al., 2012; Amores et al., 2014) and is mostly characterized by the presence of resident Atlantic water (AW) (Salinity >37.5 psu) (Balbín et al., 2014). Conversely, the southern slope is influenced by the sporadic arrivals of mesoscale structures detaching from the Algerian current and from the instability of the Almería-Oran front (Millot, 1987), which is characterized by the presence of recent AW (Salinity <37.5 psu) (Balbín et al., 2014). During spring and summer a density front that separates the resident AW from the recent AW develops south of the archipelago when western intermediate water (WIW) is present in the Ibiza and Mallorca channels (Balbín et al., 2014). Under these conditions anticyclonic gyres

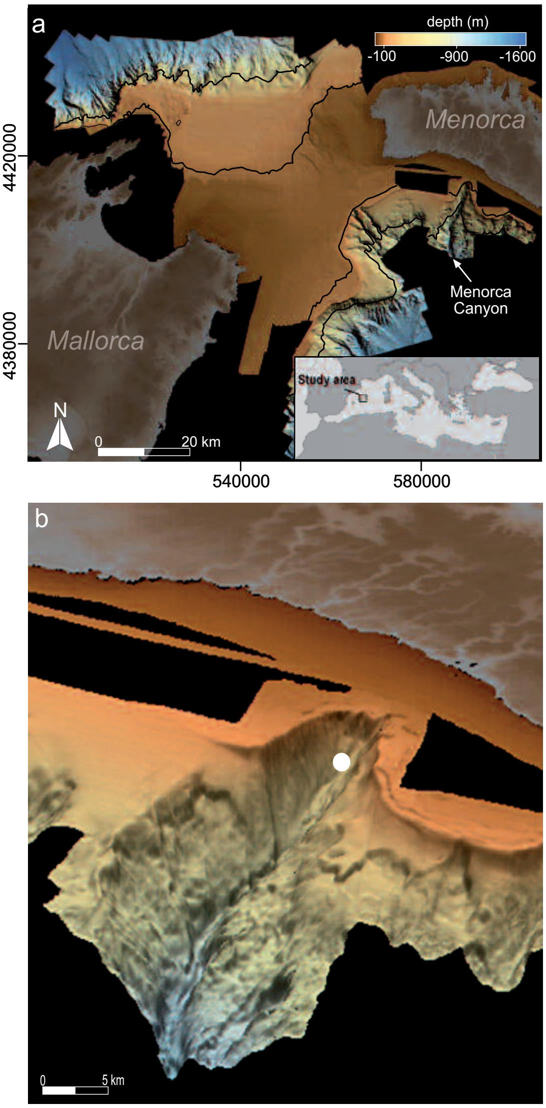

Fig. 1. (a) Three-dimensional bathymetry of the northern Balearic Archipelago and its position in the western Mediterranean Sea. (b) Detail of the Menorca canyon. The white dot indicates the position of the sediment trap.

have been detected in the southern slope of the Menorca Island (García et al., 2005; Balbín et al., 2014). Waters surrounding the archipelago are considered oligotrophic (Fernández de Puelles et al., 2007) as they receive little quantities of nutrients from land runoff due to low precipitation and the absence of rivers (Estrada et al., 1996). In some areas of the archipelago these conditions are enhanced by the intrusion of nutrient-poor recent AW including the southern slope of Menorca Island (García et al., 2005).

2. MATERIAL AND METHODS

2.1 Field work and instrumentation

One mooring array was maintained for two consecutive years in the Menorca Canyon (39º50.6601'N, 004º01.2600'E) on a site with approximately 430 m water depth. The mooring array was equipped with one cylindrical-sediment trap Technicap PPS3 and a Aanderaa current meter RCM9, tethered 30 m above the seabed and 25 m above the seabed, respectively. The sediment trap was set with 24 sequential collecting cups filled with a buffered 5% formaldehyde solution. The first study period (T1) continuously operated for 412 days (09/15/2010 – 10/11/2011). Sediment trap cups sampled during sequential intervals of 17-day except the last cup that sampled for 21 days. The second study period (T2) continuously operated for 365 days (11/03/2011 – 11/02/2012) and the sediment trap cups sampled for 15-day intervals except for the last five cups that sampled for 16-days intervals. The current meter was equipped with oxygen, turbidity, temperature and conductivity (salinity) sensors and it acquired measurements every 10 min.

2.2. Processing of sediment trap samples

Refrigerated (4 °C) sediment trap samples were processed in the laboratory according to the methodology described in Heussner et al. (1990) to produce aliquot sub-samples to carry out different analyses. Sub-samples were sieved with 0.4 µm-filtered seawater through a 1 mm nylon mesh. All "swimmers" (organisms that swam actively into the trap and died) were removed with forceps from the mesh. Sieved material was poured into a 2000-ml flask and filled up with 0.4 µm-filtered seawater. The flask was placed in a shaking table to generate homogenized aliquots separated with a robotized peristaltic pump. Aliquots were filtered onto pre-weighted 0.45 µm mesh nitro-cellulose white HAWP Millipore filters and onto pre-weighted Whatman GF/F filters both kinds, 47 mm diameter. Total mass was calculated as the dry mass weight of the filtered subsamples multiplied by the fraction of the aliquot. The total mass flux (TMF) expressed as, mg m^{-2} d^{-1}, was calculated from the total mass weight divided by the trap collecting area (0.5 m^2) and the sampling period in days.

2.3. Geochemical analyses

Total and organic carbon and total nitrogen were measured with a True Spec Carbon Nitrogen analyser LECO. Organic carbon (OC) was measured in samples pre-treated in a 1 M HCl vapour-bath for 24 h. Inorganic carbon was calculated as the difference between total and organic carbon. The inorganic carbon value was multiplied by 8.3331 to determine the calcium carbonate ($CaCO_3$) concentration. Carbon related analyses were performed with samples filtered onto Whatman GF/F filters. Biogenic opal content was obtained by alkaline extraction following the Mortlock and Froelich (1989) method modified by DeMaster (1981). Biogenic opal analyses were performed on samples filtered onto nitro-cellulose white

HAWP Millipore filters. Lithogenic content equals the difference between the total mass and the sum of the main biogenic components: biogenic opal, $CaCO_3$ and OM content (2x the OC content). Total concentrations of each geochemical component was calculated as the concentration of each subsamples multiplied by the fraction of the aliquot.

2.4. Macroscopic components

Fecal pellet, *Posidonia oceanica* detritus and foraminifera abundance were counted in aliquots using a Wild, Heerbrugg, (Switzerland) stereomicroscope (10x). Fecal pellets and *P. oceanica* detritus were measured with an eyepiece micrometer (± 10 µm). Fecal pellet volume was calculated with the formulas for cylindrical, ellipsoidal and spherical bodies, $V = \frac{4}{3}\pi r^3$, $V = \pi r^2 h$ and $V = \frac{4}{3}\pi r_a r_b r_c$ (where V is volume, r is radius and h is height, assuming that r_a and r_b had the same length in the case of ellipsoidal shapes), respectively. The volume of *P. oceanica* detritus was calculated with the formula of cylindrical bodies. Foraminifera tests were counted as single units regardless of their individual size. Pelagic and benthic species were differentiated based on the existing taxonomic works on Mediterranean foraminifera (Colom, 1974).

Additionally, images of selected samples were obtained with a Scanning Electron Microscope (SEM) HITACHI S-3500 N at 5.0 KV.

2.5 Wind and precipitation

The Spanish Meteorological Agency (AEMET) provided hourly data on precipitation, wind velocity and heading, obtained from the Maó Airport meteorological station (39°51’50.04” N, 004°13’26.04 E, 91 m above the sea level) located ~17 km from the Menorca canyon’s head. For each sampling cup interval we calculated: total precipitation, heading percentages (based on individual observations over the total) and maximum wind velocity values. Additionally, for each sampling interval, hourly wind velocity percentages were grouped from 0 to 60 km h^{-1} into 10 km h^{-1} intervals.

2.5 Wave height

Large wave height events (*Hs*) were obtained from an oceanographic buoy of the “Puertos del Estado” (Spanish Ports Authority) located at 50 km from the canyons head (39º39’00’ N, 003°29’2” E). Although there is another buoy closer to the canyon’s head (Maó), it was discarded due to multiple absences of registers during the winter and spring months.

2.7 Chlorophyll-a concentration and sea surface salinity:

Daily superficial chlorophyll a (Chl-a) concentration of waters surrounding Menorca Island and monthly average Chl-a concentration maps of the southern part of the Balearic subbasin and most of the Algerian subbasin were obtained from the satellite database of the Ocean Color Climate Change Initiative project (oceancolor.gsfc.nasa.gov/cms/). Daily satellite-derived Sea Surface Salinity (SSS) maps from the Marine Copernicus Environmental Monitoring Services (http://marine.copernicus.eu/web/69-interactive-catalogue.php) were revised in order to identify fronts or eddies in the study area.

2.8 Statistical analyses

For statistical comparisons two sampling intervals that lasted one-year approximately, and covered the same seasonal range were defined. The first interval, from hereby referred as 2011, ranged from 9/15/10 – 9/23/11. The second interval, from hereby referred as 2012, ranged from 9/24/11 – 9/12/12. Due to the mismatch between sampling cup intervals in T1 and T2 both sampling intervals do not comprise the same duration, being 2012 18 days shorter than 2011. Distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was used to test the null hypothesis of no significant differences between 2011 and 2012 fluxes.

Each term of the analysis was tested using 9.999 permutations. Previous to the analysis data were standardized respect to their mean absolute deviation ($MAD = \frac{1}{n} \sum_{j=1}^{n} [\ x_{ij} - \bar{x}_i\ ]$, where x_i is the value of the *i* variable observed in the *n* colonies) (García Pérez, 2005). The PERMANOVA was performed with the PaST software (Hammer et al., 2001). In order to explore the relationship among the TMF magnitude and the fluxes of its geochemical ($CaCO_3$, the lithogenic fraction, OC and biogenic opal) and macroscopic components (fecal pellet, *P. oceanica* detritus and foraminifera) simple linear regression analyses were performed using the lm function (Chambers, 1992) of the R software platform (R Development CoreTeam, 2011) for both 2011 and 2012 intervals separately.

3. RESULTS

3.1 Total mass flux and geochemical components

Forty-seven samples were obtained from mid-September 2010 (9/15/2010) to mid October 2012 (10/17/12). The 24th sampling cup of T1 (October 2011) was lost during the recovery process. During T1 three peaks in TMF were observed, a first one during mid autumn 2010, a second one during late spring and early summer 2011 and the third one during late summer 2011 (Fig. 2). The largest peak was the one registered during late spring and early summer 2011 (Fig. 2). During T2 four peaks in TMF were observed (Fig. 2). The first two peaks occurred during spring 2012, the third one during late summer 2012 and the fourth and largest during mid autumn 2012 (Fig. 2).

During both T1 and T2 $CaCO_3$ fluxes were the largest fraction contributing 44% to 70% of the TMF, and the lithogenic fraction was the second most abundant component of the TMF ranging between 22% to 45%. OC contributed between 2% and 3% of the TMF. Biogenic opal fluxes contributed between 0.7% and 4% to the TMF.

Fluxes of $CaCO_3$ and the lithogenic fraction were were significantly correlated with TMF ($p<0.01$) in both T1 and T2 (Fig. 3). OC fluxes were also significantly correlated with TMF. However, correlations were weaker during T1 ($R^2 = 0.678$) than during T2 ($R^2 = 0.922$) (Fig. 3).

During T1 biogenic opal fluxes presented two peaks, one in autumn and a second one in late spring that coincided with TMF peaks. During T2, this coincidence did not occur and biogenic opal fluxes progressively increased from early spring to early summer (Fig. 2). Biogenic opal fluxes were significantly correlated with TMF in both T1 and T2 ($p<0.01$ and $p<0.05$, respectively) but correlations were weaker than other geochemical components (Fig.3). However, during T1 and T2, the highest proportions of biogenic opal took place during the transition between autumn and winter (Fig. 2).

No significant differences were found, between the two annually defined periods of 2011 and 2012, when comparing the magnitude of the TMF and the fluxes of the geochemical components (Table 1).

3.2 Macroscopic characterization

During the T1 fecal pellets volumes (Fig. 4g) presented four peaks (Fig. 5). The first and second peaks occurred during mid autumn 2010 and late winter 2011, respectively (Fig. 5). The third and fourth peak occurred during late spring 2011 and mid summer 2011 and presented higher magnitudes than the earlier two (Fig. 5).

During T2 fecal pellet volumes were one order of magnitude larger than in T1. The highest fecal pellet volumes during T2 were registered during the autumn 2012 (Fig. 5).

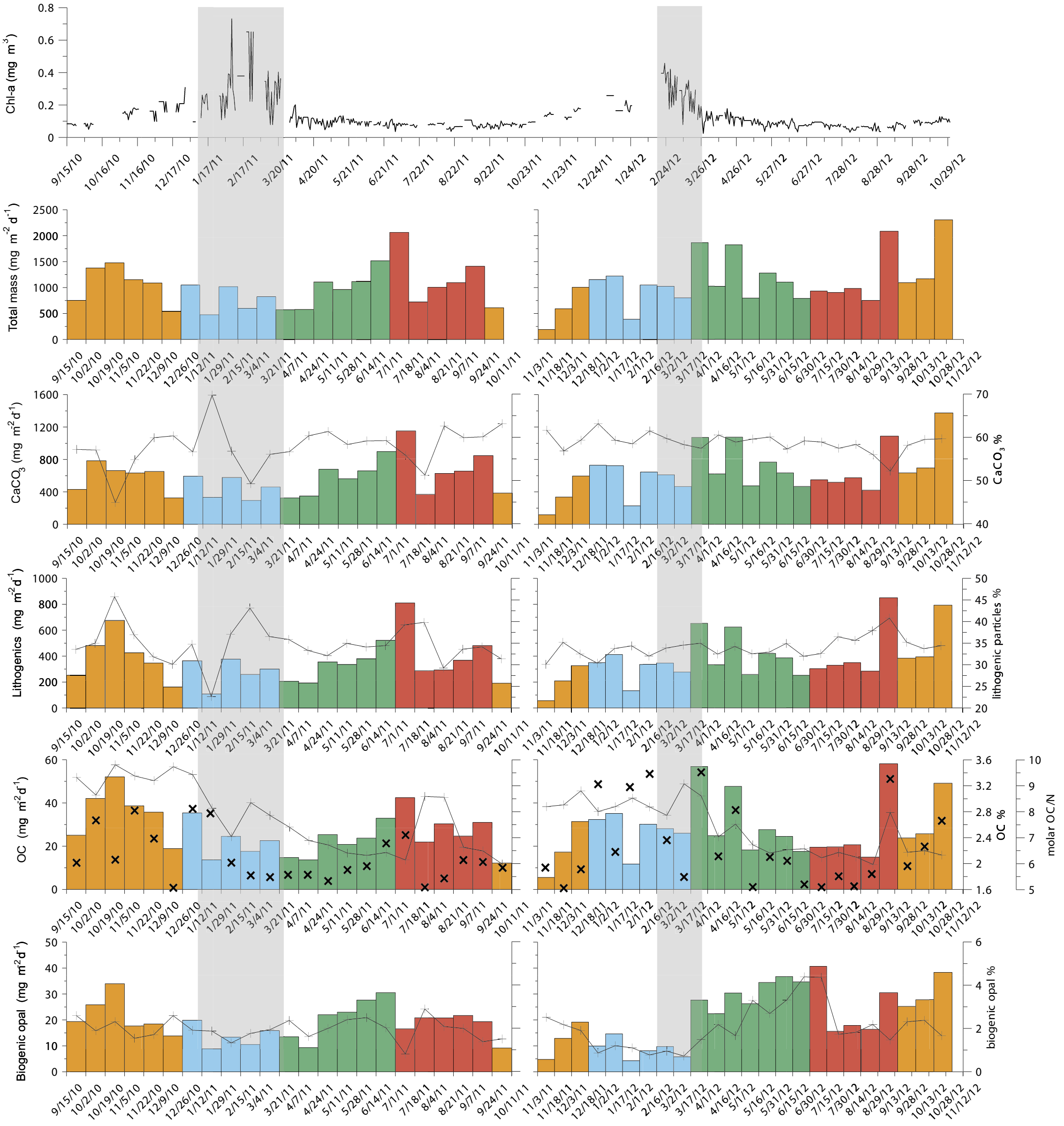

Fig. 2. Time series of surface Chl-a concentration, total mass, and the major constituent fluxes. Gray bands indicate the period with highest surface Chl-a concentration. Connected crosses represent each constituent percentage and the black crosses represent the OC/N (mol) values. The different bar colours indicate the sampling season (fall = orange, blue = winter, green = spring, summer = red).

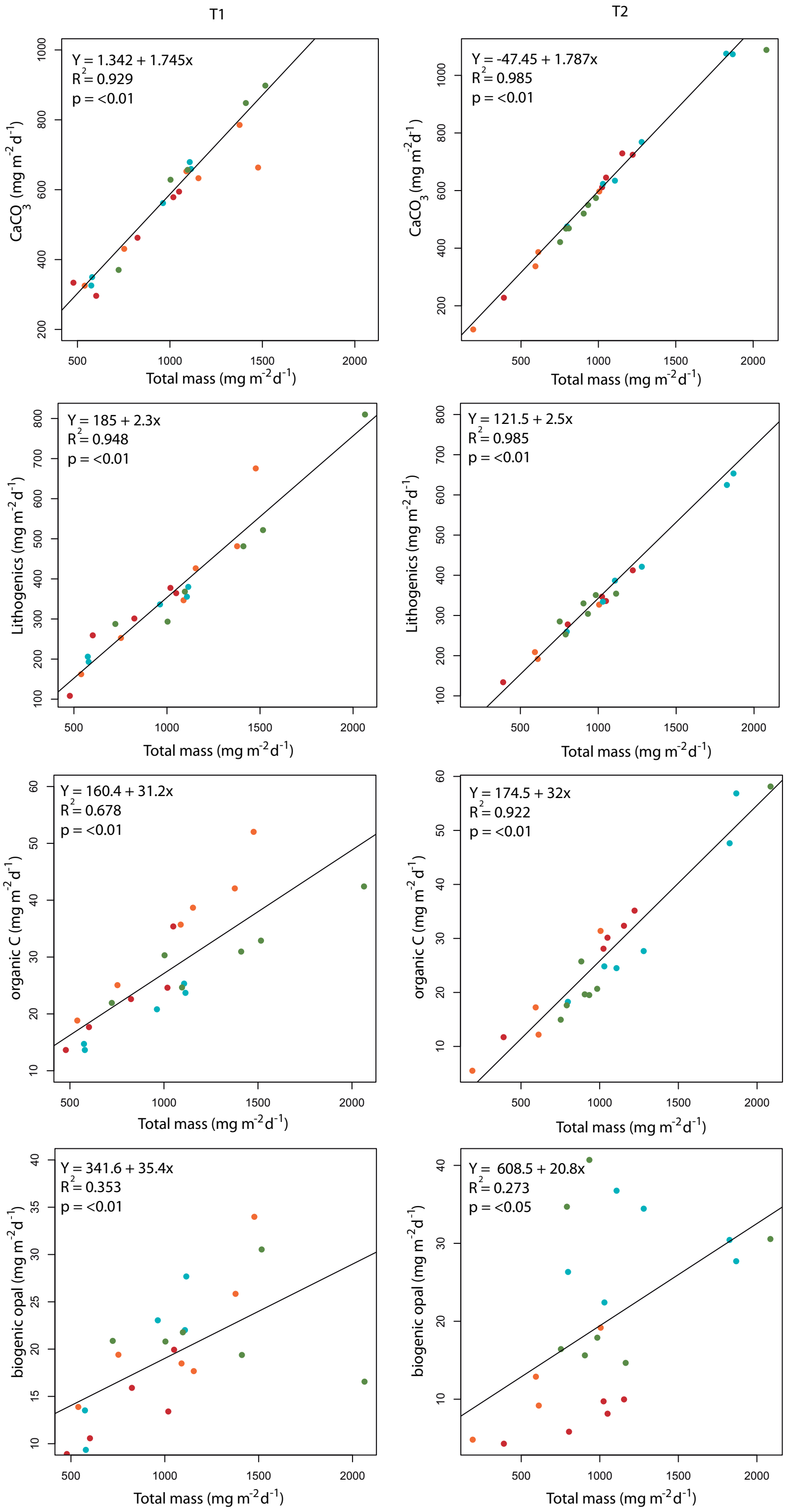

Fig. 3. Linear correlations between fluxes of $CaCO_3$, the lithogenic fraction (Litho), OC, biogenic opal and total mass flux.

Table 1 Total mass and geochemical component mean fluxes for the annually defined periods of 2011 and 2012 and PERMANOVA analysis. SD = Standard deviation, Litho. = lithogenic.

Fluxes	2011		2012		PERMANOVA	
	Mean	SD	Mean	SD	Pseudo-F	p value
Total Mass (mg m^{-2} d^{-1})	1023.3	388	1018.4	453	0.001	0.971
CaCO$_3$ (mg m^{-2} d^{-1})	585.6	216.6	596.2	251.6	0.022	0.833
Litho. (mg m^{-2} d^{-1})	362	166	349.8	174	0.069	0.792
OC (mg m^{-2} d^{-1})	579	225	665	316	0.17	0.687
Biogenic opal (mg m^{-2} d^{-1})	404	144	513	270	0.022	0.883

During T1 *P. oceanica* detritus (Figs. 4e and f) volumes were comparatively low with no remarkable peak. During T2 several peaks were observed during winter, one occurring in December 2011 and two occurring in February 2012 (Fig. 5).

During the T1 both pelagic (Figs. 4a and 4b) and benthic foraminifera (Figs. 4c and 4d) fluxes were low from early autumn until late winter (Fig. 5). In early spring their values increased and remained more or less stable until early autumn (Fig. 5). During T2 both pelagic and benthic foraminifera fluxes remained low except for two isolated peaks during winter (Fig. 5).

Fecal pellets and *P. oceanica* detritus volumes were significantly larger during the annual period of 2012 (Table 2). Conversely, the fluxes of pelagic foraminifera were significantly larger during the annual period of 2011 (Table 2). No significant differences were found among benthic foraminifera fluxes.

None of the macroscopic components fluxes were significantly correlated with TMF (Fig. 6). Microplastic fibres were found in all sampling cups for both T1 and T2 (Fig. 4h); however, due to their irregular shape and small dimensions they were not quantified.

3.3 Environmental factors

3.3.1 Water currents

During both sampling periods current mainly followed a north–south direction (Fig. 7). Average current velocity during T1 was 3.5 cm s^{-1} and 4.4 cm s^{-1} during T2, and current velocity rarely exceeded 20 cm s^{-1}. During T1, turbidity values were very low (<0.27 NTU); however, after mid spring the values substantially increased and remained above 0.28 NTU for most of the remaining sampling period. Simultaneously to this turbidity increment a substantial reduction in dissolved oxygen was observed. In T2 turbidity was comparatively very low for the whole sampling period with two peaks, one in mid–summer and a second one in early winter (Fig. 7).

3.3.2 Wind and precipitation

During T1 the highest precipitation rates were registered during mid-September 2010 to mid-November 2010 ranging from 818 ml/m^3 to

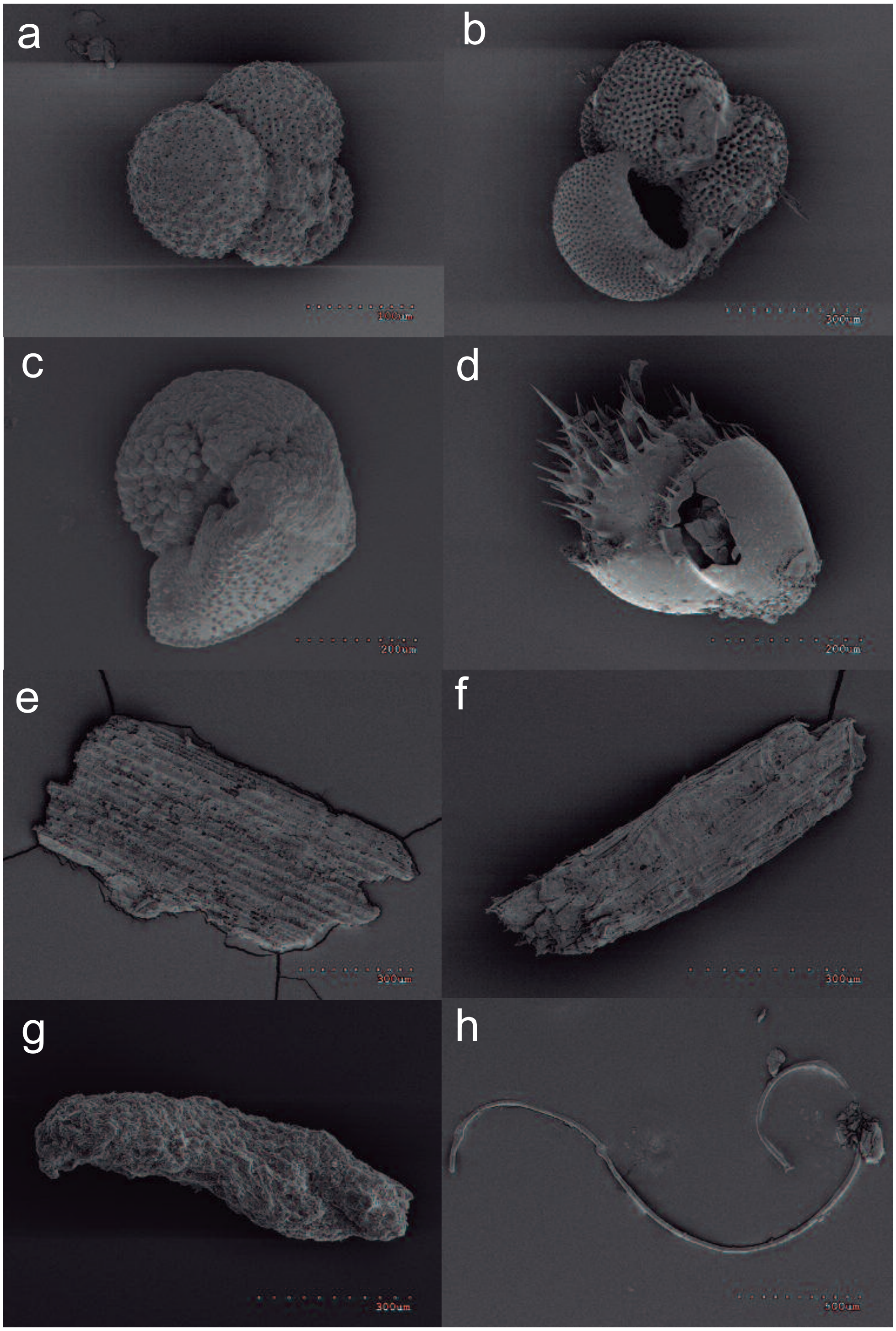

Fig. 4. Scanning electron microscope images of pelagic foraminifera of the genus a), b) *Globigerina*; benthic foraminifera of the genus c) *Cibicides*, and d) *Bulimina*, e), f) *P. oceanica* detritus, g) fecal pellet and h) microplastic fiber.

Table 2 Mean fluxes of the macroscopic components for the annually defined periods of 2011 and 2012 and PERMANOVA analysis. SD = Standard deviation, detr. = detritus.

Fluxes	2011		2012		PERMANOVA	
	Mean	SD	Mean	SD	Pseudo-F	p value
Fecal Pellets ($\mu m^3\ m^{-2}\ d^{-1}$)	10.1 10^5	486 10^6	57.4 10^6	8.4 10^6	6.85	0.002
P. oceanica detr. ($\mu m^3\ m^{-2}\ d^{-1}$)	10.7 10^5	6.5 10^5	27.4 10^5	27.7 10^5	7.54	0.002
Pel. Foram. (ind. $m^{-2}\ d^{-1}$)	1615	1620	697.7	148.3	5.95	0.016
Bent Foram. (ind. $m^{-2}\ d^{-1}$)	729	634	938	1169	0.543	0.524

2430 ml/m^3. During T2 the highest precipitation rates were registered during November 2011 and 2012 ranging between 912 ml/m^3 to 3112 ml/m^3 and in February 2012 reaching precipitation values of 1090 ml/m^3. Northerly winds were dominant during both sampling periods representing 47.9% and 45.9% of total hourly wind registers for T1 and T2, respectively. Windiest episodes were registered during January and February for both T1 and T2. During this period the percentage of the hourly winds exceeding 30 km/h ranged between 10% and 14% in T1 and between 7% and 46% in T2 and presented a northerly component.

3.3.3 Wave height

Wave measuring between 1.0 – 1.5 m were the most abundant size class and represented 36% of total wave registers for T1 and T2. Several wave height events ranging between 3.5 – 5 m were registered. Most events occurred during winter months and were associated to high wind speeds (> 40 km h^{-1}) (Table 3), few coincided with heavy precipitation (Table 3). Most registered waves presented a NNE orientation.

3.3.4 Chlorophyll-a concentration and sea surface salinity

Chl-a superficial concentration progressively increased from mid-October to early February when it reached its maximum values of ~0.7 mg m^{-3} and ~0.4 mg m^{-3} for T1 and T2, respectively (Figs. 2 and 4). For both T1 and T2, the lowest Chl-a concentrations ranging between ~0.2 mg m^{-3} to ~0.1 mg m^{-3}, were registered from early April to early October (Figs. 2, 5, 8 and 9).

Sea surface salinity maps revealed that from September 2010 until February 2011 resident AW occupied the southern slope of Balearic archipelago (Fig. 10). However, from March 2011 until August 2011 fresher recent AW progressively moved northward from the Algerian sub-basin and reached the southern slope of the archipelago (Fig. 10). Moreover, an eddy was present in the southern slope of Menorca from June to August 2011 remaining in this area for approximately 75 days (Fig. 10).

During the T2, sea surface salinity was comparatively stable and no major intrusions of fresher recent AW were observed in the southern slope of the archipelago (Fig. 11).

4. SC SS

4.1 Particle flux into the Mediterranean context

TMF in the Menorca canyon were smaller than those registered elsewhere in western Mediterranean submarine canyons (Table 4). The exceptions were the TMF registered in canyons and slopes in the Cretan margin (Table 4) and some open sea environments (Zúñiga et al., 2007; Khripounoff et al., 2012). The TMF range in the Menorca Canyon was comparable to those observed in the deep continental margin of the Alboran Sea (Sanchez-Vidal et al., 2005) and the open slope of the southern Adriatic Sea (Miserocchi et al., 1999). However, TMF in the Menorca canyon exceeded those collected in the southern and northern Balearic slope (Danovaro et al., 1999; Pasqual et al., 2015), which suggest that the canyon acts as a conduit of sediment from the continental shelf to deeper environments agreeing with the general

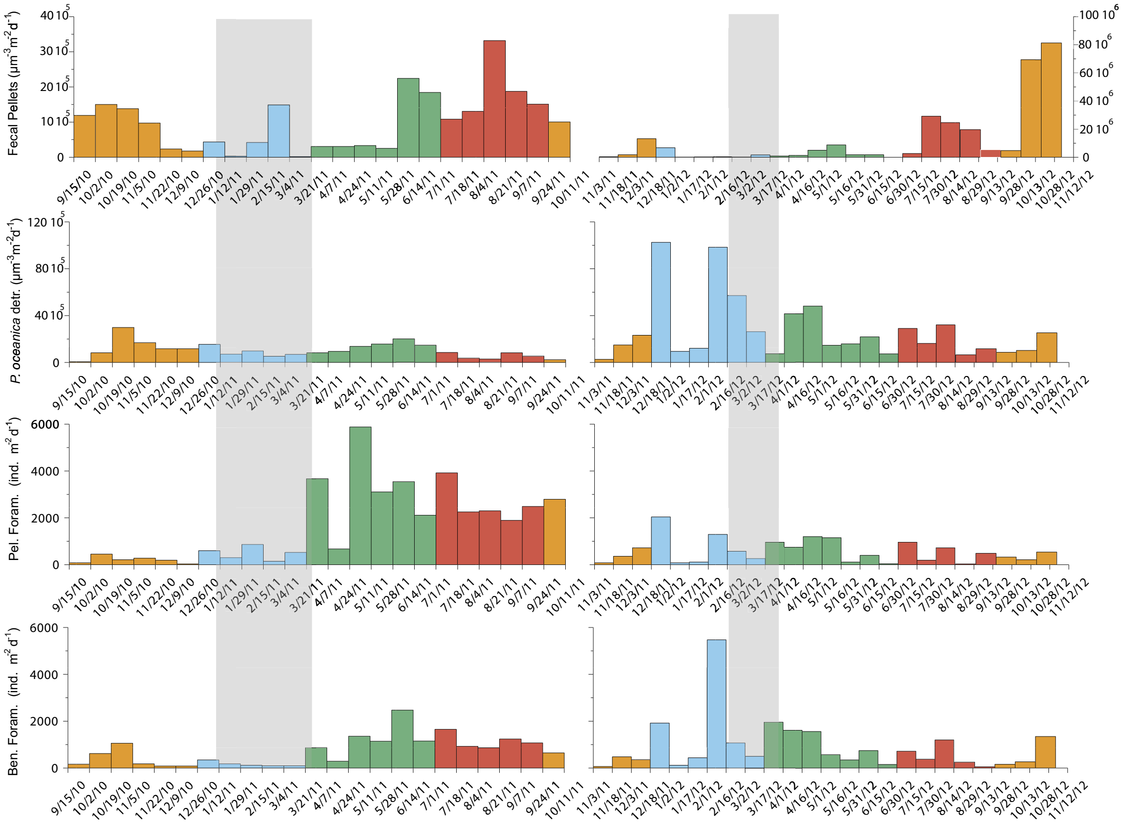

Fig. 5. Time series of fecal pellet, *P. oceanica* detritus and pelagic and benthic foraminifera fluxes. Gray bands indicate the period with highest surface Chl-a concentration. detr = detritus, Pel. Foram. = pelagic foraminifera, Ben. Foram. = benthic foraminifera. The different bar colours indicate the sampling season (fall = orange, blue = winter, green = spring, summer = red).

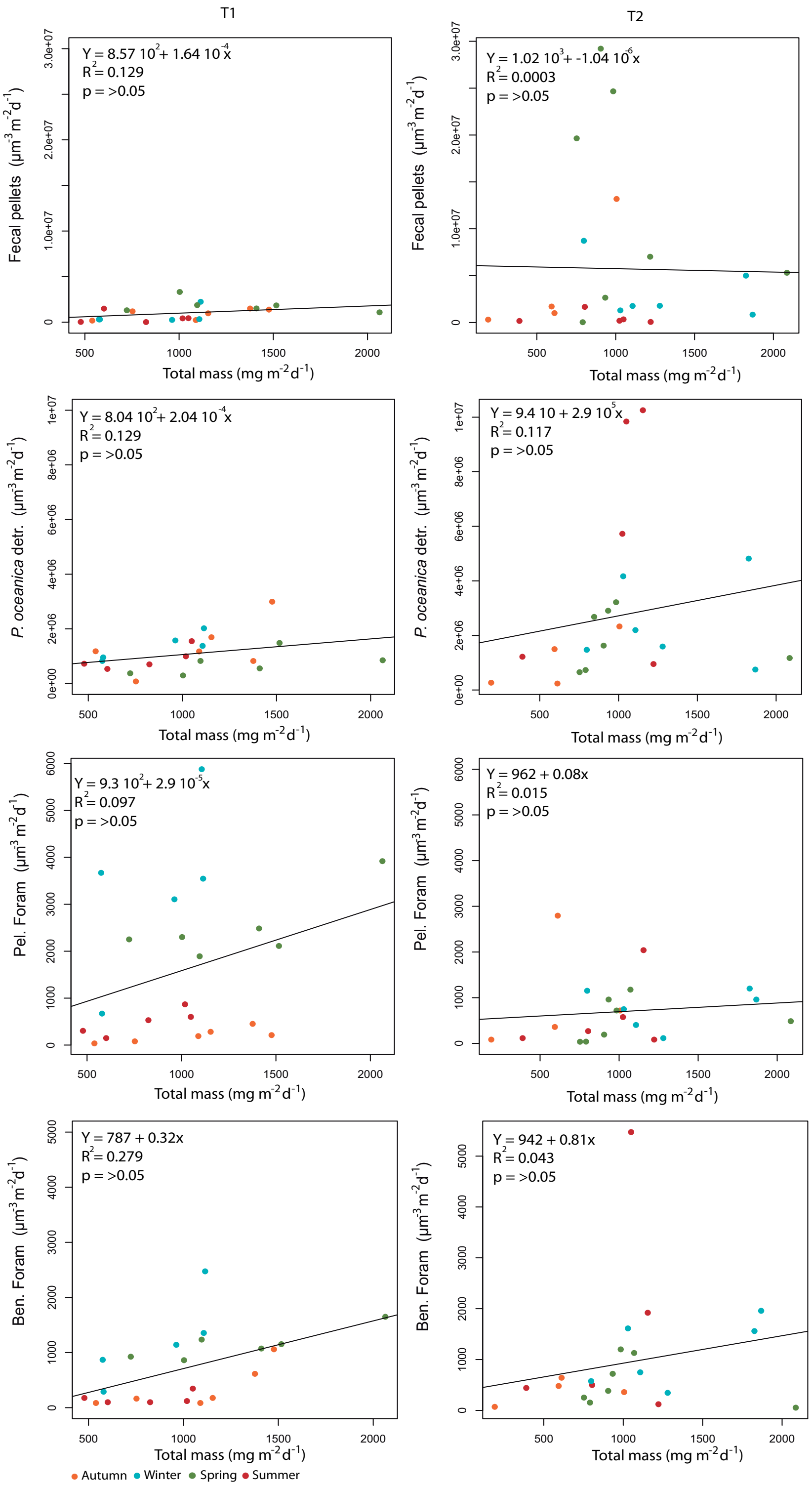

Fig. 6. Linear correlations between fluxes of fecal pellets, *P. oceanica* detritus, pelagic and benthic foraminifera and total mass flux. detr = detritus, Pel. Foram. = pelagic foraminifera, Ben. Foram. = benthic

Table 3 Large wave height events during both sampling intervals. Max WH = maximum wave height. Gray rows indicate those events that coincided with particle flux peaks. Max WS = maximum wind speed.

Date	Max WH (m)	Max WS (km/h)	Precipitation (ml/m^3)
10/18/10	4.07	44	0
10/25/10	4.25	50	41
11/02/10	4.08	46	46
12/15/10	5	53	0
12/26/10	3.8	53	18
01/21/11	3.48	36	0
02/02/11	3.78	51	0
03/01/11	4.02	47	194
08/03/11	3.98	36	0
08/10/11	4.57	57	0
12/19/11	4.13	28	0
12/24/11	4.17	51	0
12/30/11	4.23	41	0
01/06/12	4.69	60	0
02/03/12	4.88	58	62
02/07/12	4.86	49	0
03/05/12	3.57	61	2
04/16/12	4.31	40	314
09/14/12	3.74	55	0
10/27/10	4.70	59	611

pattern observed in Mediterranean canyons (e.g. Monaco et al., 1990; Martín et al., 2006).

The temporal evolution of the TMF within the Menorca Canyon showed a marked seasonal variability. During both sampling periods there were particle flux peaks during spring, summer and fall (Fig. 2) and their individual contribution to the TMF in T1 and T2 ranged between 4% and 6%. Winter fluxes were comparatively low (Fig. 2). The seasonal pattern and low contributions of the winter peaks contrast with the seasonal trends reported in submarine canyons related to fluvial systems and affected by intense across slope current events (Table 4). In these kind of canyons, enhanced particle fluxes mostly occur during fall and winter (e.g. Martín et al., 2006; Lopez-Fernandez et al., 2013b), can account for very large proportions (40% – 60%) of the TMF input (Durrieu de Madron et al., 1999; Turchetto et al., 2007) and mainly derive from brief and intense forcing mechanisms (e.g., river floods, cascading, storms) that trigger sediment resuspension and down canyon particle mobilization (Pasqual et al., 2010; Khripounoff et al., 2012). Overall, these differences suggest that the seasonal meteorological attributes of the Mediterranean basin (e.g., winter precipitation and storms) have little effect in the TMF collected at the southern slope of Menorca Island, accentuated by the absence of large fluvial systems.

4.2 Particle flux triggering factors

In the Menorca Canyon only few particle flux peaks coincided with atmospheric or hydrodynamic events that could act as forcing mechanisms to resuspend and trigger the transport particulate material. The peaks reported during mid autumn 2010 (October 2010) and mid spring 2012 (April 2012) (Fig. 2) coincided with large wave high events (*Hs* = > 4 m) (Table 3) that could potentially cause inner shelf bottom sediment resuspension (Guillén et al., 2006; Ulses et al., 2008). These peaks were charac-

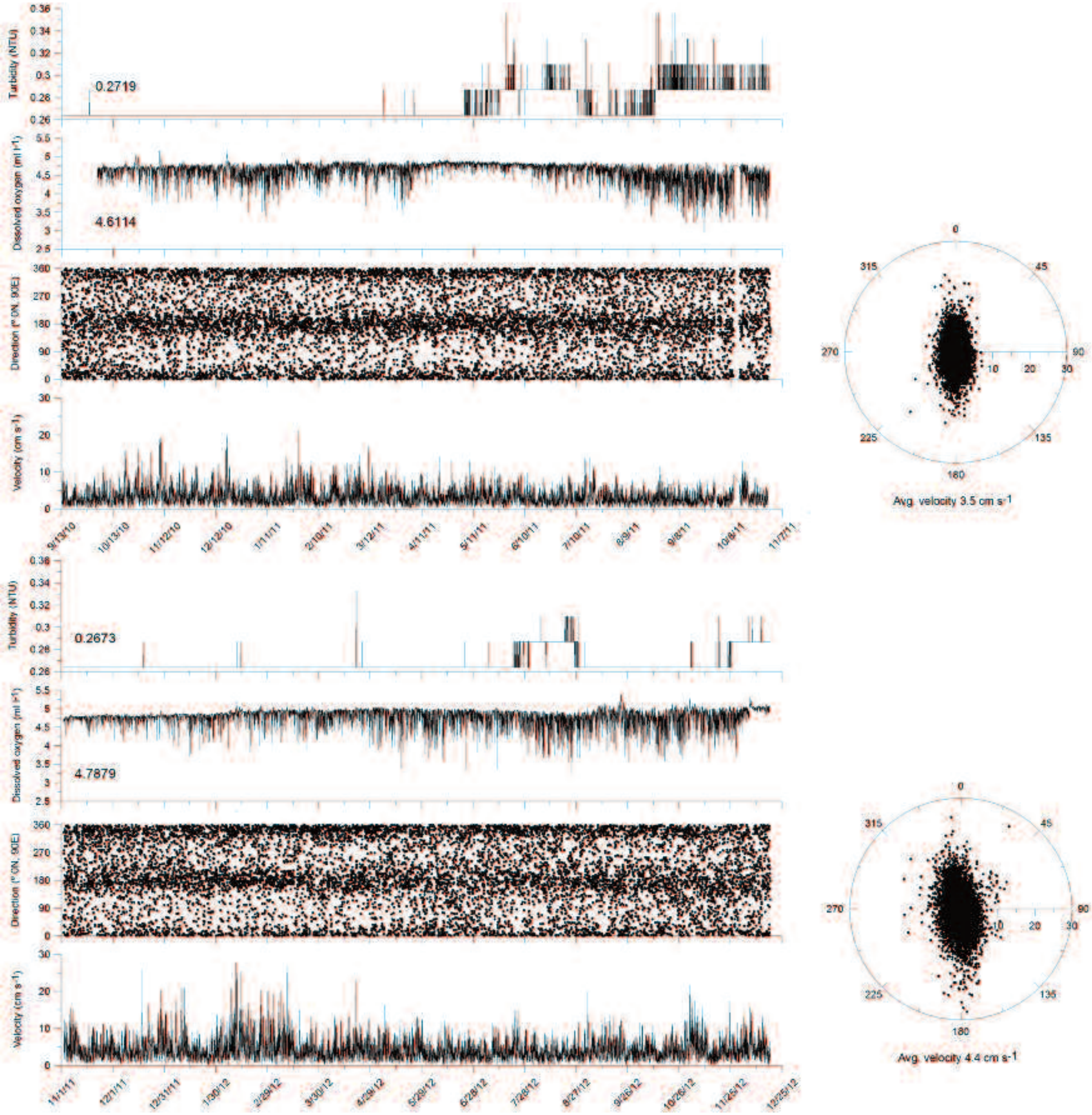

Fig. 7. Times series plots of turbidity, dissolved oxygen and current direction and velocity during T1 and T2 sampling intervals.

terized by a decrease in the $CaCO_3$ fraction and a slight increment in turbidity, in OC/N ratio (6.5 – 8) and the lithogenic fraction (Figs. 2 and 7), which provides further support to this hypothesis (Martín et al., 2006; Zúñiga et al., 2009).

From mid June to mid August 2011 an anticyclonic eddy was detected over the southern slope of Menorca (Fig. 9). Although it is unclear whether the effect of the eddy reached the mooring site (it was not clearly detected in the currentmeter), and during this period a peak in TMF was collected (Fig. 2). This peak coincided with a slight increment in turbidity (Fig. 7), the lithogenic fraction and a punctual decrease in the $CaCO_3$ (Fig. 2). During this peak OC/N ratio presented intermediate values (7.5). This association would agree with previous observations in the Balearic Archipelago, that reported similar

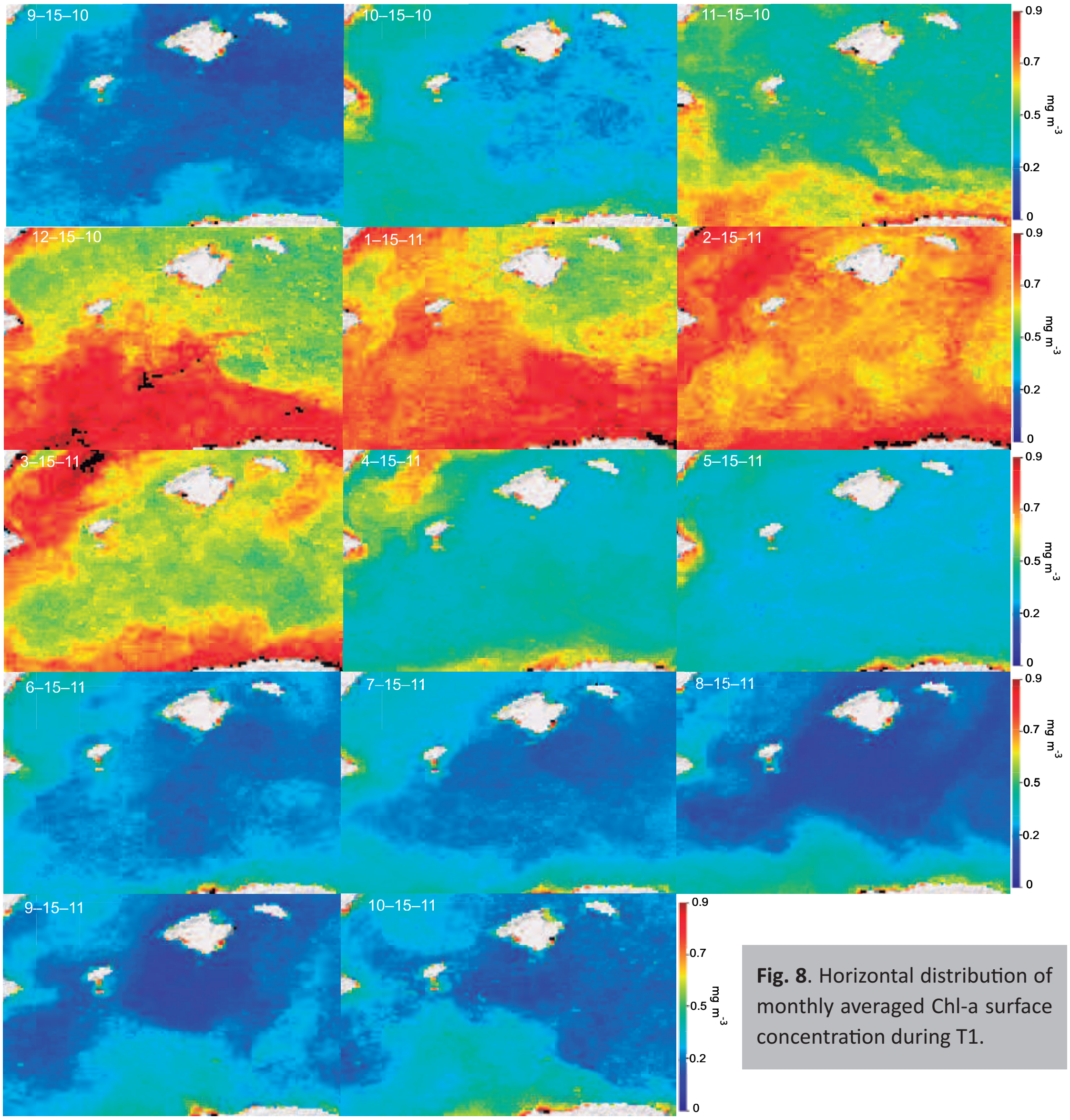

Fig. 8. Horizontal distribution of monthly averaged Chl-a surface concentration during T1.

TMF patterns in relation to anticyclonic eddies (Amores et al., 2013; Pasqual et al., 2014).

TMF peaks occurring during late summer 2011 (September 2011), and late summer and mid autumn 2012 (Fig. 2, August and October 2012) were associated with slight increments in the lithogenic fraction and turbidity, indicating a possible association with resuspension processes (Martín et al., 2006). However, the forcing mechanism that triggered these processes remains unclear given that no strong meteorological or hydrodynamic event was observed (Figs. 7, 10, 11 and Table 3).

In other areas of the Mediterranean, TMF peaks that did not present any evident relationship with natural forcing mechanisms were found to derive from trawling activities (Martín et al., 2006; Palanques et al., 2006). Indeed, trawling has been identified as a forcing mechanism that can cause abrupt increments in turbidity and particle fluxes (Martín et al., 2006; Palanques et al., 2006). However, it is unlikely that the observed particle flux peaks derive from this fishing activity given that in the study area, trawling is mostly conducted below 500 m (Moranta et al., 2014).

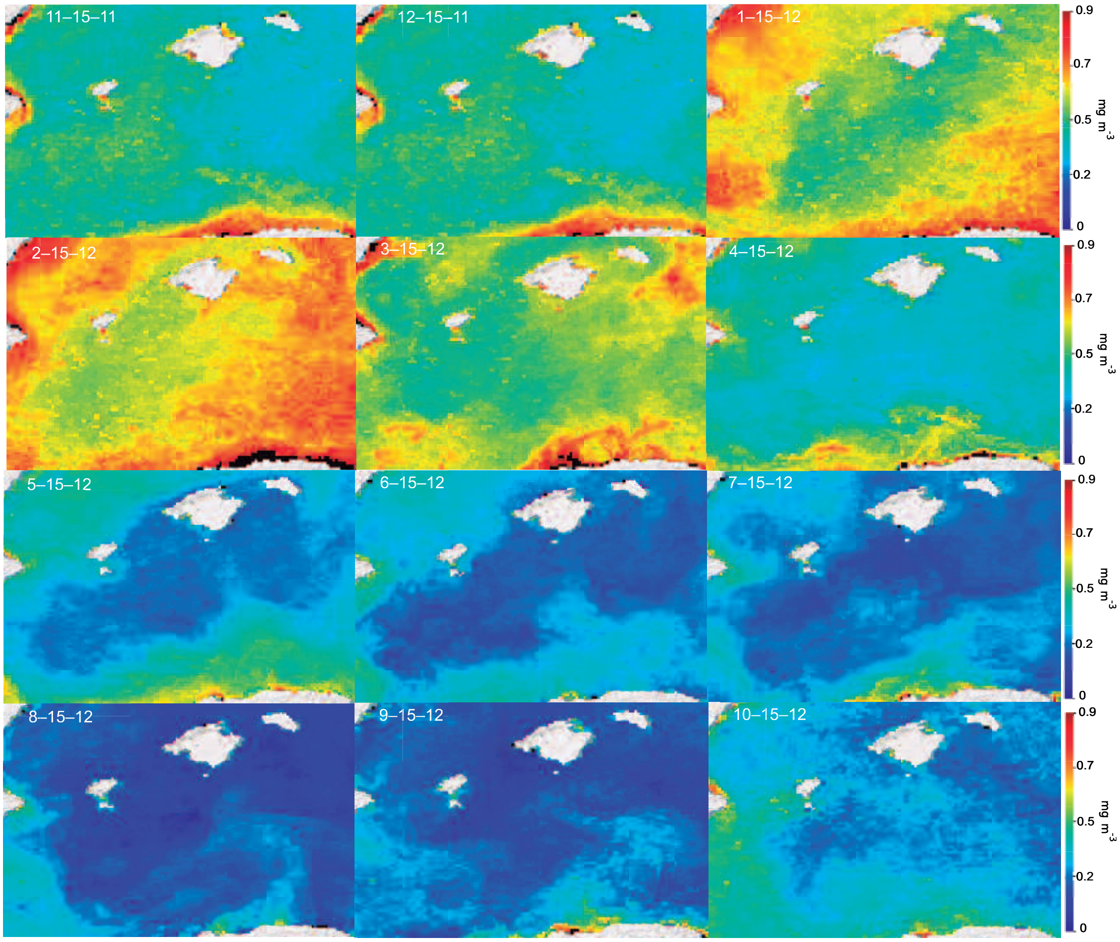

Fig. 9. Horizontal distribution of monthly averaged Chl-a surface concentration during T2.

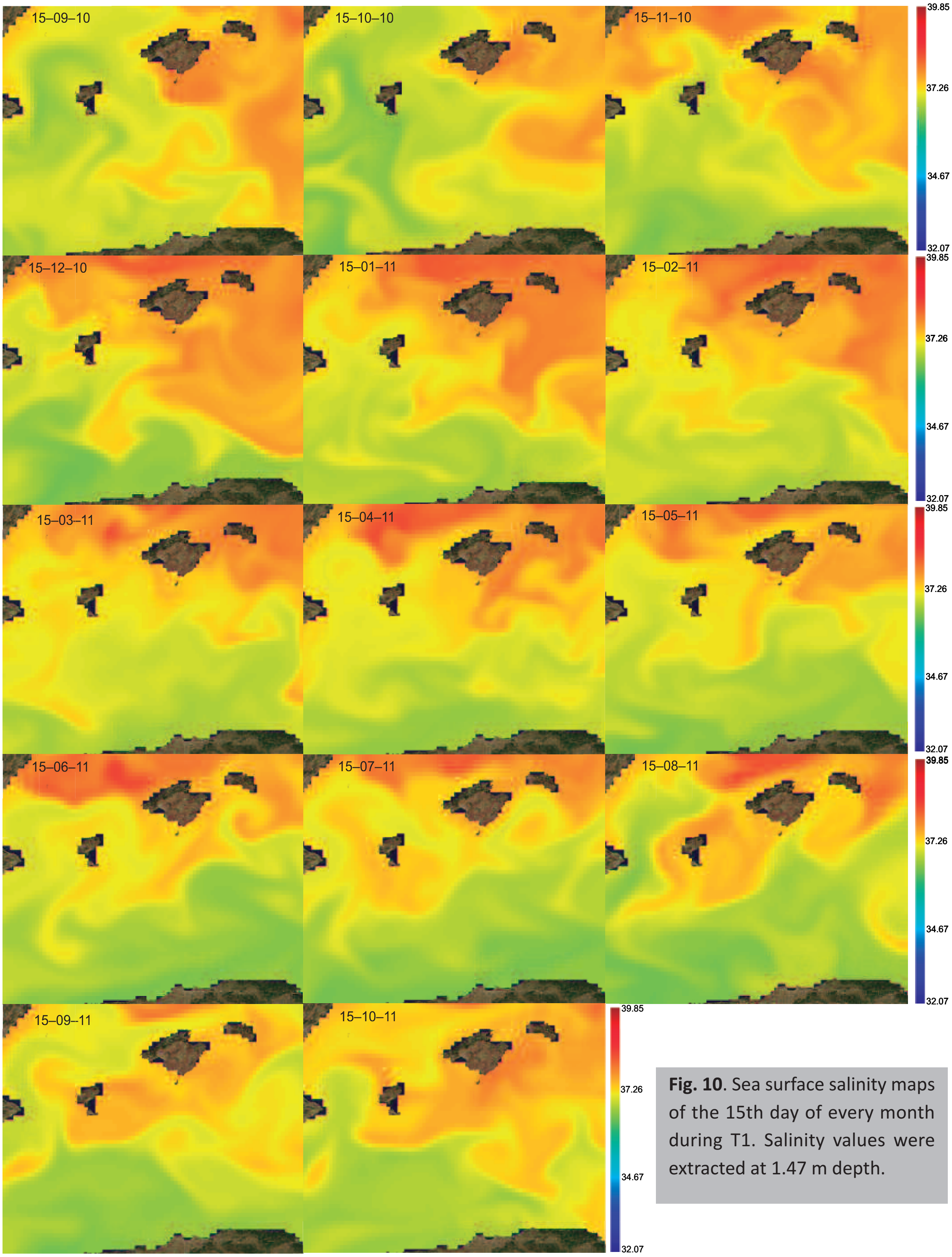

Fig. 10. Sea surface salinity maps of the 15th day of every month during T1. Salinity values were extracted at 1.47 m depth.

Spring and summer TMF peaks have been associated to primary production blooms (Sanchez-Vidal et al., 2005; Stavrakakis et al., 2013). These TMF peaks are characterized by material with relative high organic carbon and opal concentrations (Sanchez-Vidal et al., 2005) derived from exports from the euphotic zone (Rigual-Hernandez et al., 2013). In our study only the peak in March 2012 coincided with the winter-spring bloom and presented high organic carbon but low opal percentages (Fig. 2), suggesting that non-siliceous phytoplankton dominated

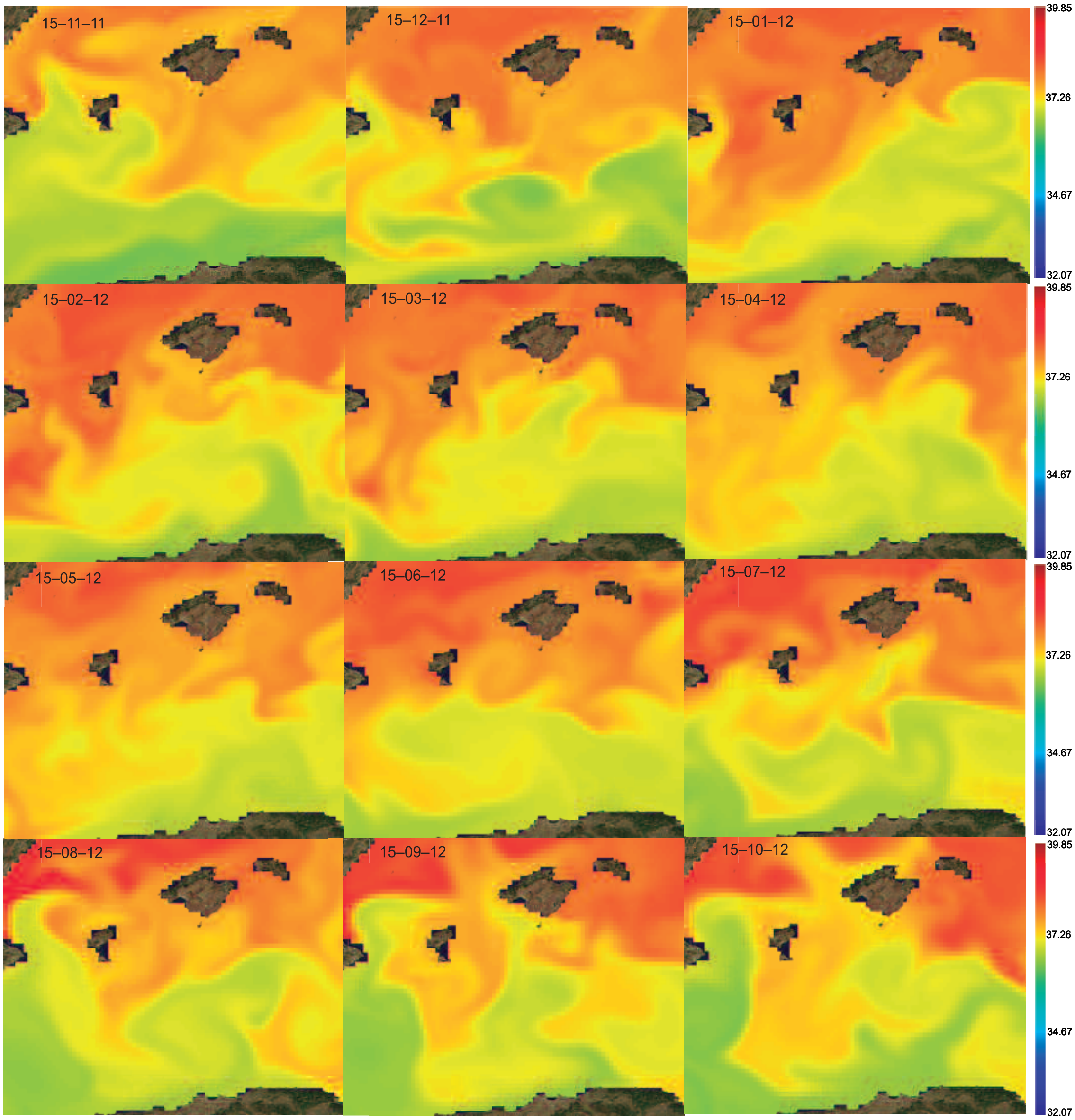

Fig. 11. Sea surface salinity maps of the 15th day of every month during T2. Salinity values were extracted at 1.47 m depth.

the bloom. The high OC/N ratio (9.5 molar OC/N) registered during this peak (Fig. 2) suggests that the presence of resuspended sediments, with remineralized organic matter diluted the bloom signal (Martín et al., 2011). This peak appears to be related to both, resuspension processes and primary production and suggests that the fraction of resuspended material in the TMF is larger than the input of fresh organic matter from the euphotic zone as probably happened in the earlier spring and summer (2011) samples.

4.3 Geochemical components

The high $CaCO_3$ fraction (58±4% and 59±2% for T1 and T2, respectively) observed in the Menorca canyon's TMF (Fig. 2) contrasts with most studies performed in Mediterranean canyons, slopes and open sea environments, where the lithogenic material was the highest fraction comprising more than 50% of the total (Table 4, Stavrakakis et al., 2013; Fabrés et al., 2002). Conversely, in the Menorca canyon the lithogenic material barely represents 35% of all collected material. These unusual patterns in the $CaCO_3$ and lithogenic fractions may derive from the synergy of low continental inputs (Estrada et al., 1996) and the high benthic carbonate productivity occurring in the adjacent continental shelf, derived from thriving communities of calcareous algae (Canals and Ballesteros, 1997). The relatively high $CaCO_3$ fraction values resembled the surface sediment composition of the adjacent slope (Alonso et al., 1988) suggesting that most of the $CaCO_3$ was resuspended and transported along the canyon, supporting previous hypothesis on particle transport in the area (Canals and Ballesteros, 1997). This observation implies that resupended material might be the most important source of lithogenic matter in this area, as expected for a setting without substantial river inputs.

Moreover, during mid January 2011 there was a sharp increment in the $CaCO_3$ concentration (70%) coinciding with the Chl-a concentration maximum (Fig. 2). It has been observed that coccolithophore blooms may trigger rapid increments in $CaCO_3$ and OC (Malinverno et al., 2009). Although coccolithophores have been identified as one of the most representative groups during early stages of the phytoplankton bloom in the Balearic Sea (Valencia-Vila et al., 2015), the observed $CaCO_3$ peak was not associated with an increment in the OC fraction and the OC/N ratio (8) (Fig. 2), and was rather high to be associated with a phytoplankton bloom (Middelburg and Nieuwenhuize, 1998). Thus, with the present information it cannot be distinguished the potential contribution of lithogenic $CaCO_3$ from that of phytoplanktonic origin.

The OC and opal were the two constituents with the highest seasonal fluctuation (Fig. 2). Both components presented similar percentages to those observed in other Mediterranean submarine canyons (Table 4; Bonnin et al., 2008; Pasqual et al., 2013) but were lower than values from oligotrophic continental slope environments where pelagic inputs are the main source of particulate matter (Pasqual et al., 2014). The Menorca canyon is also located in an oligotrophic environment. However, the pelagic inputs are probably diluted by continental shelf and slope inputs with lower OC and opal contents due to the funnel effect of the canyon's shape in concert with the general trend in Mediterranean canyons (Table 4).

Highest organic carbon percentages were registered from early autumn to early winter during T1 and from early autumn to early spring during T2. During these periods the $CaCO_3$ and lithogenic fractions remained constant except for an abrupt increment in the $CaCO_3$ fraction and a simultaneous decrease in the lithogenic fraction during January 2011 (Fig. 2). The OC/N ratio widely fluctuated, ranging from 5 to 9.5 (Fig. 2). The highest OC/N ratios (8 – 9.5) were registered in periods were large wave height (*Hs* = > 4 m) events associated to strong

Table 4. Register of TMF and geochemical constituents in Mediterranean submarine canyons and slopes. Med. = Mediterranean, S Adr. = South Adriatic, Lig. = Ligurian, GL = Gulf of Lions, Cat. Mar. = Catalan Margin, Bal. Arc. = Balearic Archipelago, Alb. = Alboran Sea, Forc. Mech. = forcing mechanism, am = annual mean, twf = time weighted flux, CCC= Cap de Creus canyon, LDC = Lacaze-Duthiers canyon, PP = primary production, R = resuspension, NADW = North v H tti ~ v

Med. Area	Sub-Area	Depth (m)			Duration (days)	TMF (mg m^{-2} $^{-1}$			x				c c	R f c
			C				x	x	L %	C CO$_3$%	OC%	O l%		
Crete	Samaria Canyon	1246	-	1231	350	0.332	~1.2	March	-	-	-	-	PP	Malinverno et al., 2009
Crete	Samaria Canyon	1316	-	1301	350	0.056	~0.1	September	-	-	-	-	PP	Malinverno et al., 2009
Crete	Samaria Canyon	1982	-	1967	350	0.077	0.225	July	-	-	-	-	PP	Malinverno et al., 2009
Crete	Samaria Canyon	3568	-	3553	350	0.060	0.11	March	-	-	-	-	PP	Malinverno et al., 2009
Crete	N Cretan Margin	1150	1135	1515	363	0.209	0.459	March	68.3	52.7	3.57	16.1	R / PP	Stavrakakis et al., 2000
S Adr.	Bari Slope	642	570	607	~365	1.7 am	6.8	March – April – May	-	-	~2	-	NADW	Turchetto et al., 2007
S Adr.	N Bari Canyon	600	570	565	~365	3.1	15.6	March – April – May	-	-	~2	-	NADW	Turchetto et al., 2007
S Adr.	S Bari Canyon	595	570	560	< 150	8.1	24.6	March – April – May	-	-	~1.4	-	NADW	Turchetto et al., 2007
Lig. Sea	Var Canyon	1280	1245	1235	121	-	120	December	-	-	2	-	RF	Khripounoff et al., 2012
Lig. Sea	Var Canyon	1575	1545	1555	121	-	218	February	-	-	1.5	-	RF	Khripounoff et al., 2012

Table 4 (cont.)

v H tti ~ v

						$^{-2}$ $^{-1}$			x				c c	R f c
			C				x	x	L %	C CO_3%	OC%	O l%		
GL	CCC	302	297	84	84	7.5	~30	Spring	64.8	29.4	1.8	2.2	S–DSWC	Bonnin et al., 2008
GL	LCD	309	304	140	140	11.7	~110	Autumn	66.1	31.0	1.9	3.2	S–DSWC	Bonnin et al., 2008
GL	Aude Canyon	309	304	84	84	7.8	~25	Autumn	65.3	31.4	1.3	1.3	S– DSWC	Bonnin et al., 2008
GL	Hérault Canyon	302	297	168	168	9.6	~75	Autumn	64.6	30.4	1.6	0.3	S– DSWC	Bonnin et al., 2008
GL	Petit Rhône Canyon	302	297	168	168	8.6	~30	Autumn	63.8	33.1	1.9	2.8	S– DSWC	Bonnin et al., 2008
GL	Planiere Canyon	307	302	84	84	3.1	3.1	Autumn	45.1	35.1	2.5	0.9	S– DSWC	Bonnin et al., 2008
GL	LDC	645	620	600	304	6.02	20	February	68	30	7	15	S	Courp and Monaco, 1990
GL	LDC	300	295	270	360	6.7	18.1	November	71.6	36.6	4.1	3.7	DSWC	Pasqual et al., 2010
GL	LDC	1000	995	970	300	6.7	11.2	January	68.6	42.9	5.5	5.2	DSWC	Pasqual et al., 2010
GL	LDC	1500	1495	1470	345	1.4	11.9	March	65.8	34.9	5.4	10.8	DSWC	Pasqual et al., 2010
GL	CCC	300	295	270	150	7.0	20	October	67.6	29	3.2	3.5	DSWC	Pasqual et al., 2010
GL	CCC	1000	995	970	360	11.7	90.1	January	73.1	29.9	3.4	9	DSWC	Pasqual et al., 2010
GL	CCC	1500	1495	1470	345	1.3	6.3	January	68.6	36.3	9.5	13.1	DSWC	Pasqual et al., 2010

Table 4 (cont.)

v H tti ~ v

						$^{-2}$ $^{-1}$			x				c c	R f c
			C				x	x	L %	C CO_3%	OC%	O l%		
Cat. Mar.	Blanes Canyon	300	275	275	181	12.06	82.67	December	78.27	43	7.68	4.19	S–RF	Lopez-Fernandez et al., 2013b
Cat. Mar.	Blanes Canyon	900	875	875	359	14.75	35.29	December	75.63	30.1	4.44	2.17	S–RF	Lopez-Fernandez et al., 2013b
Cat. Mar.	Slope	900	875	875	359	3.43	7.81	April	74.01	26.2	10.3	3.39	SD	Lopez-Fernandez et al., 2013b
Cat. Mar.	Blanes Canyon	600	568	570	319	14 twf	40.78	December	-	24	5.5	-	RF	Zúñiga et al., 2009
Cat. Mar.	Palamós Canyon	470	~446	448	~274	28.75	94	November	77	33	3.5	12.5	S	Martín et al., 2006
Cat. Mar.	Foix Canyon	600	-	570	~91	3.8 am	19	July	-	27.5	2	4	S–RF	Puig and Palanques, 1998
Cat. Mar.	Foix Canyon	1180	-	1150	~516	12 am	7.5	October	-	28	~2.5	5	S–RF	Puig and Palanques, 1998
Cat. Mar.	Slope	980	-	950	~516	0.8 am	1.8	April	-	30	5.9	8.75	S–RF	Puig and Palanques, 1998
Bal. Arc.	Menorca Canyon T1	430	405	400	412	1	2.06	July	45.7	70	3.5	3	R–HS	Present Study
Bal. Arc.	Menorca Canyon T2	430	405	400	365	1.09	2.30	October	40.7	63	3.2	4.3	R–HS	Present Study
Bal. Arc.	N slope Mallorca	900	870	870	344	0.31	0.61	March	65.2	43	5.8	17.1	S –RF	Pasqual et al., 2015
Bal. Arc.	S slope Mallorca	900	870	870	225	0.16	0.337	February	66.4	47.3	7.1	9.1	PP	Pasqual et al., 2015
Alb.	Guadiaro Canyon	592	567	567	155	6.1	27.4	May	~80	~14	~2.3	~8	RF	Palanques et al., 2005
Alb.	Channel	717	692	692	346	1.87	5.7	December - January	~82	~15	>5	~12	RF	Palanques et al., 2005
Alb.	Oppen Slope	720	695	695	191	3.03	6	December	82	~15	3	~8	RF	Palanques et al., 2005

winds (> 40 km h^{-1}) occurred, suggesting a resuspended (remineralized) origin of the collected OC (Fabres et al., 2008) (Fig. 2 and Table 3). Conversely low OC/N ratios (5 – 7) may have resulted from biogenic exports from the photic zone (Middelburg and Nieuwenhuize, 1998). In this regard, strong winds and large wave, registered during these periods, can induce turbulent mixing, increasing nutrient supply in shallow environments that can result in a posterior increment of phytoplankton production and biogenic exports (Marty et al., 2008; Isla et al., 2009). Thus we suggest that the wide fluctuation of the OC/N ratio, registered during these periods, may have resulted from the alternated supply of resuspended and pelagic OC.

During spring and summer of 2011 and 2012, we observed a decline in OC fraction (Fig. 2) that could be related to water stratification. Under these conditions nutrients undergo a rapid depletion in superficial layers limiting primary production (Marty et al., 2002) and reducing the pelagic particulate matter supply (Coma et al., 2000).

However, most spring and summer samples presented low OC/N ratios (5 – 6.5) (Fig. 2) indicating pelagic origin of OC and a low contribution of resuspended matter. This would agree with wind (14.6 ± 9.2 SD km h^{-1} and 14.5 ± 9.2 SD km h^{-1} T1 and T2 respectively) and wave height (Table 3) that were relatively low during these periods.

Highest opal percentages did not coincide with surface Chl-a maximum and were detected during spring and early summer (Fig. 2). This temporal pattern contrasts with previous studies in Mediterranean submarine canyons (Martín et al., 2006; Pasqual et al., 2010) and slopes (Sanchez-Vidal et al., 2005; Stavrakakais et al., 2013) that detected a simultaneous increment in OC and opal associated with the winter–spring phytoplankton bloom. In this sense, the low opal concentrations registered in the present study during the winter surface Chl-a maximum (Fig. 2) may imply that siliceous phytoplankton was overshadowed by other groups. This would agree with Estrada et al. (1999) who observed that during the winter spring bloom phytoplankton biomass was not associated with diatom dominance in the Balearic Sea. The weak relationship between surface Chl-a concentrations and spring and early summer opal maximums may be explained by the development of a deep chlorophyll maximum (~60 m) closely related with the nutricline (Crombet et al., 2011). In other oligotrophic areas of the Mediterranean Sea it has been reported that under high stratified conditions, diatoms can dominate the deep chlorophyll maximum (Crombet et al., 2011). Nonetheless, the low values of OC during opal maximums (Fig. 2) could indicate that opal came from resuspended biological remains as it has been suggested in other areas of the Mediterranean (Lopez-Fernandez et al., 2013b). However, the low OC/N ratio (5 – 6.5), the absence of other sediment resuspension indicators (enhanced current velocity, enhanced turbidity, and increments in the lithogenic fraction), (Figs. 2 and 7) and the poor correlation of opal with TMF (Fig. 3) do not support this environmental approach. Consequently, the mechanisms involved in both opal maximums remain unclear.

Moreover, T1 maximum opal percentages, registered during early May to late June 2011, were 47% lower than T2 maximum opal percentages, registered during early May to late June 2012 (Fig. 2). This could be related with the characteristics of the water masses influencing the study area during both sampling periods. During spring and early summer 2011 the study area was influenced by recent AW (Fig. 10) considered highly oligotrophic (García et al., 2005). Conversely during 2012 spring and summer the study area was influenced by resident AW (Fig. 11). Resident AW has higher H_4SiO than recent AW (Crombet et al., 2011). In this regard, we hypothesized that higher opal fluxes observed during T2 could result from higher H_4SiO_4 availability, which could trigger the deep Chl-a peaks and sustain larger siliceous phytoplankton populations.

4.4 Macroscopic components

Throughout both sampling intervals fecal pellet fluxes (Fig. 4g) presented several peaks with only one peak coinciding with the 2011 winter spring bloom (Fig. 5). This temporal pattern contrasts with zooplankton dynamics in the Balearic archipelago (Cartes et al., 2008) and long–term fecal pellet monitoring in other areas of the western Mediterranean that showed a clear relationship between the winter–spring phytoplankton bloom and the fecal pellet maximums (Fowler et al., 1991). During T2, fecal pellet fluxes were one order of magnitude larger than during T1. This is surprising as surface chlorophyll satellite observations revealed that during T2 winter-spring bloom was less intense and shorter than the one observed during T1 (Figs. 8 and 9). However, it has been observed that zooplankton abundances may experience a wide variability under different hydrographic settings (Guerrero et al., 2016). In the Balearic Archipelago higher zooplankton abundances have been observed under the prevalence of resident AW (Fernández de Puelles et al., 2007). This would agree with our results as during 2012 resident AW strongly influenced the southern slope of the archipelago (Fig. 11). Moreover, fecal pellets may induce rapid vertical transports enhancing biogenic components fluxes (Monaco et al., 1990; Stone and Steinberg, 2016). In the Menorca canyon fecal pellet fluxes were not significantly correlated with $CaCO_3$ ($p=0.51$, $p=0.34$ during 2011 and 2012 respectively), OC ($p = 0.32$, $p = 0.34$ during 2011 and 2012 respectively) and opal fluxes ($p = 0.06$, $p = 0.07$ during 2011 and 2012, respectively) suggesting a low contribution to biogenic fluxes. This could also reflect the strong diluting influence of resuspended material into the sediment trap.

The fluxes of *P. oceanica* fragments (Figs. 4e and 4f) presented high variability throughout both sampling periods and were poorly correlated with TMF (Figs. 5 and 6). During the T1 fluxes of *P. oceanica* detritus remained quite stable and could not be related to any environmental pattern. Conversely, during the T2, fluxes were more variable and there seemed to be a relationship between relatively high *P. oceanica* detritus fluxes and large wave high events (Fig. 5 and Table 3). Indeed, shelf resuspension processes have been identified, as a major source of macrophyt and sea grass detritus to deeper environments (Britton-Simmons et al., 2012). In North Pacific canyons macrophyt and seagrass detritus have shown to be a major carbon source, accounting for 20–83% of the particulate organic carbon (Harrold et al., 1998). *P. oceanica* detritus does not seem to widely contribute to the bulk OC flux as both variables were not significantly correlated ($p = 0.33$ and $p = 0.35$ during 2011 and 2012 respectively). Despite this lack of correlation, *P. oceanica* detritus cannot be ruled out as major carbon sink in deep Mediterranean environments due to its refractory nature (Danovaro et al., 1994) and high carbon contents (Romero et al., 1992). In this regard, peaks in *P. oceanica* detritus registered during mid December 2011 and early February 2012 coincided with high OC/N ratio (9 – 9.5) (Figs 5 and 2). However, it should be mentioned that very small, reworked fragments represented the *P. oceanica* detritus fraction and this material should behave as a particle lighter than $CaCO_3$ or lithogenic sediment of the same size. That implies a more complicated transport mechanisms related to the coincidence of *P. oceanica* fragments availability at the beach and shallow coastal areas and resuspension and downslope transport mechanisms. With the available information, explaining the presence of this fraction represents the strongest challenge in this study and it cannot be clarified.

Benthic and pelagic foraminifera presented similar trends during both sampling periods, with poor correlation with the TMF pattern (Figs. 5 and 6). From early spring to late summer 2011 there was a substantial increment in the fluxes of both foraminifera groups (including resuspended dead pelagic foraminifera). Coinciding with this increment, we observed a turbidity enhancement, suggesting that these peaks in foraminifera fluxes derive from resuspension processes (Figs. 5 and 7). Moreover, in the water column pelagic foraminifera reached its maximum concentrations during winter months (Pujol and Grazzini, 1995), which points to resuspension as the main responsible process to explain the spring and summer 2011 pelagic foraminifera peaks (Fig. 5). During the T2 foraminifera fluxes presented two peaks during mid December 2011 and early February 2012 coinciding with *P. oceanica* detritus fluxes. Pelagic foraminifera may produce high densities during winter months (Pujol and Grazzini, 1995) and probably the signal of these developments was recorded in the trap collection. Coinciding with both peaks several large wave height events (Table 3) were registered, which may indicate that the observed peaks during the second sampling periods could also derive from resuspension events, especially that of benthic foraminifera. Nonetheless these peaks were not associated with increments in turbidity nor in the lithogenic fraction (Figs. 2 and 7). Moreover, benthic ($p = 0.11$ and $p = 0.17$ during 2011 and 2012, respectively) and pelagic ($p = 0.17$ and $p = 0.07$ during 2011 and 2012, respectively) foraminifera fluxes were poorly correlated with $CaCO_3$ suggesting that the contribution of foraminifera to the $CaCO_3$ is diluted by other $CaCO_3$ sources. During both sampling periods microplastic fibres (Fig. 4h) were found in all samples. Future research should quantify the amount of carbon that these artificial polymers introduce into the carbon flux and the possible bias to the budget of naturally produced OC carbon.

4.5 TMF: general approach

The southern area of the Balearic archipelago has been described as a sediment-starved margin where no major sources of continental sediments are present (Lo Iacono et al., 2014). Consequently, lithogenic fluxes within the insular system of the Menorca canyon were rather poor within the Mediterranean context and were far exceeded by fluxes registered in canyons and slopes that receive sediment inputs from fluvial systems and where intense across slope current occur (Table 4). To our knowledge, the registered fluxes were the lowest ever registered in a western Mediterranean canyon head. These observations suggest that Mediterranean submarine canyons related to insular environments, without important fluvial networks, receive particle fluxes similar to those collected in deep-sea environments (Sanchez-Vidal et al., 2005). No clear relationship was found between environmental parameters and TMF temporal variation. Unlike other studies in the Mediterranean, where the relation between hydrodynamic, meteorological and biological processes and TMF peaks was more evident, we advocate that in the present study, the low magnitude of the TMF difficults the identification of a main mechanism that explained its magnitude and temporal variation (Table 4). The fraction represented by biogenic material was smaller than the resuspended, degraded material. Nonetheless, our results suggest that physical mechanisms, (e.g., resuspension) were more important than biological processes in the control of the temporal variation and chemical composition of the TMF. TMF were poorly related with phytoplankton blooms in contrast to the patterns observed in other Mediterranean oligotrophic environments with similar surface Chl-a concentrations (Malinverno et al., 2009). Although TMF and geochemical components varied between both sampling periods (Fig. 2), the absence of significant differences between both periods (Table 1) indicates that overall, the magnitude and composition of the TMF showed little interannual variation between 2011 and 2012 indicating that overall sediment sources remain constant throughout the year. Except for benthic foraminifera, macroscopic components significantly differed between 2011 and 2012 (Table 2) suggesting that the fluxes of the macroscopic particles may develop according to a different combination of sources and physical-biological mechanisms than the TMF and its main components.

The amount of OC collected in the Menorca Canyon (~3%) is similar to that of areas with higher TMF that receive continental inputs such us the Gulf of Lyons or the Catalan Margin (Table 4). This suggests that the dilution effect of the lithogenic fraction, in the Menorca Canyon, is less intense than in areas adjacent to continental landmasses. Submarine canyons have been identified as macrobenthic community hotspots of diversity and biomass (e.g. Fabri et al., 2014; De Leo et al., 2010). However, high sedimentation rates may generate instability (erosion, smothering) restricting the development of macrobenthic communities to areas with low sedimentation rates (Sousa, 2001). Thus, the synergy of the magnitude of the OC fluxes and low TMF measured in the Menorca Canyon presumably are sufficient to maintain the high biodiversity of the benthic communities in the Menorca Canyon and surrounding areas (Barberá et al., 2012, Grinyó et al., 2016). The results of the present work stimulate further research on the interesting relationship between benthos and particle fluxes in oligotrophic environments.

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Conclusions

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● Chapter 1

Six different assemblages of macrobenthic species were identified in the Menorca Channel, mainly segregated by substrate and depth. Hard substrates hosted coral gardens and sponge grounds. Both assemblages presented a very similar sponge species composition, being basically differentiated by the absence of gorgonians and antipatharians in sponge grounds. Soft sediments were characterized by vast extensions of the crinoid *Leptometra phalangium* and the brachiopod *Gryphus vitreus*. *L. phalangium* mostly occurred on the shelf edge, whereas *G. vitreus* was mostly found in deeper areas. The highest diversity of benthic macrofauna was concentrated in the shelf edge. This high diversity most likely results from the synergy of habitat heterogeneity and hydrodynamic conditions in the shelf edge. The good preservation of most of the observed assemblages is probably related to the low pressure of bottom trawling fishing, which is mainly concentrated above 100 m on the continental shelf, and below 500 on the continental slope.

● Chapter 2

Gorgonian diversity at 40–360 m depth in the Menorca Channel was comparatively higher than observed in Mediterranean coastal areas. Gorgonian assemblages on the continental shelf and upper slope were mostly monospecific, whereas shelf edge assemblages were highly multispecific. Gorgonian populations were dominated by small size colonies throughout the studied bathymetric range. However, in deeper environments intermediate and large colonies were more abundant. More stable environmental conditions and food availability in the shelf edge may allow gorgonians to reach larger dimensions. The high diversity and abundance of deep gorgonian assemblages in the rocky areas of the shelf edge, and the vast area covered by high densities of *Paramuricea macrospina* on the continental shelf most likely result from low trawling pressure and the exceptional fishing practices exerted by local artisanal fishermen. These high-density deep gorgonian assemblages may provide an approximate idea of how Mediterranean continental shelves and upper slopes stood before decades of bottom trawling.

● Chapter 3

The three chromatic forms of the gorgonian *Paramuricea macrospina* observed on the continental shelf and upper slope of the Menorca Channel, significantly differs in terms of sclerite size and shape, but not in terms of molecular markers and colony shape. Indeed, most of the analyzed colonial morphological features barely changed among the three forms indicating that colonial morphology is a conservative character that experiences little variation across the different environments. Both environmental factors and genetic differentiation may be conditioning differences in sclerite size and shape among the three different forms. Considering the similar coloration in M2 and M3 colonies and the less marked differences in sclerite size and shape, it could be suggested that M2 and M3 are a shallow and a deep forms of a single morphotype occurring over a wide depth range (continental shelf and shelf edge), whereas M1 is a morphotype restricted to the continental shelf. The lack of resolution in mtMutS is not necessary indicative of species identity, and thus recent speciation process that separated M1 versus M2+M3 forms of *P. macrospina* cannot be discarded. In this regard, new genetic markers are needed to clarify the fine line between species and morphological variation among Mediterranean gorgonians.

● Chapter 4

Paramuricea macrospina reproductive cycle is delayed 2–3 months respect to the shallow Mediterranean gorgonian species. Late increase in seawater temperature occurring on the Mediterranean continental shelf could be the main driver of this delay. Internal brooding in *P. macrospina* contrasts with surface brooding of the congeneric *P. clavata*. The differences in the habitats where the two species occur, arises the question about the possible adaptive advantage of their reproductive strategies. *P. macrospina* lipid content was much lower and more constant than observed in shallow Mediterranean gorgonian species. These differences between depths may reflect a lower but more constant food availability on the Mediterranean continental shelf than in littoral environments. A general stability in food availability for gorgonians on the Mediterranean continental shelf is also supported by the lack of seasonality in the $\partial^{13}C$ and $\partial^{15}N$ composition of *P. macrospina* tissue. Seasonality in the energetic requirements of *P. macrospina* is highlighted by the seasonal changes observed in the FFA content and composition

● Chapter 5

Particle fluxes collected in the Menorca canyon head were comparatively low in the Mediterranean context, reflecting the lack of intense continental and fluvial supply to this insular and oligotrophic study area. There was not a clear parameter, which controlled the temporal variation of the TMF. However, resuspension and biochemical characteristics associated to alternating water masses (resident and recent AW) seemed to be the main drivers controlling the magnitude and composition of the TMF. The findings of the current study also suggest that that Mediterranean submarine canyons related to insular environments, without important fluvial networks, receive as small fluxes as in some deep-sea environments.

Future perspectives

Results presented in this thesis derive from the study of Mediterranean macrobenthic assemblages of the continental shelf and upper slope by means of non-destructive techniques. Quantitative information about species abundance and distribution over large extents were obtained applying quantitative video analyses, complemented with the sampling of a limited number of organisms to confirm species identification. Non-destructive, video-based samplings are currently considered a valid alternative to traditional sampling methods to study deep benthic environments. When larger number of samples was needed to address the characterization of specific aspects of the species taxonomy or biology, they were carefully and selectively collected by means of the arm of the manned submarine, or as by-catch of traditional fishery. This approach is especially preferable for the study of vulnerable species with slow growth and high longevity, such as most of those investigated in this thesis.

Many of the results obtained within this thesis have also represented the scientific base for the ecological characterization of the Menorca Channel for its inclusion in the Natura 2000 Network from the European Union (LIFE07/NAT/E/000732). More in detail, results about species abundance and distribution (chapters 1 and 2) represent the ground zero for the future monitoring of the effectiveness of the management and conservation measures that will be undertaken in the frame of the new established special area of conservation (http://www.magrama.gob.es/es/costas/temas/proteccion-costa/actuaciones-proteccion-costa/illes-balears/LIC-ESZZ16002-Canal-de-Menorca.aspx). Moreover, future exploration of areas adjacent to the Menorca Channel, such as Serra de Tramuntana (Northern Mallorca) or the rocky gullies on the northern slope of Menorca Island, could unveil similarly reach and well preserved macrobenthic assemblages. These areas are currently under low trawling pressure, and artisanal and recreational fishing by-catch observations suggest the presence of rich and diverse benthic communities.

In situ growth experiments of the main bioengineering species (e.g. gorgonians, large massive sponges, antipatharians) would be beneficial to understand the recovery capacity of the observed benthic communities, and set the base for the restoration potential of impacted areas. In order to elucidate the phylogenetic relationship of the three forms of *Paramuricea macrospina* observed (chapter 3), future studies should address this question by means of high through put DNA sequencing (e.g. RAD sequencing, restriction-site associated DNA). Indeed, these techniques have shown to be useful in delimiting deep gorgonian species. Furthermore, the possible role that asexual reproduction could play to complement sexual reproduction (chapter 4) in the population dynamic of *P. macrospina* could be addressed by sampling colonies at different spatial scales and perform microsatellite genotyping analysis.

Finally, the high oligotrophy of the Balearic Sea apparently contrasts with the observed presence of high-density aggregations of passive and active suspension feeders. Future studies should consider monitoring particle fluxes and seasonal pattern of the main environmental conditions in the continental shelf and upper slope, to understand the main drivers of species distribution.

Annex I

GRINYÓ J (2016) PhD Thesis pp 161-173

LÓPEZ-GONZÁLEZ, P.J., GRINYÓ, J. AND GILI, J.M. 2012
Rediscovery of *Cereopsis studeri* Koch, 1891, a forgotten
Mediterranean soft coral species, and its inclusion in
Nidalia Gray, 1835 (Octocorallia, Alcyonacea, N
Marine Biology Research, 8: 594–604

Annex II

GRINYÓ J (2016) PhD Thesis pp 175-198

LÓPEZ-GONZÁLEZ, P.J., GRINYÓ, J. AND GILI, J.M. 2012
Chironephthya mediterranea **n. sp. (Octocorallia, Alcyonacea,**
Nidaliidae), the first species of the genus discovered
in the Mediterranean Sea.
Marine Biodiversity**, 45: 667–688**

SUMMARY

Technological development and increased availability of remotely operated vehicles (ROVs) and manned submersibles have significantly increased accessibility to deep environments revealing the presence of rich and diverse macrobenthic assemblages dominated by suspension feeders. These assemblages have been largely exposed to the impacts of fishing activities (especially bottom trawling) in the Mediterranean Sea. Consequently, it is currently urgent to increase knowledge on their distribution, as well as on the ecology and biology of the main species that constitute these macrobenthic assemblages in order to implement effective management and conservation measures. This thesis addressed the characterization of macrobenthic assemblages in the continental shelf and upper slope (40–360 m depth) of the Menorca Channel through a multidisciplinary approach at different ecological levels.

Six macrobenthic assemblages mainly segregated by substrate and depth. Hard substrates hosted sponge grounds and coral gardens, whereas crinoid and brachiopod beds occurred on soft sediments. Highest diversity values were found in the shelf edge, probably as a consequence of the bottom heterogeneity and the constant hydrodynamic conditions. Gorgonians were one of the most important and diverse bioengineering organisms in the Menorca Channel, forming dense assemblages that extended over vast areas. Gorgonian assemblages on the continental shelf and upper slope were mostly monospecific, whereas shelf edge assemblages were highly multispecific. Small colonies were dominant throughout the studied bathymetric range, but in deeper environments intermediate and large colonies were more abundant. The good preservation state of most of the observed benthic assemblages and gorgonian populations is probably related to the low pressure of bottom trawling, which is mostly concentrated in deeper areas of the continental and the exceptional fishing practices exerted by the local artisanal fisherman. *Paramuricea macrospina* is the most abundant gorgonian species in the Menorca Channel. Throughout its distribution, three different chromatic forms were observed. Two forms occurred on the continental shelf, and a third occurred on the shelf edge. Colony shape, sclerite size and shape, and the genetic variability of mitochondrial markers were compared to elucidate if these chromatic forms represented different taxonomic units. Colony morphology barely changed among the three forms resulting as a high conservative character. Conversely, sclerite size and shape significantly differed amongst the three forms, possibly conditioned by genetic and environmental factors. However, no significant differences were observed in the studied mitochondrial markers. The reproductive cycle, energy storage and metabolic requirements of *P. macrospina* showed significant differences compared to Mediterranean coastal gorgonians. Its reproductive cycle was delayed 2–3 months respect to shallow species, possibly following the late summer increase in seawater temperature occurring on the Mediterranean continental shelf. Moreover, internal brooding in *P. macrospina* contrasts with surface brooding in the congeneric *Paramuricea clavata*. Lipid content was lower and more constant in *P. macrospina* than in shallow species, and ∂13C and ∂15N composition showed almost no seasonal variation, suggesting that food availability in the continental shelf is lower but more constant than in shallower environments. The high oligotrophic conditions of the Balearic Sea apparently contrasts with the high abundance and diversity of active and passive suspension feeders observed in the Menorca Channel. Indeed, the downward particle fluxes quantified during two consecutive years in the Menorca Canyon were comparatively low within the Mediterranean context, reflecting the oligotrophic nature of the study area and the lack of continental inputs of particulate matter. Hydrodynamic settings and physical processes that cause sediment resuspension appeared to be more important than surface primary production in the control of the magnitude and composition of the total mass flux in the study area, likely playing a major role in determining the distribution of the macrobenthic assemblages.