UNIVERSITAT DE BARCELONA

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Carlos Domínguez Carrió

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ROV-based ecological study and management proposals for the offshore Marine Protected Area of Cap de Creus (NW Mediterranean)

Carlos Dominguez Carrió **2018**

UNIVERSITAT DE BARCELONA **FACULTAT DE BIOLOGIA**

Programa de doctorat en Ciències del Mar

ROV-based ecological study and management proposals for the offshore Marine Protected Area of Cap de Creus (NW Mediterranean)

Memòria presentada per Carlos Dominguez Carrió per optar
al grau de doctor per la Universitat de Barcelona

Tesi doctoral realitzada a l'Institut de Ciències del Mar de Barcelona (CSIC)

El doctorand

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Dans des secteurs au relief tourmenté, où les techniques de prélèvements aveugles sont rendues difficiles, les engins d'exploration comme la soucoupe SP 300 sont exceptionnels. Et, si l'observation directe seule ne suffit pas pour connaitre un milieu, elle devient particuliérement précieuse quand on peut la coupler avec des prélèvements, chaque méthode complétant l'autre et permettant de mieux comprendre les faits. Il sera indispensable, dans un avenir proche oú de nouveaux engins d'exploration existeront, capables de descendre plus profondément, de faire systematiquement des observations directes en même temps que toute étude qualitative et quantitative du benthos par les méthodes classiques.

Daniel Reyss, 1971

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Quan un decideix fer una tesi doctoral, mai s'imagina tot el que comportarà una aventura d'aquestes característiques. Aquesta tesi doctoral ha estat molt més del que jo inicialment hagués pogut imaginar. Ja no només per tot allò que he après, que és molt, sinó per la gran quantitat de moments bons que m'ha proporcionat, les situacions fantàstiques que he pogut viure (sí, baixar en submarí és una experiència increïble) i la gran quantitat de gent meravellosa amb qui he tingut el plaer de trobar-me i treballar-hi aquests últims anys.

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Una tesi de la magnitud d'aquesta, on s'han utilitzat dades recollides en campanyes oceanogràfiques que tenien objectius molt diversos, no hagués estat possible sense la col·laboració de moltíssimes persones, cadascuna especialista en el seu camp, que d'una manera o altra han posat el seu granet de sorra per a què la feina es portés endavant de forma exitosa. Intentaré no oblidar-me a ningú.

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The vast majority of the work performed during the completion of this PhD thesis is image based, which means that most of its results could have not been obtained without the high-quality underwater videos that were recorded on the continental shelf and submarine canyon off Cap de Creus. I can only thank the great effort placed by Gavin Newman in always getting the best image quality possible while performing all ROV transects following our demands. Spending time on board with you was always a great opportunity to learn from your knowledge and experience about video recording and post-processing. I would also like to thank Karen and Jurgen for their contribution towards the acomplishment of this PhD thesis by providing unvaluable images from the steepest parts of the submarine canyon with the manned submersible JAGO. It would have been very difficult to get those images without your help, together with the large set of samples that we required to correctly id most of the species. It was fantastic to share those cruises with you, I will always remember your enthusiasm, kindness and willingness to help.

Un cop a Barcelona, amb les campanyes ja finalitzades, la feina de processament de les imatges i dades obtingudes a bord, així com el seu anàlisi estadístic, ha requerit de la col·laboració d'un elevat nombre de persones. Susana, gràcies per tota la feinada que vas fer amb el tractament de les dades de VMS i en tota l'organització del projecte Indemares. Sense la teva ajuda no haguéssim pogut arribar mai fins aquí. Claudio, gracias por encontrar siempre tiempo en tu agenda para ayudarme con la determinación del tipo de sustrato a partir de los videos, que en el Cap de Creus no es tarea fácil. Thank you Kat for introducing me into the world of predictive modeling and providing me with the necessary tools to develop distribution maps that will be of key importance for the correct management of the future MPA of Cap de Creus. Claude and Thomas, thank you for your generosity. Your bottom current data have improved significantly the results of this PhD thesis, and I am very grateful that you always had the time to answer my e-mails and provide me with all the data that was required. Andrea, gràcies pel teu entusiasme, bondat i per ser-hi sempre que t'hem necessitat (no només jo, tot l'equip). Tu vas ser el primer que em vas ensenyar com funcionava l'anàlisi d'imatges per extreure dades ecològiques, i des de llavors no hem deixat de treballar junts. És un luxe poder comptar amb algú com tu en un equip de recerca, i espero que poguem continuar treballant com fins ara durant molts més anys. Alejandro, gracias por haber dedicado tanto tiempo a que los muestreos en el Cap de Creus consiguieran todos los objetivos propuestos. Contigo uno aprende que siempre se puede construir algun artilugio que permita recolectar aquellas muestras que a priori parecen imposible de conseguir. Stefano, gracias por haber ayudado siempre en todo aquello que fuera necesario, dejando de banda lo que tuvieras entre manos. Los dos empezamos juntos a analizar los vídeos del Cap de Creus, y mira hasta donde hemos llegado. Siempre recordaré aquellas primeras anotaciones tan sofisticadas, donde teníamos que dejar los corales blandos como "antozoo de pólipos blancos". Maria M, obrigado por aquellos primeros años de análisis de imagen, cuando yo aún estaba haciendo el máster. Me ayudaste mucho con la organización y análisis de los videos de la primera campaña oceanográfica que hicimos en el Cap de Creus. Guillem, gràcies per l'ajuda en l'anàlisi de les imatges del canyó i per la currada que et vas fotre en digitalitzar les cintes en HD i arreglar els arxius del Final Cut. David, gràcies per compartir la teva visió crítica de la ciència i per tota l'ajuda desinteressada que sempre ens has proporcionat. No oblidaré aquella conversa camí de Cadaqués on em vas deixar clar que acabar una tesi era molt més del que jo m'havia imaginat, i crec que a partir de llavors és quan vaig començar a ser conscient del llarg camí d'escriptura que em quedava.

Una de les parts més difícils de la tesi ha estat la identificació fins a nivell d'espècie de la gran majoria d'organismes que apareixien a les imatges. Quan un ha bussejat una mica, creu que més o menys coneix la fauna marina Mediterrània. Però tot això canvia quan comences a veure imatges gravades a majors fondàries. Descobrir la diversitat d'invertebrats de la plataforma i el talud mediterrani ha estat un dels millors aprenentatges que m'enduc d'aquesta tesi. Iosune, gràcies per les hores interminables al teu despatx intentant determinar les esponges que apareixien a les imatges en base a les mostres que teniem recol·lectades. Sense la teva ajuda, més del 90% de les esponges haguéssin acabat com a "Unidentified Porifera". Pablo, muchas gracias por haber compartido tu conocimiento conmigo y haberme ayudado con la identificación de la gran mayoría de antozoos. Siempre que hablamos aprendo alguna cosa nueva sobre corales y gorgonias. Rafa, gracias por tu ayuda en la identificación de los poliquetos. Yo solo no hubiera sido capaz de diferenciarlos. Xavier, gràcies per donar-me un cop de mà amb les ascídies. Sé que a partir de les imatges no era senzill, però vam arribar a determinar-ne unes quantes. La part de peixos semblava que havia de ser més senzilla, però també va portar les seves complicacions. Vull agrair a totes les persones que em van ajudar a la seva identificació, especialment al David, al Miquel S i l'Andrea de Lucia. A tots tres, gràcies. Grazie mille!

Una tesi basada en l'anàlisi d'imatges necessita d'ordinadors potents amb softwares específics. Quan jo vaig arribar a l'ICM mai havia fet anar un Mac, i des del primer moment vaig entendre que per treballar amb imatges, més valia no perdre el temps amb un Windows. Em van inocular el virus del Mac, i ara ja no hi ha marxa enrere. I fer anar un Mac de manera que en puguis aprofitar tot el seu potencial necessita d'algú que t'ajudi a fer-lo anar correctament. Lluís Alfons, gràcies per deixar-me l'ordinador sempre a punt i per la teva disponibilitat a l'hora de sol·lucionar problemes. I no només això, sinó que gràcies també per la teva generositat, sempre ensenyant-nos una millor manera de treure'n el màxim rendiment a les màquines.

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E quando uma etapa acaba, outra etapa começa. Nos últimos meses de escrita da tese foram no Faial, já a participar num projeto novo. Telmo e Marina, eu estou muito agradecido pela oportunidade de poder trabalhar convosco no estudo do mar profundo das Açores, pela boa acolhida que tive no vosso grupo e pelas possibilidades que tenho ali de crescer profissionalmente. E gostaria também de agradecer a todas as pessoas que me ajudaram para que minha adaptação à vida Faialense fosse

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I finalment, només em queda donar les gràcies a la persona més important que m'ha portat aquesta tesi. Només pel fet que ens haguem conegut, ja ha valgut la pena tota aquesta aventura. Tu has viscut les parts més bones, però també les més difícils. I no han estat poques. Però sempre hi ets, amb un somriure a la cara. Em fas molt feliç Eli, suposo que ja ho saps. Gràcies per permetre'm compartir tot això amb tu, i sobretot per haver-me aguantat aquests dos últims anys d'escriptura. Ara que tots dos haurem acabat, crec que ens mereixem aquelles vacances que durant molt de temps no ens hem pogut regalar. Aquest any és el bo, ja voràs. Gràcies per ser-hi, t'estimo molt! Ah, i no em vull oblidar del Nuk, segurament qui ha passat més estona al meu costat mentre escrivia la tesi. De forma incondicional, sempre estirat als meus peus, fos al pis de Sants o a casa dels meus avis. Cada dia de cada dia. I per sort, qui em feia sortir una estona de casa. Ens agradava dir que era jo qui el treia a passejar, però en realitat era ell qui em treia a mi. Quines passejades fantàstiques que fèiem entre vinyes i camps la primavera passada, quan estava immers de ple en l'escriptura. Us estimo molt als dos!

Per acabar i un altre cop, moltíssimes gràcies a tots!

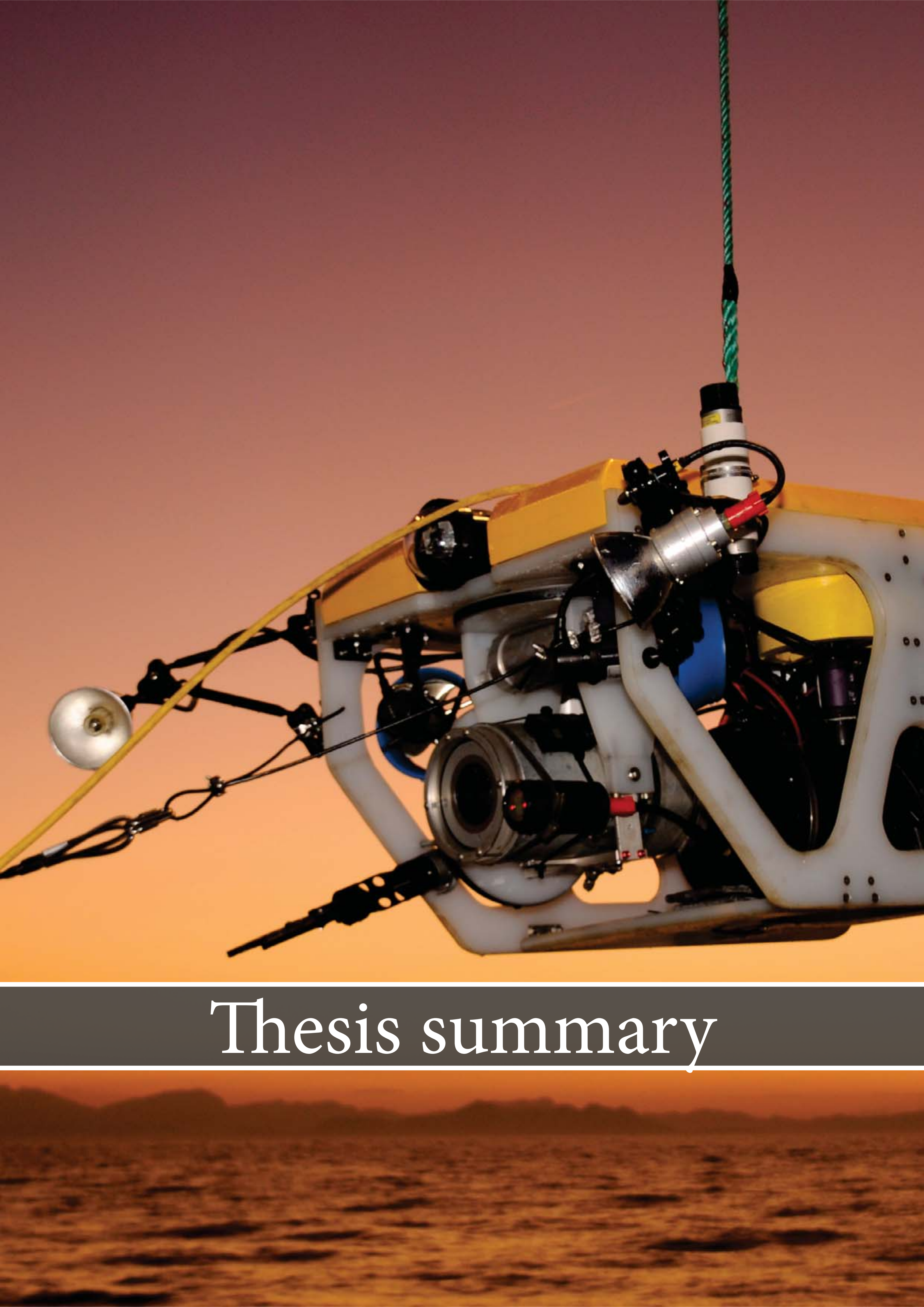
Table of Contents

List of acronyms used

ANOVA – Analysis of variance
ALDFG – Abandoned, Lost or otherwise Discarded Fishing Gear
AUV – Autonomous Underwater Vehicle
BACI – Before/After and Control/Impact
CEAB – Centre d'Estudis Avançats de Blanes
CSIC – Consejo Superior de Investigaciones Científicas
dbRDA – distance-based Redundancy Analysis
ECMWF – European Centre for Medium-Range Weather Forecasts
EFH – Essential Fish Habitat
EU – European Union
EUNIS – European Union Nature Information System
FAO – Food and Agriculture Organization of The United Nations
FI – Fishing Intensity
GES – Good Environmental Status
GIS – Geographic Information System
HD – High Definition
ICM – Institut de Ciències del Mar
IndVal – Indicator Value Index
IEHEM – Inventario Español de Hábitats y Especies Marinos
LGM – Last Glacial Maximum
LPC – Liguro-Provençal Current
MAGRAMA – Ministerio de Agricultura, Alimentación y Medio Ambiente
MAP – Mediterranean Action Plan
MPA – Marine Protected Area
MSP – Marine Spatial Planning
MSFD – Marine Strategy Framework Directive
OSPAR – Convention for the Protection of the Marine Environment of the North-East Atlantic
PCA – Principal Component Analysis
PCoA – Principal Coordinates Analysis
RF – Random Forest
ROV – Remotely Operated Vehicle
RAC/SPA – Regional Activity Centre for Specially Protected Areas
SD – Standard Definition

List of acronyms used

SH – Sensitive Habitat
SPAMI – Specially Protected Area of Mediterranean Importance
SSE – Sum of Squared Error
TM – Trawl marks
TPI – Topographic position index
TRI – Terrain Ruggedness Index
UPGMA – Unweighted Arithmetic Average Clustering
UNEP – United Nations Environmental Program
USBL – Ultra Short Baseline
UTM – Universal Transverse Mercator
UVC – Underwater Visual Census
VME – Vulnerable Marine Ecosystems
VMS – Vessel Monitoring System
WGS – World Geodetic System



Thesis summary

0. Thesis summary

There exists a general consensus among scientists, managers and politicians about the poor conservation status of the world's oceans and the need to regulate human activities that threaten their ecological integrity. The European continent, and the Mediterranean Sea in particular, have long suffered the strong influence exerted by human populations, which has led to the impoverishment of most of its marine habitats. Aiming to overcome this situation, the European Commission adopted in 2008 the Marine Strategy Framework Directive (MSFD). Such regulation establishes a general framework for all EU countries in the field of marine environmental policy, pursuing a sustainable use of the seas and the conservation of its marine ecosystems.

The MSFD enforces all Member States to adopt an **adaptive management strategy** in their territorial waters grounded on (1) an **ecosystem-based approach**, (2) the creation and management of **Marine Protected Areas** (MPAs) as essential tools to reach a (3) previously designed **Good Environmental Status** (GES), to which they must evolve as the result of (4) **policy measures** that would only be enforced after the launching of a (5) **monitoring program** based on (6) previously selected **indicators/parameters**. The MSFD also details a precise schedule, which should at present be reaching the end of its run. For this reason, selecting indicators sensitive to human threats and feasible enough to routinely monitor the status of the seas at an ecosystem level has become a pressing and challenging goal that needs to be urgently addressed.

Shallow benthic ecosystems, which by their higher persistence seem much more informative than pelagic biota, can be assessed by direct access (SCUBA diving). At the present state of technological development, Remotely Operated Vehicles (ROVs) and manned submersibles seem to be ideal tools to perform biological samplings at greater depths. Although the use of such technology has been available for a long time, there are still very few tests on the ability of ROVs to provide useful evaluation techniques, which must be robust and simple, in a way that could support complex experimental designs (i.e. largely replicated samplings repeatedly made over long periods of time) and also quantitative, repeatable and sensitive enough to support accurate statistical testing. This PhD thesis is intended to provide a significative advancement in such field.

In the case of Spain, the MSFD was legally transposed as a state law in year 2010, leading to the designation of 10 new offshore MPAs. One of these 10 areas, evaluated under the framework of the Life+ Indemares project, sets the spatial limits of this PhD thesis: the offshore area of Cap de Creus, which encompasses a submarine canyon and its adjacent continental shelf. The main objective of this thesis consists in a proposal of a monitoring protocol using ROVs to assess the evolution of its benthic communities in front of commercial fishing activities, one of their most important human threats to date. In order to fulfill such goal, the thesis has been structured in a series of Parts, with its main findings summarized as follows:

Part 1 evaluates the structure of the **megabenthic invertebrate assemblages** present on the continental shelf and submarine canyon off Cap de Creus. In order to do so, 60 ROV transects were performed between 80 and 400 m depth, aiming to survey all the potential habitats, from the flat muddy substrates of the continental shelf to the rocky outcrops of the submarine canyon. A total of 167 different taxa of the invertebrate megafauna were identified, revealing the extraordinary ecological value of the new MPA of Cap de Creus. The clustering analysis led to the identification of 9 different invertebrate assemblages: (A) gorgonian shelf assemblage with *Eunicella cavolini*; (B) soft-bottom shelf assemblage with *Pteroeides spinosum*, *Alcyonium palmatum* and *Pennatula rubra*; (C) low-diverse soft-bottom assemblage with *Sabella pavonina*; (D) sandy bottom shelf assemblage with *Leptometra phalangium*; (E) deep-shelf and shelf-break assemblage with *Lanice conchilega*; (F) cold-water coral assemblage with *Madrepora oculata*; (G) 'Roche du large' assemblage with a high diversity of sponges; (H) coarse substrates of the shelf break and slope with *Cerianthus membranaceus* and *Echinus acutus*; and (I) massive aggregation of the brittle star *Ophiothrix fragilis*.

Part 2 aims to determine the relative role played by **abiotic parameters** as drivers of the spatial patterns observed. For this reason, a set of environmental parameters and the commercial fishing activity of bottom trawlers are used to explain the spatial distribution of such assemblages. Depth, current speed and substrate type are the most important factors determining the spatial patterns detected at the scale of the whole study area, although fishing intensity plays a predominant role in the soft-bottom areas of the continental shelf, where fishing activities are known to occur.

Data extracted from the ROV images is generally limited in its spatial cover. For this reason, Part 3 makes use of the driving power of the environmental factors to elaborate **predictive distribution maps** of the assemblages identified in Part 1. The algorithm Random Forest was used to predict the distribution of megabenthic assemblages and the biological diversity over the whole study area (species richness and Exponential of Shannon diversity index). The maps generated displayed a good correlation between the environmental parameters and biological data, with two hotspots of biodiversity identified on the continental shelf (gorgonian assemblage at 100 m depth and a very well preserved sponge assemblage close to the shelf break, at 150 m) and inside the canyon head, at depths of 200-300 m (a well established cold-water coral assemblage with *Madrepora oculata*).

Part 4 focuses on the impact of **commercial fishing activities** on the benthic invertebrate megafauna. In particular, it quantitatively evaluates the potentially deleterious effects of overfishing on the structure of the benthic assemblages of the soft-bottom continental shelf. After proving the non-independence between fishing effort and the remaining environmental parameters (Part 2), only a subset of ROV transects located in areas susceptible of being fished were used to elucidate the response of biological diversity to varying degrees of fishing pressure. Increasing levels of bottom trawling not only had a negative impact on species richness and diversity, it also affected the structure of the community, producing a change in the dominant species. In terms of key megafauna species, two contrasting patterns were observed: some anthozoans showed a decrease in their mean abundance as fishing intensity increased and two polychaete species displayed a positive response to increasing levels of fishing effort. Such relationships gave us the possibility to identify a set of indicator species (i.e. *Pteroeides spinosum*, *Alcyonium palmatum*, *Lanice conchilega* and *Sabella pavonina*) that could potentially be used in the monitoring program that should be implemented in accordance to the demands set by MSFD.

Based on such findings, Part 5 proposes a **monitoring protocol** to assess the evolution of the benthic ecosystem once management measures are put in practice. The proposed protocol extensively describes the sampling methodology to be applied at sea, the video analysis, the processing of the data and the interpretation of the results. Furthermore, in accordance with the adaptive strategy proposed by the MSFD, which asks for Before/After and Control/Impact (BACI) experimental designs, Part 5 provides, for the very first time, baseline data for a set of descriptors from the continental shelf. In essence, the results of Part 5 provide local managers with the necessary tools to immediately implement a monitoring program based on reasoned hypotheses, an experimental design, a sampling protocol, a baseline dataset and statistically conclusive analyses.

Part 6 places its focus on the **fish community**. Since the ichthyofauna constitutes the most valuable resource obtained by the fishing industry, it would seem advisable to use fishes as the first choice when looking for indicators to asses fishing impacts, even when applying an ecosystem-based approach to conservation. When compared to other methodologies, the use of ROVs for long-term monitorings of fish stocks in deep waters has not been implemented. This might be caused by the inherent problems that relate to such technology: there is still no suitable way to accurately evaluate deep-sea fish abundances using underwater images. The set of ROV images used in this PhD thesis allowed us to identify two fish assemblages, one on the shelf and shelf break area and one in the submarine canyon of Cap de Creus. The shelf assemblage was characterized by highly-mobile small-sized fishes, such as *Trisopterus* spp., *Serranus cabrilla*, the triglidae *Chelidonichthys* *cuculus* and *Trigloporus lastoviza*, and different species of the genus Scorpaena. The submarine canyon assemblage presented more mobile and larger fishes, in which to include *Trachurus* spp., *Helycolenus dactylopterus*, *Lepidopus caudatus*, *Conger conger* or *Phycis phycis*. The low number of fishes identified in the ROV footage makes us very pessimistic with the possibility of using of fish species as indicators of fishing activity, and they were not included in the proposed monitoring protocol.

Finally, and following the demands of the MSFD, Part 7 makes use of the extensive ROV footage to evaluate the amount of **marine litter** that is currently accumulated over the seafloor of the continental shelf and submarine canyon off Cap de Creus. The footprint derived from human activities in the study area is not only restricted to commercial fishing activities, but also related to the disposal of human-drived items that reach the seabed. In this regard, the quantity of domestic litter items observed on the continental shelf and submarine canyon was relatively low, with only 28 items observed in 1 ha explored. The origin of such items was diverse, with components made of plastic, metal, clothing and glass. On the other hand, a large number of abandoned, lost or otherwise discarded fishing gears (ALDFG) were observed, with long lines particularly abundant in areas of the submarine canyon, at depths of 200-400 m. On average, 8 longlines were registered every 100 linear meters on the canyon head, with density peaks of 25 lines per 100 m in two ROV dives. Approximately 10% of cold-water coral colonies had longlines entangled around them, giving an idea of the damaging effects produced by abandoned fishing gears over key structuring species in deep-sea environments.

The results of this PhD thesis have to be considered an attempt to simplify the implementation of the MSFD in offshore areas that can only be explored using remote sampling techniques. In essence, the work presented here aims to provide the necessary information to implement an ecosystem-based approach to management in the marine area off Cap de Creus, while providing the necessary tools to support the continuous monitoring of the benthic ecosystem demanded by the MSFD. Futhermore, the monitoring program proposed here is image-based, aiming to reduce the impacts to the marine realm that traditional, more destructive techniques involve.

Introduction

1. Introduction

1.0 General introduction

The development of human societies has traditionally given rise to a wide number of activities that exert differential degrees of pressure on the world's oceans. The list of activities known to produce negative effects on marine ecosystems is long, and in most cases linked to economically lucrative endeavors. Their detrimental effects have historically been reported in estuarine and coastal systems, mostly in the vicinities of human settlements. The rapid growth experimented by human populations in the last decades and the constant improvement of technology have contributed to the expansion of the anthropogenic pressures further away from shore and further down into the deep ocean (Morato *et al.* 2006). Human influence on marine ecosystems has become so large that the number of areas that remain unspoiled can be considered negligible, and a large fraction of marine habitats are simultaneously being disturbed by multiple threats (Halpern *et al.* 2008).

The continuous exposure of marine habitats to varying degrees of human pressures is having severe ecological and societal consequences, which inevitably depend on the vulnerability of the ecosystem and the strength of the potential threat (Halpern *et al.* 2007). Human activities have led to habitat transformation, depletion of key species, ecological changes in the structure of the marine communities and increased rates of species invasions, among others (Lotze *et al.* 2006). One of the most worrying consequences over the functioning of marine ecosystems is the depletion of populations and species, which may limit the ocean's capacity to provide food, maintain water quality and recover from perturbations in an rapidly changing world (Worm *et al.* 2006).

The foundation of the European Union favored an intense debate on how to overcome this situation, searching ways to promote economic growth in commitment with the conservation of its natural resources. The first step taken by the European Commission to favor the protection of its natural heritage consisted in the application of the Birds (Council of the European Union 2009) and the Habitats Directive (Council of the European Union 1992), which were adopted by Member States in 1972 and in 1992, respectively. Both regulations established the basis for the implementation of the EU Natura 2000 Network of protected areas, a powerful tool designed to promote the maintenance of biodiversity while considering economic, social, cultural and regional requirements.

Most of the initial efforts made by EU member states to apply the Habitats Directive focused on terrestrial habitats, and little attention was placed on the marine environment. To overcome this situation, the EU parliament approved the Marine Strategy Framework Directive (MSFD) in 2008, aiming to (1) "protect and preserve the marine environment, prevent its deterioration or, where practicable, restore marine ecosystems in areas where they have been adversely affected" as well as to (2) "prevent and reduce inputs in the marine environment (…) to ensure that there are no significant risks to marine biodiversity, marine ecosystems, human health or legitimate uses of the sea" (Council of the European Union 2008).

The main objective of the MSFD is to develop and establish thematic strategies aimed at the conservation of marine ecosystems across Europe. According to the Directive, the adopted marine strategies "shall apply an ecosystem-based approach to the management of human activities, ensuring that the collective pressure of such activities is kept within levels compatible with the achievement of *good environmental status* and that the capacity of marine ecosystems to respond to human-induced changes is not compromised, while enabling the sustainable use of marine goods and services by present and future generations". The MSFD defines 'good environmental status' (GES) as a condition reached when "seas and oceans are ecologically diverse and dynamic, clean, healthy and productive, their use is at a sustainable level, safeguarding the potential for uses and activities by current and future generations".

The need to reach GES in different areas of the continent embraces a certain degree of complexity since management measures have to be applied to a wide range of marine ecosystems. Not unexpectedly, the term 'good' has generated considerable debate, and its interpretation varies across countries (for an extensive review, see Mee *et al.* 2008). To overcome this situation, the European Commission proposed a set of criteria and methodological standards to assess the extent by which GES was being achieved in the different marine areas under consideration. Such assessment is based on a list of 11 related indicators, which are designed to summarize the way in which the whole system functions, to make the criteria operational and allow subsequent progress (Borja *et al.* 2010). The application of the criteria specified for each indicator should be carried out keeping in mind the need to develop assessment and monitoring programs, as well as providing prioritized actions in order to diminish the threats to the marine environment. The complete list of indicators that should be considered to determine GES can be found in the Commission Decision 2010/477/EU (European Commission 2010).

One of the most important tools provided by the MSFD to ensure the conservation of marine biodiversity corresponds to the creation of a coherent and representative network of Marine Protected Areas (MPAs) across Europe, which should adequately cover the diversity of the constituent ecosystems (Article 13). According to the IUCN, MPAs can be defined as "any area of intertidal or subtidal terrain, together with its overlying water and associated flora, fauna, historical, and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment" (Kelleher 1999).

Theoretically, the creation of a well-designed network of MPAs should provide a series of benefits, both to the ecosystem and to society, in which to include (1) improvements in the functioning of the ecosystem, (2) recovery of exploited populations and fisheries, (3) a better scientific understanding of marine ecosystems, and (4) enriched opportunities for non-extractive activities with an economical return (Murray *et al.* 1999). In terms of resource management, MPAs have historically proven satisfactory in enhancing the conservation status of species and habitats, consistently resulting in an increased density, biomass, individual size and diversity of any functional group, regardless of the size of the area being protected (Halpern 2003).

The MSFD states that the spatial protection measures proposed for each new MPA have to integrate conservation objectives, management measures, monitoring programs and assessments of human activities. In order to design a program of measures to fulfill all the objectives set by the MSFD, a series of steps should be specifically taken in every single MPA:

1. A profound analysis of the characteristics of the area, as well as the pressures and impacts on its waters (base line)
2. Determination of a set of characteristics that correspond to a good environmental status
3. Establishment of environmental targets and monitoring programs, necessary to develop an evaluation on a regular basis
4. Implementation of the programs and measures designed to achieve a good environmental status

The MSFD was adopted by the Spanish state in 2010 after the approval of the law 41/2010 by the Spanish parliament (Ley de Protección del Medio Marino; BOE 2010). Until then, Spain lacked a comprehensive regulatory framework in terms of protection of the marine environment. The approval of the law 41/2010 ensures that human activities at sea are managed in a way that do not compromise the conservation of the natural resources, in agreement with the ecosystem approach proposed by the MSFD. The most important instrument by which Spain contributes towards the conservation of its marine ecosystems is through the designation of a series of MPAs. In order to identify and characterize a number of marine areas that could potentially be included in the Natura 2000 Network, the EU and the Spanish Government implemented the Life+ Indemares project.

Its main objective was to gather sufficient information about habitats and marine species in 10 offshore areas that are considered Sites of Community Interest (SCI) are currently proposed to the European Commission in order to be declared Special Areas of Conservation (SAC) (see Box I for further details).

One of the 10 areas selected in the frame of the Life+ Indemares project corresponded to the offshore marine area of Cap de Creus, which frames the experimental setting of this PhD thesis. This marine area is located in the southernmost part of the Gulf of Lions, and it includes the submarine canyon off Cap de Creus as well as the continental shelf delimited between the canyon and the coastline (further information about its main environmental features is given in Section 3.1). The Marine Biodiversity, Ecology and Conservation Group from the Institute of Marine Sciences (ICM-CSIC) produced a detailed evaluation of the physical and ecological characteristics of the seabed and water column of the marine area of Cap de Creus, providing the necessary scientific knowledge for its proposal to become an SCI by the European Union. The marine area proposed by the Spanish Parliament to the European Commission in 2014 to become part of the Natura 2000 Network was further extended to include the submarine canyon of Lacaze-Duthiers and its adjacent continental shelf, reaching a total surface of 987 km^2. The SIC was designated in year 2014 under the name of "South-West Gulf of Lions Canyon System" (Region code ESZZ16001; BOE 2014a).

The application of specific management measures in the different areas selected across the Spanish territory as part of the Natura 2000 network includes a set of technical difficulties related to the particularities of working at sea. As it can be expected, such difficulties increase in the case of offshore MPAs, mainly due to the complexity of accessing the seabed on a regular basis when the lower limit of SCUBA diving is exceeded. For this reason, the methodology selected to determine the basal state and the definition of the monitoring protocol demanded by the MSFD for each of the 10 newly declared MPAs has to be addressed individually.

The use of Remotely Operated Vehicles (ROVs) seems to be an excellent solution to overcome the limitations of working passed certain depths, since there are now models capable of reaching depths of 6000 m (Smith & Rumohr 2013). The number of studies that make use of this rapidly developing technology has risen exponentially in the past few years, and an important part of shelf, slope and even deep-sea research is nowadays based on the knowledge produced by means of these devices. There is a large list of publications in which ROVs are used as the main sampling device, covering a wide range of topics, such as single species distributions (e.g. Mortensen & Buhl-Mortensen 2004; Dolan *et al.* 2008), community structure (e.g. Post *et al.* 2011; Davies *et al.*

2014), habitat mapping (e.g. Kendall *et al.* 2005; Neves, Preez & Edinger 2014) the effects of fishing activities (Buhl-Mortensen *et al.* 2016; Smith, Banks & Papadopoulou 2007; Smith *et al.* 2007) and the distribution of marine litter (e.g. Tubau *et al.* 2015; Pham *et al.* 2013).

Box I

The Life+ Indemares Project (LIFE 07/NAT/E/00732)

The Life+ Indemares project, which was 50% co-financed by the European Commission, aimed to understand the natural and socioeconomic values of different marine areas scattered around the territorial waters of the Spanish territory in order to complete the Natura 2000 Network for marine environments. A total of 10 large areas were selected, covering a surface of almost 5 million hectares (Fig. B1.1). The selection of those areas followed a proposal made by WWF in 2005 (Marcos 2005), responding to a set of criteria which included (1) their wide representation, (2) the presence of endangered species or habitats as defined in the annexes of the Habitats and Birds Directives and (3) high ecological values with good conservation status.

The different surveys programmed throughout the 6 years of the Indemares project (2009-2014) explored the shelf and deep-sea habitats as well as the pelagic environment of areas located in the 3 marine biogeographic regions of Spain:

- Mediterranean region: Marine area of Alborán Island, the Marine area of Columbretes Islands, South-West Gulf of Lions canyons system and the Menorca Channel
- Atlantic region: Avilés Canyon System, Galicia bank and the Mud Volcanoes of the Gulf of Cadiz
- Macaronesic region: Conception Bank and the Eastern and southern marine area of Lanzarote-Fuerteventura.

The results of the project have led to the protection of more than 7 million hectares of marine environment, which represent more than 8% of Spanish territorial waters. The 10 areas selected have already been added to the Natura 2000 Network, a significant step towards fulfilling European Directives on habitats and birds (see BOE 2014a and BOE 2014b). The project has also contributed to fulfill the objective set out by the UN's Convention on Biological Diversity of designating at least 10% of the planet's seas and oceans as protected areas.

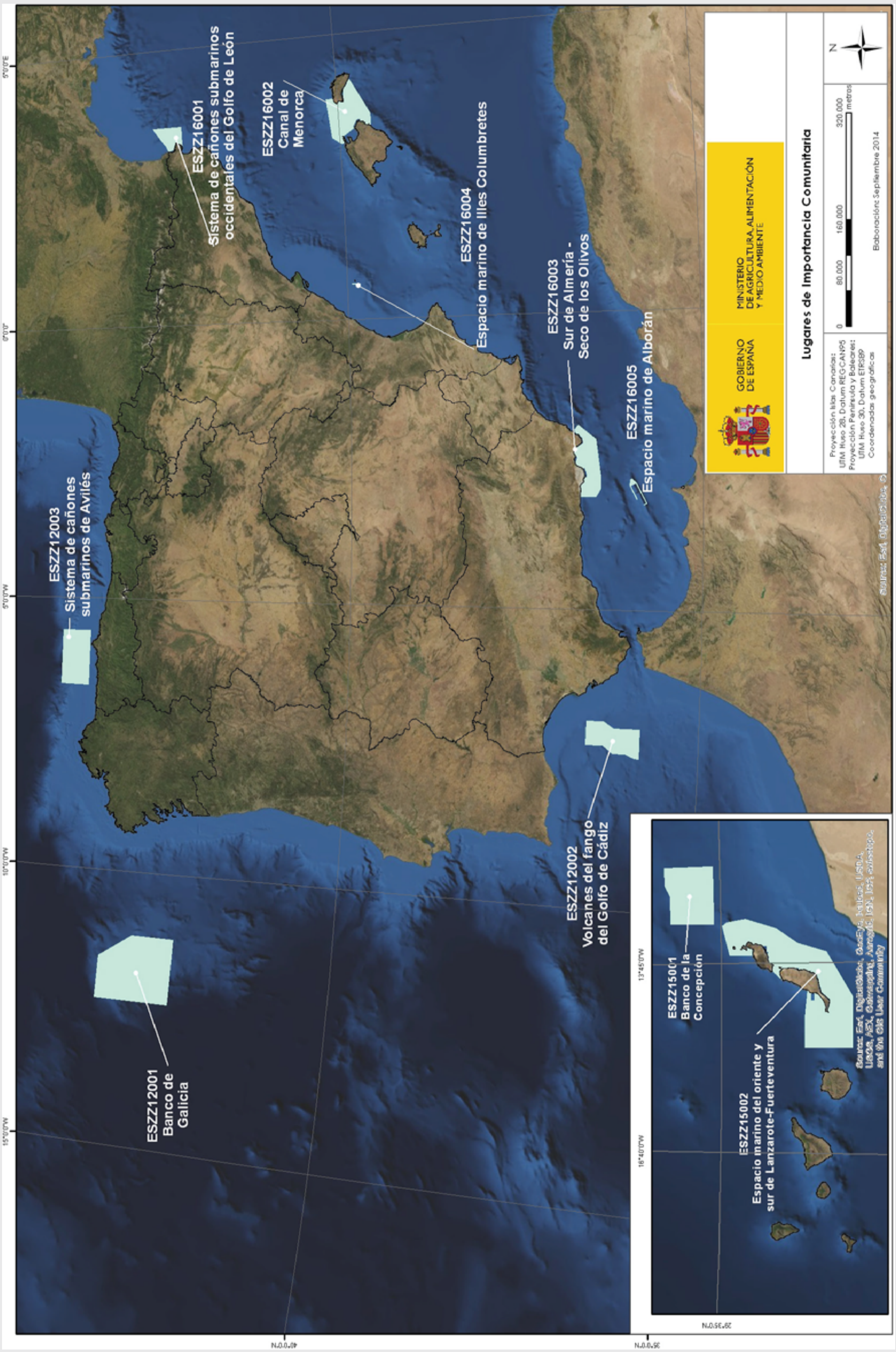


Figure B1.1. Location of the 10 sites of community importance (SCI) that were explored during the Life+ Indemares project. From http://www.mapama.gob.es.

1.1 Biological diversity and benthic assemblages

1.1.1 Classifying benthic communities for marine management

The application of an ecosystem approach to marine management implies a shift from the traditional resource-based evaluation to a more complex assessment of the different components that make up the whole ecosystem, including a wide range of ecological, environmental and human factors (Curtin & Prellezo 2010). Conservation strategies implemented in the different MPAs established as a response to the MSFD should incorporate an ecosystem-based approach to the management of human activities, aiming to eventually reach a good environmental status in their waters. Since every MPA generally presents particular problems that require specific solutions, the MSFD demands a thorough assessment of the marine ecosystems that are to be managed, including an analysis of the "essential features and characteristics, and current environmental status of those waters, (...) covering the physical and chemical features, the habitat types, the biological features and the hydro-morphology" (Council of the European Union 2008). One of the key aspects of this exhaustive assessment corresponds to the description of the main biological features, in particular, the benthic communities associated with the predominant seabed habitats, including their species composition (see Annex III of the MSFD).

This type of assessment is not new, and the study of marine benthic communities is a long-standing discipline within benthic ecology. The idea of communities was embraced in the 1800s by several authors to differentiate between the distribution of fauna in geographical zones and distinctive associations of organisms. Möbius (1880) gave one of the first references about how different species group with each other to form benthic communities, which he defined as biocenoses. It was Petersen (1913), however, who gave relevance to the concept after studying the benthic fauna of the Danish waters. His analyses of the soft-bottom ecosystem determined a series of infauna and epifauna communities, which he considered to be "statistical units" due to the recurring combination of a few conspicuous animals, strongly represented numerically.

Defining assemblages of species into clearly defined ecological units is not a straightforward task since ecological communities range from loose aggregations to highly stable co-adapted groups in equilibrium with the environment for long periods of time (Mills 1969). In fact, most studies have demonstrated that the role played by environmental factors in determining the groupings is large, and no benthic community can be thought of as a unit bound together merely because of its biological components. Mills (1969) defined community as a "group of organisms occurring in a particular environment, presumably interacting with each other and with the environment, and separable by means of ecological survey from other groups". In this sense, it is now perfectly accepted that classifying faunal associations in identifiable communities highly relies on the combination of external factors (i.e. the 'habitat'), but the actual classification should be derived from a study of the distribution of the organisms concerned (Jones 1950). For that reason, modern classifications have a tendency to combine both the physical environment (habitat) and its distinctive assemblage of conspicuous species (community) into a higher level entity, originally defined as 'biotope', even though some directives and conventions also referred to it as 'habitat' (Olenin & Ducrotoy 2006). Box II summarizes the most important habitat catalogues that incorporate information about Mediterranean shelf and slope environments.

Box II

Classification systems for marine benthic communities

There has been an increased interest by the scientific community, governing bodies, stakeholders and policy-makers to develop exhaustive lists of biological communities found within the boundaries of a given region. These classifications are of key interest in terms of marine management and conservation, since they simplify the reporting of assemblage data in a comparable manner across regions, create a consistent database of communities and species and also help towards the organization of data in maps and reports (Costello 2009). The implementation of EU strategies, such as the Habitats Directive, favored the development of a standardized reference system across Europe that integrates all available information about biotopes found along the European continent. The result was the EUNIS Habitat classification (European Union Nature Information System), designed to provide a common European reference list of habitats (note that habitat and biotope are considered synonyms here).

EUNIS provides a detailed description of all habitats currently identified and provides a common hierarchical classification to help discriminating between different situations, facilitating its use for scientific and management purposes (for more information, visit http://eunis.eea.europa.eu/index.jsp). The regulatory system used for selecting conservation areas and conducting environmental assessments in Europe makes EUNIS the most convenient classification system to be used, primarily because it provides the most integrated and ecologically relevant methodology of available systems, with detailed information about how to conduct field sampling, identify and map each level in the classification and describe new biotopes (Costello 2009).

In the case of the Mediterranean Sea, the first attempt to agree on a classification system of marine habitat types was proposed by the Regional Activity Centre for Specially Protected Areas (RAC/SPA) after a meeting of experts held in Hyères (France) in 1998, a list later revised by the Fourth Meeting of National Focal Points for SPAs held in Tunis in 1999. The Reference List of Benthic Habitat types proposed by RAC/SPA as a tool to implement the agreements reached in the Barcelona Convention and its SPA/BD Protocol (UNEP/MAP 1994) uses the typologies defined by the extensive work of Pérès & Picard (1964) and the granulometric nature of the sea bed classified by Dauvin *et al.* (1994). The final document aims to serve as a reference list for the identification of sites of conservation interest and it includes a total of 18 biotopes (defined as biocenoses), from which 55 facies (understood as local species associations) can be derived (UNEP 2006). The main objective of the agreed document was to serve as a common reference for the establishment of national inventories of natural sites of conservation interest in the Mediterranean region.

From a more regional perspective, the Spanish Government, following the demands of the law 42/2007 regarding the natural heritage and biodiversity of the national territory (BOE 2007), encouraged a profound collaboration between the Ministry of Environment, local authorities and the scientific community to elaborate an inventory of the marine habitats and species found in Spanish jurisdictional waters. The result consisted in an extensive list of habitats, hierarchically organized, named *Lista Patrón de Hábitats Marinos,* which is included in the *Inventario Español de Hábitats y Especies Marinos*, IEHEM. Its main objective is to serve as a source of knowledge about the distribution, abundance and conservation status of marine habitats in Mediterranean and Atlantic Spanish waters, contributing towards the designation and better management of a coherent network of MPAs (Templado *et al.* 2013).

These hierarchal classification systems are designed for administrative purposes and not for scientific research, since they respond to the need of selecting conservation areas and conducting environmental assessments by governing bodies (Costello 2009). However, their use has become so widespread in recent years that it is of crucial interest to detect the similarities and divergences between the results of research surveys and the different habitats described in national and international classification systems.

1.1.2 Cap de Creus as a case study

The Life+ Indemares project gave the opportunity to perform 4 oceanographic cruises and several littoral surveys with smaller vessels in order to gather substantial information about the ecological features that characterize the marine area of Cap de Creus (see Section 4.1.1 for further details). A large sampling effort was placed to fully comprehend the biodiversity patterns and the structure of the faunal assemblages that define the benthic environment.

Although the work presented in this PhD thesis might be the largest exploratory assessment made in the region during the last 40 years, it is not the first attempt to evaluate its ecological features. There exist references that describe the diversity of the marine invertebrate fauna and its distribution in areas of the Gulf of Lions that go back more than 100 years, the most relevant work of that time being that of Pruvot (1895). It was not until the 1960s and 1970s, however, that an intensive evaluation of the marine benthic communities and the sedimentary features of the gulf was performed by researchers from the Musée Oceanographique de Monaco and the Laboratoire Arago in Banyuls-sur-Mer (e.g. Got *et al.* 1968; Guille 1970). In terms of our study area, the most relevant assessment that was made over the continental shelf belongs to Desbruyères, Guille & Ramos (1972), who focused on the analysis of macrofauna species living in the sediment. At the same time, Reyss (1971) made the first attempt to characterize the invertebrate fauna living in the submarine canyons of Cap de Creus and Lacaze-Duthiers (see Box III for further details).

Box III

Shelf and slope communities of Cap de Creus: a historical background

A few years before the bionomy of the continental shelf off Cap de Creus was elaborated by Desbruyères *et al.* (1972), Alain Guille and his collaborators (Guille 1965) performed two dives in a submersible in the southern part of the shelf, in depths between 120 and 140 m. Their observations did not lead to an accurate description of the epibenthic assemblages, since fauna was not quantitatively assessed and the number of dives was very limited. However, 3 different environments with distinct megafauna species could be derived from their observations.

I. Flat areas dominated by muds and very fine sands, full of burrows of rounded edges. The dominant species were two sea anemones *Ilyanthus diaphanus* (species now accepted as *Andresia parthenopea*) and *Eloactis mazeli*, accompanied among others by the echinoderms *Echinus acutus*, *Anseropoda placenta* and *Stichopus regalis* (*Parastichopus regalis*) and the cnidarians *Alcyonium palmatum*, *Veretillum cynomorum* and *Pteroeides griseum* (*Pteroeides spinosum*), all three species observed facing the current. Also remarkable was the presence of high-density aggregations of the ophiuroid *Ophiothrix quinquemaculata*. This species is most likely the species identified as *O. fragilis* in Atlantic waters, also known for its aggregative behavior. Morphological and DNA analyses performed recently also support this hypothesis, so following the recommendations given by Pérez-Portela, Almada & Turon (2012), all *Ophiothrix* specimens identified in this work are considered to be *O. fragilis*.

II. Muddy detritic, with shells and small stones as a common feature. The dominant species were *Echinus acutus*, *Holothuria forskali*, *Stichopus regalis* and *Cidaris cidaris*.

III. Rocky outcrops covered by sand and biogenic detritus, mostly shells. These outcrops were commonly observed as stretched banks tens of meters long and some 2-3 m in width. They were commonly covered by tube forming worms, probably belonging to the genus *Protula,* accompanied by a series of sponge species such as *Axinella damicornis*, *Poecillastra compressa*, *Suberites carnosus* (*S. syringella*), *Ciocaliptra penicillus*, *Geodia* sp. and *Calyx nicaeensis*. Massive formations of the polychaete *Salmacina dysteri* were also common. To a smaller extent, but still remarkable, was the presence of *Lophogorgia sarmentosa* (*Leptogorgia sarmentosa*) and *Eunicella stricta* (probably *E. cavolini*).

It was not until the work led by Desbruyères *et al.* (1972) that a complete characterization of the continental shelf was developed. Their work was part of a larger study aimed to produce the sedimentologic and faunistic cartography of the Mediterranean continental margin. The authors analyzed the composition of the major infauna assemblages found along littoral areas and the continental shelf from Cerbère in France to Palamós in the Catalan coast, aiming to determine the biogeographical role played by the prominent position of Cap de Creus cape. The study qualitatively and quantitatively evaluated the invertebrate fauna present in 114 samples collected using a Van Veen grab, following the methodology applied in previous works.

The authors identified and described a set of benthic assemblages, from which only the community of muddy bottoms with *Amphiura filiformis* was represented over the continental shelf in front of Cap de Creus (Fig. B3.1a). This community is characterized by the dominance of pelites in the sediment, and hence by polychaetes adapted to muddy environments. Four different variations of this community were identified coexisting in the continental shelf off Cap de Creus:

a. **Sub-community of muds with *Nucula sulcata***. A bathymetrically dispersed assemblage (20-185 m), generally found in areas where sediments were largely dominated by pelites (>50%). All characteristic species were polychaetes, mostly sedentary. Its most common species was *Nucula sulcata*, but other species could also be considered characteristic, such as *Terebellides stroemi, Sternaspis scutata, Oestergrenia digitata* and *Trachythyone tergestina*. Different variations were identified within this sub-community:

    i. **Variation of littoral muds (VC)**. Found in depths of 50-105 m, in a substrate largely dominated by pelites (>75%). It was characterized by the disappearance of all eurytherm littoral species and increasing numbers of circalittoral species such as *Chaetozone setosa*, *Marphysa bellii* and *Nephtys histricis*.

    ii. **Variation of shelf muds (VL)**. Found in depths of 87-185 m. It showed a reduction in the overall number of species, but an increase in strictly stenotherm species.

b. **Sub-community of muddy detritic with *Venus ovata* (DE)**. Only observed in depths of 30 to 90 m, where the substrate was made of a portion of pelites mixed with coarse sands and gravels. Common species were *Epizoanthus arenaceus*, *Cardium papillosum*, *Drilonereis filum*, *Amphiura apicata*, *Ophiotrix quinquemaculata* (*O. fragilis*), *Owenia fusiformis*, *Venus ovata* and *Tellina donacina*. Some epifauna was also recorded, mainly hydrozoans and erect bryozoans.

c. **Sub-community of offshore detritic with *Auchenoplax crinita* (DL)**. Found in detritic areas between 82 and 163 m, although the dominant fauna was from a muddy origin. Dives performed in a manned submersible by Guille (1965) allowed to characterize the common epibenthic species found in this sub-community: the sea anemones *Ilyanthus diaphanus* (*Andresia parthenopea*) and *Eloactis mazeli*, the alcyonarians *Alcyonium palmatum*, *Veretillum cynomorinum* and *Pteroeides griseum (P. spinosum)*, as well as the echinoderms *Leptometra phalangium*, *Echinus acutus*

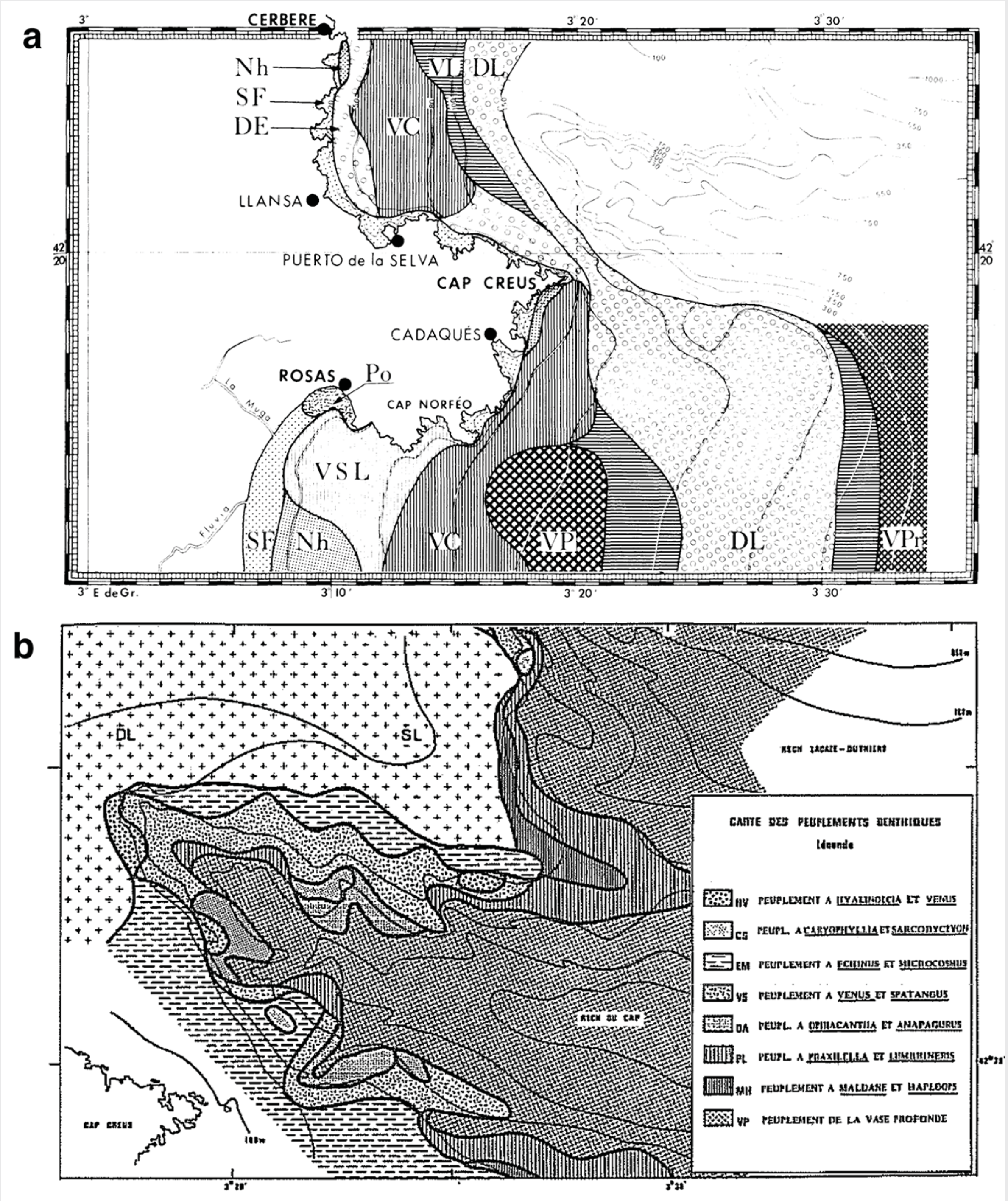


Figure B3.1. Spatial distribution of the main benthic assemblages dwelling on the (a) continental shelf and (b) submarine canyon off Cap de Creus described during the 1970s. Codes in map (a), translated to English, originally in French: SF: fine sands with *Spisula subtruncata*; Nh: transition facies of muddy sands with *Xephtys hombergii;* VSL: variation of the littoral sandy muds with *Nucula sulcata*; VC: variation of the coastal muds; VP: variation of the pure muds; DE: subcommunity of muddy detritic with *Venus ovata*; DL: subcomunity of shelf detritc with *Auchenoplax crinita*; PO: harbour sands and muds; VP: deep-sea muds. Map (a) modified form Desbruyères (1972) and map (b) modified from Reyss (1971).

and *Ophiotrix quinquemaculata* (*O. fragilis*). This last three species were observed to form dense aggregations in certain areas of the shelf, always oriented towards the dominant north-to-south bottom current.

These benthic assemblages showed certain modifications in terms of the relative abundances of certain species when compared to those identified on the French continental shelf in earlier works. The authors attributed such changes to the particular physiography of the continental shelf off Cap de Creus, although there were no major changes in the composition of the different assemblages when compared to other areas of the Gulf of Lions.

The evaluation of the macrobenthic communities of the submarine canyon off Cap de Creus, alongside with its adjacent canyon Lacaze-Duthiers, were performed by Reyss (1971). This work was complemented by a sedimentologic evaluation carried out by Got, Monaco & Reyss (1969). The area explored extended from the end of the continental shelf (limit defined by the author between 120-150 m) all the way down to depths of 1000 m. The methodologies to evaluate the benthic communities, in this case, were different to the methods used by Desbruyères *et al.* (1972) due to the varying nature of the substrate. The authors used two different epibenthic sledges (type "Picard" and type "Charcot"), designed to sample benthic macrofauna both in soft and hard substrates. Assemblages were described using the species identified after a minimum volume of sediment was sorted, and data was complemented using the information extracted from visual observations of the seafloor during several submersible dives from a previous study (Reyss & Soyer 1965), which helped catalogue larger species that rarely got caught by the epibenthic sledge.

The authors identified and described 8 different communities, based on the type of substrate and the dominant fauna (Fig. B3.1b). Those assemblages were as follows:

- **Bottoms with *Kophobelemnon* and *Funiculina***. Corresponded to the deepest part of the canyon, its shallowest distribution being at 300-350 m. This assemblage was observed as a characteristic muddy compact bottom dominated by polychaetes. When slopes were steep, the most conspicuous species were the sponges *Thenea muricata* and *Rhyzaxinella pyrifera*, as well as the octocorals *Kophobelemnon* sp. and *Funiculina quadrangularis*. For these last two species, densities estimated were of about 1 organism per 100 m^2. The characteristic species in the deepest part of the canyon, where the seabed is almost flat, was *Isidella* sp.

- **Bottoms with *Leptometra phalangium***. Found in areas with fine sands, generally between 200-300 m depth. It was observed in various locations within the canyon, mostly appearing in the flanks and also in areas between canyons. 73 species were identified in this assemblage, more than 50% of them being polychaetes. However, *Leptometra phalangium* was the most conspicuous and abundant species, with densities reaching 30-50 ind·m^{-2} in some areas.
- **Bottoms with *Salmacina dysteri***. Found in three different areas of the canyons, all of them being relatively shallow (150-200 m) and dominated by sandy muds and muddy sands. The polychaete *Salmacina dysteri* was the dominant species, forming aggregations of a large number of individuals fixed to the substrate over small stones or large shells. Other large species observed alongside with *Salmacina dysteri* were *Cidaris cidaris*, *Echinus echinus* and *Pteria hirundo*.
- **Bottoms with *Brissingella coronata***. Found in detritic areas with a particularly heterogeneous mixture of muds and sands, where strong currents dominated. The dominant fauna corresponded to large echinoderms, mainly *Mesothuria intestinalis*, *Brisingella coronata* (*Hymenodiscus coronata*) and *Cidaris cidaris*, sometimes accompanied by a relatively large number of ceriathids and the bivalves *Arca tetragona* and *Venus casina*.
- **Bottoms with *Ophiacantha setosa* and *Ophiothrix fragilis***. Found in areas with a mixture of fine sands, coarse sands and gravels in depths of 300 to 350 m. Even if they are not typical from such depths, these two ophiuroid species dominated in this assemblage. The infauna was still dominated by polychaetes, with a reduced number of mollusk species if compared to other gravelly areas.
- **Bottoms with *Microcosmus vulgaris* and *Echinus acutus***. Common assemblage found on the edges of the canyons in contact with the shelf, where the substrate was of a muddy detritic nature. It can be considered an intermediate stage between the detritic bottoms of the shelf and the muddy detritic with *Brissingella coronata* (*Hymenodiscus coronata*). Numerous *Microcosmus* sp. were observed in this assemblage, along with echinoderms of the genus *Echinus*, in this case of a smaller size than those found on the shelf.
- **Bottoms with *Caryophyllia clavus* (*C. smithii*) and *Sarcodyctyon catenata***.

Characteristics of the muddy detritic areas found around outcropping rocks, in depths of 200-400 m. Those two species largely dominated this assemblage, living fixed to shells or small stones that characterized the substrate. The fauna living on the sediment showed a dominance of mollusks, such as *Arca* spp. and *Pycnodonta cochlear* (*Neopycnodonte cochlear*).

- **Bottoms with *Hyalinoecia tubicola* and *Venus casina***. Only observed in a small gravelly area in the canyon head, where these two species dominated.
- **Rocky bottoms**. Different types of outcrops were described by the authors after a series of dives performed in a submersible.
    - **Boulders**. Rocks of large dimensions found in areas of strong currents. Species found living on their surface were the black coral *Antipathes fragilis*, the brachiopod *Gryphus vitreus*, the serpulid *Serpula vermicularis*, the echinoderms *Cidaris cidaris* and *Echinus melo*, some crustaceans of the genus *Munida* and several encrusting sponges.
    - **Slabs**. The authors found differences in fauna composition between horizontal and vertical surfaces. Horizontal surfaces held populations of *Anthipates fragilis*, *Primnoa verticillaris* (?), *Poecillastra compressa* and *Terebratula vitrea.* Vertical surfaces held very complex assemblages, mostly dominated by sponge species such as *Hymeraphia stellifera*, *Hymedesmia versicolor*, *Jaspis jonhstonii* and *Bubaris vermiculata*. Brachiopods and serpulids were also common.
    - **Terraces**. There were differences in the composition of the assemblages on the terraces located either on the northern or the southern flank of the canyon. In the northern flank, between 220 and 250 m and exposed to strong bottom currents, the assemblage was composed by a mixture of circalittoral and bathyal species, similar to the community previously described as “roche du large” (Pérès & Picard 1964). Species commonly observed were the anthozoans *Eunicella stricta* (most possibly *E. cavolini*) and *Dendrophyllia cornigera*, as well as the sponges *Poecillastra compressa*, *Rhizaxinella pyrifera*, *Axinella verrucosa* and *A. polypoides*. In the southern flank, below 250 m depth, areas with strong bottom currents and exposed rocks were characterized by a cold-water coral assemblage. Two species were most commonly observed: *Madrepora oculata* and *Lophelia prolifera* (*L. pertusa*). A set of species

were associated to this assemblage: the anthozoans *Caryophyllia arcuata*, *Desmophyllum cristagalli* and *Corallium rubrum*, the sponges *Poecillastra compressa*, *Phakelia ventilabrum*, *Axinella* spp. and *Acanthella acuta*, the polychaetes *Eunice floridana*, *Acanthicolepis cousteaui* (*A. asperrima*) and *Lagisca drachi* (*Neolagisca jeffreysi*).

The studies performed by Desbruyères *et al.* (1972) and Reyss (1971) led to the production of the first cartography ever made for the study area (Fig. B3.1), which has not been updated in the last 40 years. It is very likely that changes in the distribution of the benthic assemblages may have occurred since those maps were produced, probably due to the increased effects of human pressure and the natural dynamics of the benthic populations.

1.1.3 Methods for the study of marine benthic communities

A. Sampling

The description of benthic communities, such as those exposed in Box III, has traditionally been accomplished through the extraction, sorting and identification of species living either inside or directly above the substrate. Classic methodologies include grabs, corers, trawls and dredges, which were designed for specific purposes (see Eleftheriou & Moore 2013 for a comprehensive review about the different devices available and their potential uses). In general terms, grabs offer a replicable sampling methodology in which the analyzed area can easily be determined, although the technique is restricted to soft bottoms. Furthermore, accurate results rely on highly trained taxonomists with an extensive knowledge of the species living in the sediment. Large or more sparsely distributed epifauna organisms are usually misrepresented and their abundances difficult to estimate. For example, the work that Desbruyères *et al.* (1972) carried out on the continental shelf off Cap de Creus provided a list of 501 different species. More than 75% corresponded to crustaceans and polychaetes, most of which are of a small size and live buried inside the sediment.

The use of benthic trawls can be seen as an alternative to grabs in order to sample less frequent or larger organisms. They also provide additional information about the distribution and abundance of epifauna species, although they are inefficient when sampling large aggregations of organisms or less common species (McIntyre 1956). However, the complexity of assessing spatial changes in assemblage composition in areas of mixed substrates, the inefficiency of capturing certain species and the difficulty of accurately assessing the area sampled can limit their potential use. Furthermore, the technical difficulties already inherent to such methods exponentially increase when studying benthic communities that dwell at great depths.

In contrast to those classic methods, the capacity of ROVs to evaluate large areas of the seabed at a relatively low cost (especially if small ROVs are used) seem to make imaging technology very convenient for underwater exploration and monitoring (Sheehan, Stevens & Attrill 2010). Such methods were already proposed by McIntyre (1956) over 50 years ago after comparing the results provided by a Van Veen grab, an Agassiz trawl and a photographic camera. Recent developments in imaging technology have favored their expansion for the study of the marine benthos, since ROVs can provide in situ, non-destructive, representative and potentially repeatable samples. Video-based research relies on the capacity of the researcher to quantify epibenthic organisms, which need to be sufficiently large to be identified from the images. As expected, ROV studies embrace a certain reduction in the taxonomical resolution when compared to other methodologies, which seems compensated by the short processing time needed to generate results. In fact, the use of ROVs appears to be an ideal solution to overcome the limitations of working in areas with mixed substrates or below certain depths (Smith & Rumohr 2013).

B. Size of sampling unit

Species densities generally vary at a range of spatial scales, so selecting the size of the sampling unit is crucial to correctly identify patterns of abundance (Underwood & Chapman 2013). At the same time, detecting aggregation patterns between species and how they distribute spatially will also depend on the size of the samples selected, which could finally have an influence on the hypotheses to be tested (Andrew & Mapstone 1987). Not many studies have dealt with the problem of scale below SCUBA depths, neither on soft or hard substrates, when characterizing them using ROV images. This is probably due to the most common strategy followed by a large part of the deep-sea surveys, in which patterns in species composition and abundance are evaluated using still images taken directly by the ROV camera during navigation (or even from the video footage) at set intervals of time or distance. In these cases, fauna is individually evaluated for each image and data is then normalized to surface area in order to generate density values, generally as organisms per square meter or hectare.

In the case of this PhD thesis, the methodology that has been developed at ICM to analyze underwater video footage allows us to determine the best size of sampling unit according to the specificities of the study area. In our case, this will be accomplished by developing a specific species/area relationship, a very common methodology to determine the optimal size of sampling unit in marine benthic studies (Weinberg 1978a).

C. Statistical analyses

Even though the tendency in marine management consists in identifying biotopes or habitats through an evaluation of environmental descriptors, which would consist on a top-down approach, the methodology applied during the completion of this PhD thesis followed that of classical studies on marine benthic communities, defined by Ferrier & Guisan (2006) as 'assemble first, predict later'. This approach considers that biological survey data should be first classified, ordinated or aggregated to produce community-level entities and then modeled in relation to environmental predictors. In our case, biological communities were identified and described based on their biological composition and later related to the environmental factors in order to determine the role played by the latter in the distribution of the biological assemblages.

Methods derived from multidimensional statistics have also developed very rapidly in the last few decades, in consonance with the increase in calculation power of personal computers, which can now perform complex multivariate calculations in relatively short periods of time. Multivariate methods are capable of describing the variability of animal assemblages as a whole, taking into account the co-varying nature of ecological data and provide evidence of the structures that underlie such data (Legendre & Legendre 2012). There are numerous statistical packages that implement a variety of multivariate techniques that can be used to analyse the structure of ecological communities and are able to relate them to a set of environmental factors. Examples of these packages include CANOCO (Braak & Šmilauer 2002), one of the most powerful computational programs primarily based on ordination methods, and PRIMER (Clarke & Warwick 2001), initially designed to assess changes in marine communities but now widely used in all areas of ecology.

In the last few years, the free software R (R Core Team 2016) has rapidly become the first choice platform used by many biologist to perform statistical analyses on multivariate their data, mainly due to its versatility and the possibility to program specific routines. Packages like *vegan* (Oksanen *et al.* 2016) provide most of the multivariate techniques needed to understand the underlying processes behind species aggregations and the drivers that determine them.

1.1.4 Objectives of Part 1

Part 1 pursues the following three objectives, which were addressed successively:

1. *Test of the methodology employed.* Even though we are fully aware that ROVs are nowadays very popular tools to evaluate the ecology of benthic organisms, assemblages and habitats, we still wanted to assess the pros and cons of using these vehicles to characterize deep fauna in Mediterranean shelf and slope environments, to finally determine their potential use in a monitoring plan in a Mediterranean offshore MPA. This aspect can be considered the first methodological goal of this PhD thesis.

2. *Description of the natural heritage of Cap de Creus continental shelf and submarine canyon.* Following the demands proposed by Descriptor 1 of MSFD, the first part of the thesis aims to characterize the invertebrate benthic assemblages of Cap de Creus continental shelf and submarine canyon. In order to achieve this goal, 2 successive steps were followed:

    a. Evaluation of the structure of the different benthic assemblages using multivariate statistical methods (how species organize themselves spatially)
    b. Identification of the characteristic species in each assemblage (which species allow to discriminate between communities)

3. *Evaluation of temporal changes.* The results obtained in the first part of the thesis were used to perform a comprehensive comparison with studies carried out in the 1970s, aiming to update the list of benthic assemblages present in the study area and to temptatively estimate potential changes occurred throughout the last 40 years.

1.2 Environmental-vs-fishing effects on assemblage distribution

1.2.1 Environmental factors as driving forces in assemblage distribution

The species composition of the different assemblages identified in a locality should be thought of as an integrative response variable, whose variability is determined by the complex interaction that occurs among several structuring factors (Dray *et al.* 2012). In general terms, differences observed in the distribution of marine species and assemblages respond to 3 types of factors, not considering historic or stochastic events: (1) the control exerted by the environmental parameters, (2) the biological interactions that take place among different organisms and (3) the set of disturbances produced by human activities (Borcard & Legendre 1994).

The structure of the different assemblages found in bottoms of the continental shelf and submarine canyon off Cap de Creus is evaluated in the first part of this thesis. The next step would be to evaluate the specific role played by human impacts, and most especifically bottom fishing, over the spatial heterogeneity patterns displayed by the megabenthic fauna. This cannot be considered a straightforward assignment: it is very likely that the response of the benthic assemblages to a set of environmental parameters might cover up the effects produced by fishing practices. Thus, the potential effects of bottom trawling cannot be assessed directly unless we have previously quantified the influence of other important environmental factors in the distribution of the benthic fauna. At the same time, we can also expect that fishing itself might not be completely independent from other physical factors, and hence, it would not be surprising to find that fishing effort changes spatially following a combination of different environmental parameters. This last aspect is of special relevance when selecting the experimental setting to find indicator species sensitive to varying degrees of fishing intensity (see Part 4).

Identifying the magnitude by which each structuring factor determines the observed spatial structures can provide valuable information when assessing the potential distribution of marine species and assemblages over large spatial extents. Obviously, not all potentially important factors, both physical and biological, can be considered in ecological studies, and there exist economical and technical limitations to the quantity and quality of the data gathered, especially at great depths. In this sense, biotic interactions like predation and competition are very difficult to measure, so they tend to get ignored or disregarded in broad-scale ecological studies. Furthermore, within the set of accessible abiotic variables, choosing which factors are relevant and should be included in the analyses must never be a random decision. But even if in most cases it responds to the knowledge that has historically been collected about the type of communities under study, it also depends on the specific measuring gears on board of the research vessels. In the case of continental margin biota, we would expect its distribution and abundance to be strongly related to environmental parameters of a geological, hydrographical and anthropogenic nature.

1.2.2 Environmental and human factors considered in this PhD thesis

A. Geomorphological factors

It is widely accepted that depth and substrate composition are two very strong driving forces dictating the spatial distribution of marine benthic assemblages. Although depth cannot be considered a factor as such, but a major ecological axis where a compound of environmental factors converge, it is the first to be identified, since changes in species composition along depth gradients have long been observed in benthic environments. In fact, clear depth-related zonation patterns have been reported from shallow littoral environments (Chappuis *et al.* 2014) all the way to the deep sea (Vinogradova 1961).

In a similar way, local changes in community structure have long been related to changes in the composition of the seabed substrate (see the early work of Gray 1974 for a review). Rocky outcrops tend to have completely different assemblages than in sedimentary areas, and even within soft sediments, particle size plays a key role in determining the dominance of certain species over others (Etter & Grassle 1992). Besides depth and substrate type, sea bed complexity has also been identified as a source of heterogeneity for benthic assemblages, which can play a fundamental role in the distribution of marine fauna at greater scales (Mortensen *et al.* 2009). For this reason, the geological parameters selected to understand differences in assemblage composition and species abundance include not only depth and substrate type, but also slope, rugosity of the seabed, its topography and the main orientation of the seabed.

B. Hydrographical factors

Although water currents have not received as much attention as other environmental parameters, their influence on megafauna abundance has been identified by different authors (Mohn *et al.* 2014; Genin, Paull & Dillon 1992). Cap de Creus is known to be a very active area in terms of water fluxes, with recurrent episodes of dense shelf water cascading being reported every so often (Canals *et al.* 2006). Considering the effects of bottom currents in shaping the seabed and their relation to sediment transport to the deep sea, we wanted to explore their role in the structuring of the benthic assemblages. Making use of current speed data generated through the free surface, generalized sigma vertical coordinate 3D hydrodynamic model SYMPHONIE, mean annual velocities, yearly variability and current direction were obtained to further understand the importance of water fluxes in the distribution of megafauna species.

C. Anthropogenic factors

The influence of fishing activities over benthic fauna has been evaluated for decades in different habitats of the world's seas. Bottom trawling can be considered a strong driving force in shaping benthic assemblages since it can destroy habitats and reduce biodiversity, especially when key structuring species are affected (Coleman & Williams 2002). The availability of Vessel Monitoring

System (VMS) data from our study area gave us the possibility of understanding to what extent commercial activities with mobile bottom gears are shaping the benthic assemblages of the continental shelf and submarine canyon off Cap de Creus.

1.2.3 Objectives of Part 2

In summary, the second part of the thesis aims to:

1. Evaluate the overall effect of the selected environmental parameters over the distribution of the benthic fauna in the study area, incorporating the activity of commercial bottom trawling as an additional factor.
2. Hierarchically ordinate the relative role played by each factor.
3. Analyze the relationship between the intensity of fishing activities and the remaining environmental parameters.

1.3 Modelling the distribution of species and assemblages

1.3.1 The need for distribution maps

A growing number of marine ecosystem managers believe that state-of-the-art management measures should always consider the spatial component, which needs to be particularly well defined if areas of conservation priority are to be identified or if long-term changes in species composition and abundances are to be monitored (Cogan *et al.* 2009). Ideally, management plans and the implementation of conservation measures should be based upon detailed knowledge about the geographical distribution and ecological characteristics of the resource of interest, be that a single keystone species, a particular assemblage or even a preferred habitat for reproduction (Reiss *et al.* 2015). In this sense, implementing a coherent legislation to protect the oceans and developing measures to effectively manage marine resources cannot be achieved unless we produce accurate maps displaying the distribution of benthic species, assemblages and habitats, as well as the potential threats to the welfare of the ecosystem due to human activity (Brown *et al.* 2011).

Understanding the relationship between the benthic fauna and the abiotic environment not only provides relevant ecological information about species and assemblages, it also sets the basis to potentially estimate their distribution along large spatial scales using mathematical models (Guisan

& Zimmermann 2000). Since maps can be regarded as spatially explicit data syntheses of different habitat conditions and can integrate a series of basic indicators (Cogan *et al.* 2009), they are an excellent way to communicate information and should be considered an essential requirement when managing human activities in the marine environment for conservation purposes.

One of the key aspects of the ecosystem-based approach that has been endorsed by the MSFD is the consideration of the spatial component in the decision-making through an integrated process, which should lead to location-specific management measures and the delimitation of areas that require a special protection (Council of the European Union 2008). Such an approach has recently received much attention by the scientific community under the discipline of Marine Spatial Planning (MSP), which aims to establish a more rational organization of how the marine space is used by determining the spatial and temporal distribution of human activities in a way that balances the demands for development with the need to protect the marine ecosystem (Ehler & Douvere 2009). Ecosystem-based MSP is based upon four key ecological principles, two of which place their focus in the maintenance or restorement of native species diversity and habitat diversity and heterogeneity (Foley *et al.* 2010). For this reason, the EU Commission Decision 2010/477/ EU (European Commission 2010) requested Member States to use GIS modeling tools to map a wide range of biodiversity features and human pressures in the different MPAs created under the MSFD. These maps should include species and assemblage distributions, directly setting the objectives proposed for Part 3 of this PhD thesis.

1.3.2 Elaboration of maps

Distribution maps of benthic fauna exist since the nineteenth century, but the methodologies to produce them have evolved very rapidly in the past two decades. Initial maps of marine benthic assemblages were based on the information extracted from large sets of biological samples collected using remote sampling techniques, such as grabs or epibenthic sleds. This type of approach requires large amounts of time and effort to process the material, it requires trained taxonomists and it also has a very limited spatial coverage (Eleftheriou & Moore 2013). In fact, our knowledge about the extent, geographical range and ecological functioning of many marine habitats dwelling below certain depths is still very scarce due to the limitations imposed by traditional methods to survey the bottom of the ocean and the lack of full-coverage environmental data (Brown *et al.* 2011).

The challenge at the turn of the century was to demonstrate that video recordings of the seabed using ROVs could provide the necessary information to build species/assemblage distribution maps in a more precise an efficient way than those offered by classic methodologies. The study of benthic megafauna via imaging techniques presents several advantages over other remote methods, with their non-intrusive character being its fundamental difference. The spatial coverage provided by ROV surveys can be considered relatively larger than, for example, Van Veen grabs, but it is still very limited if vast areas of the continental shelf or the slope are to be evaluated.

It has only been recently that scientists have considered the possibility of combining data extracted from ROV images with abiotic information to produce extensive maps of the seafloor. The methodology employed basically uses the statistical correlation found between the biological components and different environmental parameters, such as depth, substrate type or regional oceanography (Reiss *et al.* 2015). There exists now a wide range of publications that have used underwater images to produce valuable distribution maps for keystone species (e.g. Dolan *et al.* 2008; Fosså *et al.* 2005), commercial fishes (Robinson *et al.* 2011b) and benthic assemblages (e.g. Gonzalez-Mirelis & Lindegarth 2012; Buhl-Mortensen *et al.* 2014; Preez, Curtis & Clarke 2016), which are of key importance to implement ecosystem-based management measures in different marine protected areas of the world's seas.

In a synthetic way, the production of species and habitat maps requires not only a strong relationship between the biological variable(s) under study and a set of environmental parameters, but also a statistical method to predict its expected distribution in areas for which only environmental data is available (Brown *et al.* 2011). Notwithstanding the widespread use of abiotic maps as surrogates for species occurrences, biological distribution maps are currently drawn based upon three general strategies (further details in Ferrier & Guisan 2006 and Brown *et al.* 2011):

I. 'assemble first, predict later'. This methodology requires two successive steps. First, the biological data obtained from samples or video images is classified into community-level entities without making use of the environmental information, using multivariate classification or ordination techniques. Secondly, those entities are spatially modeled according to their relationship with environmental factors.

II. 'predict first, assemble later'. This method models the spatial distribution of individual species as a function of the environmental variables, generating a set of maps, one for each species. Once all grids have predicted information for each species, multivariate classification or ordination methods are then applied.

III. 'assemble and predict together'. This technique simultaneously uses the data for all species in a single integrated modeling process, generating a "multiresponse" model.

Any of these three strategies requires specific predictive modeling techniques to produce full coverage maps of benthic species, assemblages or habitats. There is a wide array of methodologies at the disposal of any researcher, which include general additive models (GAM), classification trees (e.g. Random Forest), maximum entropy models (MaxEnt), or even simple ordination methods (Reiss *et al.* 2015). One of the advantages of using these modeling techniques is the possibility to assess their predicting power based on the available biological and environmental data, which provides estimates of the accuracy of the map. This will be very valuable to determine if the ROV images obtained in Cap de Creus shelf and submarine canyon can be considered suitable to feed predictive mapping models.

For this PhD, the strategy 'assemble first, predict later' was employed. The community-level entities defined with multivariate clustering techniques (Part 1) and the set of environmental variables selected were combined using the algorithm Random Forest (Breiman 2001) to generate predictive maps. Although this algorithm has only been implemented in ecological studies during the last 10 years, it is now widely used due to its high classification accuracy, the possibility to determine the relative importance of the different abiotic factors and the ability to model complex interactions among predictor variables (Cutler *et al.* 2007). Furthermore, one of the advantages of predicting with RF is that it can be used to model continuous variables and categorical data, which gave us the possibility to use the same algorithm to generate all predictive maps.

1.3.3 Objectives of Part 3

The third part of the thesis aims to generate a series of predictive maps to show the potential distribution over the continental shelf and submarine canyon off Cap de Creus of

1. the biological diversity of the benthic megafauna (species richness and α-diversity using the exponential of Shannon index)
2. the biological assemblages that derive from the species by sites dataset, which are described in Part 1.

The validity of the maps created was assessed based on the quality of the data employed and the accuracy of the methods used. Finally, the potential use of those maps in the future ecosystem-based management plans to be implemented in the offshore MPA of Cap de Creus is also discussed.

1.4 Effects of commercial fishing activities on megafauna assemblages and selection of descriptors

1.4.1 Assessing the effects of bottom trawling

Even though the number of human activities that have a negative impact on the marine environment is large, overfishing can still be considered a key factor in the collapse of many coastal and deep-sea ecosystems (Jackson *et al.* 2001). There exists a strong consensus among the scientific community, together with a certain degree of concern, about the destructive role that commercial bottom trawling has had on the distribution and diversity of benthic fauna in the different seas and oceans (Clark *et al.* 2016). In fact, some researchers have compared this type of fishing practices to forest clear-cutting, since it crushes, buries and removes all structures of the marine benthic environment, becoming a major threat to biodiversity and economic sustainability (Watling & Norse 1998). Although its severe consequences for the natural environment are currently well understood and there exists a wide array of examples that report the depletion of certain commercial stocks, specially in deep environments (Norse *et al.* 2012), the activity of commercial fleets has not ceased during the past few decades, and a large part of Europe's continental shelf and slope is currently being trawled by mobile bottom gears (Eigaard *et al.* 2016). In fact, the steady reductions in the landings of exploitable species has moved fishing practices further offshore in the search of more profitable resources, increasing the mean depth of fishing from 100 m in the 1950's to below 150 m in the 2000's (Morato *et al.* 2006). In the case of the Mediterranean Sea, no large-scale areas have escaped the activity of commercial trawlers, and heavily exploited sites can be found in areas of the Adriatic Sea and south of Sicily, among others (UNEP/MAP 2012a).

The first studies that evaluated the effects of bottom trawling on the marine biota placed their focus at the level of the target resource, either demersal fish or shellfish species. In general terms, their results showed that overfishing was causing a transition from long-lived, high trophic level, piscivorous fishes towards short-lived, planktivorous pelagic fishes, which indicated that a change in the structure of marine food webs was taking place (Pauly *et al.* 1998). It was not until the 1990s that the scientific community started to evaluate the potential changes caused by mobile gears on the benthic environment, first by assessing the changes in the composition of shallow infauna and macrofauna assemblages using extracting techniques (see the review of Jennings & Kaiser 1998 for further references). Since then, the number of studies that have evaluated the negative impacts of commercial fishing activities over the benthic realm has increased and moved towards deeper areas (Clark *et al.* 2016), taking advantage of modern technologies such as sidescan sonars, the

Vessel Monitoring System (VMS) and high definition video cameras. Results from recent studies show that long-term bottom trawling activities not only have negative effects over the structure and diversity of benthic communities (see for example the work of Buhl-Mortensen *et al.* 2016), but it can also alter deep-sea sediment fluxes and modify the shape of the upper continental slope (Puig *et al.* 2012).

To quantitatively demonstrate the effects of bottom trawling on the diversity and structure of benthic assemblages, the experimental setting has to be designed in a way that allows us to differentiate between the effects of mobile fishing gear and those caused by other abiotic factors. As a matter of fact, different natural factors such as depth and substrate type are also drivers of the structure of benthic and demersal assemblages, and they directly compete with fishing practices in influencing the global distribution of epibenthic species. Furthermore, trawling may have more severe and long-lasting effects on deep assemblages than on shallow communities due to their slow individual growth rates and their limited capacity of recovery (Thrush & Dayton 2002). Similarly, benthic assemblages found on hard substrates might find fishing effects more devastating that those dwelling on soft sediments. In this sense, Part 2 of this thesis looks at the combined effect on the diversity and structure of benthic assemblages of all measurable abiotic factors for which there is data available in Cap de Creus area (including fishing intensity), in order to determine the relative role played by trawling activities among the other parameters.

Testing the specific effects of bottom trawling on the distribution of marine organisms, however, requires an experimental design that limits the potential influence of other driving factors, and would largely benefit from a BACI (Before/After and Control/Impact) experimental design. Since there tends to be a limitation in the number of areas where this type of surveys can be implemented, a 'compare-and-contrast' type of experiment is generally more suited to examine biological differences between areas that have similar environmental conditions but are known to have historical differences in their fishing intensity. This is the approach taken in Part 4 of this thesis, which looks at how equivalent areas in terms of the main abiotic factors may support distinct faunal compositions and diversities depending on the intensity of the commercial fishing practices.

1.4.2 Searching for descriptors of bottom trawling activities

The adaptive management approach promoted by the MSFD requires long-term evaluations to determine if GES is being achieved or maintained in the different marine areas of Europe (Council of the European Union 2008). Such evaluation has to be based on a set of robust indicators fully implemented in a well-defined monitoring program, which will determine the effectiveness of the different spatial and temporal management measures that are being applied (Van Hoey *et al.* 2010). It seems reasonable that a critical aspect in any monitoring program is the selection of the right indicators.

In essence, any ecological indicator used in a long-term monitoring program should be able to capture the complexity of the ecosystem, provide a measure of change caused by an environmental phenomenon and also be simple enough to be monitored on a regular basis (Heink and Kowarik 2010). For this reason, a good biological indicator has to fulfill at least the following requirements, as proposed by Dale & Beyeler (2001):

1. Statistic practicality: it has to be abundant, representative or easy to identify/quantify and also capable of generating robust long-time numerical series;
2. Sensitive to the disturbance under study: in our case, its value has to respond to differences in the intensity of bottom trawling;
3. Provide an unambiguous response to changes in the magnitude of the disturbance;
4. Display a low variability in the response, so background processes do not cover the variability produced by the disturbance;
5. Be cost-effective, in a way that its measure at sea (or sample collection and treatment, if necessary) should not represent an excessive expenditure;
6. Socially comprehensible and easy to explain: in this case, biological species are preferable to abstract biological parameters, such as indices of community structure or diversity, which are more complicated to understand and explain to the general public.

When dealing with continental shelf environments and how to monitor the effects of overfishing at the ecosystem level, the scientific community is still relatively far from reaching an agreement on what descriptors are best suited to accomplish such tasks, and further research is needed to determine the techniques to be used in an effective monitoring program. From this point of view, and once the role played by each abiotic factor in the distribution of the marine benthic fauna is determined, as well as the interaction between the different factors and fishing intensity, Part 4 aims to go a step further and determine:

1. the capacity of underwater filmings made by ROVs to detect the effects produced by different intensities in the activity of bottom trawling, isolated from all other factors,
2. what descriptors (indices, assemblages and/or species) are sensitive to fishing practices and can be used as indicators in the future monitoring program of Cap de Creus.

To evaluate the potential effects of commercial fishing practices, Part 4 will limit the experimental setting to those video sequences recorded in areas that present similar topographic conditions and, at the same time, are also susceptible (or adequate) to fishing activities that use mobile gear. Furthermore, in order to determine the effects of increasing intensities, there will be a search for a gradient in the frequency of activity of bottom trawlers, which will be determined using georeferenced data provided by the Vessel Monitoring System (VMS).

Using the subset of ROV transects that accomplish the requirements of this experimental design, there will be a search for evidence (including correlational evidence) of the sensitivity to fishing intensity of two types of indicators:

1. abstract descriptors at the level of community, including species richness, biological diversity and community structure
2. specific indicator species, which are far less ambitious but readily measured and easier to communicate

1.4.3 Objectives of Part 4

Part 4 of this thesis aims to evaluate the impact of commercial fishing activities over the benthic fauna of the continental shelf off Cap de Creus using an experimental design that attempts to limit the effects produced by other environmental factors. After selecting a set of environmental conditions to restrict the influence of other parameters, several biological descriptors were tested against different levels of fishing intensity. The effects of fishing were examined at three different levels of complexity: (1) species richness and biological diversity, (2) the structure of the invertebrate community as determined by species composition and abundance and (3) selected key species commonly found on the continental shelf and that can be easily identified in the video images.

1.5 Monitoring design and baseline definition

1.5.1 Adaptive management of MPAs unde the MSFD

According to the Marine Strategy Framework Directive (MSFD), Member States must identify and put into practice a series of management actions in order to achieve or maintain good environmental status (GES) in their territorial waters (Article 13) based on a comprehensive set of environmental targets and associated indicators (Article 10). In order to assess through time the rate at which the environmental targets are being achieved, the MSFD requires Member States to implement an adaptive approach to management, which requires the development of specific monitoring programs in the MPAs declared under the MSFD (Article 11; Council of the European Union 2008). Adaptive management means that decisions should be taken in an iterative way between management actions after evaluating their effects on the ecosystem (Parma 1998). The launching of monitoring programs should facilitate the evaluation of the efficacy of the management measures implemented in each MPA in a rigorous and efficient manner during the forthcoming decades, in a way that management practices are periodically reviewed or updated, and new measures can be incorporated depending on the information available or if circumstances change. The biological diversity of the benthic ecosystem was included under Descriptor 1 of the MSFD, and a set of parameters have already been proposed as potential indicators to determine the rate at which GES is being achieved (see Zampoukas *et al.* 2012 for further details).

In the case of the Spanish State, the protection of 10 offshore areas as part of the Indemares project came along with an evident difficulty: monitoring the evolution of their benthic ecosystem through time requires access to the sea bed in a cheap, simple and replicable manner. Long-term monitoring on a continuous basis can become a complex and expensive task, especially when dealing with depths below 100 m (Day 2008). The use of classical methodologies to extract biological samples for monitoring seems now less adequate compared to the potential of modern techniques, primarily due to the invasive nature of some traditional sampling gears (e.g. grabs and sleds). Furthermore, classic methods often demand large efforts to sort samples and require trained taxonomists to correctly identify the different infauna/epifauna species, especially if every individual is to be accounted for (Hartley 1982). For this reason, one of the objectives of this PhD thesis is to develop a monitoring protocol suitable to evaluate changes to the megabenthic communities dwelling on the continental shelf off Cap de Creus using data obtained from ROV images. The implementation of the proposed methodology should allow stakeholders and managers to document long-term changes in the composition and diversity of benthic fauna after management measures have been applied.

1.5.2 Monitoring the evolution of MPAs using ROVs

Monitoring benthic ecosystems through time after the application of protection measures is a common practice currently implemented in many MPAs around the world. Most of the long-term evaluations in shallow areas are SCUBA based, making use of a wide array of techniques: visual censuses (Sala, Garrabou & Zabala 1996), photographs (Linares *et al.* 2012), baited underwater video (BUV) (Stobart *et al.* 2015) and even underwater video transects performed by SCUBA divers (Tilot *et al.* 2008). Deeper areas, on the contrary, have received less attention in this respect and very few monitoring programs currently assess the evolution of benthic assemblages dwelling on continental shelves, submarine canyons or sea mounds. There exist experimental bottom-trawl surveys aimed to asses the status of demersal fish stocks on areas that are subject to fishing practices, as it is the case of the MEDITS program in the Mediterranean Sea, which evaluates the state of various shelf populations (Bertrand *et al.* 2002). Similarly, a large scale monitoring program based on experimental beam trawls has recently been designed for the Barents Sea with the objective of evaluating the evolution of invertebrate populations dwelling on the soft bottoms of the continental shelf (Jorgensen *et al.* 2015).

The use of underwater images and videos to monitor the seabed seems to be a very convenient technique, primarily due to (1) its non-destructive nature, (2) its greater affordability in recent years, (3) its quick data retrieval, (4) the possibility of obtaining quantitative information of species abundances and (5) its use in outreach programs, where the efficacy of protection measures can be shown to a wider audience of non-scientists (Bicknell *et al.* 2016). To our knowledge, no official ROV-based monitoring program is currently implemented in offshore MPAs in order to evaluate temporal changes in the abundance of deep-sea benthic invertebrate species. Certain studies have, however, used imaging technology to evaluate changes in the condition of the deep-sea fauna after the closure of marine areas to fishing practices (e.g. Huvenne *et al.* 2016; Bennecke & Metaxas 2017) or even after an oil spill (Etnoyer *et al.* 2015). Also, recent developments in technology have brought new possibilities to perform a continuous monitoring of deep-sea areas, including the use of Automated Underwater Vehicles (AUVs) (Morris *et al.* 2014) and the implementation of a network of permanent deep-ocean monitoring stations (Danovaro *et al.* 2017). None of these two alternatives, however, seems currently applicable to Mediterranean offshore MPAs due to their complexity and expensive costs.

1.5.3 Experimental design and baseline information

One of the pillars of a correct monitoring program consists in providing robust evidence that there exists a causal relationship between the creation of a new protected area and the eventual recovery of the communities that were exposed to the disturbance, in our case, bottom-trawling activities. Such demonstration requires the formulation of very clear hypotheses together with an adequate experimental design (Yoccoz, Nichols & Boulinier 2001). An intense debate among ecologists took place in the 1990s to determine what statistical analyses were best suited to demonstrate the effects of human impacts on wild fauna. There seemed to be a certain consensus that impacts on natural populations should be evaluated using a Before/After and Control/Impact (BACI) design, which compares at different times (before and after the impact occurred) a Control site (unaffected by the human activity) and an Impact site (closed to the activity and showing an effect if it exists). Such experimental design aims to separate the effect of the human activity from other sources of spatial and temporal variability (Osenberg & Schmitt 1996). The idea behind a BACI design is that a different pattern of change in the abundance of, let's say, a certain species should be observed between disturbed (impact) and non-disturbed (control) areas when comparing data gathered before and after the disturbance started. This change in pattern should be detected in an analysis of variance (ANOVA) as a statistically significant interaction between the effects of the factor "time" and the factor "impact" (Underwood 1992). Further developments of this approach led to Beyond-BACI sampling designs, which consider not one but several control locations. This asymmetrical design is capable of reliably detecting a variety of environmental impacts, including those that do not affect long-run mean abundances but generate a change in the temporal variability (Underwood 1994).

When the "human impact" to be tested corresponds to the application of protection measures, such as the creation of an MPA where human activities (i.e. fishing) are prohibited, the treatments in the design are reversed: "control" treatments are represented by localities where fishing activities continue as usual and localities inside the new MPA, where fishing is forbidden or strictly regulated, become the "impact" condition. In many cases, the unpredictable nature of natural and anthropogenic disturbances could limit the application of BACI designs due to a lack of data reported before the "impact" (i.e. the "Before" condition). This is not the case of management initiatives that are planned in advance, such as the demands listed in the MSFD. The legal background imposed by the EU establishes the obligation by Member States to implement their monitoring programs prior to executing the management or protection measures, such as the creation of MPAs, to generate data to be compared with future evaluations (Council of the European Union 2008).

In our case, the experimental design behind the proposed monitoring protocol aims to test two main hypotheses. Our primary hypothesis deals with the direct effects of fishing on the benthic populations. We expect that, as fishing intensity inside the limits of the new MPA is drastically reduced, the abundance of large megabenthic species will increase, together with the overall diversity and species richness of the community. Our second, more ambitious hypothesis, deals with the capacity of the benthic fauna to recover in areas that have been subjected to different fishing pressures. We expect the recovery rate of benthic species to be directly related to the activity of bottom trawling, with areas heavily transformed by years of intense fishing activity showing a reduced capacity of recovery, both for populations of key species and the overall community.

The attention placed on extracting robust quantitative data from the video images gathered in Cap de Creus has been useful not only to satisfy the demands of the Indemares project (description of the ecological features of the benthic environment) but also to offer a database of numerical records that can be replicated in future sampling events during the forthcoming years. The descriptors that show some correlative evidence of being sensitive to different degrees of fishing intensity (Part 4) are proposed as the "Before" data for the monitoring of the MPA for the next years. Furthermore, a selected group of transects has been sorted and a step-by-step protocol is provided in order to set the basis for this experimental testing. The future surveys aimed to repeat the ROV transects will have to be performed by the ruling authorities in order to obtain the "After" data, which will be crucial to determine if the management measures applied in the area can be considered a success.

1.5.4 Objectives of Part 5

This PhD thesis cannot provide the results of the BACI experiment described above due to the lack of the "After" data, but it can provide the most valuable information for the future monitoring program of Cap de Creus offshore MPA:

1. The BACI experimental design to be adopted, spatially explicit and properly replicated
2. The baseline data for the species/descriptors in each selected locality to be used for comparison with new data after management measures are implemented (the "Before" data).

We are aware that the application of this proposal might become rather complex if only the experimental design and the "Before" data are made available. For this reason, this thesis provides an unambiguous protocol with a detailed explanation of the technique to be used to monitor the evolution of the benthic fauna of the continental shelf off Cap de Creus using an ROV. An exhaustive description of the different stages required to successfully implement a monitoring program is provided, from sampling at sea all the way to image analysis, data treatment and interpretation. The implementation of the proposed protocol will provide a direct answer to our primary hypothesis about the beneficial effects for the benthic fauna of creating a marine protected area. Regarding the second hypothesis, a BACI design will be proposed to test for hypothesized changes in key descriptors (richness, diversity) and a set of megafauna species before and (several times) after the implementation of fishing restrictions in a set of control (left unprotected) and impacted (protected) sites. Since current fishing intensity can be recognized as a factor that may condition the response to the treatment, separate test are proposed for three levels of fishing intensity.

1.6 Characterization of demersal fish populations

1.6.1 Management of fish stocks in the frame of the MSFD

Fishing is one of the most important industries worldwide. Only in the Mediterranean region, landings exceed 1.7 million tons per year, with Italy, Turkey, Greece, Spain, Tunisia and Algeria accounting for more than 85% of the catches (UNEP/MAP 2009). Target species vary locally, but fish remain the main focus of attention for the fishing industry in the Mediterranean Sea due to its high economical return. Fishing activities occur mostly in coastal waters since the continental shelf is the preferred habitat for a large number of commercially exploited species, including the hake (*Merluccius merluccius*), different species of mullet (*Mullus* spp.), the whiting (*Micromesistius poutasou*), several species of angler fishes (*Lophius* spp.) and the sea bream (*Pagellus* spp.) among others (UNEP/MAP 2012b). Most Mediterranean demersal fisheries are currently going through a difficult situation, with large part of the stocks fully exploited or overexploited, a trend that can be observed as a decrease in the average individual lengths of the fishes and in the number of fish caught per unit of effort (Papaconstantinou & Farrugio 2000).

Due to the poor conservation status of some economically important species in the Mediterranean and other Atlantic areas, the MSFD also set as a priority the recovery of commercial fish stocks through a sustainable fishing industry. Specifically, the MSDF demands Member States to ensure that "populations of all commercially exploited fish and shellfish be within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock" (Descriptor 3; Council of the European Union 2008). Besides the specific management measures enforced by the EU to improve the conservation status of commercial fish species in traditional fishing grounds (not discussed here), the creation of a network of MPAs as part of the demands included in the MSFD should also benefit populations of a wide array of demersal species.

Many littoral marine reserves have historically proven their positive effects on local fish populations by contributing to increase the abundance and the overall species richness of the fish community when compared to other adjacent fished areas (e.g. García-Rubies & Zabala 1990; Claudet *et al.* 2006). In theory, the creation of no-take zones should allow exploited populations to reach a higher degree of maturity, not only through an increase in their abundance but also in the average size of the individuals due to a significant reduction in adult mortality (García-Charton *et al.* 2008). Furthermore, the establishment of MPAs not only favors the recovery of commercial species inside the limits of the no-take zone, it could also promote the export of adult fishes and larvae to neighboring non-reserve sites, a phenomenon defined as spillover. The effectiveness of marine reserves to provide surrounding areas with fish exported from no-take zones has proven relatively limited in the Mediterranean region (e.g. Harmelin-Vivien *et al.* 2008; García-Rubies, Hereu & Zabala 2013), but this might be due to increases in the fishing pressure around the boundaries of the different MPAs.

1.6.2 Monitoring fish abundances using ROVs

To evaluate the success of MPAs designated under the framework of the MSFD, long-term monitoring programs should not only place their focus on the invertebrate fauna, but also on the composition and abundance of the fish community (Cochrane *et al.* 2010). Many shallow marine protected areas have already implemented monitoring programs using the abundance of certain fish species as their main ecological indicators, some of which proving to be the most evident, constant and easily recorded indices of the 'reserve effect' that is being tested (Harmelin, Bachet & Garcia 1995). In these situations, fish abundances are generally estimated via underwater visual censuses, perhaps the most accepted non-destructive technique to evaluate temporal changes in shallow areas (Pelletier *et al.* 2011).

In the case of offshore MPAs, monitoring plans designed to evaluate the dynamics of benthic assemblages could benefit from visual techniques (like ROVs), which could be used to assess the evolution of the invertebrate fauna and the fish stocks at the same time. This could result in a reduction in the number of surveys necessary to implement the monitoring plan (reducing the overall cost), and would also limit the use of conventional, more intrusive techniques (like experimental trawling), which would probably be regarded as unacceptable inside newly declared MPAs (Cappo, Harvey & Malcolm 2003).

Long-term monitoring programs require ecological indicators that provide consistent data and display very low variability in the response, so statistical analyses can provide conclusive results (Dale & Beyeler 2001). Unlike sessile fauna, the great mobility of fishes can generate important biases when evaluating their abundance and distribution. The capacity of ROVs to provide true fish densities has been assessed in recent studies, with varying results. Some experimental trials in shallow tropical coral reefs suggested that video images can underestimate fish abundance by up to 40-50% in some cases, and might detect 30% fewer species compared to underwater visual census (UVC) or underwater camera systems operated by SCUBA divers (Pelletier *et al.* 2011).

At greater depths, the use of vehicles equipped with powerful lights and noisy engines, attached to the surface by an umbilical, might disrupt the normal behavior of demersal fishes and generate an extra bias in the estimate of deep-sea fish abundance (Stoner *et al.* 2008). Although some authors have experimentally proven that ROVs can provide higher estimates of deep-sea fish abundance compared to experimental trawls (up to one order of magnitude higher than an Agassiz trawl; Ayma *et al.* 2016), some fish species have been observed to intentionally avoid the ROV, displaying a very marked disruptive behavior (Trenkel *et al.* 2004).

Despite these caveats, two American MPAs have already implemented monitoring programs using ROVs to evaluate the recovery of fish populations after the application of management measures: California Channel Islands (Karpov *et al.* 2012) and British Columbia's Rockfish Conservation Areas (Haggarty, Shurin & Yamanaka 2016). In both sites, monitoring plans include an assessment of the density of different fish species inside and outside the limits of the MPA for several years after their creation, although no "before" data is available. In both cases, no differences could be detected in between years in the abundance of any of the selected fish species due to the 'reserve effect', and differences in density must be basically attributed to other environmental factors, such as substrate type.

1.6.3 Objectives of Part 6

While the ability of ROVs to provide true estimates remains under debate, Part 6 of this thesis aims to tentatively evaluate the possibility of using the same ROV images recorded to characterize the benthic megafauna to monitor the evolution of fish populations through time. The results of the video analysis will be compared with data obtained in a previous study performed in the area that evaluated the catches of commercial trawlers at different times of the year (see the work of Mallol 2005). An attempt will be made to determine what proportion of the fish diversity can be captured using ROV images and whether the recorded densities are sufficient to sustain a robust monitoring protocol.

At the same time, making use of the extensive footage, this PhD thesis aims to go a step further and provide detailed information about the diversity of the demersal fish communities that is captured by the ROV, aiming to (1) produce a detailed catalogue of the fish species observed in the ROV images and (2) classify the fish fauna in distinguishable assemblages to finally determine their relationships with the set of environmental parameters selected.

1.7 Presence of marine litter

1.7.1 Marine litter in the MSFD

The negative effects produced by commercial fishing activities over benthic communities have long been a focus of attention by the scientific community, and a large number of studies have addressed this issue (Hiddink *et al.* 2017). Nevertheless, oceans are not only exposed to the impacts caused by the direct extraction of biological resources, many other human activities have also been identified as potential threats to the marine realm. The number of activities that put at risk the stability of the marine ecosystem has increased steadily in the last century and the list of impacts to be considered is fairly large: oil drilling, dredging, chemical pollution of rivers and seas, rise in temperature and acidification due to climate change, introduction of invasive species or dumping of marine litter, among others.

Not surprisingly, the disposal of materials manufactured by human societies that reach the seabed or get washed onto the shore is steadily becoming a critical issue in certain areas, with 4.8 to 12.7 million tones of plastic entering the ocean every year (Jambeck *et al.* 2015). The rate at which marine litter accumulates in different areas of the world has alerted researchers, stakeholders as well as national and international authorities worldwide, which foresee the consequences of not addressing a problem that generates not only negative environmental impacts, but also has economic, health and aesthetic implications (UNEP 2009).

For this reason, the MSFD intends to reduce such problem by including the presence of marine litter as an issue to be taken into account by each Member State when implementing management plans in their marine waters (Descriptor 10; Council of the European Union 2008). Following the definition proposed by UNEP, the Working Group on GES for the implementation of MSFD defined marine litter as ‘any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment’ (Galgani *et al.* 2010). The MSFD provides the legal framework to encourage Member States to report and subsequently monitor the abundance of marine litter in their territorial waters. The application of the MSFD should lead to the implementation of area-specific management measures in order to reduce the amount of marine litter that reaches the marine environment and accumulates over the seabed, taking specific actions to minimize the land-based sources when necessary (Galgani *et al.* 2013). The application of such measures, however, must be based upon a comprehensive knowledge of the actual composition, abundance and spatial distribution of the marine litter.

Box IV

Harmful effects of marine litter on the benthic fauna

There is a general consensus that marine litter of an anthropogenic origin is a serious threat to the marine ecosystem, in particular to wildlife. Besides the aesthetic, health and economical impacts derived from the accumulation of human derived objects in shores and over the seabed, marine litter produces a variety of negative effects on the environment, since it can kill or maim all sorts of marine organisms, from algae to birds (UNEP 2009).

The number of deleterious effects that marine litter produces on wildlife is extensive, but it could be summarized following the classification provided by Kühn, Bravo Rebolledo & van Franeker (2015):

1. Entanglement of marine organisms, which is generally associated to lost fishing gear. The term ghost fishing was introduced to describe the lost or abandoned fishing gears that continue catching after fishermen have lost control over them (Breen 1989). Ghost fishing occurs over most taxa, including sessile organisms such as corals and sponges, which can get entangled by the monofilaments of gillnets or longlines, or even by trammel nets moved by the bottom currents (e.g. Yoshikawa & Asoh 2004). Global estimates of the total amount of lost or abandoned fishing gears are complicated to produce, since the number of gears that get lost every year varies locally (Macfadyen, Huntington & Cappell 2009).

2. Smothering of sessile organisms, producing the suffocation of the animal. This effect is usually caused by plastic bags or large cloths, which can cover large parts of the animal, like corals or gorgonians, generating a mechanical damage while limiting their capacity to feed (Richards & Beger 2011). Accumulation of litter over the seabed can also produce anoxia, which could lead to a change in the infauna community (Mordecai *et al.* 2011).

3. Dispersal of species attached to floating litter. Almost 400 species from various Phyla have been reported attached to floating debris drifting around in all oceans (Kiessling, Gutow & Thiel 2015). Presence of large quantities of litter, particularly plastics, can be a potential vector for the introduction of allochthonous species, mainly invertebrates with planktonic larvae, to a large number of habitats all around the globe.

4. Ingestion of plastics by a wide range of heterotrophic organisms. The ingestion of plastics can block the animal's digestive tract or affect its metabolism due to their chemical composition, which can end up causing the organism's death. Numerous marine animals have been reported with pieces of plastic in their stomachs, from cetaceans (Baulch & Perry 2014) to seabirds (Codina-García *et al.* 2013) or even corals.

Most harmful effects caused by the presence of marine litter may have very long-lasting consequences, since the vast majority of discarded items are primarily made of plastic or have plastic components (see (UNEP 2009) for further references). The low cost of manufacturing materials derived from plastic, its light weight and its durability make plastic elements very desirable for trade and packaging, but also extremely dangerous to marine pollution: they are very likely to end up being disposed, their floatability favors their dispersal capacity, and once they reach the seabed, they are very likely to remain in the marine environment for decades (Laist 1987).

Furthermore, after litter items have reached the marine environment, measures to retrieve them from the bottom of the sea are currently difficult to execute and can be rather expensive, especially at great depths (Iñiguez, Conesa & Fullana 2016).

1.7.2 Presence of marine litter on the seabed

In deep areas of the European seas, an estimated 30% of all marine litter accumulated on the shelf, slope and submarine canyons can be attributed to lost, abandoned or discarded fishing gears (Pham *et al.* 2014b), although this percentage can reach local values of about 50-80%, especially in traditional fishing grounds such as Sardinia or Sicily (Angiolillo *et al.* 2015). These high percentages may be explained by the rapid increase of the fishing effort during the past decades, together with a steady transition to synthetic and more durable materials used in the manufacturing of fishing nets and lines (Gilman *et al.* 2016).

The remaining 70% of marine litter observed over the seabed belongs to a wide range of items of a human origin, most of them made of glass, metal, paper, clinker, but mostly plastic (Pham *et al.* 2014b). In fact, it is becoming more common to find plastic bottles and bags, glass bottles, metallic objects and clothes half buried in the sediment or entangled around rocks (see examples from different parts of Europe in van den Beld *et al.* 2016; Moriarty *et al.* 2016 and Neves, Sobral & Pereira 2015).

In the case of the Mediterranean Sea, studies performed over shelf and slope environments have found litter in almost all surveyed sites. Its distribution, however, is somewhat irregular, with densities ranging from very low number of items per ha in certain areas, such as the continental shelf and certain submarine canyons of the Gulf of Lions, to sites where more than 30 items ha^{-1} have been registered, such as the Blanes Canyon (Tubau *et al.* 2015). In general terms, higher densities tend to be found in areas closer to shore or immediately adjacent to large cities, but this was not always the rule, since some deep areas inside submarine canyons can act as accumulation zones of marine liter.

An evaluation of the marine litter present in Cap de Creus submarine canyon has already been performed as part of a wider study, with 8 ROV dives made at depths between 150 and 1500 m (Tubau *et al.* 2015). A total of 415 items were identified in an area of 0.05 km^2, which represents an average density of 80 items ha^{-1}, a value relatively higher than that found in similar studies performed in other European areas (see for example Pham *et al.* 2014b, who found an average of 0.4 items ha^{-1} in submarine canyons of the Gulf of Lions). Tubau *et al.* (2015) found that most marine litter items concentrated on the canyon floor at depths below 1000 m, and its density largely decreased in depths above 500 m. More than 70% of all items were of a plastic origin and only 11% were identified as lost fishing gear, almost exclusively composed by fishing nets and longlines.

1.7.3 Objectives of Part 7

For the reasons explained above and following the demands of the MSFD, an efficient regulation of the activities that can potentially increase the amount of litter accumulated over the seabed of Cap de Creus marine area should be based on a comprehensive catalog of the predominant human pressures defined under Descriptor 10 (Council of the European Union 2008)

In this sense, Part 7 of this PhD thesis aims to:

1. Evaluate the quantity of human-derived items in order to determine the main sources of marine litter in the study area.
2. Determine the effects of ALDFG over the fragile cold water coral species of the submarine canyon.

Aims

2. Aims of this PhD thesis

All aspects covered in this thesis have been developed following the demands of the MSFD, aiming to give specific answers to its application in the continental shelf and submarine canyon off Cap de Creus. Descriptor 1 of the MSFD focuses on biological diversity, stating that a GES can only be reached when "quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climate conditions" (European Commission 2010). There is a demand to assess the biological diversity at 3 ecological levels: ecosystems, habitats (and related communities) and species. For this reason, a very large effort is placed in Part 1 of this thesis to evaluate the diversity and structure of the **invertebrate benthic assemblages** of the continental shelf and submarine canyon off Cap de Creus using the video images obtained during the Indemares cruises. The dataset used to produce such a large bulk of information was obtained from the analysis of 60 ROV and manned submersible dives performed between 80 and 400 m depth, in which all invertebrate megafauna species that could be identified are accounted for.

Once the benthic assemblages are described, the **role that the environmental factors play** in determining the observed patterns is evaluated by means of multivariate statistical techniques. This part of the thesis makes use of a large dataset obtained from a variety of sources:

1. substrate typologies, directly determined from the video footage
2. bathymetry, obtained by means of a multibeam echosounder, and its derived metrics such as slope, orientation, rugosity, etc.
3. bottom current flow, derived from a 3D hydrodynamic model, and
4. fishing intensity, obtained from the Vessel Monitoring System (VMS) installed on board of the commercial trawlers that operate in the area

Finding strong relationships between the benthic fauna and the different environmental parameters is of high interest in terms of establishing the distribution patterns of species and assemblages. In this sense, the design and application of efficient management measures would strongly benefit from **species and assemblage distribution maps**. Although a significant effort was made during the different oceanographic surveys to cover as much of the study area as possible, the amount of seabed explored by the ROV constituted a relatively small part of the total area under consideration. Manually producing distribution maps merely using the biological information obtained from scattered observations incorporates a high degree of uncertainty and deeply relies on the experience of the researcher. For this reason, a series of complex computational techniques have been developed in recent years to overcome the limitations of working with scarce spatial data, which tends to be the general rule in the case of underwater studies, where expensive surveys generally produce incomplete datasets. Mathematical models have the ability to objectively predict, with a certain degree of accuracy, the distribution of species, biotopes or habitats using the interactions between the physical environment and the biota. The capacity of these models to correctly predict spatial distributions largely depends on the strength of such interactions, and how comprehensive the environmental dataset is. Making use of the predictive power of the Random Forest (RF) algorithm, this thesis aims to generate a full coverage map of the benthic assemblages in the whole study area. In parallel, predictive maps of how the species richness and biodiversity distributes over the study area were also generated, aiming to detect the location of relevant biodiversity hotspots.

The MSFD also considers that a GES is met when "sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected" (European Commission 2010). In this sense, Descriptor 6 of MSFD determines that human pressure should not limit the capacity of the different ecosystem components to maintain their natural diversity, productivity and ecological processes. For this reason, the next step of this thesis consists in evaluating the **chronic effects of the commercial fishing activities** over the structure and distribution of the benthic fauna of the continental shelf off Cap de Creus.

A detailed analysis of the relationship between fishing activity and the different environmental factors showed that a homogenous distribution of the trawling effort along the survey area by the local fleet is highly unlikely. Indeed, the intensity of commercial fishing activities largely depends on a combination of factors, mainly depth, substrate type, slope and distance to port. Such an irregular distribution of the fishing effort implies that certain limits in the set of experimental conditions must be considered, which would minimize the number of undesirable interactions and, hence, the effects produced by other environmental factors. For this reason, a subset of samples was selected to test the long-term effects that different degrees of fishing intensity have had on the distribution of the marine benthic megafauna. In this sense, the effects of such interactions were evaluated at 3 levels:

1. biodiversity level, where we included species richness and the Exponential of Shannon diversity index, which not only considers the number of species present, but also their evenness
2. community level, where the whole species-by-sites matrix is taken into account
3. species level, where a selection of conspicuous and frequent species is used

The final objective of these analyses is to identify megafauna **species sensitive to varying degrees of fishing intensity**, either through a positive or a negative response. Such species can be considered indirect descriptors of the trawling activity in the study area. From an applied perspective, the identification of these descriptors sets the basis for the **proposal of a long-term monitoring protocol**, which would be indispensible to assess the evolution of the area once management mesures are put into practice. Considering the importance of having the necessary tools to detect changes in the benthic environment during the forthcoming years, this thesis proposes a **detailed and easy to replicate methodology to monitor the evolution of a set of species** that dwell on the continental shelf, where most of the fishing activity takes place.

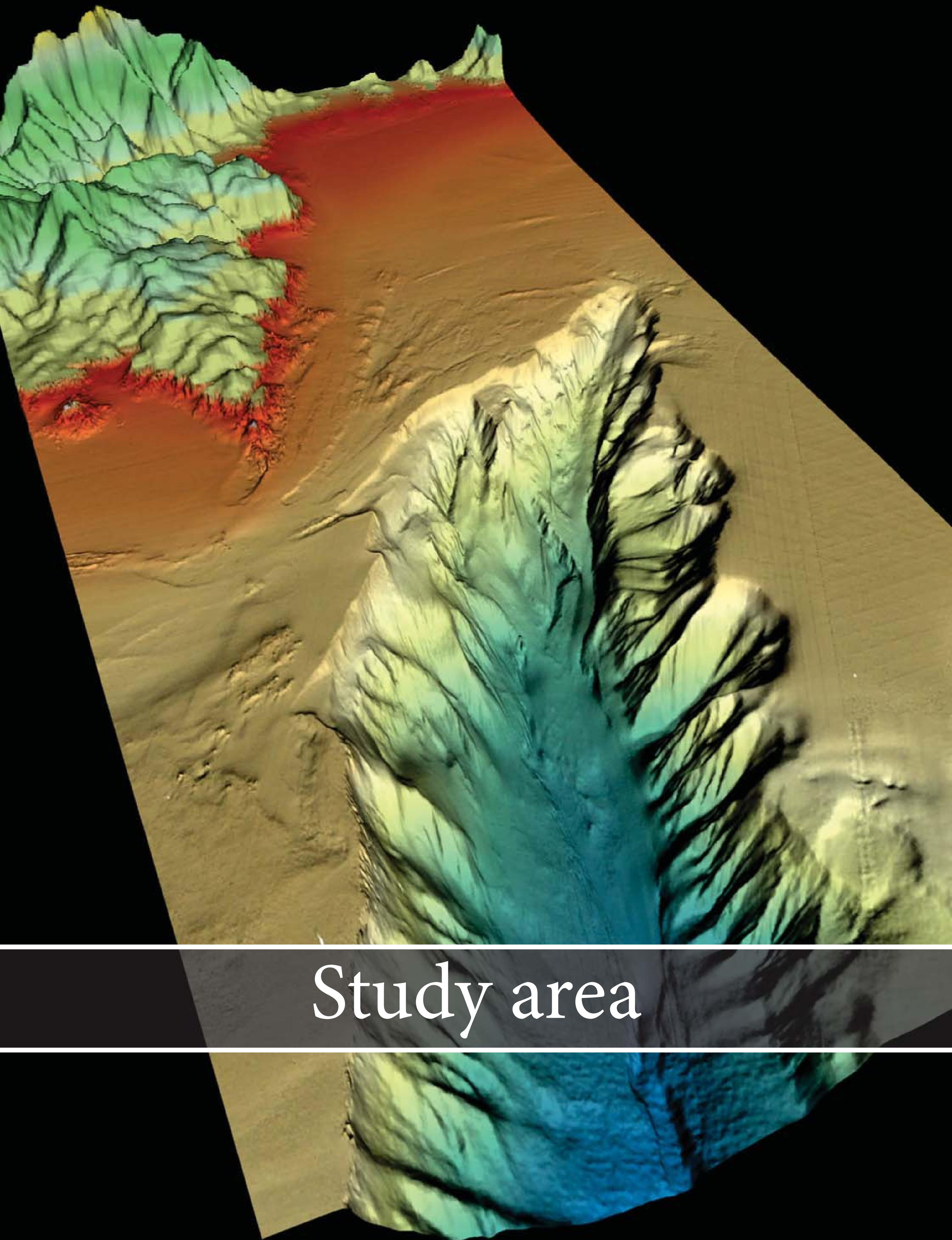
In order to evaluate the results of the management plans proposed by the governing bodies following the demands of the MSFD, the sampling methodology should follow a Before/After and Control/Impact (BACI) design, which would experimentally test the effectiveness of the conservation measures by assessing changes in the density of selected species. This BACI design also requires quantitative robust information about species that can be used as baseline data of the study area in 2012. For this reason, another objective of this thesis is to provide density data calculated from the video recordings of the species identified as sensitive to the commercial fishing activities. This **baseline information** would allow for the monitoring program to be set based on robust hypotheses and a sound experimental design, a well-defined sampling protocol and concluding statistical data analyses.

It is interesting to point out that the set of analyses presented so far only put the focus in the study of the invertebrate megafauna. It is true, however, that most species targeted by the fishing industry with a substantial economical value belong to the ichtyofauna. In this sense, it seemed only logical to question the **use of certain fish species as descriptors** to evaluate the effectiveness of the management measures implemented in the future MPA of Cap de Creus. In comparison to other sampling techniques, such as experimental trawl surveys or SCUBA diving, the effectiveness of ROVs to evaluate fish stocks has not been extensively examined. Although recent studies suggest that video techniques may be an efficient way to asses the species composition and abundance of the fish fauna in deep waters, specially in those areas where trawling is unfeasible, some authors have found that certain fish species react to the presence of underwater vehicles, concluding that estimates of their population abundances can be biased.

Being aware that true population densities might be underestimated due to the presence of an underwater vehicle, this PhD thesis aims to **identify the main fish assemblages** of the continental shelf and submarine canyon off Cap de Creus using ROV video images. Analyzing species richness, the abundance of each species and the structure of the fish community will also provide the tools to determine the potential use of fish species as quantitative descriptors of the fishing effort in a future monitoring program, complementing the use of megabenthic invertebrate species.

The **presence of marine litter** over the seabed is the final aspect evaluated in this PhD thesis. The MSFD considers that good environmental status is met when "properties and quantities of marine litter do not cause harm to the coastal and marine environment" (European Commission 2010). Descriptor 10, in this sense, encourages governing bodies not only to take them into consideration for monitoring programs, but also to identify the activity to which they are linked, whenever possible. Since the area explored over the continental shelf and canyon off Cap de Creus is relatively large, this thesis provides an exhaustive catalogue of the marine litter identified in the ROV images and its main sources of origin. It is true that small objects cannot be detected by means of ROVs, but the results shown here can still be considered a relevant approximation to the volume of marine litter present in the continental shelf and submarine canyon off Cap de Creus.

Study area



Cover image by Claudio Lo Iacono (National Oceanography Center, Southampton).

3. Study area

The marine area of Cap de Creus is located in the southernmost part of the Gulf of Lions (Fig. 3.1), a passive, prograding continental margin incised by a complex network of submarine canyons that developed during the Quaternary period (Canals & Got 1986). The unusually broad continental shelf of the gulf, with a shelf break defined at 100-200 m depth, is incised by 12 submarine canyons that coalesce towards the deep basin, at depths below 2000 meters (Canals *et al.* 2004). Due to differences in shelf width along the margin, central submarine canyons initiate relatively far from shore (70 km in the case of Sète and Montpellier canyons), while canyons located on both ends of the gulf initiate particularly close to land. This is the case of Cap de Creus canyon, whose head can be found at a distance of just 4 km from the cape promontory, producing a very marked narrowing of the continental shelf (Lastras *et al.* 2007). This complex morphological setting greatly influences sediment dispersal and accumulation, making Cap de Creus canyon a very active link between the continental shelf and the deep basin (Canals *et al.* 2006).

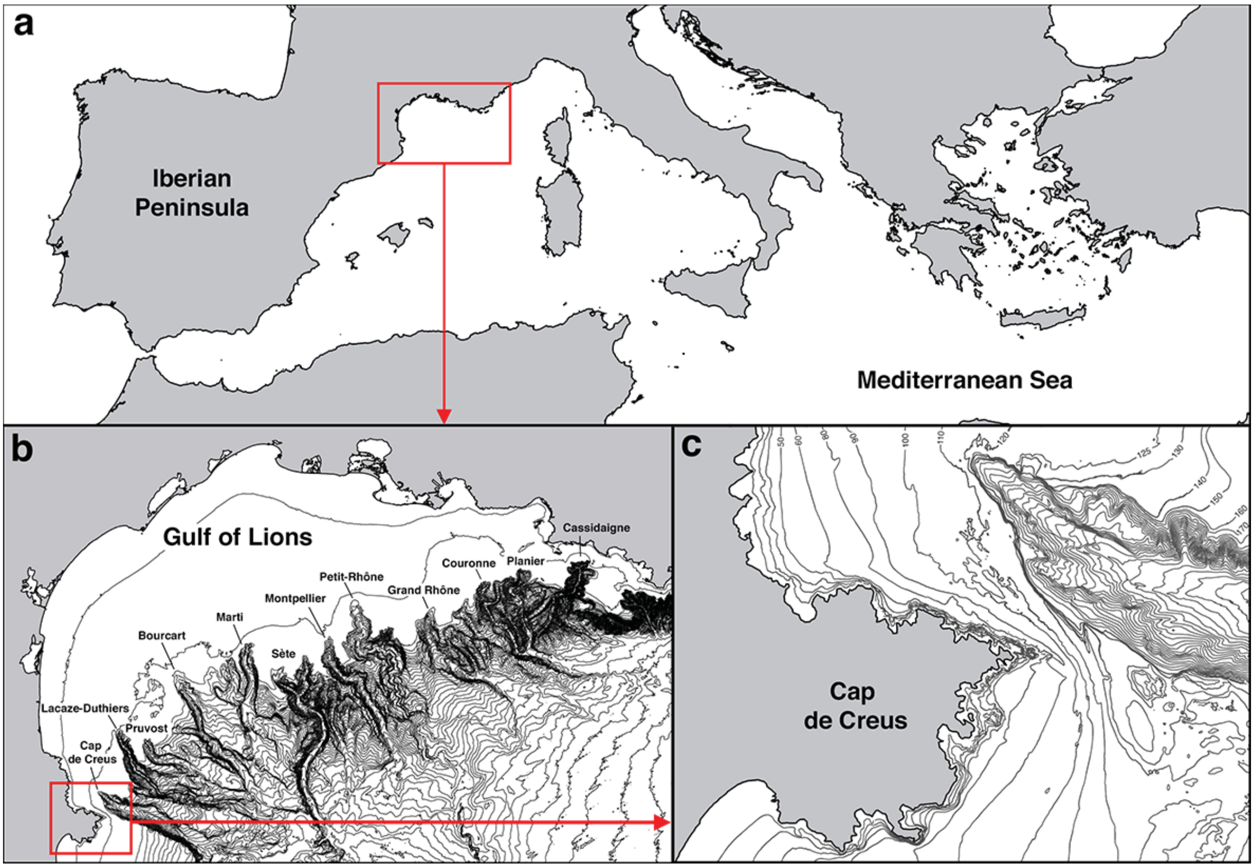


Figure 3.1. Location of Cap de Creus continental shelf and submarine canyon. The study area is found on the southernmost part of the Gulf of Lions, in Spanish territory. Names given in (b) correspond to the most important submarine canyons found in the Gulf of Lions.

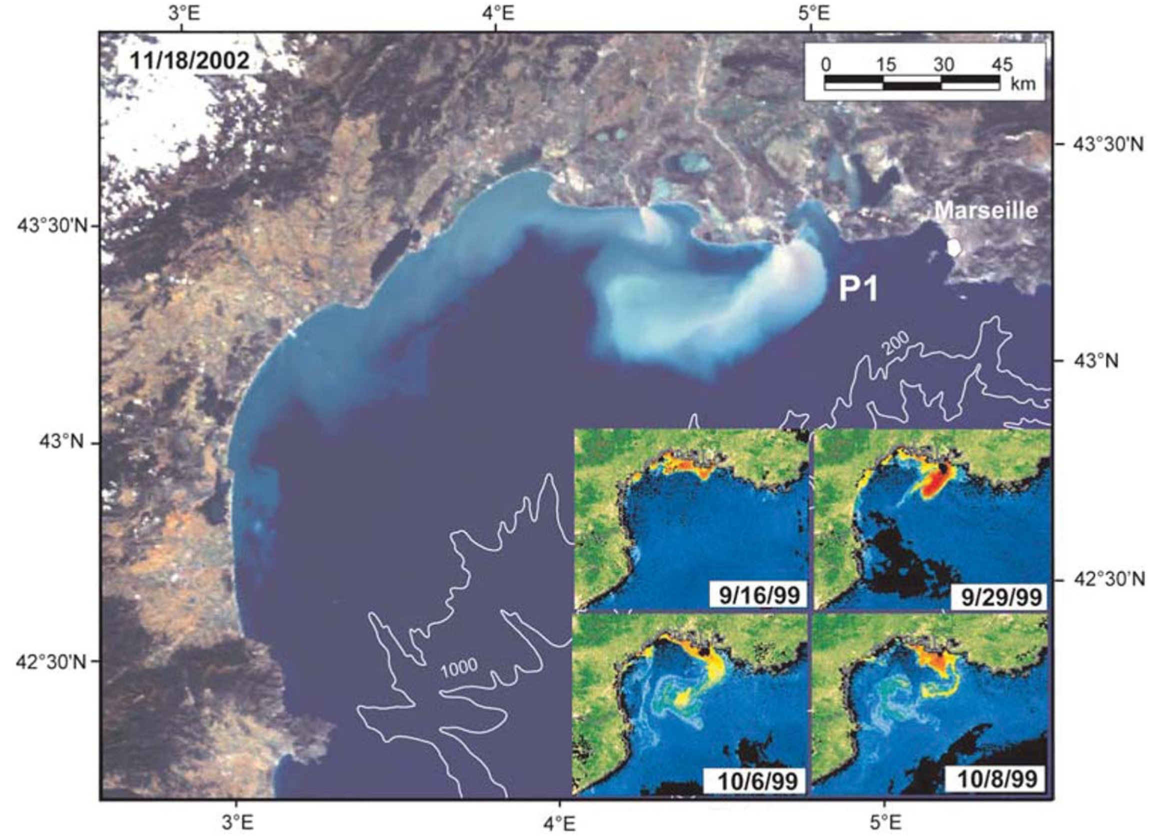


Figure 3.2. Satellite image showing a maximum discharge episode from the Rhone river in year 2002. The large plume of fine-grained suspended sediments is marked as P1. Heatmap false color images (inset) show the evolution of chlorophyll concentration on surface waters during the formation and spreading of the river plume. From Arnau *et al.* (2004).

A total of 16 rivers drain from southeastern France into the Gulf of Lions. The marked seasonality of the Mediterranean climate generates a strong intra-annual variability, with highest river discharges recorded in spring and autumn, sometimes with large amounts of sedimentary material reaching the sea in very short periods of time (Bourrin & Durrieu de Madron 2006). The continental shelf of the gulf is mainly composed by terrigenous sediments provided by the Rhône river, which supplies almost 80-90% of the terrigenous input to the gulf, depending on the year (Courp & Monaco 1990). More than 107 tons of fine-grained sediment are discharged annually by the Rhône, with an annual discharge of freshwater and sediment one order of magnitude higher than the remaining coastal rivers (Bourrin & Durrieu de Madron 2006). The freshwater plume produced by the Rhône river expands over the continental shelf at a relatively short distance from the coast, being rapidly deflected southwestward by the general water-mass circulation (Fig. 3.2), which moves the terrigenous input along the coastline (Arnau, Liquete & Canals 2004).

Shelf water displays a strong stratification from spring to autumn due to high summer temperatures, with differences in temperature that can exceed 10 °C, forming a marked thermocline at 10-20 m depth. When the recurrent cold and dry northern winds (Tramontane and Mistral) blow for long periods of time, shelf waters lose heat, gain density and sink, and the water column gets homogenized. The presence of the Northern Current, which flows southwestwards along the continental slope as part of the cyclonic circulation of NW Mediterranean Sea, creates a thermal front between the cold waters of the shelf and the warm waters of the open sea, beyond the shelf break (Millot 1990). The constrained morphology of the coastline and the narrow shelf off Cap de Creus deviate shelf waters towards the nearby submarine canyons, where the downslope movement of cold and dense water largely contributes to the export of sediments and carbon from the shelf to the deep basin (Canals *et al.* 2006). This phenomenon, named "dense shelf water cascading", is particularly intense in Cap de Creus submarine canyon, mostly because of its position at the end of the gulf (Fig. 3.3). Cascading of dense waters has a profound effect in bottom current dynamics and the sedimentary regime of the canyon, not only due to increased shelf-slope sediment transport, also for the sustained seafloor current speeds that can lead to furrow formation and the transport of coarse sediment particles (Puig *et al.* 2008). The implications of such intense cascading events for the normal development of benthic fauna have yet to be examined. Stronger off-shelf sediment transport in Cap de Creus has also been recorded during intense eastern storms, which are associated to large waves and rapid river floods. When both events concur, off-shelf export of sediment reaches maximum values (Palanques *et al.* 2006).

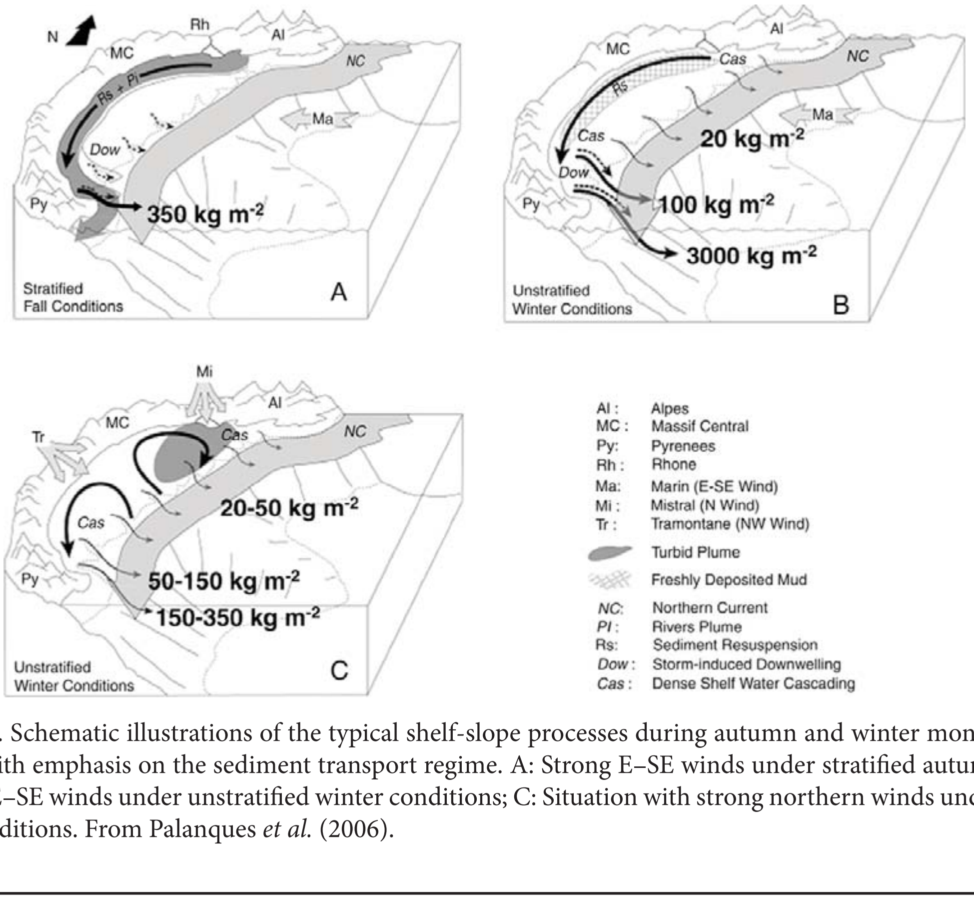


Figure 3.3. Schematic illustrations of the typical shelf-slope processes during autumn and winter months in the Gulf of Lions with emphasis on the sediment transport regime. A: Strong E–SE winds under stratified autumn conditions; B: Strong E–SE winds under unstratified winter conditions; C: Situation with strong northern winds under unstratified winter conditions. From Palanques *et al.* (2006).

Materials & Methods

Cover image by JAGO Team (GEOMAR).

4. Materials and Methods

4.1 Data acquisition

4.1.1 Oceanographic surveys

Most of the data used in this PhD thesis was obtained in the frame of the Life+ Indemares project during 4 oceanographic cruises on board of the R/V García del Cid between 2007 and 2012, as well as during two other complementary surveys with smaller vessels. The main objective of the Indemares project was to characterize with great detail the physical and ecological features of the continental shelf and submarine canyon off Cap de Creus. For that reason, all surveys had a clear multidisciplinary approach, covering a wide range of disciplines.

Samples and data collected during the cruises were of the following types:

- Multibeam bathymetry data, to complement those areas for which information was not available
- CTD profiles, to characterize the physical properties of the water column
- Water samples, to analyze the chemistry of the water column at different depths
- Pelagic plankton, to characterize the planktonic community of the water column
- Van Veen samples, to characterize the meiofauna assemblages and determine the grain size of the sediment
- Box corer samples, to evaluate the chemical properties of the sediment
- Epibenthic sled samples, to identify macrofauna species of the continental shelf
- ROV and manned submersible dives, to characterize megafauna communities using video images

Table 4.1. Number of deployments of each instrument in the different oceanographic surveys performed in Cap de Creus marine area as part of the Indemares project.

Survey	Vessel	Dates	ROV dives	Sleds	Van Veen grabs	Box corers
Ind 0	Dolores (CEAB-CSIC)	23-27/08/09	12	8	16	-
Ind 1	R/V Garcia del Cid	23-29/09/09	17	17	17	-
Ind 2	R/V Garcia del Cid	7-18/06/10	11	15	37	-
Ind 6	R/V Garcia del Cid	6-10/07/12	21	27	-	4

The number of deployments of each device in the different Indemares cruises is summarized in Table 4.1. Since one of the main objectives of this PhD thesis is to evaluate the composition of the benthic megafauna assemblages using underwater video images to later relate such results to different environmental and anthropogenic factors, not all data gathered in the Indemares surveys was included in the analyses.

4.1.2 Video recordings

Filming underwater images of the seabed is a complex procedure, especially if quantitative data is to be extracted from the video recordings. For this reason, a general protocol was applied to all ROV dives performed during the Indemares cruises. Some of the aspects that were taken into consideration when performing dives to record video footage can be summarized as follows:

- Underwater images were recorded following a rectilinear trajectory, avoiding as much as possible sudden changes of direction or loops around the same point.
- Vehicle speed was kept as constant as possible, preferably between 0.2-0.3 knots.
- Each dive aimed to record between 45 minutes and one hour of valid video footage. Certain variability in dive length occurred due to weather conditions, the state of the sea or technical difficulties.
- The vehicle was kept as close as possible to the seabed, with a camera angle of approximately 30-45º, in order to maximize taxonomic resolution.
- All vehicles were equipped with two parallel laser pointers, preferably emitting green light, which gave scale to the images during its subsequent quantitative analysis.
- The distance between laser beams was not equal in all vehicles, but a minimum distance of 10 centimeters was preferred to facilitate the analysis.
- Close-up images of interesting/unknown organisms were recorded in some dives. These images were used to improve the identification process and also for outreach activities. As much as possible, close-up images were taken at the beginning or at the end of the dives.
- All vehicles were equipped with an Ultra Short Baseline (USBL) system, which accurately determined their GPS position of the vehicle underwater.

The correct application of these recommendations allowed for a simple yet effective quantitative analysis of the images, maximizing the amount of information extracted from each video transect. There was no need to select video frames or sequences since images were analyzed in a continuous manner.

4.1.3 Underwater vehicles

The underwater filming was obtained by means of 3 different vehicles:

1. Nemo ROV, operated by Gavin Newman. Medium sized ROV, full HD camera, unlimited dive time, maximum operational depth of approximately 300 m, equipped with a hydraulic grabber to collect one sample per dive (Fig. 4.1a)
2. JAGO submarine, operated by Jürgen Schauer and Karen Hissman (IFM/GEOMAR). Three-tone manned submersible, two-person capacity, full HD camera, maximum operational depth of 400 m, recommended dive time of approximately 3-4 hours, equipped with a hydraulic grabber and a basket to collect multiple samples per dive (Fig. 4.1b)
3. Bleeper EVO, directly operated by our research team (ICM-CSIC). Small sized ROV, SD camera, maximum operational depth of 150 m (Fig. 4.1c)

4.1.4 Geopositioning of the ROV tracks

Obtaining quantitative data from video images heavily relies in an accurate estimation of the area surveyed. For this reason, one of the most important aspects of the video analysis corresponded to determining the geographical positioning of the underwater vehicle with the highest accuracy possible. Positioning data from the ROV and manned submersible was collected using a LinkQuest Tracklink USBL positioning system, which recorded the georeferenced position of the vehicle every *ca.* 20 seconds. The position of the underwater vehicle was calculated from its range and bearing with respect to the boat, measured by a transceiver mounted on a pole in the ship's hull (Jamieson, Boorman & Jones 2013). All data points were then used to define the trajectory made by the vehicle over the seabed.

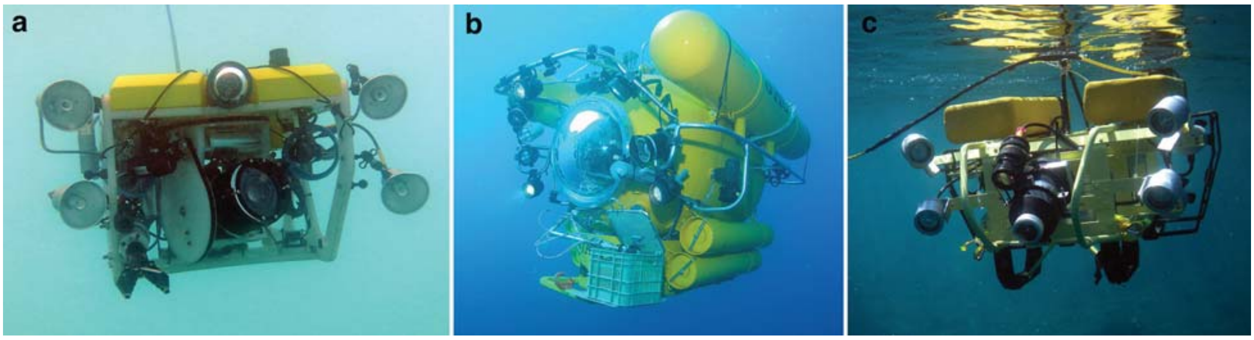


Figure 4.1. Underwater vehicles used to obtain the video footage in Cap de Creus continental shelf and submarine canyon. (a) Nemo ROV, operated by Gavin Newman, (b) manned submersible JAGO, operated by Jürgen Schauer and Karen Hissman and (c) Bleeper EVO, owned by the Benthic Suspension Feeders group at the Institute of Marine Sciences (ICM-CSIC).

Although the number of incorrect points produced by the tracking system was low in general, a certain post-processing of the data was carried out to remove positional noise. To obtain a smooth trajectory of the vehicle, outlier points were manually removed using the geospatial software QGIS v.2.12-Lyon (QGIS Development Team 2016). Outlier points were identified as those positions too distant from the previous positioning point if ROV speed was kept constant. The processing of the data provided accurate information about the length of each ROV transect, which was later converted into area explored to generate a quantitative database for all organisms identified.

4.2 Video processing

4.2.1 Video format

All underwater images were recorded in HD format (1920x1080), except those recorded using the ROV Bleeper Evo, which was equipped with a SD camera (720x480). Images were recorded in MiniDV tapes in the case of the submarine JAGO and the ROV Bleeper EVO, and directly to a digital support in the case of Nemo ROV. Since all analyses were performed using the editing software Final Cut Pro 7 (Apple Inc.), video sequences were directly recorded in .mov format when possible, or later digitized to .mov if recorded onto tapes.

4.2.2 Selection of ROV tracks

The first step to quantitatively analyze the video footage was to select those sequences in which image quality was adequate to identify benthic megafauna. This initial filtering was done at the transect level. The criteria used to select useful ROV transects were simple: (1) laser beams had to be visible on screen, (2) most of the dive had to be considered valid in terms of distance from the seabed and image quality, and (3) the vehicle was correctly geopositioned of over the seabed. From the total number of ROV dives performed in the different oceanographic surveys, 60 transects were finally selected to become the basis over which this thesis is built upon.

Fig. 4.2 shows the spatial distribution of the 60 selected ROV and manned submersible dives over the study area, numbered chronologically following the date and time in which they were recorded. Table 4.2 summarizes the main features of the video transects, indicating their geographical position, start and end depths, valid amount of time recorded, distance covered and total area analyzed.
All video transects were performed over the continental shelf and on the southern side of the

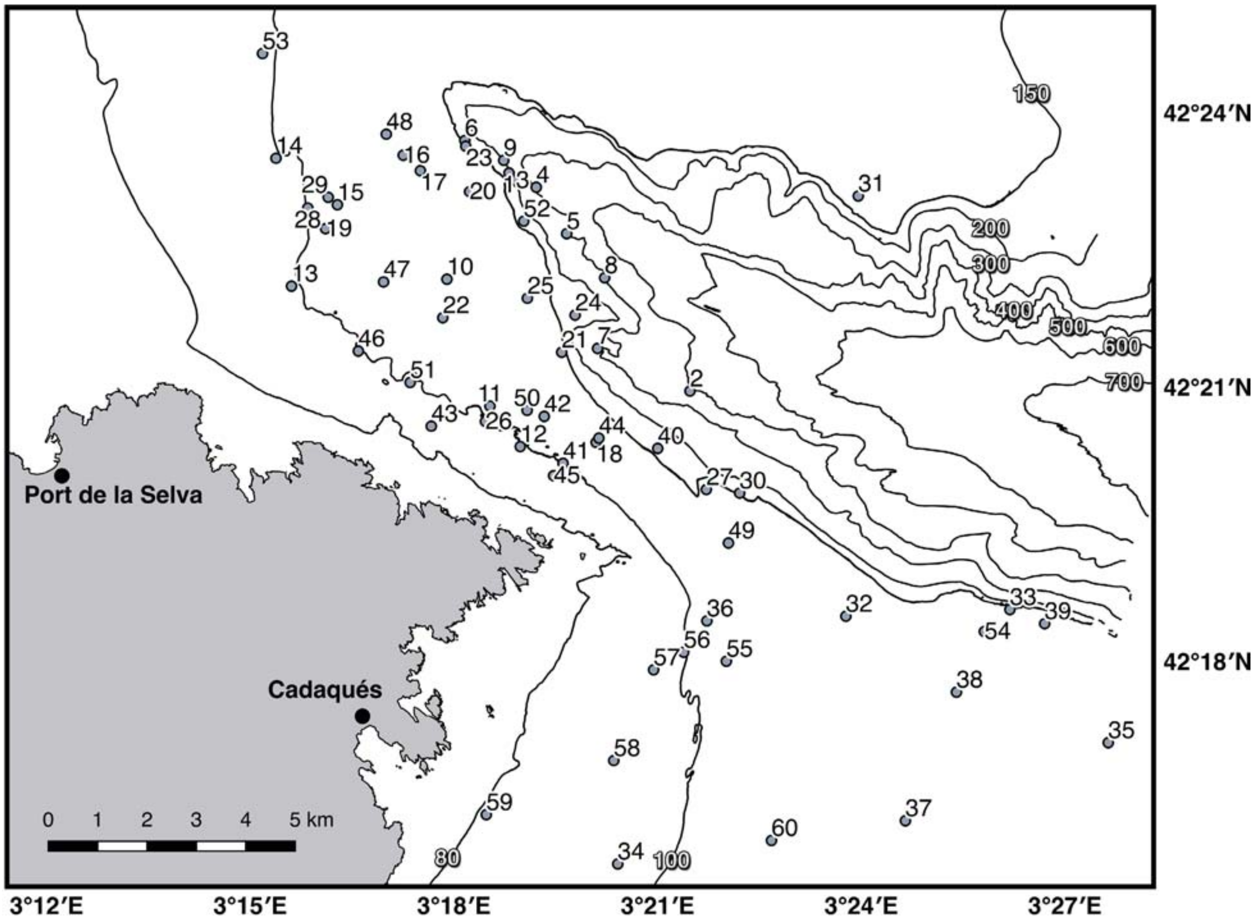


Figure 4.2. Location of the 60 ROV and manned submersible dives to record the video footage used for the different analyses performed throughout this PhD thesis. Detailed information about all dives is given in Table 4.2.

submarine canyon, in depths that ranged between 80 and 390 m. The effective length of each ROV dive was extremely variable, but on average they displayed a length of approximately 500 m. Some dives were relatively short (smallest distance in a single dive was 80 m) while others were significantly larger (longest transect is almost 1500 m long). Overall, the sum of all dive lengths was around 33 km.

4.2.3 Valid video sequences

The footage from the selected video transects was edited using the video editing software Final Cut Pro 7 (Apple Inc.). The methodology employed required that all video sequences where the ROV was kept stationary had to be removed, together with those initial sequences before it began to cruise at a constant speed over the seabed. Once all video transects were edited, the next step consisted in a second filtering that identified the poor-quality images due to sediment resuspension,

Table 4.2. Main characteristics of the 60 ROV dives performed on the continental shelf and submarine canyon off Cap de Creus analyzed for this PhD thesis. Date is given in dd/mm/yy. Duration of dives is given in hh:mm:ss. SU, number of valid 5-m^2 sampling units in each ROV transect.

ROV	Date	Vehicle	Start position		End position		Depth (m)		Duration	Length	SU
dive			x	y	x	y	start	end		(m)	
1	09/09/07	JAGO	3.315	42.390	3.314	42.390	215	186	00:28:58	83	6
2	09/09/07	JAGO	3.357	42.350	3.360	42.350	386	377	00:32:48	250	18
3	10/09/07	JAGO	3.317	42.387	3.314	42.389	199	173	01:38:59	675	50
4	12/09/07	JAGO	3.321	42.386	3.320	42.387	302	302	00:33:03	157	8
5	13/09/07	JAGO	3.329	42.379	3.329	42.380	316	302	01:27:00	386	23
6	13/09/07	JAGO	3.304	42.395	3.302	42.396	168	153	00:28:09	177	15
7	14/09/07	JAGO	3.334	42.357	3.334	42.356	234	236	01:40:39	597	41
8	15/09/07	JAGO	3.338	42.369	3.337	42.371	293	264	01:14:54	334	17
9	15/09/07	JAGO	3.315	42.393	3.313	42.390	282	165	01:40:20	544	44
10	25/08/09	EVO	3.298	42.374	3.302	42.366	109	110	00:48:56	1027	56
11	26/08/09	EVO	3.309	42.347	3.310	42.347	115	112	00:13:15	122	2
12	26/08/09	EVO	3.313	42.342	3.318	42.337	98	95	01:18:03	718	33
13	26/08/09	EVO	3.260	42.373	3.261	42.366	99	99	00:59:50	1347	46
14	27/08/09	EVO	3.256	42.395	3.258	42.389	97	99	00:49:10	676	39
15	27/08/09	EVO	3.272	42.386	3.272	42.382	102	103	00:45:18	476	28
16	27/08/09	EVO	3.289	42.394	3.288	42.392	107	107	00:25:10	173	9
17	27/08/09	EVO	3.293	42.392	3.297	42.389	106	107	00:19:42	438	26
18	27/08/09	EVO	3.332	42.340	3.339	42.341	111	117	00:32:35	675	25
19	23/09/09	Nemo	3.269	42.386	3.270	42.375	102	101	00:48:33	1463	77
20	23/09/09	Nemo	3.306	42.388	3.303	42.384	111	111	00:28:37	504	30
21	23/09/09	Nemo	3.327	42.358	3.327	42.355	151	118	00:32:12	436	21
22	24/09/09	Nemo	3.299	42.364	3.297	42.362	111	108	00:45:08	291.5	17
23	26/09/09	Nemo	3.304	42.394	3.303	42.394	148	142	00:23:21	83	4
24	27/09/09	Nemo	3.333	42.363	3.328	42.363	166	160	00:59:39	475	22
25	27/09/09	Nemo	3.322	42.367	3.316	42.365	121	115	00:58:27	625.4	37
26	27/09/09	Nemo	3.309	42.347	3.307	42.342	111	94	00:56:37	587.1	32
27	28/09/09	Nemo	3.364	42.333	3.361	42.331	130	148	00:18:26	321.1	13
28	28/09/09	Nemo	3.267	42.382	3.260	42.384	100	99	01:08:29	533.5	26
29	28/09/09	Nemo	3.272	42.385	3.268	42.385	102	101	00:44:26	405.9	24
30	29/09/09	Nemo	3.371	42.331	3.370	42.331	160	150	00:17:34	91.3	2
31	29/09/09	Nemo	3.400	42.385	3.400	42.385	137	137	00:19:07	90	5
32	13/06/10	JAGO	3.393	42.311	3.397	42.306	116	117	01:11:26	936.2	93
33	13/06/10	JAGO	3.434	42.310	3.439	42.309	132	128	01:04:42	636.3	55
34	14/06/10	JAGO	3.338	42.266	3.343	42.260	94	94	00:52:43	659.6	66
35	17/06/10	JAGO	3.460	42.289	3.463	42.282	127	125	01:33:19	1117.3	95
36	18/06/10	JAGO	3.363	42.307	3.364	42.308	108	114	01:24:56	892.64	85
37	06/07/12	Nemo	3.409	42.270	3.412	42.273	126	126	00:28:59	505	30

Table 4.2. (Continued)

ROV dive	Date	Vehicle	Start position		End position		Depth (m)		Duration	Length (m)	SU
			x	y	x	y	Start	End			
38	06/07/12	Nemo	3.424	42.293	3.423	42.296	127	126	00:43:44	348	20
39	06/07/12	Nemo	3.444	42.310	3.449	42.306	147	129	01:17:14	760.3	43
40	07/07/12	Nemo	3.450	42.304	3.347	42.337	128	125	01:17:01	830	49
41	07/07/12	Nemo	3.329	42.336	3.326	42.336	99	96	00:29:40	347	20
42	07/07/12	Nemo	3.323	42.343	3.322	42.347	108	109	00:27:43	432.5	21
43	07/07/12	Nemo	3.296	42.343	3.294	42.343	90	88	00:13:50	178	10
44	07/07/12	Nemo	3.336	42.340	3.336	42.342	115	116	00:11:25	178	10
45	08/07/12	Nemo	3.325	42.337	3.327	42.331	98	91	00:38:45	784.7	44
46	08/07/12	Nemo	3.277	42.361	3.277	42.354	103	97	00:38:34	865	51
47	08/07/12	Nemo	3.286	42.371	3.280	42.369	109	105	00:29:08	574	34
48	09/07/12	Nemo	3.286	42.398	3.281	42.397	108	104	00:32:02	583.1	30
49	09/07/12	Nemo	3.368	42.325	3.366	42.319	127	123	01:07:41	872	52
50	09/07/12	Nemo	3.321	42.351	3.316	42.343	107	102	00:50:10	949	56
51	09/07/12	Nemo	3.290	42.353	3.289	42.350	96	98	00:29:48	284	17
52	09/07/12	Nemo	3.324	42.377	3.312	42.384	210	115	01:42:13	1319	62
53	09/07/12	Nemo	3.253	42.410	3.254	42.413	98	98	00:23:40	292	15
54	09/07/12	Nemo	3.427	42.307	3.435	42.304	124	129	00:47:40	791	47
55	10/07/12	Nemo	3.367	42.300	3.367	42.301	116	118	00:11:09	184.1	11
56	10/07/12	Nemo	3.358	42.300	3.355	42.305	101	96	01:07:17	627	33
57	27/01/13	EVO	3.345	42.299	3.353	42.299	90	96	00:29:04	639	38
58	27/01/13	EVO	3.336	42.283	3.342	42.281	90	92	00:40:00	601	36
59	28/01/13	EVO	3.308	42.270	3.308	42.276	83	79	00:29:59	704	19
60	28/01/13	EVO	3.382	42.268	3.383	42.264	121	121	00:48:38	1235	53

excessive distance from the seabed and laser beams absent from the image. Only those sequences identified as valid were considered in the successive statistical analyses. The combination of all valid sequences represented approximately 80% of the total amount of video footage recorded, which summed up to more than 47 hours of filming. Considering only the valid sequences, the total area sampled reached almost 1000 m^2.

Once all valid video footage was determined, an initial exploratory analysis was carried out to get familiarized with the different substrate types and the fish and invertebrate species that compose the benthic fauna of Cap de Creus continental shelf and submarine canyon.

4.3 Biological data

4.3.1 Species identification

The statistical analyses performed for this PhD thesis were based on the identification of all megabenthic organisms that appeared in the valid video sequences, both invertebrate and fish species, together with any other object of a human origin. Megafauna species, defined as those animals or colonies large enough to be identified in still pictures of the sea floor, were categorized to the lowest possible taxon. In order to generate a consistent database with all the invertebrate species that could be identified from the video images, a visual inventory was produced (see Annex I). The first step taken towards the completion of this visual guide consisted in an exhaustive search for high quality close-up images of all invertebrate and fish species. From those sequences, still pictures were extracted to start building the main body of the inventory. The rest of the organisms for which close-up images were not available were also photographed, after a thorough search for good quality shots. Once all images were gathered, organisms that could unquestionably be identified to species level directly from still pictures were immediately added to the inventory. The remaining images were classified into the different Phyla and later taken to renowned taxonomists for further examination.

4.3.2 Biological sampling

Even though some of the pictures could be directly assigned to a certain species, a large number of specimens needed live biological samples to be correctly identified from the still images. For this reason, a set of biological samples from the continental shelf and the submarine canyon were collected in each of the oceanographic surveys by means of two different devices:

i. A Rauschert epibenthic sled (Fig. 4.3a). This compact sled was towed over the soft bottoms of the continental shelf at 1-2 knots for 5-10 min during every deployment, with the objective of collecting macrofauna organisms that either live over the sea floor or half-buried in the sediment (Fig. 4.3b).

ii. The hydraulic grabber of the submersible JAGO (Fig. 4.3c) or the ROV Nemo (Fig. 4.3d). This selective sampling devices were used to collect organisms that were found on hard substrates or at great depths, where deploying an epibentic sled was complex.

Samples collected were catalogued and taken to specialists of different taxa for further identification: Porifera (Dr. Iosune Uriz, CEAB/CSIC), Cnidaria Hydrozoa (Dr. Josep-Maria Gili, ICM/CSIC), Cnidaria Anthozoa (Dr. Pablo López-González, Universidad de Sevilla), Bryozoa (Dr. Mikel Zabala, Universitat de Barcelona), Polychaeta (Dr. Rafael Sardá, CEAB/CSIC) and Ascidia (Dr. Xavier Turón, CEAB/CSIC).

Once all samples were identified to species level, they were linked to the organisms observed in the video footage with the help of the taxonomists. Names of all the organisms identified down to species/genus level were matched to the reference list provided by the World Register of Marine Species (WoRMs, www.marinespecies.org). Annex I at the end of this document shows images of all species/morphospecies that make up the fauna inventory produced for this PhD thesis.

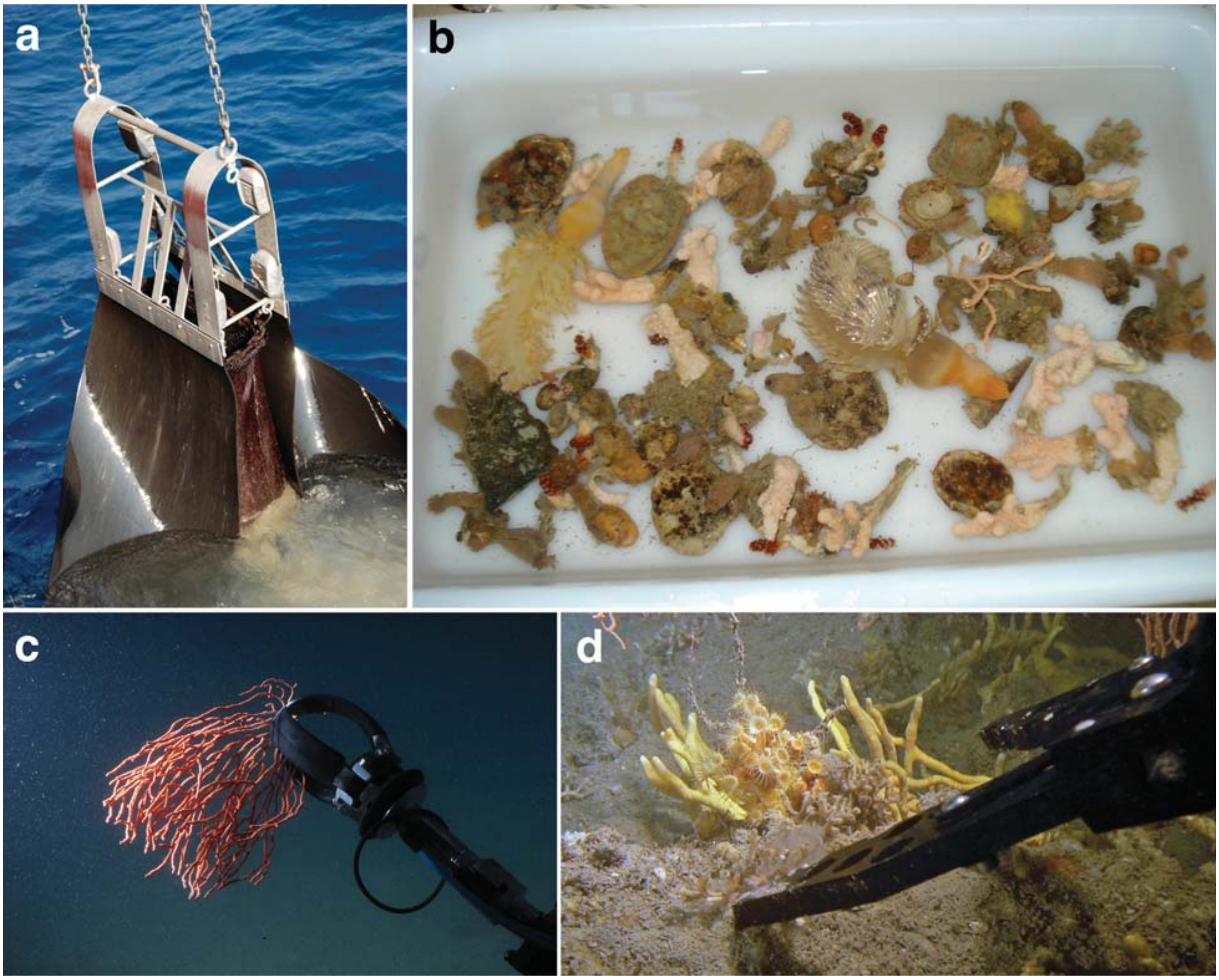


Figure 4.3. Methods used to collect live samples of benthic invertebrates to improve the identification of those organisms observed in the video footage. (a) Rauschert sled, a small but effective bottom trawl, used to capture live organisms dwelling on the soft bottoms of the continental shelf. (b) Aspect of some species collected with the Rauschert sled. (c-d) Grabbers of the submarine JAGO and Nemo ROV used to collect organisms dwelling on hard substrates or in deeper locations. Image (c) by JAGO Team (GEOMAR) and image (d) by Nemo ROV (Gavin Newman).

4.4 Data processing

To maximize the amount of information extracted from each dive, data was registered as a continuous string of species occurrences along the video transect. To convert species occurrences into quantitative data, the total area surveyed in each ROV dive was indispensible. For this reason, the projection of the laser beams over the seafloor was used to determine a fixed transect width inside which all organisms were counted. Transect width was kept constant along the whole dive, representing a section of the seafloor of either 30 or 50 cm, depending on the situation. The syncing between the tracking system and the video images allowed us to accurately estimate the distance travelled by the ROV in each dive, which was later converted into total area surveyed.

The position of each organism along the video transect was determined using the time elapsed since the beginning of the video transect. To do so, the time code generated by the video editing software was annotated at the moment the organisms crossed the section provided by the laser beams. Time was then converted into distance from the first georreferenced point, which generated a succession of distances along the lenght of the video transect for all species. Each video transect was then subdivided into a continuous sequence of sampling units of an equal size, for which the density of each species was calculated. The process of assigning each organism to a sampling unit is currently automatized via a web-based software specifically designed for this purpose (see Box V). Organisms from all species were identified and catalogued individually one by one, except those belonging to the species *Ophiothrix fragilis* when found in dense aggregations. In this case, 5 density categories were generated (see Fig. 4.4), which were then used to estimate density values along those video transects.

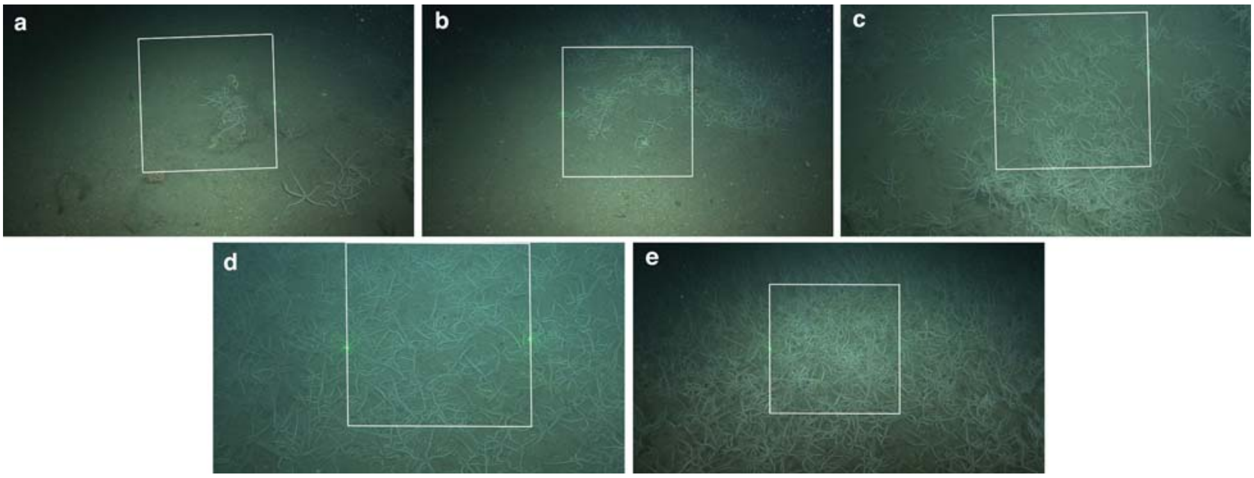


Figure 4.4. Ilustrations of the five categories into which the density of the brittle star *Ophiothrix fragilis* was divided in order to estimate their numbers when massive aggregations were found. Images correspond to transect 36. Estimated densities are, in ind·m^{-2}: (a) 50, (b) 125, (c) 300, (d) 440 and (e) 600. Images by JAGO Team (GEOMAR).

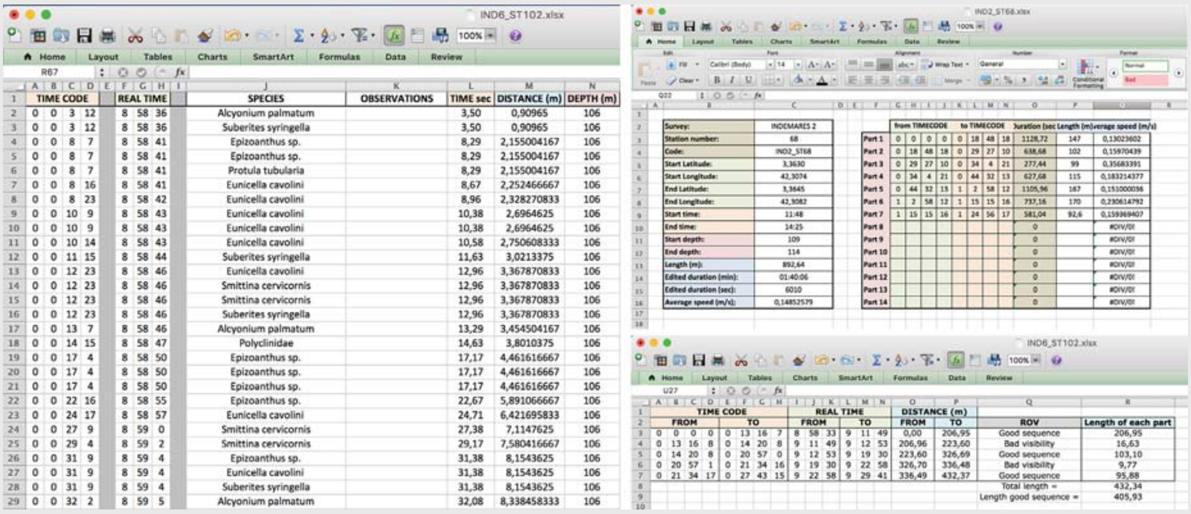
Box V

New processing software for ROV video data

Quantitative analyses of benthic assemblages are based on counts of organisms, so the number of individuals belonging to each species/taxonomic group must be assigned to sampling units of a selected size. Manually processing this type of data when working with long ROV transects can become an extremely time-consuming task, especially when the number of species and/or the sampling effort is very large. For this reason, during the development of this PhD thesis, I searched for external assistance in order to develop a new piece of software to automate the counting process. The collaboration between Aine Informática, SL and our research group gave rise to a specifically designed web-based software that has automated a large part of the data treatment. The idea behind the new software was rather simple: we aimed to create an interactive tool that transformed the raw data obtained from each ROV dive into quantitative measures in a single Excel matrix, giving the user the possibility to determine the sampling size. The final version of the software performs a series of algorithms over the data in a way that assigns the position of each organism along an ROV transect and then counts the number of individuals of each species in the different sampling units in which the transect length can be divided.

The researcher has to provide information regarding the characteristics of each ROV transect (total length, location, width of the analyzed area, speed of the ROV at different intervals, etc.) and the position of each specimen in the video footage using the timecode provided by the video editing software. From that point onwards, the web-based software performs an automated counting for each ROV dive and then incorporates all the data into a single table.
Data to be provided is presented in the form of simple Excel files, which have a series of spreadsheets to incorporate as much information as necessary (some examples given in Fig. B5.1). The different sheets are summarized as follows:

Sheet 1: General data & positioning. It contains general information about the dive and the position of the ROV along the track. The panel on the left is used to store the metadata of the dive: survey name, date, transect number, start and stop position, length, width of the lasers, depth range, etc. The panel on the right contains the GPS information about the transect, and will be used by the software to properly assign the position of each organism along the track.



TIME CODE				REAL TIME			SPECIES	OBSERVATIONS	TIME sec	DISTANCE (m)	DEPTH (m)
0	0	3	12	8	58	36	Alcyonium palmatum		3,50	0,90965	106
0	0	3	12	8	58	36	Suberites syringella		3,50	0,90965	106
0	0	8	7	8	58	41	Epizoanthus sp.		8,29	2,155004167	106
0	0	8	7	8	58	41	Epizoanthus sp.		8,29	2,155004167	106
0	0	8	7	8	58	41	Protula tubularia		8,29	2,155004167	106
0	0	8	16	8	58	41	Eunicella cavolini		8,67	2,252466667	106
0	0	8	23	8	58	42	Eunicella cavolini		8,96	2,328270833	106
0	0	10	9	8	58	43	Eunicella cavolini		10,38	2,6964625	106
0	0	10	9	8	58	43	Eunicella cavolini		10,38	2,6964625	106
0	0	10	14	8	58	43	Eunicella cavolini		10,58	2,750608333	106
0	0	11	15	8	58	44	Suberites syringella		11,63	3,0213375	106
0	0	12	23	8	58	46	Eunicella cavolini		12,96	3,367870833	106
0	0	12	23	8	58	46	Smittina cervicornis		12,96	3,367870833	106
0	0	12	23	8	58	46	Smittina cervicornis		12,96	3,367870833	106
0	0	12	23	8	58	46	Suberites syringella		12,96	3,367870833	106
0	0	13	7	8	58	46	Alcyonium palmatum		13,29	3,454504167	106
0	0	14	15	8	58	47	Polyclinidae		14,63	3,8010375	106
0	0	17	4	8	58	50	Epizoanthus sp.		17,17	4,461616667	106
0	0	17	4	8	58	50	Epizoanthus sp.		17,17	4,461616667	106
0	0	17	4	8	58	50	Epizoanthus sp.		17,17	4,461616667	106
0	0	22	16	8	58	55	Epizoanthus sp.		22,67	5,891066667	106
0	0	24	17	8	58	57	Eunicella cavolini		24,71	6,421695833	106
0	0	27	9	8	59	0	Smittina cervicornis		27,38	7,1147625	106
0	0	29	4	8	59	2	Smittina cervicornis		29,17	7,580416667	106
0	0	31	9	8	59	4	Epizoanthus sp.		31,38	8,1543625	106
0	0	31	9	8	59	4	Eunicella cavolini		31,38	8,1543625	106
0	0	31	9	8	59	4	Suberites syringella		31,38	8,1543625	106
0	0	32	2	8	59	5	Alcyonium palmatum		32,08	8,338458333	106

Survey:	INDEMARES 2
Station number:	68
Code:	IND2_ST68
Start Latitude:	3,3630
Start Longitude:	42,3074
End Latitude:	3,3645
End Longitude:	42,3082
Start time:	11:48
End time:	14:25
Start depth:	109
End depth:	114
Length (m):	892,64
Edited duration (min):	01:40:06
Edited duration (sec):	6010
Average speed (m/s):	0,14852579

	from TIMECODE				to TIMECODE				uration (sec	Length (m)	verage speed (m/s)
Part 1	0	0	0	0	0	18	48	18	1128,72	147	0,13023602
Part 2	0	18	48	18	0	29	27	10	638,68	102	0,15970439
Part 3	0	29	27	10	0	34	4	21	277,44	99	0,35683391
Part 4	0	34	4	21	0	44	32	13	627,68	115	0,183214377
Part 5	0	44	32	13	1	2	58	12	1105,96	167	0,151000036
Part 6	1	2	58	12	1	15	15	16	737,16	170	0,230614792
Part 7	1	15	15	16	1	24	56	17	581,04	92,6	0,159369407
Part 8									0		#DIV/0!
Part 9									0		#DIV/0!
Part 10									0		#DIV/0!
Part 11									0		#DIV/0!
Part 12									0		#DIV/0!
Part 13									0		#DIV/0!
Part 14									0		#DIV/0!

TIME CODE								REAL TIME						DISTANCE (m)			
FROM				TO				FROM			TO			FROM	TO	ROV	Length of each part
0	0	0	0	0	13	16	7	8	58	33	9	11	49	0,00	206,95	Good sequence	206,95
0	13	16	8	0	14	20	8	9	11	49	9	12	53	206,96	223,60	Bad visibility	16,63
0	14	20	8	0	20	57	0	9	12	53	9	19	30	223,60	326,69	Good sequence	103,10
0	20	57	1	0	21	34	16	9	19	30	9	22	58	326,70	336,48	Bad visibility	9,77
0	21	34	17	0	27	43	15	9	22	58	9	29	41	336,49	432,37	Good sequence	95,88
																Total length =	432,34
																Length good sequence =	405,93

Figure B5.1. Aspect of the template especifically designed to register the main features of an ROV dive, including the position of all species along the transect and the abiotic parameters that can be derived from video images (in this case, substrate type, depth and seabed slope).

This panel incorporates a series of partitions that can be made to the ROV track to increase the accuracy of the analyses: each partition contains its length in meters and also the time it has taken the ROV to complete it, by adding the start and end time given by the video editing software. This way, the web-based software can calculate the average speed of the ROV in each partition.

Sheet 2: Sequence. It contains the information about the amount of video footage that can be considered of a good quality, and hence, can be incorporated in the statistical analyses. The software determines what sampling units can be considered valid based on the information provided here.

Sheet 3: Depth. It contains the depth information along the ROV dive, in one-meter depth intervals.

Sheet 4: Substrate type. It contains information about the type of substrate that can be observed along the ROV transect. There exists the possibility to include two different classifications, one coarser (hard and soft substrate) and one more specific.

Sheet 5: Slope. It contains information about how slope varies along the transect. This sheet can be filled either categorically directly from the video images (e.g. flat, sloping and vertical) or if the GPS positioning is accurate enough, in degrees derived from the bathymetry.

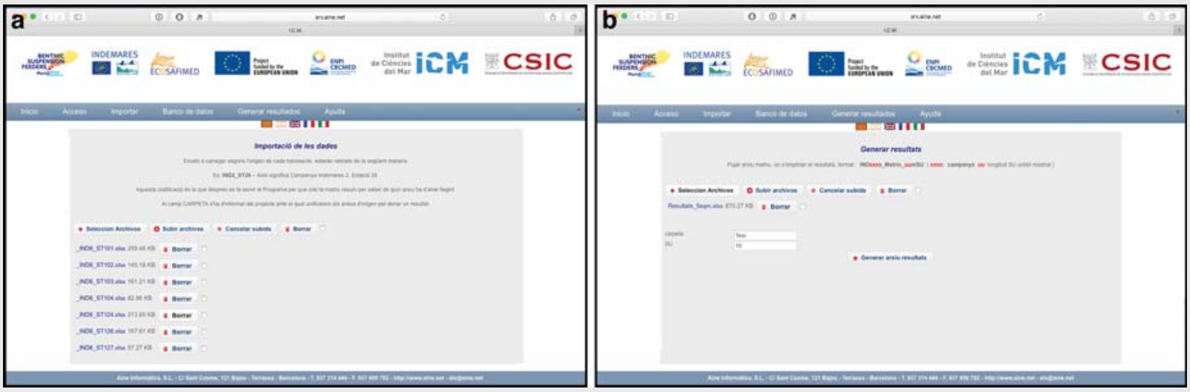


Figure B5.2. Aspect of the web-based software designed to automatically calculate species density in sampling units of a selected size. (a) Screenshot of the upload section of the web application and (b) screenshot of the output generating section, where the user can input the size of the sampling unit.

Sheet 6: Organisms. It contains information about the different organisms identified along the transect, which are introduced according to the frame in which they are observed. The software will then convert the timecode of each frame in distance from the begining of the track. There is also the possibility to add additional information about each organism, such as body measures (size, width), behavior, color, etc.

Once the Excel file of each ROV dive has been completed, the user can upload them into the web-based software to obtain the quantitative data. The user only has to provide the size of the sampling unit and the software does the counting automatically, providing an Excel file that contains the following information:

A. A summary of the number of organisms of each species in each ROV dive
B. The number of organisms of each species in each of the sampling units of a selected size.
C. The value of density of each species in each sampling unit given in organisms per square meter.

There is no need to download and install the software since all the process is made online (Fig. B5.2). At the moment, the software is for internal use only, but there is a plan to open it to the general public so it can be used free of charge. The web page will have detailed information on how to edit and analyze the video footage and how to use the software. After a simple registration, the user will be given a password to gain access to the software privately, in a way that the data introduced is securely handled.

4.5 Environmental factors

A series of environmental factors were considered to explore the role of external drivers, both natural and anthropogenic, on the composition and structure of the benthic assemblages in Cap de Creus. Bearing in mind the practical approach of this PhD thesis, the interest was basically placed in opposing the role played by the abiotic factors against the effect (supposedly) exerted by the commercial fishing practices. The factors selected can be divided in 4 groups according to the way they were acquired: (1) substrate type, (2) depth and geomorphological attributes, (3) bottom currents and (4) the activity of bottom trawlers. Table 4.3 summarizes all environmental parameters employed, their typology, how they were obtained and their actual range.

4.5.1 Substrate type

Substrate type was directly evaluated from the video footage. After an initial examination of the images together with a senior geologist (Dr. Claudio lo Iacono, National Oceanography Center, Southampton), substrate type was divided in 10 categories in order to incorporate the existing variability of substrates present on the continental shelf and submarine canyon off Cap de Creus (see Annex II for more detailed information).

The complexity of discriminating between similar categories when visibility was not great, together with the difficulty of interpreting results that include so many categories, led to a further reduction in the number of typologies. The 10 initial categories were grouped to produce a final classification based on 5 different substrate types easily distinguishable from one another.

The final substrate types, which are shown in Fig. 4.5, can be summarized as follows:

i. **Muddy fine to medium sands.** It corresponds to soft bottoms with the largest proportion of fine sediments. If clay and mud were dominant, the substrate became compact and little bioturbation was observed. Due to the configuration of Cap de Creus, there always existed a certain degree of sand in the soft bottoms of the continental shelf, although the ratio mud:sand varied in the different areas. When sand was found in large quantities, small mounds of a few cm were observed. Holes can be present either with mud or sand, although burrowing organisms find it easier to live in sandy bottoms. In general, the finest sediments tended to appear in deep areas inside the canyon.

ii. **Medium sands to fine gravel.** This category is rather broad, but it responds to the complex environmental conditions found off Cap de Creus. The strong prevailing currents tend to wash out the finer particles, leaving small stones and shells exposed. There actually existed very few places on the continental shelf where sand was found alone, and it was common to find sandy areas with a certain amount of small bioclastic gravels.

iii. **Coarse gravels to pebbles.** It corresponds to bottoms dominated by large numbers of dead shells, which generally increased in proportion close to the shelf break or in the upper part of the continental slope. Large deposits of bioclastic gravels were also found in the canyon walls due to dominant bottom currents, which move large amounts of sediment towards deeper areas. The large number of shells present in Cap de Creus continental shelf can be considered the remains of ancient fauna, now partially extinct, that developed in a past era (Pruvot & Robert 1897).

iv. **Suboutcropping rock.** This is probably the most complicated type of substrate to identify. In most cases, the actual rock was barely visible and was covered by a fine layer of sediment. Biological structures that need hard substrates to develop were used for discrimination. In some cases, when the suboutcropping rock was difficult to identify, the nature of the sediment covering it was used.

v. **Outcropping rock.** This category includes exposed mother rock and also large boulders and slabs, which most times were found on top of outcropping or suboutcropping rocks. In any case, this category was very distinctive in all situations.

At the same time, to have a detailed idea of how the different substrates distribute along the whole study area, a substrate map produced by the Spanish Fisheries General Secretary was also used. This map was based on the information gathered in a specific survey performed off Cap de Creus,

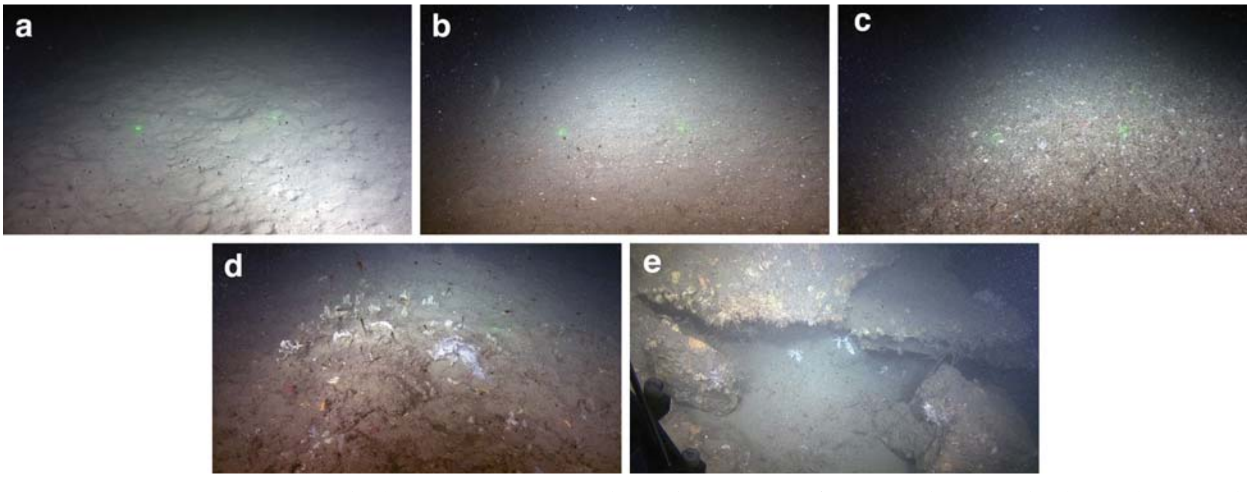


Figure 4.5. Main categories in which substrate type was divided: (a) Muddy fine to medium sands, (b) Medium sands to fine gravel, (c) Coarse gravels to pebbles, (d) Suboutcropping rock and (e) Outcropping rock. All images by JAGO Team (GEOMAR). Further details are provided in Annex II.

Table 4.3. Explanatory variables used to environmentally characterize the megabenthic assemblages and used as raster layers for the predictive modelling of diversity and benthic assemblages. TPI: Topographic position index; TRI: Terrain ruggedness index; FI: Fishing Intensity; n/a: Not applicable.

Variable	Source	Resolution	Type of variable
Substrate type	Video footage	5 m^2	Categorical (5 levels, described in the text)
Depth	Multibeam bathymetry	10 x 10 m	Numerical, continuous (range: 80 to 400 m)
Slope	Multibeam bathymetry	10 x 10 m	Numerical, continuous (range: 0 to 90º)
Surface Ratio	Multibeam bathymetry	10 x 10 m	Numerical, continuous (range: 1 to 1.55)
TPI	Multibeam bathymetry	10 x 10 m	Numerical, continuous (calculated at 3 scales)
TRI	Multibeam bathymetry	10 x 10 m	Numerical, continuous (calculated at 3 scales)
Northness	Multibeam bathymetry	10 x 10 m	Numerical, continuous (range: -1 to 1)
Eastness	Multibeam bathymetry	10 x 10 m	Numerical, continuous (range: -1 to 1)
Latitude	GPS position	n/a	Numerical, continuous (range: 42.261º N to 42.412º N)
Longitude	GPS position	n/a	Numerical, continuous (range: 3.253º E to 3.463º E)
Current, avg. speed	SYMPHONIE model	400 x 300 m	Numerical, continuous (range: 0 to 0.17 m/s)
Current, max. speed	SYMPHONIE model	400 x 300 m	Numerical, continuous (range: 0 to 0.70 m/s)
Current, sd	SYMPHONIE model	400 x 300 m	Numerical, continuous (range: 0 to 0.05 m/s)
Current, direction	SYMPHONIE model	400 x 300 m	Numerical, continuous (range: 0 to 360º)
FI, nº pings	Vessel Monitoring System	250 x 250 m	Numerical, continuous (range: 0 to 237 pings)
FI, qualitative	Vessel Monitoring System	250 x 250 m	Categorical (No fishing, Low, Med, High)

where backscatter data derived from swath bathymetry was groundtruthed with sediment samples collected along the whole study area. The final map provided by the Spanish Fisheries General Secretary consisted of 9 different sediment categories, which were then compared to the images recorded by the ROV. In order to be able to use the map provided, their 9 categories were reduced to 4 and groundtruthed with the video images.

The resulting substrate map is shown in Fig. 4.6a.

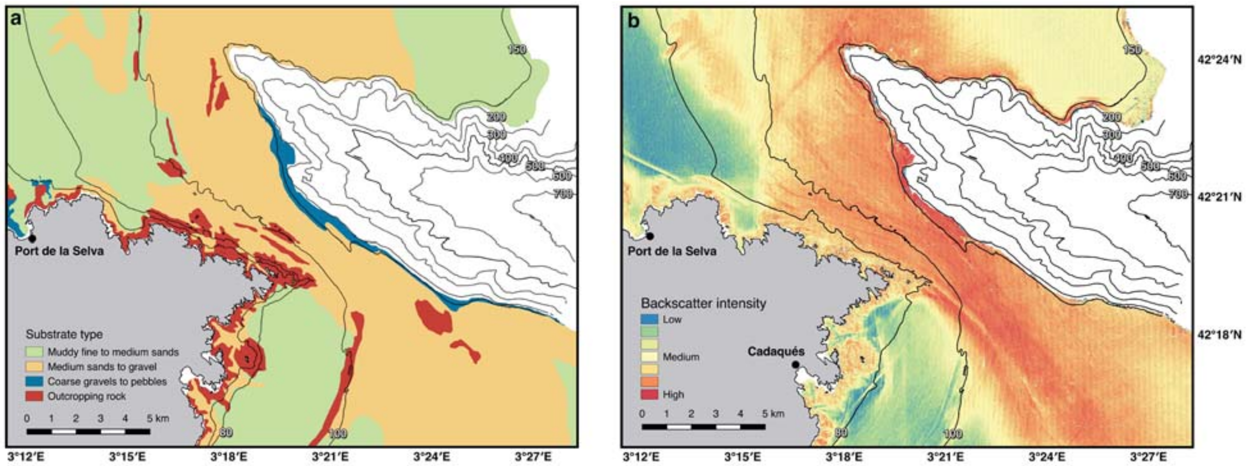


Figure 4.6. (a) Substrate map displaying the spatial distribution of the main sea-bed categories identified in the ROV video footage. The map has been adapted from the original 9-category map provided by the Spanish Fisheries General Secretary. (b) Backscatter map of the study area. Data provided by the Spanish Fisheries General Secretary.

4.5.2 Bathymetry and terrain attributes

Backscatter. Acoustic backscatter data for the continental shelf was provided by the Spanish Fisheries General Secretary and acquired in the frame of the ESPACE Project. Data was collected using a multi-beam echo-sounder at a frequency of 180 kHz, corrected with sound velocity profiles obtained by multiple CTD deployments during the cruise. Filtered and corrected backscatter data was gridded to a cell size of 10 by 10 m, obtaining the final digital map shown in Fig. 4.6b.

Depth. The depth profile for shelf area was provided by the Spanish Fisheries General Secretary and acquired in the frame of the ESPACE Project. Additional bathymetric data was acquired in specific sectors as a part of the projects DeepCoral and Life+ Indemares. Multibeam data for the submarine canyon was acquired during different surveys performed by the University of Barcelona, AOA Geophysics and the Institute of Marine Sciences (ICM-CSIC).

After combining the different datasets, a 10 by 10 meter cell size bathymetric image of the area was produced, which encompassed a depth range between 20 and 850 m (Fig. 4.7). The swath bathymetry data was processed by Dr. Claudio lo Iacono (National Oceanography Center, Southampton).

Slope. Calculated using the 'Slope' tool included in the 'Spatial Analyst Tools' from the GIS software ArcGis. This tool calculates the maximum amount of change between contiguous cells, providing a slope value in degrees for each cell in the output raster layer. More information can be found in the ESRI webpage (http://goo.gl/WpxLXD). Slope calculations were performed by Dr. Katleen Robert (National Oceanography Center, Southampton). The resulting map is shown in Fig. 4.8a.

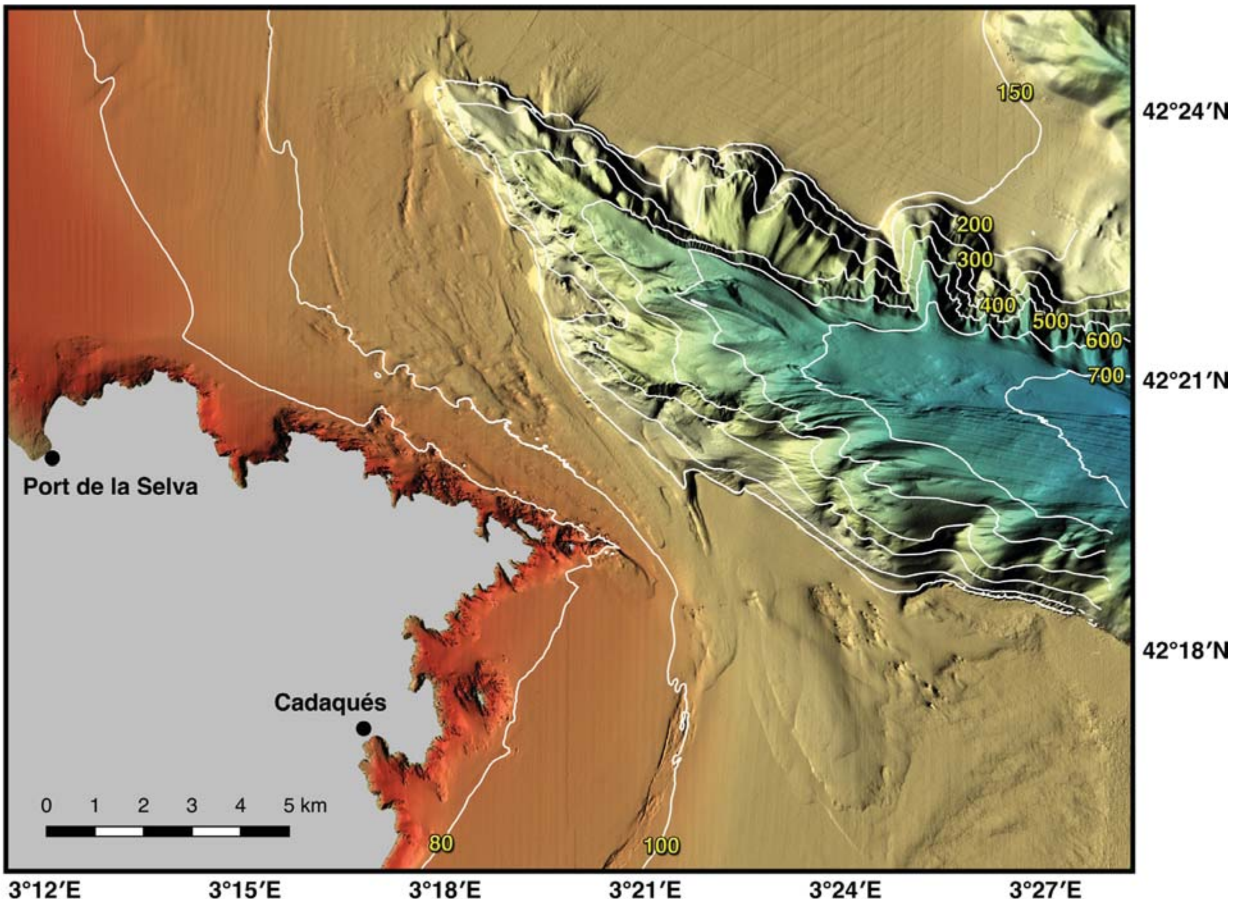


Figure 4.7. Three-dimensional bathymetric map of the study area based on a 10 m resolution grid. Data obtained by the Spanish Fishery General Secretary as part of the ESPACE Project, AOA Geophysics, University of Barcelona and ICM-CSIC during the Indemares project. Map produced by Dr. Claudio Lo Iacono (National Oceanography Center, Southampton)

Surface ratio. Surface ratio is a measure of the roughness of the landscape, since it calculates the ratio between the land area available to an animal (surface area) and the planimetric area using 9 contiguous pixels (Jenness 2004). The Surface area ratio was calculated using the Jenness Enterprises ‘DEM Surface Tools’ and ‘Land Facet Corridor Designer’ toolboxes for ArcGis. Surface Ratio calculations were performed by Dr. Katleen Robert (National Oceanography Center, Southampton). The resulting map is shown in Fig. 4.8b.

Topographic Position Index (TPI). Also named Bathymetric Position Index (BPI), this measure provides information about the presence of geological features in the terrain. TPI values give an indication of what pixels belong to positive features, such a crests or mounds, against those that belong to negative features, such canyons or gullies (Wilson *et al.* 2007). Pixels with positive values of TPI have lower depth values than their surroundings, and vice versa. Since the measure of TPI is based on the variation among cells within a specified radius, there exists the possibility to calculate this index at different scales in order to identify local or regional features. In our case, TPI values

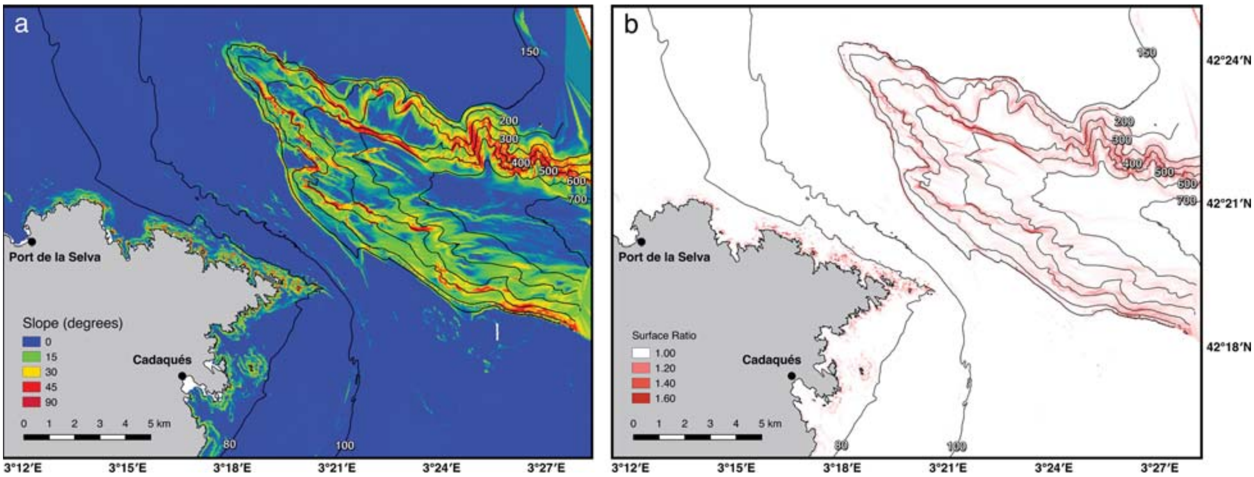


Figure 4.8. (a) Map showing slope values for the study area derived from the multibeam bathymetry. (b) Map displaying the calculated values of surface ratio, which provides an indication of the roughness of the seabed landscape.

were calculated for distances of 10, 30, 100 and 500 m radius. TPIs were calculated using the Jenness Enterprises 'DEM Surface Tools' and 'Land Facet Corridor Designer' toolboxes for ArcGis. All TPI calculations were performed by Dr. Katleen Robert (National Oceanography Center, Southampton). As an example, the resulting map selecting a radius of 500 m is shown in Fig. 4.9a.

Terrain Ruggedness Index (TRI). TRI is a quantitative measure of topographic heterogeneity, since it calculates the sum change in terrain elevation between a central cell and its eight neighboring grid cells (Riley, DeGloria & Elliot 1999). The algorithm used can calculate the index at different scales, so the same scales used for TPI were applied (10-30-100-500 m radius). TRI was computed in SAGA GIS using the tools provided by the library 'Terrain Analysis | Morphometry'. All TRI calculations were performed by Dr. Katleen Robert (National Oceanography Center, Southampton). As an example, the resulting map selecting a radius of 500 m is shown in Fig. 4.9b.

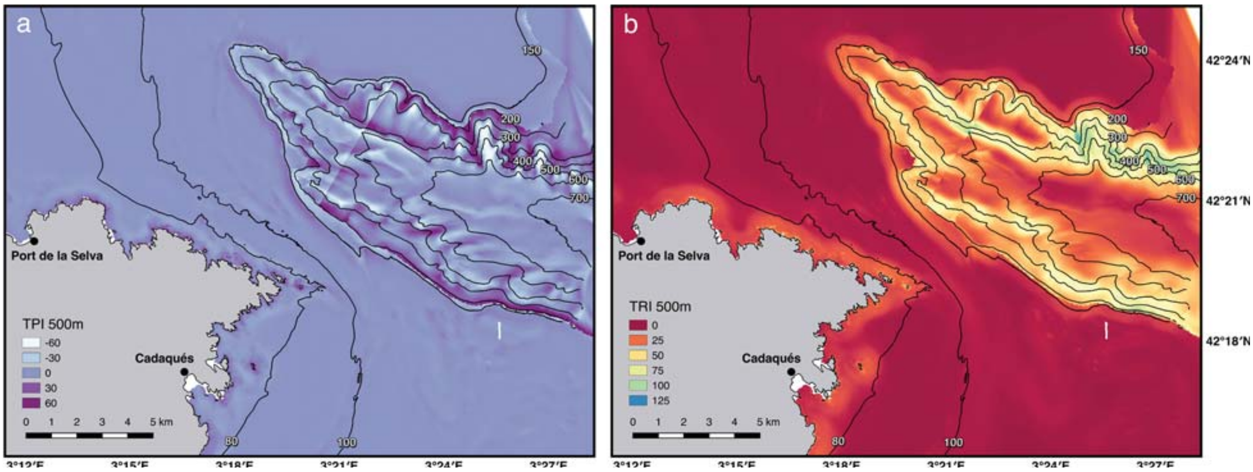


Figure 4.9. Values for Topographic Position index (a) and Terrain Ruggedness index (b) for the whole study area calculated using a 500 m radius.

4.5.3 Bottom currents

The bottom current model used in this thesis was built by Dr. Claude Estournel and her team (Laboratoire d'Aérologie, CNRS, Toulouse). It uses the free surface, generalized sigma vertical coordinate, 3D hydrodynamic model SYMPHONIE described by Marsaleix *et al.* (2008), Marsaleix, Auclair & Estournel (2009) and Marsaleix *et al.* (2012). It classically solves the equations for temperature, salinity and the two components of horizontal current starting from initial conditions and using time-dependent forcing. The vertical diffusion is parameterized following Gaspar, Grégoris & Lefevre (1990) with a prognostic equation for the turbulent kinetic energy and a diagnostic equation for the mixing and dissipation lengths. This model has previously been used in the Mediterranean to simulate several processes from the near-shore (Michaud *et al.* 2012) to the shelf (wind induced circulation, Estournel 2003; rivers plume, Estournel *et al.* 1997) and the deep sea. Dense water formation has been studied in the open sea (Herrmann *et al.* 2008a), as well as on the shelf leading in that case to cascading towards the deep environment (Ulses *et al.* 2008; Estournel *et al.* 2005; Herrmann *et al.* 2008b).

In the present application, a long run (January 2000 to December 2013) was done for different purposes linked to the study of the interannual variability of physical, biogeochemical and biological processes. The main interest was the Gulf of Lions and the Catalan region, but also the connections between these regions and the south of the basin through the dispersion of dense water. The numerical grid developed is a curvilinear grid with a pole positioned in the Pyrenees, which allows for a minimum resolution of 700 m near the French and Catalan coasts, and increases towards the south to reach 5-6 km near Algeria (Fig. 4.10).

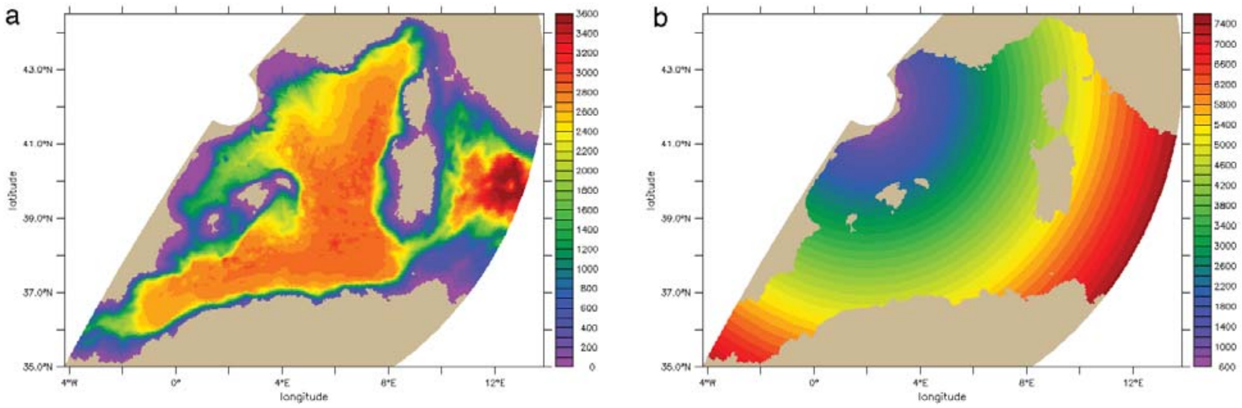


Figure 4.10. (a) Bathymetry for the whole western Mediterranean basin used to calculate bottom current speed and direction. (b) Size of curvilinear grid with a pole position at the Pyrinees, where it has the smallest resolution, which increases with distance to reach almost 7000 m in the southern part of the basin. Images provided by Claude Estournel.

The model was initialized and forced at its open boundaries by the NEMOMED8 model described in Herrmann *et al.* (2010). At the surface, the same atmospheric forcing of the model ARPERA was used (Herrmann & Somot 2008), which is a dynamic downscaling of the ERA40 climate model reanalysis (1976-2001) and the ECMWF (European Centre for Medium-Range Weather Forecasts) model reanalysis since 2001. This forcing consists in daily averaged wind stress, solar

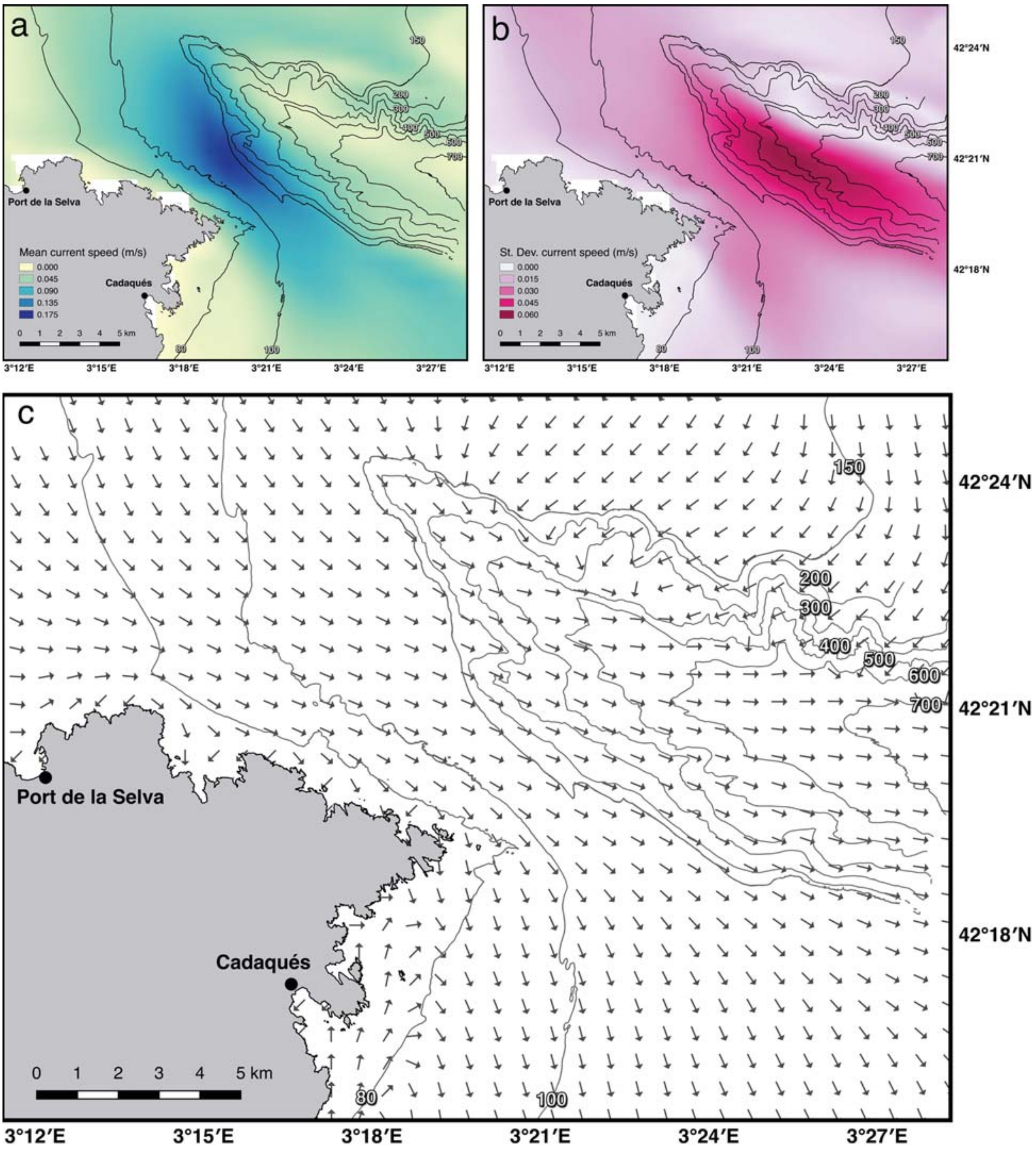


Figure 4.11. Average (a) and maximum (b) bottom current speed and average current direction (c) derived from the 3D hydrodynamic model SYMPHONIE, calculated from the montly averages modelled for the months of January 2000 to December 2003.

flux, long wave net heat flux, sensible and latent heat flux and precipitation, leaving the diurnal cycle unresolved. The sea surface temperature is nudged toward the climatological one itself used in the NEMOMED8 model. This procedure is done to ensure consistency between the two models.

The application generated for this PhD thesis used the two components of the horizontal current, which were extracted from the daily outputs of the model. As they are oriented along the axis of the model, they were first rotated to be along the WE and NS axis. The second step of the post treatment was an interpolation on a regular grid to generate 12 monthly averages for the years 2004 to 2008. From this large dataset, 3 final maps were generated: the average current speed for the whole period, the maximum average speed registered in a single month and the standard deviation, to determine areas of high and low fluctuations throughout the different years. The resulting 3 maps are shown in Fig. 4.11.

4.5.4 Fishing activity

A. Identification of trawling grounds using VMS data

Information about the distribution and intensity of the commercial fishing activity off Cap de Creus was evaluated using the locations of the different trawlers recorded by the Vessel Monitoring System (VMS), which were provided by the General Directorate of Fisheries Management of the Spanish Ministry of Agriculture, Food and Environment (MAGRAMA). VMS technology was initially implemented to aid in the monitoring of fishing activities, but it was soon used to improve the spatial management of marine areas since it provides a far more explicit description of how fisheries organize themselves temporally and spatially in comparison to data collected through catch-book statistics (Witt *et al.* 2007).

Before performing any data treatment, the original raw dataset was subjected to a validation process to exclude errors in vessel identification, position and speed, as well as the removal of any duplicated positions. A second filtering consisted in selecting those pings that corresponded to fishing activity inside the area declared as a Site of Community Importance (SCI) by following a speed-rule approach (see Lee, South & Jennings 2010). This approach consisted in removing pings with a vessel speed below 2 or above 3.5 knots, as well as those points found less than 2 nm from port. Data was then projected to ETRS89-Transverse Mercator-31N coordinate system following the Directive for the Infrastructure for Spatial Information in the European Community (INSPIRE).

To study the distribution and intensity of the fishing activity, we used an estimation of "fishing events per vessel and day per unit area", based on a point summation method over a grid of squared cells. To avoid the overrepresentation produced by vessels emitting at higher frequencies, one fishing event (ping) per vessel and day was randomly selected from January 2007 to July 2012. This approach reduced the overestimation of the fishing activity when the interval between records is large or the uncertainty of the in-between positions is considerable (Hintzen, Piet & Brunel 2010). The size of the unit area or grid cell used to display the results affects the interpretation of the fishing distribution, and hence, selecting the right scale is of key importance. The apparent area of a given fishing ground could appear too large if the cell size selected is too large (Piet & Quirijns 2009). In this sense, the predicted spatial extent of the effects produced by bottom trawling might vary depending on the size of the grid cell (Lambert *et al.* 2012), and this can also have an influence in the applicability of certain management measures (Jennings & Lee 2012).

As a starting point, we applied the MBR method (Chainey 2013), which divides the shorter side of the study area's minimum bounding rectangle by 150, and then we applied a fine tuning to match

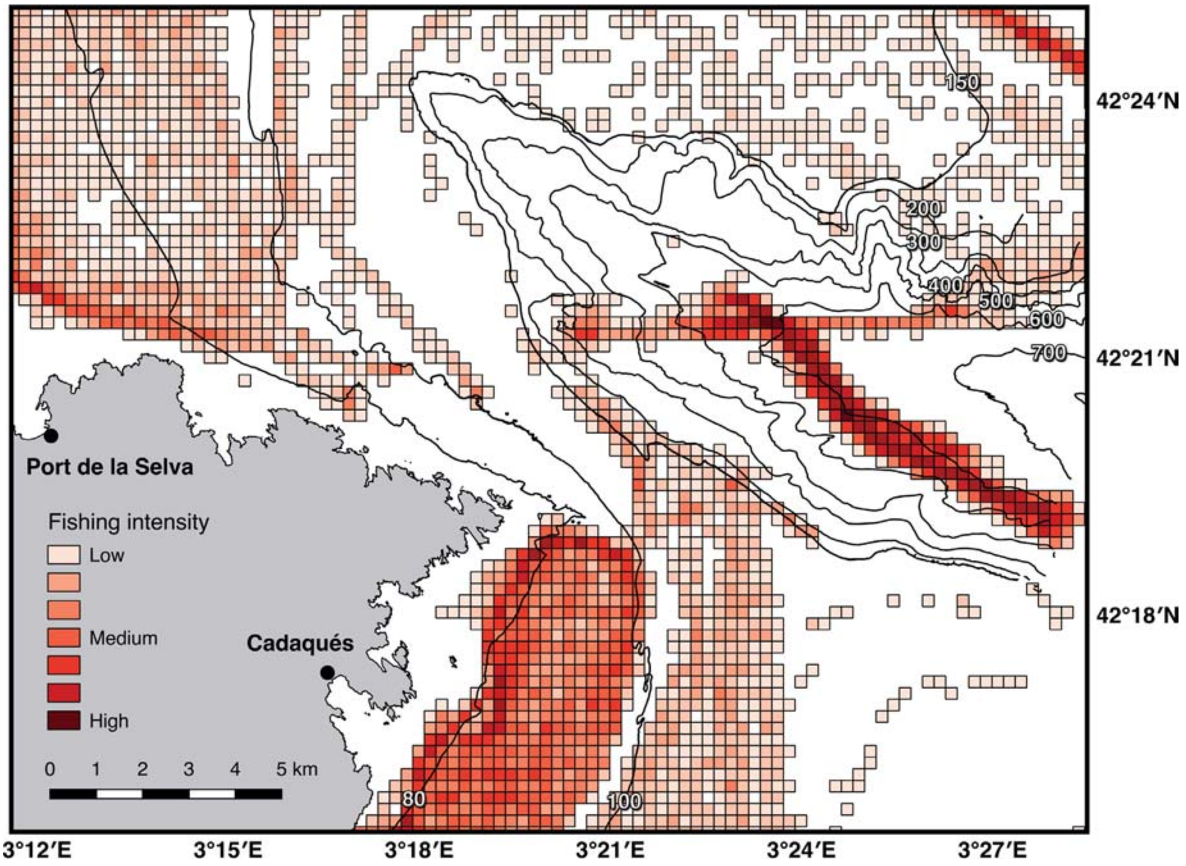


Figure 4.12. Spatial distribution of the fishing effort in the study area scaled to a grid of 250 x 250 m. Fishing intensity was calculated as the sum of pings per boat per day in each grid cell during 5 consecutive years. Details of the methodology employed are given in the main text.

cell size to the needs of this thesis. After some trials, a 250 by 250 meter cell grid provided the best fit. All valid data points inside each grid cell were then summed, to obtain a proxy for the spatial distribution and intensity of commercial bottom trawling. All the processing of the data was performed using geoprocessing techniques and tools within the QGIS (http://www.qgis.org) and the ArcGIS 10.2® (Esri software) and the Marine Geospatial Ecology Tools library. All calculations with VMS fishing data were performed by Dr. Susana Requena Moreno (Institut de Ciències del Mar, Barcelona). The final map produced is shown in Fig. 4.12.

B. Validation of VMS data with the observed trawl marks

Marks left by trawl nets and doors over the sea floor are one of the most visible consequences of bottom trawling activities. Trawl marks can be detected with imaging methods due to the large scars left on the seabed, which are mostly presented in the forms of (1) large furrow marks produced by the trawl doors, often extending to more than 20 cm inside the sediment, and (2) scrape marks produced by trawl cables and sweeps, which can be observed as parallel sets of small furrows that barely penetrate more than a few centimeters in the sediment (Smith, Banks & Papadopoulou 2007). More recent marks are usually characterized by hard edges and a lighter sediment color, while older marks tend to present softer edges and are accompanied by bioturbation features, such as holes or mounds (Smith 2000).

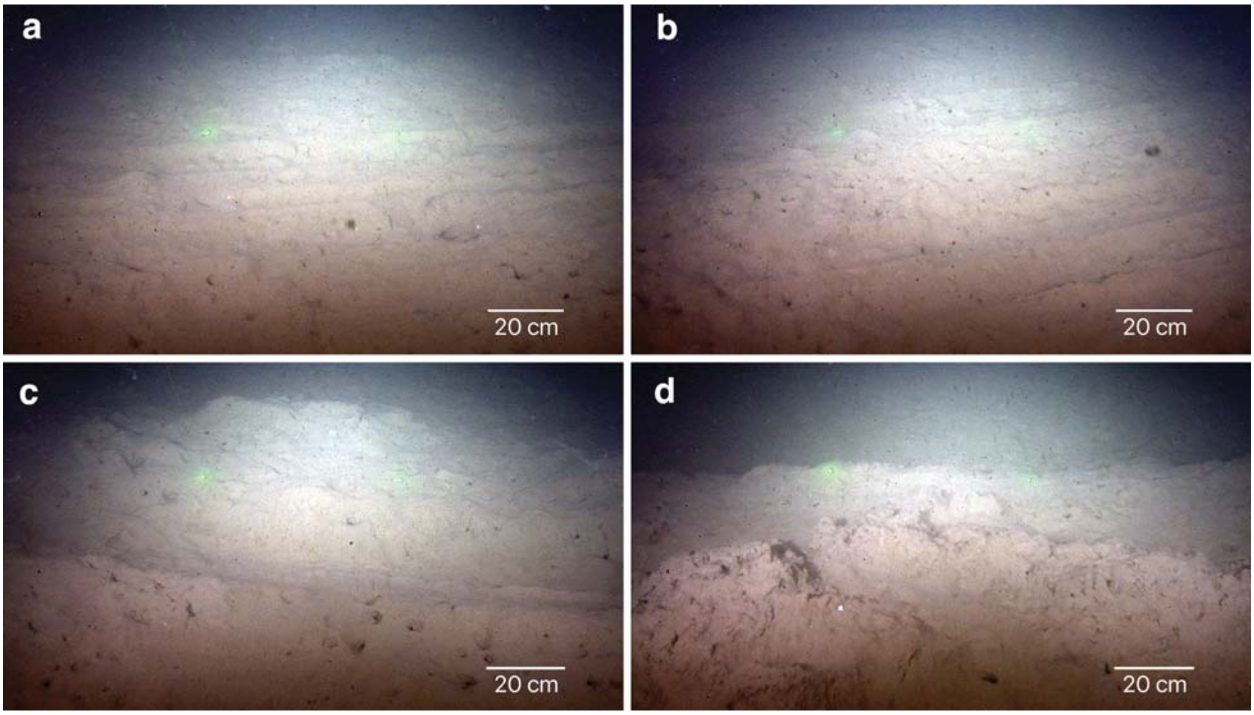


Figure 4.13. Aspect of some trawl marks observed in the ROV footage from Cap de Creus. (a-b) Small-sized parallel scrape marks left by cables or sweeps. (c-d) Larger marks left by the trawl doors. Images by JAGO Team (GEOMAR).

During the analysis of the video images to determine the species composition of the invertebrate and fish assemblages, all identifiable trawl marks were also reported, primarily all those that appeared across the image. The aspect of the scrape marks and furrows left by bottom trawlers over the continental shelf off Cap de Creus is shown in Fig. 4.13.

To estimate trawl mark density in the different areas of the shelf, and also to compare with other studies, the number of marks identified in each the ROV dive were standardized to trawl marks per 100 linear meters by simply dividing the number of trawl marks by the length of each dive ($De = TM \cdot 100 / L$). Those densities were then plotted on a map containing the estimates for the fishing intensity obtained from the VMS data in order to evaluate the degree of overlap between them.

4.6 Analysis of megabenthic assemblages

4.6.1 Discretization of the data and selection of sampling unit size

The use of ROVs for the study of the marine benthos can provide information in the form of long strips along the seabed, which generally cover relatively large areas. This type of information is rarely accessible in benthic community ecology, where data is traditionally collected in small and discrete sampling units scattered across the study area, leaving large distances with no information between samples. Without denying the possibilities offered by line transects (e.g. spatial patterns of species distributions or gradients of change in species composition), we decided to discretize the data obtained from the video images in a series of contiguous sampling units, which became the basis of all statistical analyses performed in this PhD thesis.

This approach gave the possibility (1) to compare the results with those obtained in previous studies performed either in our study area or in other areas of the Mediterranean, and also (2) to develop a simple protocol for the future monitoring programme of the marine protected area off Cap de Creus. Discretizing the information guarantees that the methodology adopted is replicable in time and space, a necessary step to be taken if the results are to be used as baseline data in future monitoring plans. Selecting the optimal sampling size when discretizing biological data, however, is of key importance if spatial patterns are to be established and quantified (Andrew & Mapstone 1987). The use of an inappropriate size of sampling unit may hide patterns of association between two or more species, limiting our understanding of the ecological processes that lie behind (Underwood & Chapman 2013). In essence, the area of the samples must be large enough to be representative but small enough to reduce the amount of effort necessary to process it. In our case, since data was extracted for the whole transect, the idea was to generate a series of sampling units within each transect that were large enough to provide an accurate representation of the number of species and their relative abundances in each of the biological assemblages present in our study area (Weinberg 1978a).

The continuous nature of the data generated by each ROV dive gave us the possibility to explore with ease the relationship between sampling effort (size of the sampling units) and the accumulated yield, expressed as biological diversity (number of species) or biomass (number of individuals). The number of organisms per sampling unit ranged from around 10 for the smallest sample (1m^2) to a little more than 100 for the largest size for which data was calculated (10m^2), increasing linearly with the size of the sampling unit. The average density of organisms, however, remained stable around 10.3-10.5 ind·m^{-2} regardless of the sampling area. In the end, we decided to establish the size of the sampling unit based upon 2 different curves: (1) the average number of species per area and (2) the maximum number of species per area. These 2 curves are presented in Fig. 4.14.

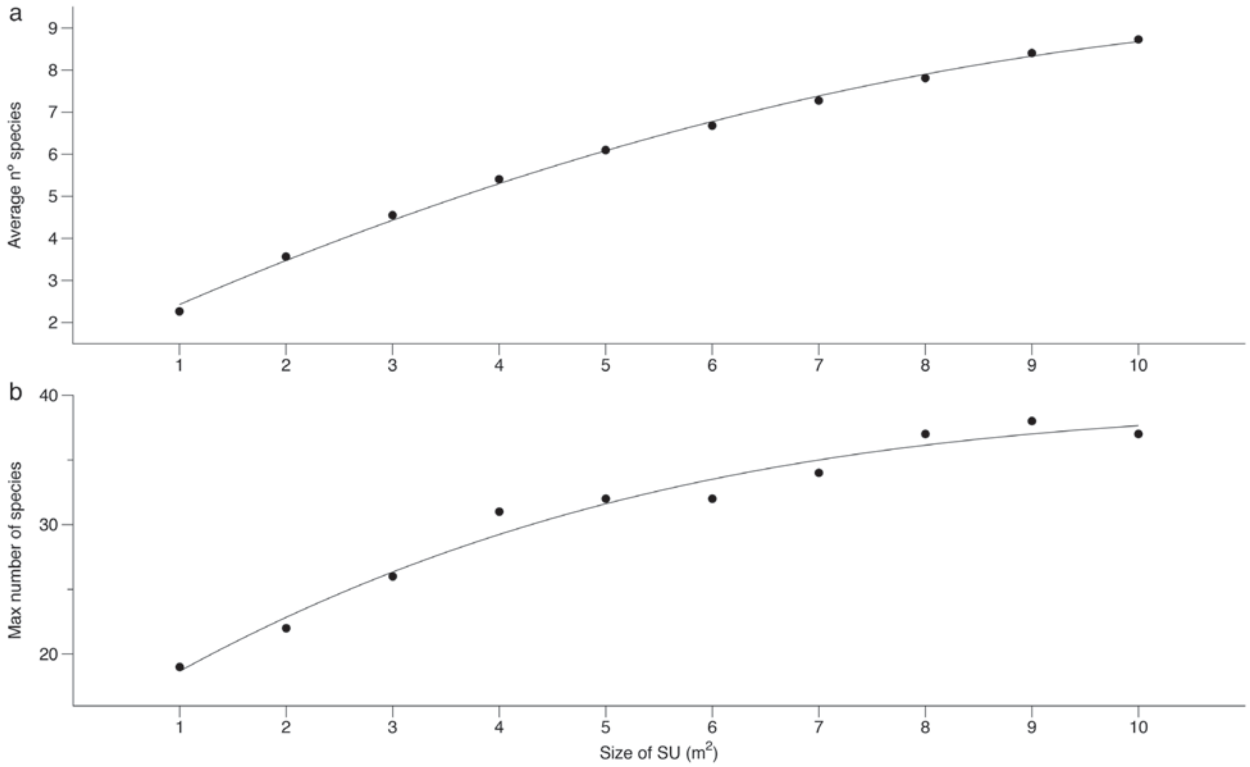


Figure 4.14. Species accumulation curves for the shelf and submarine canyon off Cap de Creus. (a) Average number of species and (b) maximum number of species per sampling unit for increasing sizes of sampling unit.

An area of 5 m^2 was finally selected for the analyses to be performed throughout this PhD thesis. The criteria used to select the size of the sampling unit can be summarized as follows:

1. The average number of species registered was approximately 2/3 the average number of species that would be identified if sampling size was made very large
2. The maximum number of species observed was very similar to the maximum number of species obtained in larger sampling units
3. The variability observed in the average number of species was not extremely large
4. The number of empty sampling units was relatively small
5. The density values obtained were large enough to support any statistical treatment

4.6.2 Selection of species for statistical analyses

The first analysis performed over the biological data obtained from the video images corresponded to an evaluation of the species composition of the benthic fauna, at two different levels: (1) their biological diversity in terms of species richness and (2) the structure it acquires in its spatial distribution, constituted in different assemblages. In order to do so, only the invertebrate fauna was selected from the whole species matrix, isolating the fish fauna (which was evaluated separatedly, see Section 4.11). Highly mobile fauna, such as cephalopod species, were also removed from the matrix to facilitate the community analyses, leaving only sessile fauna and those organisms with a restricted home range.

It is important to point out that a polychaete species from the genus *Lanicides* was also removed from the analysis. This polychaete species lives completely buried inside the sediment, only showing an external structure made with sand grains and some thin tentacles used to capture food particles (see Fig. 4.15). Although *Lanicides* specimens were relatively common in some areas, their identification became very complex due to the cryptic nature of the structure they create. In some situations, especially when the ROV flew relatively far from the sea floor o visibility was not good due to water turbidity, they were very difficult to detect.

4.6.3 Biological diversity

When working at the community level, an effort should be made to synthesize the underlying phenomena behind the data obtained. Assessing the variability among different assemblages through the measure of their biological diversity is a common practice, although there exists a

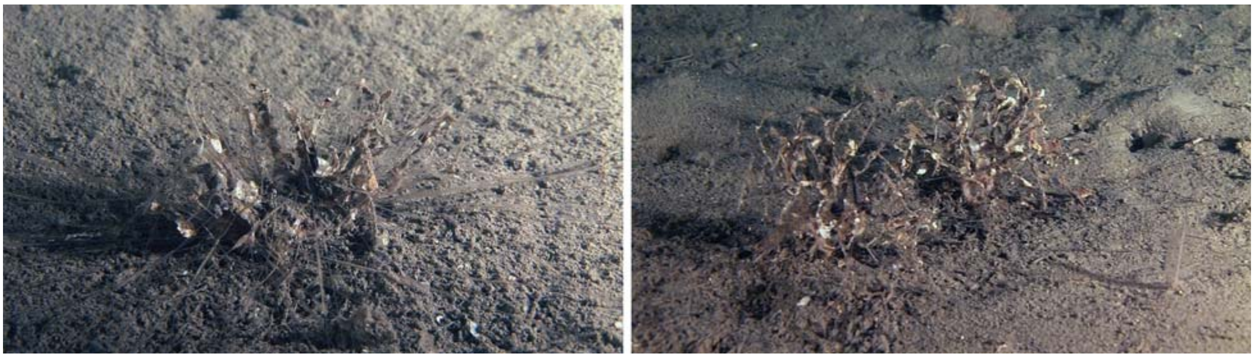


Figura 4.15. Close-up images of the polychaete *Lanicides* sp., which was removed from statistical analyses due to its cryptic nature, which made its identification almost impossible in video sequences with low visibility or filmed at a certain distance from the seabed. Images by Nemo ROV (Gavin Newman).

certain mismatch between what society regards as a rich and diverse community and the measure of diversity itself calculated through diversity indices (Leinster & Cobbold 2012). Such indices, however, have long been regarded as simple ways to summarize community information due to their univariate nature, which allows for simple statistical comparison among samples (Heip, Herman & Soetaertl 1998). They are currently used in a wide range of topics across ecology to obtain quantitative estimates of the existing variability between different biological assemblages, but not a single index can be considered correct for all situations (Peet 1974).

Species richness can be regarded as a good indicator of the relative wealth of species in a community (Peet 1974). It is probably the simplest, most interpretable and unambiguous of all indexes and it also allows for simple comparisons between areas that have been sampled in the same manner. However, since species tend to have different abundances in most datasets, species richness lacks information about the underlying assemblage. In fact, species richness is very sensitive to rare species, masking certain attributes of the community (Jost 2007).

For this reason a wide array of heterogeneity indices that incorporate the proportional abundance of each species (a term named evenness or equitability) have historically been proposed. These indices not only consider the total number of species identified in a sample, but how individuals are distributed among species, incorporating a measure of the structure of the community (Peet 1974). Traditionally, two indices have particularly been used to calculate biological diversity: Shannon entropy and Gini-Simpson Index. Shannon entropy is an index of equitability, while Gini-Simpson expresses concentration of dominance. For this reason, Shannon entropy is very sensitive to changes in the importance of middle-rank species while Gini-Simpson is more sensitive to changes in common species (Peet 1974). These two diversity indices, in fact, are not true diversity indices but entropies and their mathematical behavior does not correspond with the intuitive concept of diversity, since their measure is nonlinear with respect to species addition, even when all species are equally common (Jost 2006). In essence, this implies that their values increase in a smaller proportion as we add new species.

In our case, and making use of the extensive dataset obtained from the underwater images, this effect could be confirmed by simply plotting the species richness measured in each sample against the values of the two indices (Fig. 4.16 a-b). In both cases, diversity grew faster than species richness as we added new species, leading to a plateau where new additions did not have an effect on the diversity value. In practical terms, this implied that changes in the number of species in non-rich assemblages had a higher impact on the diversity value than changes in rich assemblages, which is counter-intuitive. To overcome this situation, both indices can be transformed into true diversities: Shannon entropy is converted by taking its exponential and the Gini-Simpson index by the formula $1/(1-H_{GS})$ (Jost 2007). In these two cases, as can be observed in Fig. 4.16 c-d, the measure of diversity has a linear relationship with species richness when all species are equally common, a result named 'effective number of species'.

For the reasons exposed above, and following the recommendations given by Jost (2007) and Gray (2000), two indices were used during this PhD thesis as proxies of alpha diversity (or intra-community diversity): (1) species richness, understood as the number of megafauna species identified in any given sample, and (2) the exponential of Shannon diversity index or $exp(H_{sh})$: $\exp(-\sum_{i=1}^{S} pi \cdot \ln pi)$

4.6.4 Identification of biological assemblages

Besides the analysis of the biological diversity, clustering and ordination are common multivariate techniques used to synthesize the variability of the data obtained from biological surveys in a format that can easily be interpreted (Legendre & Legendre 2012). There exist multiple ways to approach community analysis, but for this PhD we wanted the benthic fauna to "tell their own story", so we decided to search for patterns in the biological data first, then interpret the results based on the abiotic information available.

Following this approach, the methodology proposed by Field, Clarke & Warwick (1982) to evaluate the structure of the benthic assemblages was partly followed, which makes use of both clustering and ordination techniques. Broadly speaking, a series of steps were taken in order to identify the biological entities that derived form the video images, which were later related to the physical descriptors.

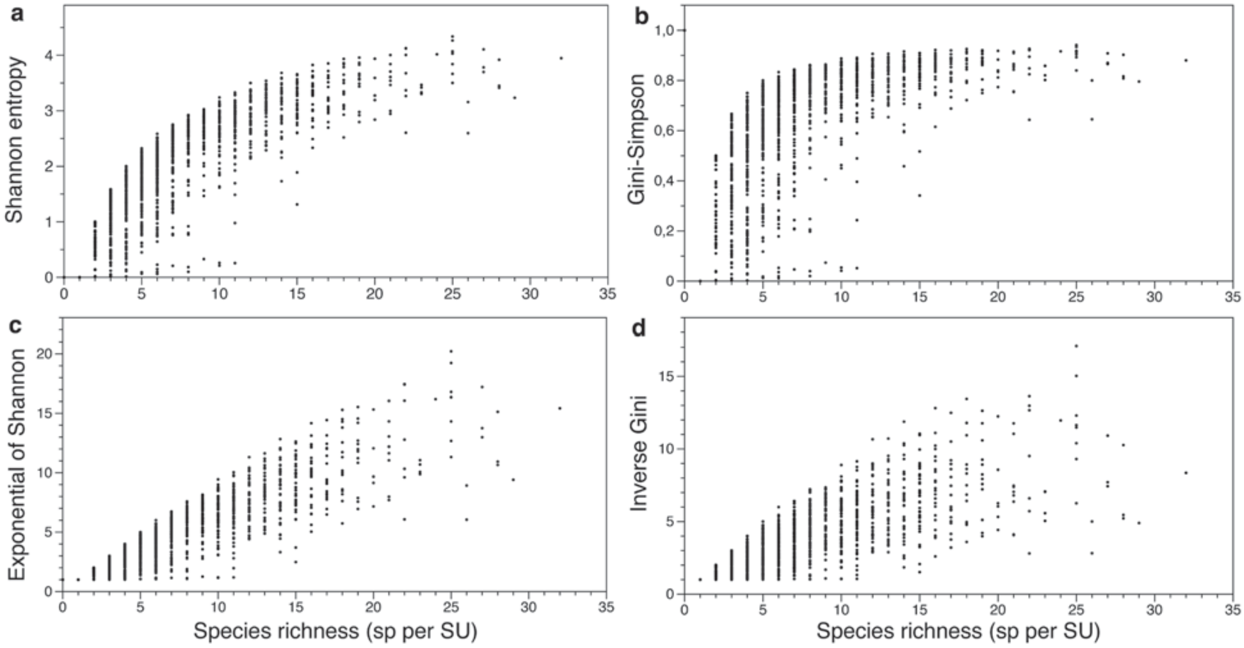


Figure 4.16. Scaterplots between species richness and 4 different diversity indices including (a) Shannon entropy, (b) Gini-Simpson index, (c) exponential of Shannon and (d) inverse of Gini using the data obtained from the video images of Cap de Creus.

Those steps can be summarized as follows:

1. transform the raw data to reduce the weight of the most abundant species
2. determine the relationships between all pairs of samples using a similarity coefficient
3. identify the optimal number of assemblages in which the data can be clustered
4. assign each sample to one of the groups by means of a hierarchical cluster classification
5. identify the most representative species for each of the groups
6. arrange the sampling units in a reduced space via an ordination method to visualize gradients among samples

The first step taken to characterize the benthic assemblages consisted in removing from the original matrix all empty data rows (sampling units) where not a single megafauna organism was found. These samples had to be excluded from the analyses, not only because of their complex biological interpretation, but also due to the mathematical difficulties they introduce to some multivariate calculations.

Another contentious aspect was the way rare species should be treated. In general terms, when large areas within the same habitat are sampled, the list of species found in small numbers becomes large, while the number of common species usually remains stable (Drury 1974). Since our sampled area was relatively large, there existed some rare species that presented very low frequencies or appeared in very low numbers throughout the whole dataset. In fact, more than 40% of the species appeared in less than 1% of the sampling units, and almost 30% of the species were observed less than 10 times in the whole video footage. Historically, there has been a tendency to remove rare species from the multivariate analyses arguing that they contribute little to the general interpretation but add noise to the statistical solution (Cao, Williams & Williams 1998). However, the results of most multivariate techniques are not affected by the presence of rare species that incorporate a small percentage of the total variance, and years ago, deleting them was probably a solution to reduce computing time (Gauch 1982). In this sense, Greenacre (2013) found that rare species appeared as outliers in correspondence analysis ordinations, which gave the impression that they could be acting as very influential points, but overall, their low weight reduced their effect on the final result.

Although it is probably true that community analyses might benefit in a certain way from the removal of very rare species, we considered that it was best to use the same dataset for all the analyses to be performed throughout this PhD thesis. Since the aims of this project were broader than merely identifying the number of benthic assemblages, and some of the analyses dealt with the effect of disturbances on the structure of the benthic fauna, no species were finally removed from the original matrix based on their low representation. On the opposite end we found the massive aggregations of the brittle star *Ophiothrix fragilis*. Density values recorded for this species were two orders of magnitude higher than the average density for all other species. In order to facilitate the interpretation of the results, especially ordinations in a reduced space, samples located on these massive aggregations were removed from the multivariate analyses. These samples were directly considered part of a separate biological assemblage and treated independently thereafter.

Another relevant aspect was the distribution of frequencies among species. While it is true that most multivariate methods do not require data to be normally distributed, they perform better if the distributions of values are not strongly skewed (Legendre & Legendre 2012). In most community studies, for any given species, very few observations often hold very high abundances, with more samples having middle-range abundances and a larger part of the observations with low or zero abundances. To reduce the skewness of the data produced by these irregular distributions, abundance data were transformed by taking the square root, the least drastic of the possible transformations, aiming to reduce the differences in total abundance while keeping the variations in the relative composition of species among sites (Legendre & Legendre 2012). The root transformation ($\sqrt{y}$) down-weights the importance of the most abundant species, so comparisons between samples will depend on less common, mid-of-the-range species (Clarke & Warwick 2001).

Determining the number of megafauna assemblages that currently coexist in our study area was, nonetheless, a crucial step to be taken before clustering the data. Selecting an optimal number of groups or clusters (a term frequently labeled as k) is of key importance if patterns of species aggregation and distribution are to be identified. Associations among species are generally the result of either common environmental requirements or biological interactions between species, and there tends to exist a certain number of species that "significantly" appear together and determine the number of groups to be made (Legendre & Legendre 2012). A simple way to identify the optimal number of clusters in which to split a dataset consists in representing the overall average silhouette width of each cluster solution and select the highest value. The silhouette of a cluster is based on the comparison of its tightness and separation, in a way that its representation displays which samples lay well within their cluster and which ones are located somewhere in between clusters (Rousseeuw 1987). In other words, the silhouette is a calculation of how close samples are to each other in a single cluster in comparison to how close they are to samples in other clusters, and calculated for a series of cluster solutions. The highest the silhouette of a cluster, the better its samples have been classified. Hence, the optimal number of clusters to be made would be indicated by that cluster solution displaying the highest overall average width of its cluster silhouettes. This was calculated using the function *silhouette* included in the *cluster* package of the R software platform (Maechler *et al.* 2017).

Ordinations in a reduced space can hide some of the relationships among samples, mainly due to the presence of intermediate points between groups (Legendre & Legendre 2012). Clustering, on the other hand, is a simple method to identify what samples are sufficiently similar to each other to be put in the same cluster category while recognizing the divisions between groups of samples (Greenacre & Primicerio 2014). Prior to clustering, a dissimilarity matrix was constructed from the abundance table of transformed data (species by sites). The coefficient selected to build the dissimilarity matrix was Bray-Curtis (also named percentage difference by some authors), a very popular semimetric (non-Euclidean) dissimilarity that has been used in many ecological studies of the marine benthos (see for example the recent works of Yesson *et al.* 2015, Davies *et al.* 2014 and Pierdomenico *et al.* 2016). This dissimilarity measure was selected to carry out all the multivariate analyses throughout this PhD thesis due to the wide array of properties that characterize it, which are effectively summarized in Clarke, Somerfield & Chapman (2006).

Here we highlight its most relevant characteristics:

- Dissimilarity values range from 0 to 1, making it easily interpretable. It takes a value of 0 when both samples are identical and a value of 100 when samples have no shared species

- Changes in the units of measurement have no effect on the relative value of the coefficient
- Double absences do not have an effect on the value of the coefficient
- It is best suited for abundance data

Samples were grouped by means of Ward's minimum variance method, a hierarchical agglomerative clustering method. Ward's algorithm finds the minimum total within-group sum-of-squares at each step of the clustering process, joining the samples or groups of samples which result in the smallest increase in the sum of the squared distances between them and the group's centroids (Legendre & Legendre 2012). The result is a hierarchical dendrogram where samples tend to be evenly distributed along the different clusters. Although it is true that this method does not necessarily find the optimal solution due to its iterative (stepwise) procedure, it is a very simple algorithm to find the lowest within-group sum of squares (Greenacre & Primicerio 2014). Ward's dendrogram was obtained using the *hclust* function of the *stats* package of the R software platform (R Core Team 2016).

A distance-based permutational multivariate analysis of variance (PERMANOVA) was employed to determine if the identified groups were significantly different from each other according to their species composition and abundance. PERMANOVA is a non-parametric hypothesis-testing method based on permutation tests that allows for direct comparisons between samples from different groups in a multivariate dataset (Anderson 2001). In general terms, PERMANOVA is suited to a wide range of situations that deal with community data, since it can be run using any type of distance coefficient (metric and non-metric), variables do not need to display a normal distribution, there is no specific assumption about the number of variables to use and it provides a P-value using permutation methods, among others. The permutational multivariate analysis of variance was made using the *adonis* function included in the *vegan* package of the R environment (Oksanen *et al.* 2016).

The most representative species from each of the benthic assemblages were identified using the indicator value index (IndVal), which takes on higher value for those species that are found mostly in a single group and present in the majority of the samples belonging to that group (Dufrêne & Legendre 1997). IndVal is calculated as the product of the relative frequency and relative average abundance in clusters, and its value is not affected by the abundances of other species (Legendre & Legendre 2012). The 10 most representative species of each group were identified using the function *indval* included in the *labdsv* package of the R environment (Roberts 2016).

Finally, the relationships between samples were mapped in a bidimensional space using a Principal Coordinates Analysis (PCoA), also known as metric multidimensional scaling (MDS). This type of ordination is able to obtain a Euclidean representation of the samples when their relationship is measured using any distance coefficient, in our case Bray-Curtis. The principal coordinates calculated are functions of the original variables, but mediated through the distance chosen to calculate the ressemblance among the different samples (Legendre & Legendre 2012). The algorithm behind a PCoA produces a series of uncorrelated (orthogonal) axes, which summarize the variability in the data set. Samples plotted closer to one another will be more similar to those further away. PCoA requires less computing time than an nMDS, and was calculated using the function *cmdscale* included in the *stats* package of the R environment.

4.7 Effects of environmental factors over benthic fauna

Following the strategy described by Field *et al.* (1982), only after groups of biologically similar samples were identified through clustering and ordination, did we proceed to quantify the influence of the environmental factors on the structure of the biotic data. Working in two separate stages, one descriptive and one interpretative, gave the possibility to analyze the biological and the environmental information independently to avoid any influence of previous assumptions, so data could then be analyzed together through an asymmetric form of canonical analysis (in our case, a distance-based redundancy analysis, dbRDA). The interpretation phase, in this sense, made use of the environmental information available to quantify the relationships between the structure of the biotic data and the different explanatory variables (Legendre & Legendre 2012).

4.7.1 Structure of the environmental data

Before exploring the relationships between the biological and the environmental datasets, a principal component analysis (PCA) was performed over the abiotic matrix. Since a PCA reduces the number of variables in large data sets, the idea behind this analysis was to visualize in a reduced space the existing interactions between the different environmental parameters in order to detect trends, groupings or key variables.

All environmental factors available were quantitative with the exception of substrate type, which was categorical. The way video images were acquired, however, allowed for a decomposition of the substrate type in percentage cover in each of the sampling units. This way, each category of substrate was introduced in the analysis as a separate factor, with values ranging from 0 to 100.

PCA

In essence, a PCA provides an overview of the linear relationships between variables, a method best suited for quantitative data in the form of continuous variables. The algorithm behind a PCA generates a set of new uncorrelated variates (named principal components) that are linear combinations of the original variables and correspond to the successive directions of the maximum variance of the data (Legendre & Legendre 2012). A biplot of the first 2 principal components of a PCA can be considered a good way to visually represent multivariate environmental data if they capture most of the variance in the original scatter, although smaller amounts can also be informative (30-40%).

Variables that were highly skewed were log-transformed in order to make their distributions more symmetric (i.e. depth, slope, TRIs and fishing intensity). Prior to PCA analysis, pairwise relationships were examined by means of scatterplot matrices. All TPIs on one side, and all TRIs on the other, were highly correlated among each other, so only TPI 500 m and TRI 500 m were left in the PCA analyses. The PCA on standardized variables was computed using the function *prcomp* included in the *stats* package of the R environment (R Core Team 2016).

4.7.2 Environmental factors as driving forces

In order to explain the patterns found in the biological data, an asymmetrical canonical analysis was performed on the two data matrices. This approach, which is widely used in ecology, employs a constrained ordination method to define a model that relates the response data (biotic information) as a function of the explanatory variables (abiotic information) (Greenacre & Primicerio 2014). The method selected was a distance-based redundancy analysis (dbRDA, Legendre & Anderson 1999), a direct extension of multiple regression analysis to the modeling of multivariate response data that allows the researcher to chose the dissimilarity coefficient, either for binary or quantitative measures. In our case, dbRDA was best suited for the type of data available since it works with continuous data and it also allowed for Bray-Curtis distance to be used.

The biological matrix (species data) was not standardized since all species were in the same physical dimension. A square root transformation was applied prior to calculating Bray-Curtis dissimilarity index between samples. Also, not all available environmental variables were used to compute the dbRDA. Following the results of the PCA on the abiotic dataset, the variables selected were depth, slope, all substrate types, geographical location, current speed (mean, maximum and standard

dbRDA

In general terms, in a RDA the ordination of the biological data is constrained in such a way that the resulting ordination axes are linear combinations of the variables in the environmental matrix (Legendre & Legendre 2012). RDA performs a multiple linear regression between the response and the explanatory variables to create a matrix of fitted values, which are then subjected to principal components analysis, which uses the Euclidean distance. In the case of dbRDA, a series of steps are taken to allow non-Euclidean dissimilarity indices to be used, with emphasis on situations where semi-metric measures are chosen (e.g. Bray-Curtis). First, the algorithm calculates a matrix of distances among replicates using the selected dissimilarity. Then, it determines the principal coordinates (PCoA), corrects for negative eigenvalues and finally performs a redundancy analysis (RDA) between the independent variables and the response variables consisting of the principal coordinates. It also implements a permutation test to determine if the explanatory variables have an effect over the variability of the biological data (Legendre & Anderson 1999).

deviation) and fishing intensity. Variables that were highly skewed were log-transformed in order to make their distributions more symmetric (i.e. depth, slope and fishing intensity) and then all variables were standardized. dbRDA was computed using the function *capscale* included in the *vegan* package of the R environment (R Core Team 2016).

4.8 Predictive mapping of benthic assemblages

Modeling the spatial distribution of the biological diversity and the benthic assemblages defined in Part 1 was achieved by means of the classification procedure Random Forest (RF), first proposed by Breiman (2001). RF is a machine-learning technique designed to construct accurate prediction models from multivariate data. RF was selected among other modeling techniques due to the following advantages: (1) a very high classification accuracy, (2) an efficient performance when dealing with large data sets, (3) an effective method to estimate variable importance, (4) the ability to model complex interactions among predictor variables and (5) an algorithm to estimate missing data (Cutler *et al.* 2007).

Distribution maps for the whole study area were generated by predicting 3 different response variables: number of species per sampling unit, exponential of Shannon diversity and the benthic assemblages defined in Part 1. All available environmental layers were used to build the 3 models,

Random Forest

The RF algorithm determines a set of rules to classify new samples based on numerical or categorical observations of a set of predictor variables. RF builds a number of classification trees (hence the name "forest") by recursively partitioning into nodes (binary partitioning) the multivariate data of the input variables according to regions that are most homogeneous in terms of the response variable (i.e. the algorithm determines which binary divisions of the explanatory variables best reduce the variability of the response variable) (Cutler *et al.* 2007). One of the main differences with other classification algorithms is that each node of the tree is split using the best predictor among a subset of predictors randomly chosen at each node, and hence the name "random" (Liaw & Wiener 2002). Trees are grown to the largest possible extent (no pruning) and predictions are then combined from all the trees (Cutler *et al.* 2007). The importance of each variable is calculated based on how much worse the prediction would be if the data for that predictor were permuted randomly, which is provided as a result table showing the relative importance of each predictor variable (Prasad, Iverson & Liaw 2006). In the case of predictions for categorical values (e.g. assemblages), results give an estimate of how much the model accuracy decreases if we drop a certain variable (mean decrease in accuracy). If we predict continuous variables (e.g. diversity), results provide an estimate of how much better the model preforms if we include each variable (% increase in mean squared errors). An important feature of classification techniques is that they split the input data into training and testing sets in order to validate the model. The set of samples not included in the model build-up, often called out-of-bag samples, were used to test the accuracy of the model using a linear regression model in the case of biodiversity data (continuous variable) and a confusion matrix in the case of assemblage predictions (categorical data).

since the number of predictor variables does not influence predicting capacity in RF. 1500 trees were built in each model run with 8 variables randomly selected at each node. Full coverage maps were created by predicting values for each pixel of the bathymetry, at a resolution of 10 by 10 m. RF was run using the functions included in the *randomForest* package of the R software platform (Liaw & Wiener 2002).

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4.9 Evaluation of fishing impact on the benthic megafauna

In order to understand the effects of varying degrees of bottom trawling intensity over the benthic megafauna off Cap de Creus, the experimental setting that framed the analyses of Parts 1-2-3 had to be limited. Samples that shared a specific set of environmental conditions were selected to avoid potential artifacts in the results derived form a wide scale analysis. The idea behind this approach was to reduce as much as possible the effects of other environmental factors, such as depth or substrate type, which could be influencing the spatial arrangement of the organisms at a larger scale and hence cover up the potential effects of commercial fishing practices.

The selection of the sampling units to be included in the analyses followed an evaluation of the spatial distribution of the fishing effort in the study area. The PCA analysis performed over the environmental matrix revealed a strong relationship between substrate type and fishing intensity, since high efforts are found in soft bottoms. At the same time, VMS data indicate that the fishing fleet performs its activities in two areas: on the continental shelf and in the deepest part of the submarine canyon, at depths below 500 m. For these reasons, sampling units selected to evaluate the effect of commercial fishing activity followed two specific criteria:

a. Samples had to display a homogenous substrate composition, so only those included under the categories “Muddy fine to medium sands” and/or “Medium sands to gravel” were considered (more details on substrate types in Section 4.5.1).
b. Samples should be located on the continental shelf, at depths between 90 and 125 meters.

The degree of fishing intensity assigned to each sampling unit was based on the analysis performed over the VMS data, which corresponds to the geographic position of bottom trawlers when working at sea displayed in a grid of 250x250m (see Section 4.5.4 for more details). Fishing activity was used both as a continuous and a categorical variable. The intensity recorded in the 250x250 m grid was categorized using Jenks clustering method in the free software QGIS. Jenks natural beaks is an algorithm capable of determining the best arrangement of values into different classes by minimizing each class average deviation from the class mean and maximizing each class deviation from the means of the other groups (Jenks 1967).

Four different categories were generated, which corresponded to:

a. No fishing. No pings recorded for 5 years
b. Low intensity. 1 to 3 pings in 5 years
c. Medium intensity. 4 to 12 pings in 5 years
d. High intensity. More than 12 pings in 5 years

The impact of commercial fishing over the distribution and structure of the benthic assemblages was evaluated at 3 different levels:

1. Species richness and diversity, by simply plotting the intensity of bottom fishing in the different sampling units against the number of species and the exponential of Shannon diversity index.
2. Community structure, by superimposing the different levels of fishing intensity over a dbRDA produced from the biological matrix and using Bray-Curtis as a dissimilarity coefficient.
3. Individual species commonly found on Cap de Creus continental shelf by simply plotting their average abundance in different fishing scenarios using bar plots.

Results of (3) set the basis of the monitoring program. Species selected as indicator species have to be sensitive to varying degrees of fishing intensity and also respond to four specific criteria: (a) they have to be able to fully developed in soft substrate types, (b) preferably, they should be sessile or with a very restricted mobility, (c) they should appear in relatively high numbers to allow robust statistical comparisons and (d) should be sufficiently large and conspicuous to be easily recognizable (Weinberg 1978b). All analyses of Part 5 were performed in the R environment (R Core Team 2016).

4.10 Experimental design of the monitoring program and selection of baseline data

Working hypotheses. The BACI experiment responds to two complementary hypotheses:

1. Impact locations where the disturbance ceases should show a different pattern of change (descriptors of natural heritage should improve) from before to after the application of management measures compared to the natural change in control locations.
2. Locations under different historical fishing intensities should evolve differently to the management measures. We consider three different levels of fishing intensity, thus simplifying the levels described in Section 4.9 by merging No fishing and Low fishing intensity.

Spatial design. Given that the spatial design of the boundaries of the future MPA of Cap de Creus are still unknown, we were forced to propose a hypothetical zonation following the recommendations given by Gili *et al.* (2011). We propose an area of enclosure where fishing activities should not be permitted (named new closure, NC) and open control areas (OC), where fishing can continue as usual and will allow for future comparisons. Furthermore, in order to satisfy the requirements of the second hypothesis, 3 sites each were selected inside and outside the new closure and for each level of fishing intensity (low, medium, high).

Baseline data. Species richness, megabenthic diversity (exponential of Shannon index) and average density for the set of species selected in Part 4 are provided for each ROV transect.

4.11 Characterization of fish populations

Fish fauna displays behavioral patterns that are drastically different to those exhibited by most benthic invertebrate species, which have very limited or no mobility at all. Even among fish species, there exists a wide array of behavioral patterns that are related to their size, diet and reproduction. It is possible that the presence of a moving vehicle underwater, either manned or remotely operated, equipped with powerful lights and noisy engines, can generate changes in their normal conduct.

There are examples of fish species that respond positively (attraction) or negatively (avoidance) to the presence of an external device (see Section 1.6), which could generate some bias in the abundance estimates. With these considerations in mind, fish fauna was evaluated using the same images used to characterize the benthic invertebrate assemblages. The first limitation of applying an equivalent methodology, besides the mobility of the target species, was found when selecting a similar size for the sampling units. The low number of species present in the video images in comparison to the sessile fauna made the 5 m^2 sampling unit impracticable in statistical terms. For that reason, the study of the fish fauna followed two methodological approaches: (1) increase the size of the sampling unit for community analysis and (2) treat each organism as an independent observation to evaluate the effect of the environmental parameters.

After evaluating the possibilities of using samples of increasing sizes, 200-meter-long sequences were selected to study the structure of the fish assemblages. All selected sequences had to fulfill 3 basic requirements, or were otherwise discarded: (1) substrate type to be as homogeneous as possible throughout the whole sequence, (2) very small variations in the depth range and (3) there should not be any visibility issues along the whole footage selected. In the end, only 56 sequences could be isolated from the total of 33 km explored. All fish identified in the images were used for the community analysis, even those that showed a highly aggregative behavior around the ROV. This was the case of *Trachurus* sp., with individuals forming medium sized schools that recurrently crossed in front of the underwater vehicle.

Abiotic data extracted from the video images was noted (depth, substrate, slope) as well as the most common benthic assemblage from the ones identified in Part 1. Community analyses followed the same methodology as that applied to benthic invertebrate fauna: (1) determine the optimal number of groups, (2) classify the samples using Ward's hierarchical clustering method, (3) identify the most representative species using the function IndVal and (4) determine if the groups are statistically different with regards to their species composition. All analyses were performed using the functions specified in Part 1 in the R environment (R Core Team 2016).

In order to get an idea of the bathymetric distribution of the fish fauna, a simple chart was created incorporating the maximum and minimum depth where each species was found. To increase the amount of information provided, the frequency distribution of each species was added to the graph. To account for the spatial variability, a simple map for the 8 most relevant species was constructed using their georeferenced position.

4.12 Presence of marine litter

The human footprint on the seafloor of Cap de Creus continental shelf and submarine canyon, besides the effects of commercial bottom trawling, was also evaluated. All human-derived objects that appeared in the video images were registered and classified in one of these two main categories: (1) abandoned, lost or otherwise discarded fishing gear (ALDFG), which included bottom trawl nets, bottom trawl cables, long-lines and trammel nets, and (2) domestic litter, which was further subdivided in plastic, metal, glass, clothing and amphorae. Although most fishing-derived items were made of plastic or metal, they were still considered under the category ALDFG.

Making use of the transect length (L) calculated using the GIS positioning of the ROV and the transect width (W) determined by the parallel laser beams, densities for each domestic litter category were calculated at the transect level as items per ha^{-1}, by dividing the number of items by the area explored ($De = items / L \cdot W$). Since fishing gears tend to be rather long (reaching lengths over 200 m or more in some cases) and usually lay flat over the seabed, especially cables and trammel nets, densities for ALDFG were calculated as items per 100 linear meters, by dividing the number of abandoned gears by the length of each dive ($De = items \cdot 100 / L$).

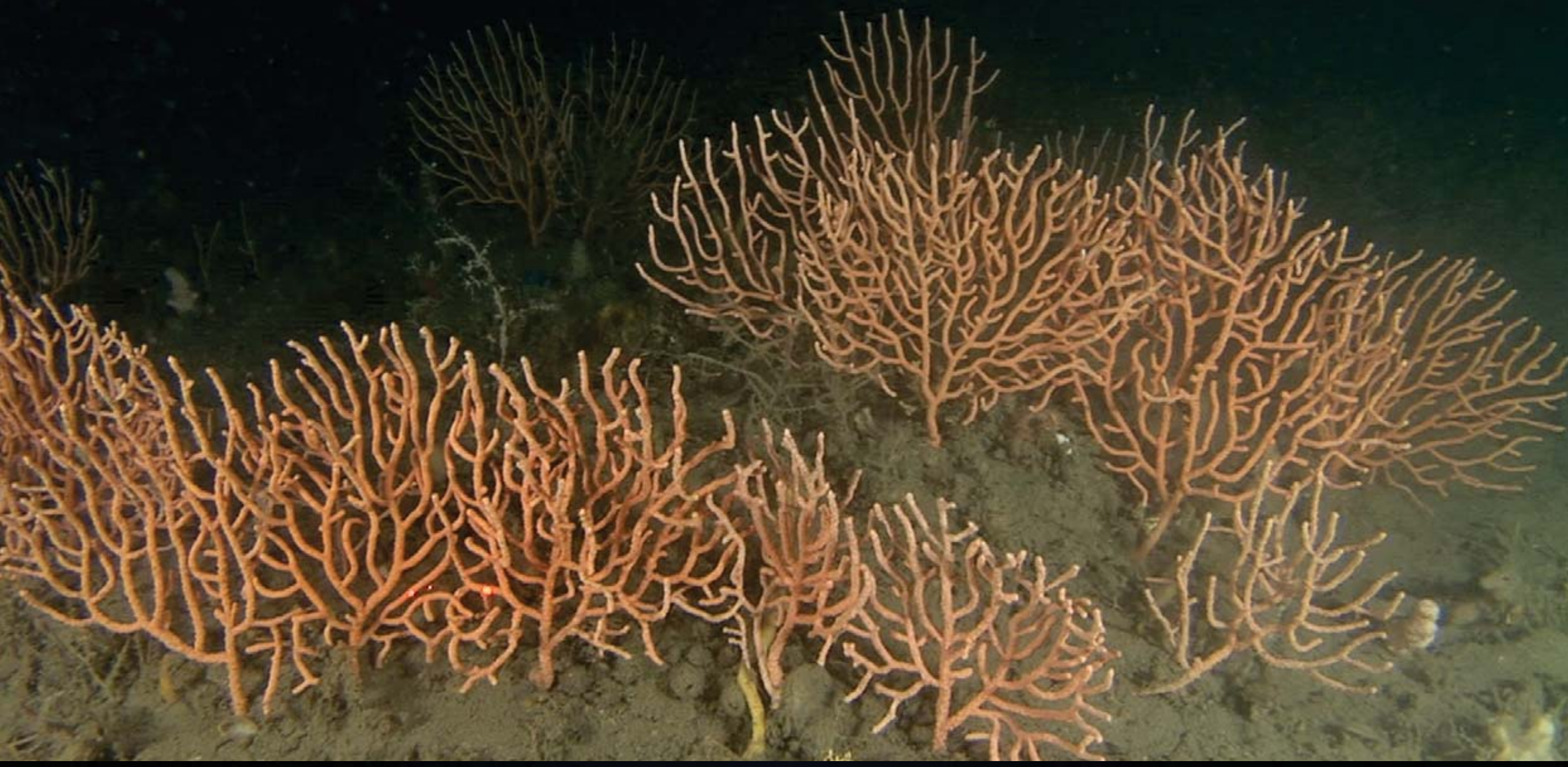
Materials and Methods

To account for the spatial and depth variability in the distribution of marine litter, 3 different geographic areas were determined, which are in agreement with those defined by Lastras *et al.* (2007):

1. Northern continental shelf (NS): the continental shelf north of the easternmost part of the cape, between 80 to 150 m depth
2. Southern continental shelf (SS): the continental shelf south of the easternmost part of the cape, between 80 to 150 m depth
3. Canyon head (CH): from the lowest part of the shelf break (150 m) down to 400 m depth

Each ROV dive was assigned to one of these 3 areas, and the density and percentage of items belonging to each category was calculated, differentiating between domestic litter and ALDFG. Results in the form of pie charts were overlaid on a map of the study area.

Finally, the impact of ALDFG on the three cold-water coral species that appeared on the video images (*Madrepora oculata*, *Lophelia pertusa* and *Dendrophyllia cornigera*) was evaluated by calculating the percentage of entangled corals of each species that was observed in every ROV dive where they were present.



Results

Cover images by Nemo ROV (Gavin Newman) and JAGO Team (GEOMAR)

5. Results

5.1 Composition and structure of invertebrate communities

5.1.1 General results: benthic megafauna

The 60 ROV and manned submersible dives performed in Cap de Creus continental shelf and submarine canyon provided a total of 2311 sampling units of a size of 5 m^2. Bad visibility sequences due to sediment resuspension or excessive distance from the seabed reduced the number of valid samples to 1991, which is equivalent to an analyzed surface of almost 1 hectare. Overall, 93,000 organisms were identified in the video footage, corresponding to 167 different taxa. A large part of these organisms could be identified down to species (54%) or genus (13%) level; the remaining 58 taxa had to be classified in higher taxonomic levels (35%). All organisms could be identified to Phylum level. Annex I provides a visual catalogue of the invertebrate species observed on the continental shelf and submarine canyon off Cap de Creus. It also shows the organisms that have not yet been identified to species level and are reported as morphospecies. The complete list of taxa identified in the images, organized by Phyla, is provided in Annex III together with information about their abundance and frequency.

For the sake of clarity, all taxa, whether species, morphospecies, genera or higher levels, will be hereafter reported as "species". Therefore, some taxa will represent a genus or a family that includes two or more species, but for which identification to species level has not yet been possible

The number of species identified in each ROV dive was very inconsistent throughout the study area, with some tracks having less than 10 species in total (e.g. dives 31-58-59) whilst others up to 70 (e.g. dives 32 and 45). Since the number of species observed did not depend only on the environmental characteristics of the sampled area, but also on the length of the video transect, species richness is reported per sampling unit. In this case, it ranged between 0 and 30 species, with an average of 5.55 including all samples. From the 1991 sampling units, up to 78 did not hold a single organism (4%), 940 had less than 5 species (47%) and 390 had more than 10 species (20%). The most common situation was to find 3 species per sampling unit (Fig. 5.1.1a). Diversity was also very variable throughout the whole study area, with values of Exponential of Shannon diversity index ranging from 1.5 to more than 10 in some areas (Fig. 5.1.1b). Information regarding species richness and diversity for each dive is provided in Annex IV.

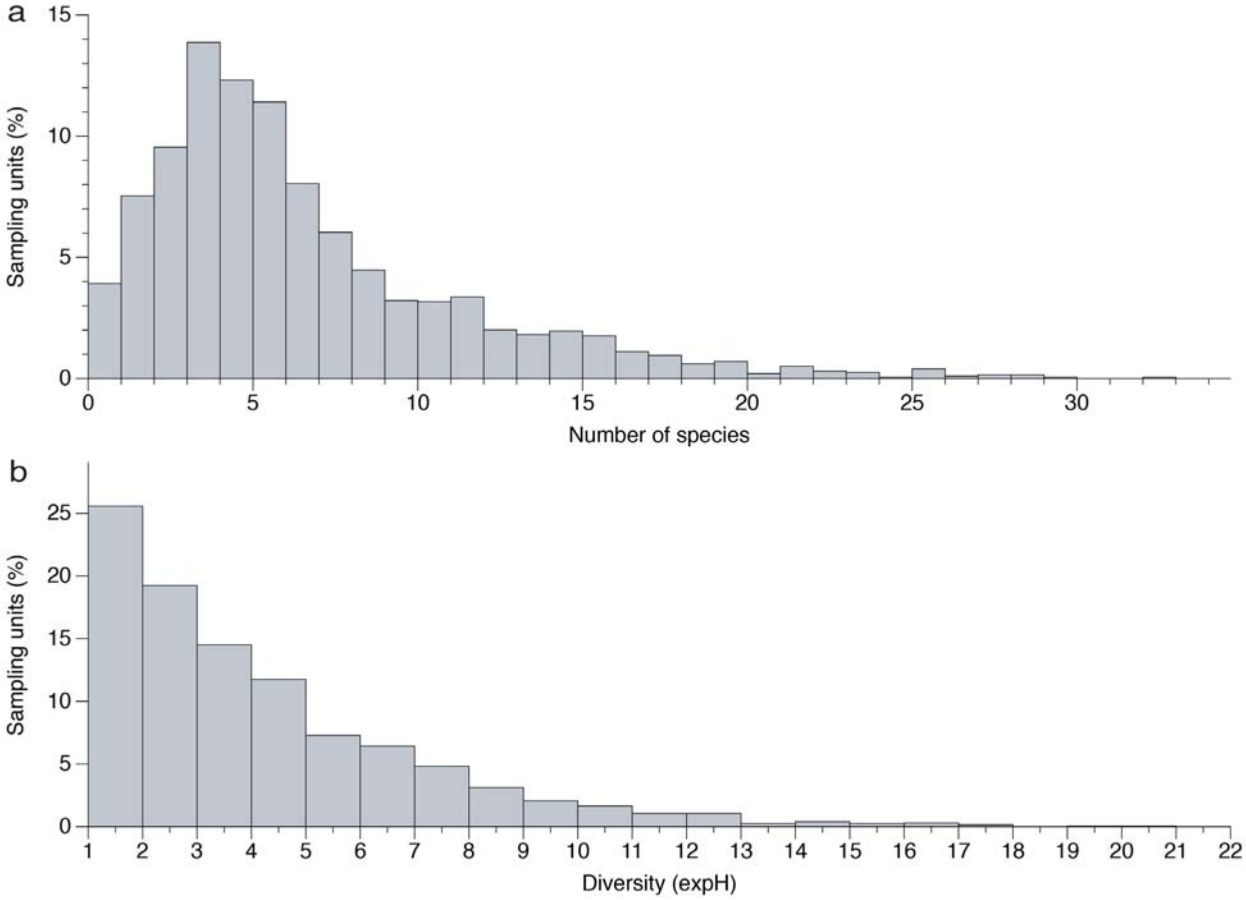


Figure 5.1.1. Frequency distribution of the number of species and Exponential of Shannon diversity values identified in the different sampling units of 5 m^2.

The whole set of species identified in the video images belonged to 10 different Phyla, two of which were especially well represented. Up to 55 species were assigned to the Phylum Porifera (33%) and almost 40 to the Phylum Cnidaria (22%). The rest of the Phyla had a relatively smaller representation, all of them with less than 10 species each (Fig 5.1.2a). In terms of abundance, almost half of the organisms reported were Echinoderms, mostly due to the high densities of the ophiuroid *Ophiothrix fragilis*. This species alone accounted for more than 45,000 records, mainly due to a large aggregation found in a specific area of the continental shelf, where abundances were as high as 600 ind·m^{-2}. Besides this massive aggregation, the three best-represented Phyla in terms of organism abundances were Cnidaria (22,959 individuals), Annelida (11,440 individuals) and Porifera (8,486 individuals) (Fig 5.1.2a).

Not taking into account the highly abundant *O. fragilis*, 7 other species alone represented more than 50% of the total number of organisms registered (Fig 5.1.2c). These species corresponded to the polychaetes *Lanice conchilega* and *Protula tubularia* (5,908 and 3,770 individuals respectively), the gorgonian *Eunicella cavolini* (4,979), the soft coral *Alcyonium palmatum* (3,905), the sea pen

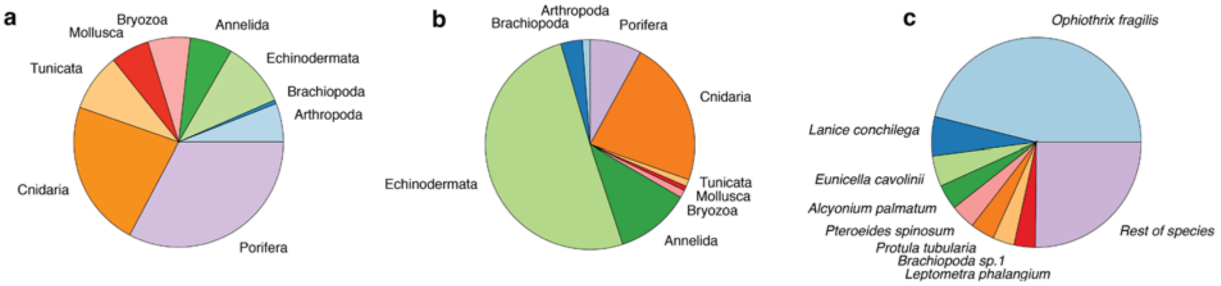


Figure 5.1.2. Summary of the main findings after analyzing the video footage recorded on the continental shelf and submarine canyon. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species.

Pteroeides spinosum (3,861), all Brachiopod species (3,455), and the sea lily *Leptometra phalangium* (3,155). The 40 most common species identified on the video images of Cap de Creus is given in Table 5.1.1, with data regarding their abundance and occurrence. The complete list is given in Annex III. Both *Alcyonium palmatum* and *Pteroeides spinosum* were the two most common species, appearing in more than 40% of the sampling units. Other representative species were the polychaete *Protula tubularia* (33%) and the cnidarians *Mesacmaea mitchellii* and *Eunicella cavolini*, present in 19% of the sampling units approximately.

5.1.2 Community analyses: description of megafauna assemblages

The optimal number of megabenthic assemblages in which to classify the ROV data was determined using the overall average *silhouette* width from 20 different cluster solutions, based on a hierarchical dendrogram constructed with Ward's algorithm. An 8-cluster solution was selected since it showed the highest overall average *silhouette* (Fig. 5.1.3a). The hierarchical dendrogram, with indication of the 8 different groups, is shown in Fig. 5.1.3b. Clusters A and B were the most frequent in the video

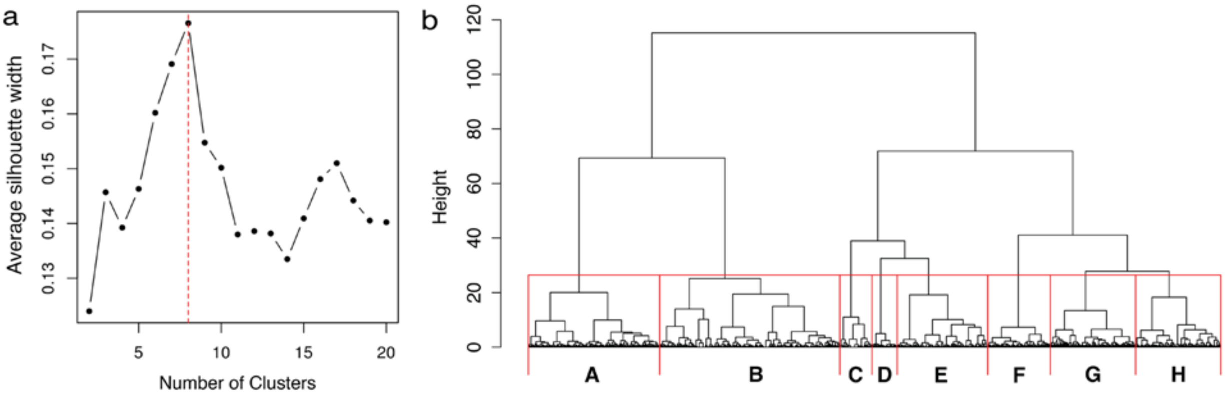


Figure 5.1.3. Cluster analysis of invertebrate megafauna. (a) Overall average silhouette for each cluster solution. The optimal number of clusters is 8, indicated by a red dashed line. (b) Ward's hierarchical dendrogram of species composition constructed using Bray-Curtis dissimilarities from square-root transformed data. Clusters are indicated by the letters A to H.

Table 5.1.1. Number of individuals, maximum density and occupancy of the 40 most abundant species or morphospecies identified in the ROV dives performed on the continentals shelf and submarine canyon off Cap de Creus. The complete list of species is provided in Annex III at the end of this document.

Phylum		Species	Total nº of organisms	Max. density (ind·m^{-2})	% of tracks	% of sampling units
Porifera		*Dendroxea lenis*	367	2.40	28.33	7.58
		Dysidea spp.	1083	5.20	33.33	11.50
		Haliclona cf. elegans	781	14	31.67	6.53
		Stelligera stuposa	604	7.40	35	7.28
		Suberites syringella	1999	14.40	45	14.57
		Unidentified encrusting Porifera sp.2	248	2.80	21.67	4.62
		Unidentified encrusting Porifera sp.4	456	6.60	31.67	7.63
		Unidentified encrusting Porifera sp.10	257	2.20	16.67	4.32
Cnidaria	Hydrozoa	*Lytocarpia myriophyllum*	314	4	31.67	5.32
		Sertularella gayi	446	4.80	55	9.69
Cnidaria	Anthozoa	*Alcyonium coralloides*	361	5.20	25	5.32
		Alcyonium glomeratum	260	17.60	20	2.36
		Alcyonium palmatum	3905	9	78.33	42.64
		Arachnanthus oligopodus	1638	26.60	20	5.63
		Capnea sanguinea	334	1.60	38.33	9.64
		Caryophyllia smithii	1432	11.40	56.67	16.27
		Cavernularia pusilla	748	4.20	36.67	10.85
		Cerianthus membranaceus	521	1.80	58.33	12.51
		Epizoanthus sp.1	1076	8.80	25	8.64
		Eunicella cavolini	4979	25.60	45	18.68
		Madrepora oculata	291	8.60	13.33	3.06
		Mesacmaea mitchellii	791	1.80	63.33	19.29
		Paralcyonium spinulosum	663	6.40	38.33	8.09
		Pennatula rubra	590	2	40	15.72
		Pteroeides spinosum	3861	6.60	65	42.54
Bryozoa		*Smittina cervicornis / Adeonella calveti*	873	4.20	28.33	12.05
Annelida		*Lanice conchilega*	5908	28.20	45	16.32
		Myxicola infundibulum	260	1	48.33	8.84
		Protula tubularia	3770	14.80	78.33	33.50
		Sabella pavonina	884	9.40	71.67	16.12
		Salmacina dysteri	527	7.20	38.33	9.59
Mollusca	Bivalvia	*Neopycnodonte* sp.1	699	9.40	16.67	3.16
Brachiopoda		Unidentified Brachiopoda spp.	3455	48	16.67	8.49
Arthropoda	Crustacea	*Munida ssp.*	632	4.20	35	8.29
		Unidentified Hermit crab	531	1	68.33	15.77
Echinodermata	Crinoidea	*Antedon mediterranea*	334	6.80	16.67	2.16
		Leptometra phalangium	3155	21.40	30	7.63
Echinodermata	Echinoidea	*Echinus acutus*	287	3.60	46.67	6.78
Echinodermata	Ophiuroidea	*Ophiothrix fragilis*	45711	*ca.* 600	33.33	6.03
Tunicata		Unidentified Polyclinidae sp.1	394	6.80	18.33	5.07

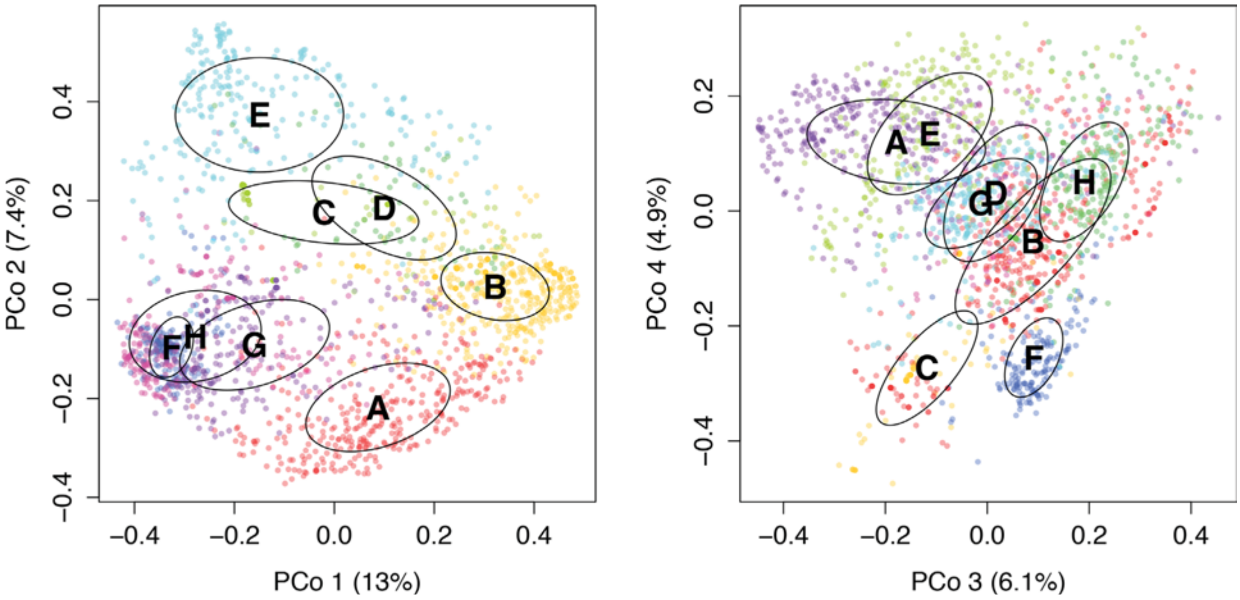


Figure 5.1.4. Principal Coordinates Analysis (PCoA) of the invertebrate community composition (ind·m^{-2}) of the different 5-m^2 sampling units obtained by means of ROVs from the continental shelf and submarine canyon off Cap de Creus. Species density data was square-root transformed prior to calculating the dissimilarity matrix based on Bray-Curtis dissimilarity index. (a) Plot of the first two PCoA axes. (b) Plot of PCoA axes 3 and 4.

images, with 353 and 484 sampling units respectively. Groups C and D, on the other hand, were the least represented, with only 86 and 68 sampling units each from a total of 1991 samples.

The PERMANOVA test indicated that groups were significantly different (Table 5.1.2), and pairwise differences between all groups were highly significant (all p-perm < 0.001, post-hoc tests not shown). The ordination of the sampling units in a reduced space through a Principal Coordinates Analysis (PCoA) is given in Fig. 5.1.4. Groups E, B and A were clearly separated from each other, whilst groups C and D on one side and F, H and G on the other appeared to overlap with each other. Groups C and D both appeared on the continental shelf, while F, H and G were typical from areas of the shelf break and the submarine canyon. Dimensions 3 and 4 of the PCoA revealed that differences between these groups were larger than the first 2 principal coordinates suggested. The 10 most important species of each assemblage, as identified by their Indicator Value index (IndVal), are given in Table 5.1.3, together with average and maximum density values of each species within each assemblage.

Table 5.1.2. Permutational multivariate analyses of variance (PERMANOVA) based on the Bray–Curtis dissimilarity measure for square-root transformed density data of all taxa identified in each benthic assemblage.

Source	*df*	SS	MS	Pseudo-F	p (perm)
Assemblages	7	263.01	37.572	134.26	0.001***
Residuals	1852	518.26	0.28		
Total	1859	781.26			

Table 5.1.3. List of indicator species for each benthic assemblage with their Indicator Value (IndVal), average and maximum density per sampling unit of 5 m^2. Density values are given in ind·m^{-2}.

Group	Species	IndVal	Avg. density ± s.d.	Max. density
A	*Eunicella cavolini*	67	2.80 ± 4.26	25.60
	Smittina cervicornis	60.05	0.51 ± 0.67	4.20
	Suberites syringella	57.24	1.20 ± 2.10	14.40
	Epizoanthus sp.1	41.26	6.87 ± 1.28	8.80
	Alcyonium palmatum	34.12	1.31 ± 1.49	9
	Unidentified Polyclinidae sp.1	26.07	0.22 ± 0.60	6.80
	Alcyonium coralloides	24.55	0.20 ± 0.56	5.20
	Paralcyonium spinulosum	23.52	0.36 ± 0.80	6.40
	Pteroeides spinosum	19.28	0.86 ± 1.14	6.40
	Distomus variolosus	15.79	0.13 ± 0.46	5.40
B	*Pteroeides spinosum*	26.63	0.82 ± 0.99	6.60
	Cavernularia pusilla	20.49	2.49 ± 0.55	4.20
	Pennatula rubra	18.78	0.18 ± 0.30	2
	Alcyonium palmatum	17.05	0.53 ± 0.74	4.60
	Caryophyllia smithii	2.59	0.10 ± 0.25	1.80
	Ophiura ophiura	1.58	0.01 ± 0.05	0.40
	Mesacmaea mitchellii	0.46	0.03 ± 0.09	0.80
	Anseropoda placenta	0.45	< 0.01	-
	Lytocarpia myriophyllum	0.41	< 0.01	-
	Alcyonium glomeratum	0.40	< 0.01	-
C	*Sabella pavonina*	35.13	0.33 ± 0.32	1.80
	Andresia partenopea	23.29	0.08 ± 0.16	0.80
	Alcyonium palmatum	2.84	0.11 ± 0.19	0.80
	Pennatula rubra	2.18	0.05 ± 0.12	0.60
	Parastichopus regalis	2.02	0.01 ± 0.04	0.20
	Ophiura ophiura	1.82	0.02 ± 0.10	0.80
	Pteroeides spinosum	1.02	0.07 ± 0.15	0.60
	Funiculina quadrangularis	0.85	< 0.01	-
	Virgularia mirabilis	0.43	< 0.01	-
	Unidentified Cucumariidae sp.1	0.24	< 0.01	-
D	*Leptometra phalangium*	88.52	6.81 ± 5.17	21.40
	Pteroeides spinosum	12.61	0.29 ± 0.30	1.40
	Pennatula rubra	10.67	0.10 ± 0.15	0.60
	Unidentified Decapoda sp.1	10.40	0.04 ± 0.11	0.60
	Caryophyllia smithii	6.92	0.13 ± 0.19	0.80
	Lanice conchilega	6.11	0.22 ± 0.32	1.40
	Alcyonium palmatum	5.74	0.20 ± 0.29	1.20
	Cavernularia pusilla	3.31	0.05 ± 0.15	1
	Unidentified Crustacea sp.2	2.82	0.02 ± 0.08	0.40
	Unidentified Hermit crab	1.71	0.04 ± 0.01	0.60

Table 5.1.3. (Continued)

Group	Species	IndVal	Avg. density ± s.d.	Max. density
E	*Lanice conchilega*	77.23	4.50 ± 4.73	28.20
	Arachnanthus oligopodus	30.73	1.26 ± 3.02	26.60
	Unidentified Sabellidae sp.2	10.83	0.05 ± 0.12	0.80
	Unidentified Anthozoa sp.1	8.24	0.03 ± 0.10	0.80
	Unidentified Anthozoa sp.2	8	0.02 ± 0.09	0.80
	Sabella pavonina	6.69	0.16 ± 0.29	1.60
	Mesacmaea mitchellii	5.64	0.11 ± 0.19	1
	Unidentified Hermit crab	5.24	0.08 ± 0.16	0.80
	Capnea sanguinea	4.95	0.06 ± 0.13	0.80
	Alcyonium palmatum	2.36	0.11 ± 0.22	1.40
F	Unidentified Brachiopoda spp.	88.91	3.68 ± 5.37	48
	Galatheoidea spp.	71.57	0.64 ± 0.70	4.20
	Caryophyllia smithii	46.60	1.03 ± 1.51	11.40
	Unidentified encrusting Porifera sp.2	44.32	0.23 ± 0.38	2.80
	Unidentified encrusting Porifera sp.10	42.98	0.26 ± 0.44	2.20
	Protula tubularia	36.43	1.88 ± 2.31	14.80
	Madrepora oculata	35.71	0.31 ± 0.88	8.60
	Unidentified encrusting Porifera sp.4	34.47	0.32 ± 0.66	6.60
	Unidentified encrusting Porifera sp.6	31.70	0.13 ± 0.26	1.80
	Unidentified Polychaete sp.1	26.13	0.12 ± 0.35	3.60
G	*Dysidea spp.*	39.72	0.62 ± 0.88	4.40
	Haliclona cf. elegans	26.08	0.46 ± 1.40	14
	Hyrtios collectrix	24.35	0.14 ± 0.37	3.60
	Axinella damicornis	23.04	0.11 ± 2.50	2
	Stelligera stuposa	22.18	2.60 ± 0.67	7.40
	Iophon sp.1	22.16	0.16 ± 0.50	5.40
	Myxicola infundibulum	19.37	0.10 ± 0.17	1
	Poecillastra compressa	17.93	0.05 ± 0.13	1
	Salmacina dysteri	16.12	0.25 ± 0.67	7.20
	Haliclona sp.1	15.85	0.09 ± 0.21	1.20
H	*Protula tubularia*	22.95	0.91 ± 1.01	5
	Capnea sanguinea	21.46	1.61 ± 0.26	1.40
	Cerianthus membranaceus	18	0.19 ± 0.32	1.60
	Unidentified Hermit crab	11.48	0.12 ± 0.19	1
	Mesacmaea mitchellii	10.87	0.18 ± 0.29	1.60
	Echinus acutus	7.22	0.10 ± 0.32	3.60
	Holothuria spp.	3.76	0.06 ± 0.17	1
	Unidentified Cucumariidae sp.1	2.47	0.03 ± 0.16	1.40
	Sagartia elegans	1.48	0.01 ± 0.06	0.40
	Myxicola infundibulum	1.42	0.03 ± 0.10	0.80

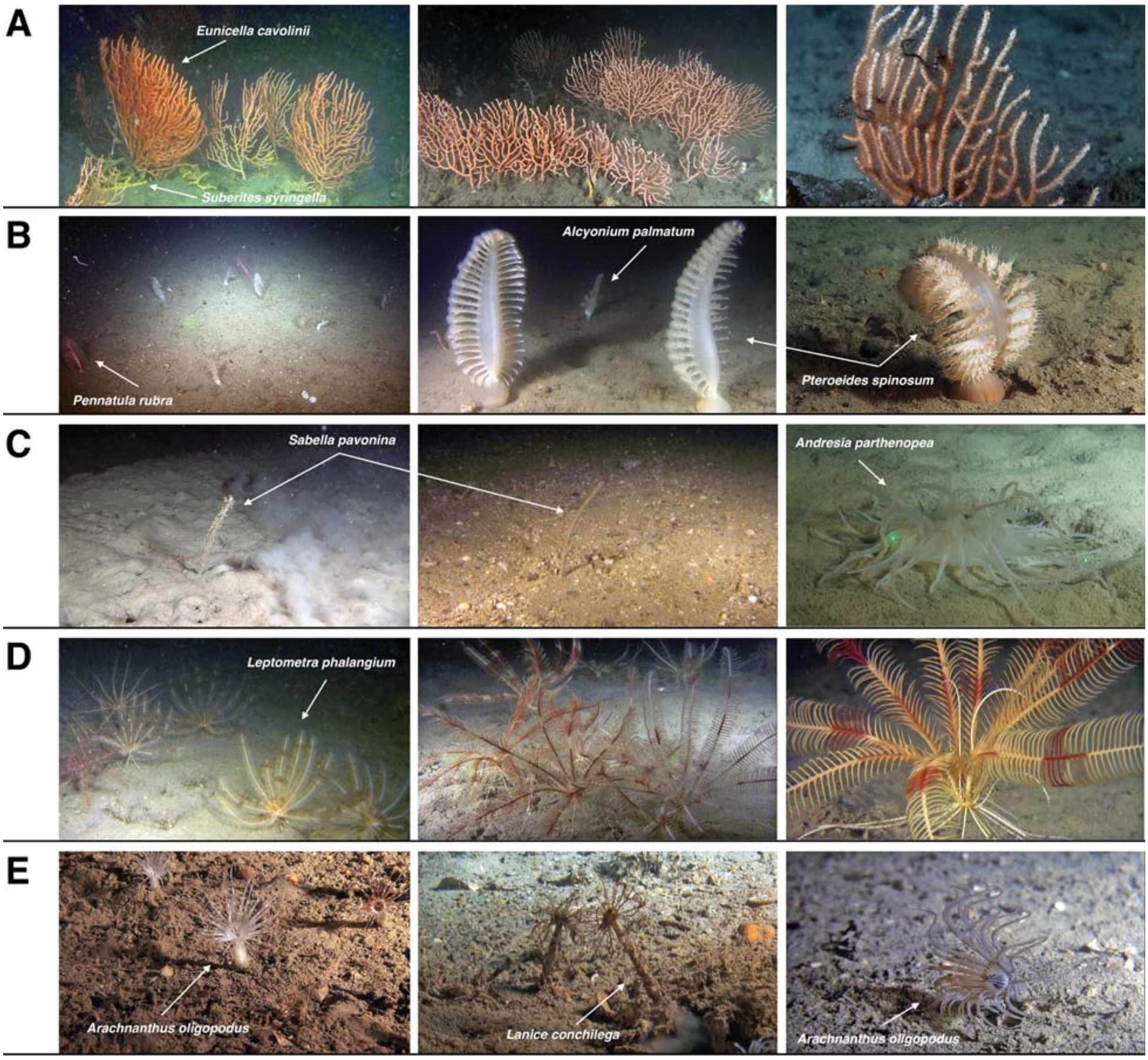


Figure 5.1.5. Selected images of the megabenthic assemblages identified in the ROV footage recorded on the continental shelf and submarine canyon off Cap de Creus during the Indemares cruises. Names of the most important species observed in the pictures are given. (A) Gorgonian assemblage dominated by the sea fan *Eunicella cavolini* together with a large number of accompanying species, including the reptant sponge *Suberites syringella* and the Bryozoan *Smittina cervicornis*. (B) Pennatulacean assemblage, characterized by the sea pens *Pteroeides spinosum* and *Pennatula rubra* and the soft coral *Alcyonium palmatum*. (C) Impoverished assemblage, with very low density values of any given species overall, but for which the polychaete *Sabella pavonina* and the sea anemone *Andresia parthenopea* could be considered characteristic. (D) Crinoid assemblage, largely dominated by the sea lily *Leptometra phalangium*, which formed aggregations of almost 20 ind·m^{-2} in certain areas of the continental shelf. (E) Shelf and shelf-edge assemblage characterized by the presence of the polychaete *Lanice conchilega*, found in association with the cerianthid *Arachnanthus oligopodus* in specific areas of the shelf break. (F) Cold-water coral assemblage largely characterized by the scleractinian coral *Madrepora oculata,* found alongside a large number of accompanying species including include brachiopods, oysters and several species of encrusting sponges. (G) Sponge assemblage characterized by the presence of a number of erect Porifera species, including include *Haliclona cf. elegans*, *Dysidea avara*, *Dysidea tupha*, *Hyrtios collectrix*, *Desmacidon fruticousm*, *Poecillastra compressa* and a few others. (H) Shelf-edge assemblage characterized by the anthozoans *Cerianthus membranaceus*, *Capnea sanguinea* and *Mesacmaea mitchellii,* as well as other species like *Echinus acutus*, *Protula tubularia* and *Holothuria* spp. (I) Massive aggregation of the brittle star *Ophiothrix fragilis,* which was found in a specific location on the continental shelf reaching local densities that were estimated to be of more than 600 ind·m^{-2}. Images from A, B, C, D, E and H by Nemo ROV (Gavin Newman) and images from F, G and I by JAGO Team (GEOMAR).

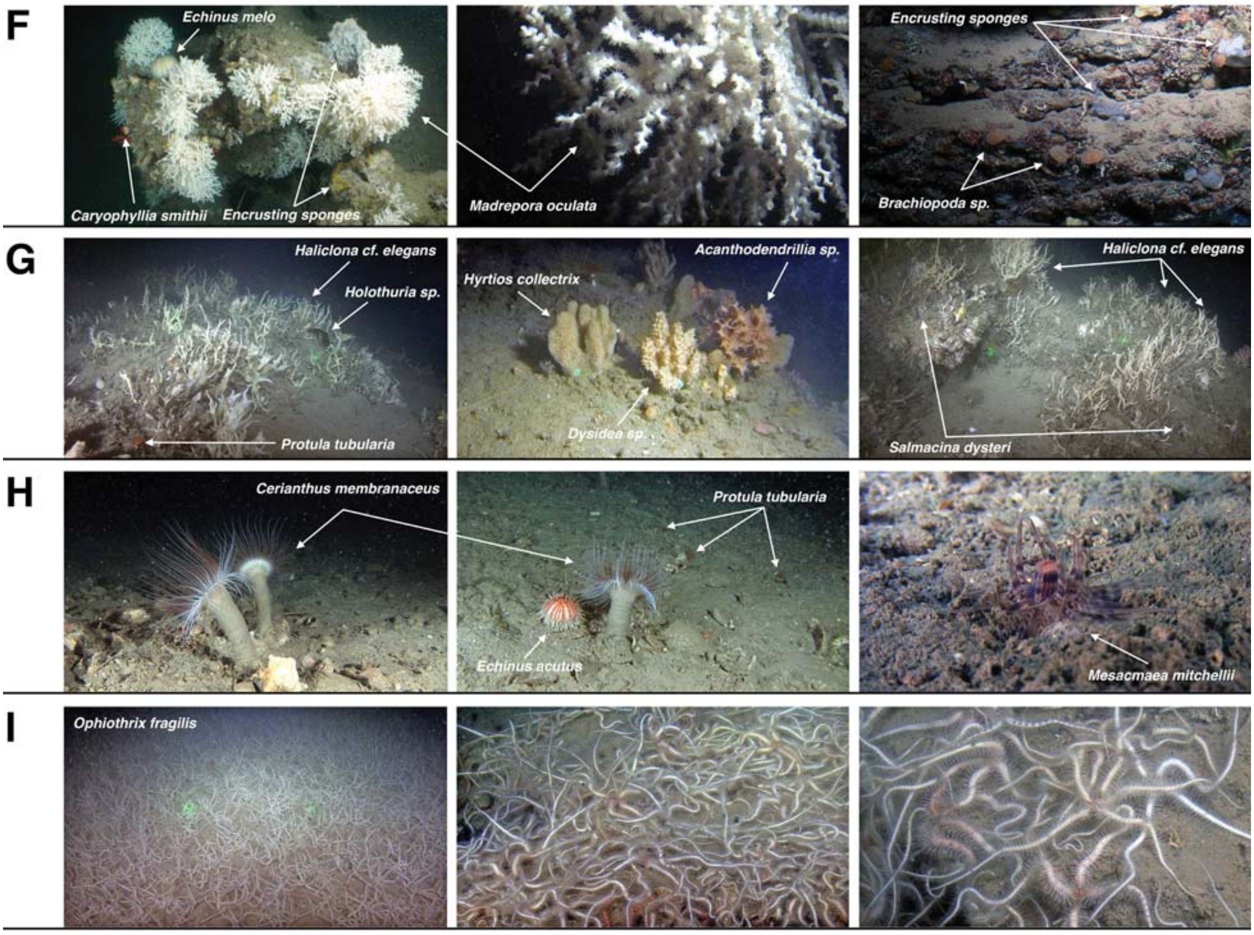


Figure 5.1.5. (Continued)

A brief description of each of the identified assemblages follows:

Assemblage A (Shelf gorgonian assemblage). Representative images in Fig. 5.1.5a. _Frequency._ Very well represented aggregation, present in 20 ROV dives with over 350 sampling units (18% of the total). _Characteristic species._ IndVal analysis identified 4 species as the most characteristic to recognize this assemblage: the orange sea fan *Eunicella cavolini*, the erecting Bryozoan *Smittina cervicornis*, the yellow sponge *Suberites syringella* and the Anthozoan *Epizoanthus* sp.1 (very likely *E. arenaceus*, although no sample could be collected) (Table 5.1.3). This assemblage is easily identifiable due to the presence of the gorgonian *E. cavolini*, which was observed forming patches of very high densities, sometimes above 25 col·m^{-2}, with an average density for the whole set of samples of 2.8 ± 4.26 col·m^{-2}. Also very conspicuous was the bright yellow sponge *S. syringella*, which formed density patches of up to 14 ind·m^{-2}. _Total species richness_. A total of 126 species were identified within this assemblage, more than 60% of which belonged to the Phyla Porifera and Cnidaria. The remaining Phyla were less well represented (Fig. 5.1.6a). In terms of abundance, more than 65% of the organisms were cnidarians (Fig. 5.1.6b) and around 20% were identified as

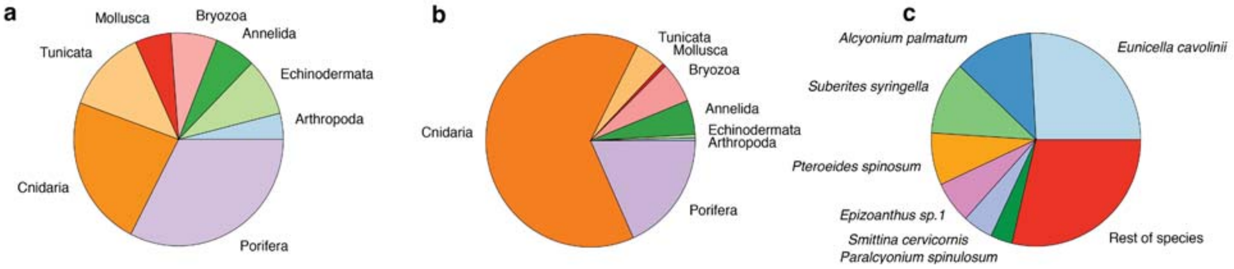


Figure 5.1.6. Summary of the main findings for assemblage A. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

sponges. *Diversity*. This group is one of the 3 most diverse assemblages found in the study area. The average diversity value measured as exponential of Shannon diversity -*exp*(H_{sh})- was 6.19, while the average number of megafauna species per sampling unit was close to 10, in some cases reaching values above 30 species per sampling unit of 5 m^2 (Fig. 5.1.7).

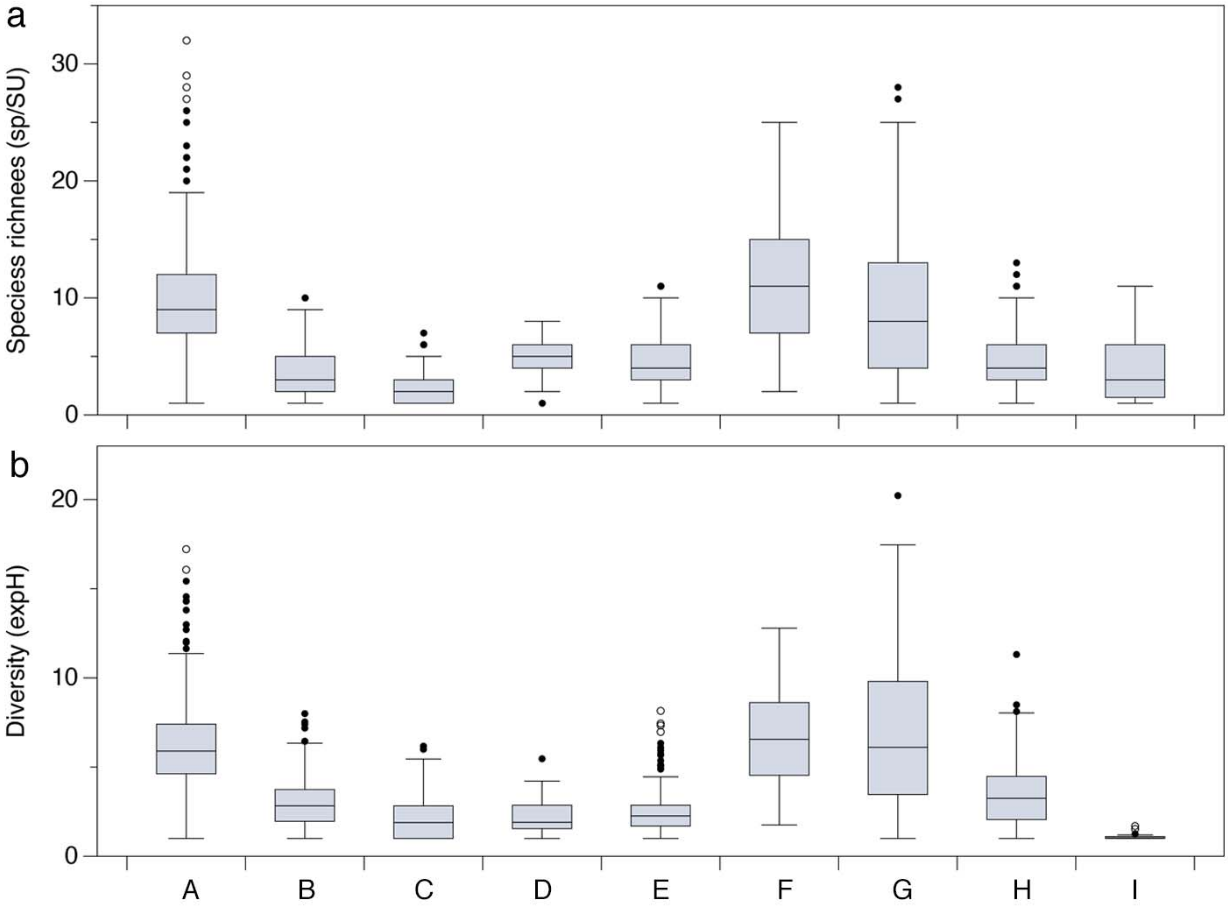


Figure 5.1.7. Box plot showing the values of (a) species richness and (b) exponential of Shannon diversity index for the 9 benthic assemblages identified in the ROV images of Cap de Creus continental shelf and submarine canyon.

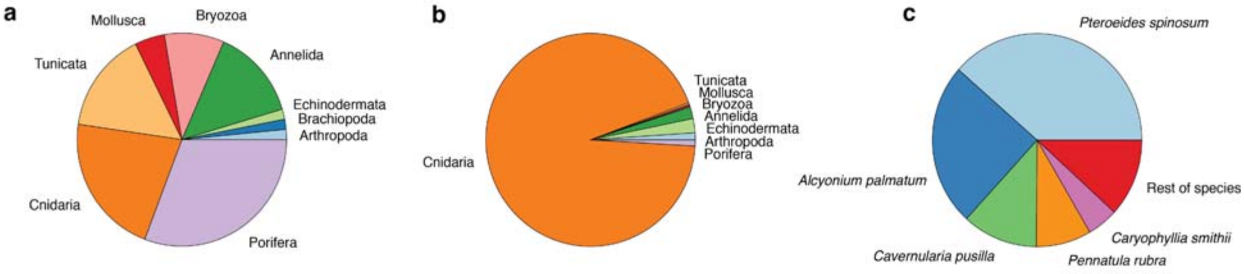


Figure 5.1.8. Summary of the main findings for assemblage B. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

Assemblage B (Pennatulacean assemblage). Representative images in Fig. 5.1.5b. _Frequency._ This is the most common assemblage of the continental shelf off Cap de Creus, with more than 480 sampling units and present in 46% of the ROV dives (28). _Characteristic species._ IndVal analysis identified 4 Cnidaria species as the most characteristic to recognize this assemblage: the sea pens *Pteroeides spinosum*, *Cavernularia pusilla* and *Pennatula rubra* and the soft coral *Alcyonium palmatum* (Table 5.1.3). *Pteroeides spinosum* was the most noticeable species in this assemblage due to its size and occurrence, present in 84% of the samples. Its overall density was rather low, with values close to 1 col·m^{-2}, although it reached local densities above 6 col·m^{-2}. _Total species richness._ The total number of species identified in this assemblage was 65, most of them belonging to the Phyla Porifera and Cnidaria (Fig. 5.1.8a). In terms of abundances, most of the organisms identified were cnidarians, and the remaining Phyla were very poorly represented (Fig. 5.1.8b). _Diversity._ The average number of species per sampling unit was around 3.5, with diversity values among the lowest recorded, with an average value of $exp(H_{sh})$ of 2.88 (Fig. 5.1.7).

Assemblage C (Impoverished assemblage). Representative images in Fig. 5.1.5c. _Frequency._ It is one of the least common groups of Cap de Creus, only present in 86 sampling units (around 4% of the total area sampled). _Characteristic species._ IndVal analysis identified the polychaete *Sabella pa-*

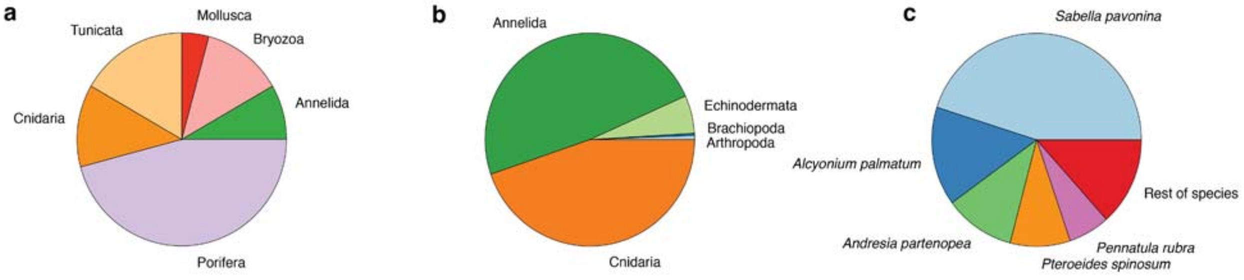


Figure 5.1.9. Summary of the main findings for assemblage C. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

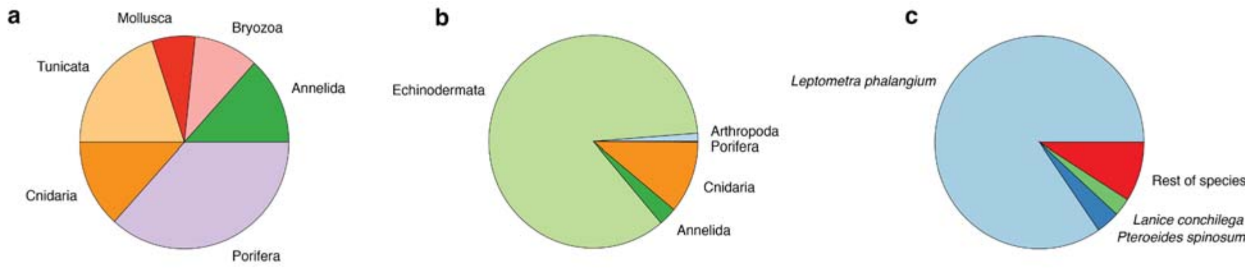


Figure 5.1.10. Summary of the main findings for assemblage D. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

vonina and the anemone *Andresia parthenopea* the most characteristic species of this assemblage, both showing very low densities overall (Table 5.1.3). Total species richness. The number of species identified in this assemblage was rather low (24). This group was mainly characterized by Cnidaria and Annelida species, which represented more than 90% of the total number of organisms (Fig. 5.1.9b). Diversity. This assemblage had on average the lowest number of species per sampling unit and some of the lowest diversity values recorded for the whole study area (Fig. 5.1.7).

Assemblage D (Crinoid assemblage). Representative images in Fig. 5.1.5d. Frequency. This assemblage only appeared in 5 ROV dives and in less than 70 sampling units throughout the whole study area. Characteristic species. This is a very homogenous assemblage largely dominated by the sea lily *Leptometra phalangium*, which had and Indicator Value of almost 90 (Table 5.1.3). The distribution of this species was very localized in the form of dense aggregations, with patches reaching some hundreds of meters in length. *L. phalangium* was very abundant where present, with local densities reaching values up to 20 ind·m^{-2}. There was a series of accompanying species, all very common on the continental shelf, such as the sea pens *P. spinosum* and *P. rubra*, the soft coral *A. palmatum* and the Polychaete *Lanice conchilega*, all of which appeared in rather low densities when *L. phalangium* was present. Total species richness. A total of 30 species were recorded throughout this assemblage, belonging to 7 different Phyla (Fig. 5.1.10a). More than 80% of the organisms identified, however, corresponded to *L. phalangium*, and only a small percentage could be attributed to the remaining species (Fig. 5.1.10c). Diversity. The average number of species per sampling unit was low, between 4 and 5. Due to the dominance of *L. phalangium*, the density values of the remaining species were very low, providing very low diversity values overall (Fig. 5.1.7).

Assemblage E (*Lanice conchilega* assemblage). Representative images in Fig. 5.1.5e. Frequency. Relatively common assemblage present in 243 sampling units (15 ROV dives). Characteristic species. This assemblage was mainly characterized by two different species: the Polychaete *L. conchilega* and

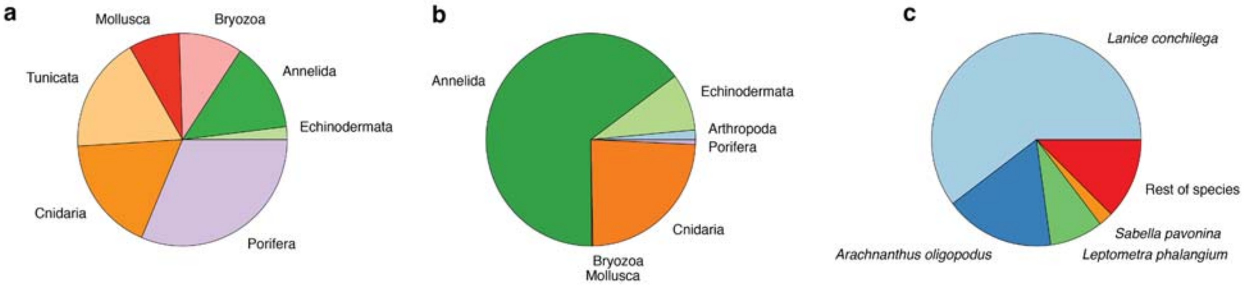


Figure 5.1.11. Summary of the main findings for assemblage E. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

the cerianthid *Arachnanthus oligopodus*. Both were present in relatively high numbers, with local densities reaching values above 25 ind·m^{-2} in both cases (Table 5.1.3). Accompanying species were mostly small burrowing Polychaetes and Anthozoans, some of which have not yet been identified due to lack of live samples. *Total species richness.* More than 50 species were identified in this assemblage belonging to 7 Phyla. More than 65% of the organisms were Polychaetes and almost 25% Cnidarians, with the remaining Phyla being poorly represented (Fig. 5.1.11b). *Diversity.* On average, 4.5 species were identified in the sampling units, a relatively low number compared to other assemblages, becoming one of the least diverse assemblages overall (Fig. 5.1.7).

Assemblage F (Cold-water coral assemblage). Representative images in Fig. 5.1.5f. *Frequency.* This assemblage only appeared in 8 ROV dives, in 168 sampling units (less than 10% of the surveyed area). *Characteristic species.* IndVal analysis identified Brachiopoda spp. as the most characteristic taxa (Table 5.1.3). It was impossible to distinguish the actual species of all Brachiopod individuals (the genera *Terebratulina* and *Gryphus* are nearly impossible to tell apart from such a distance), so they were all considered under the same taxonomic group. Some walls were partially covered by these organisms, in some areas reaching densities of almost 50 ind·m^{-2}. Galatheids were also very common hiding in between rocks. Both species *Munida rugosa* and *Munida intermedia* were

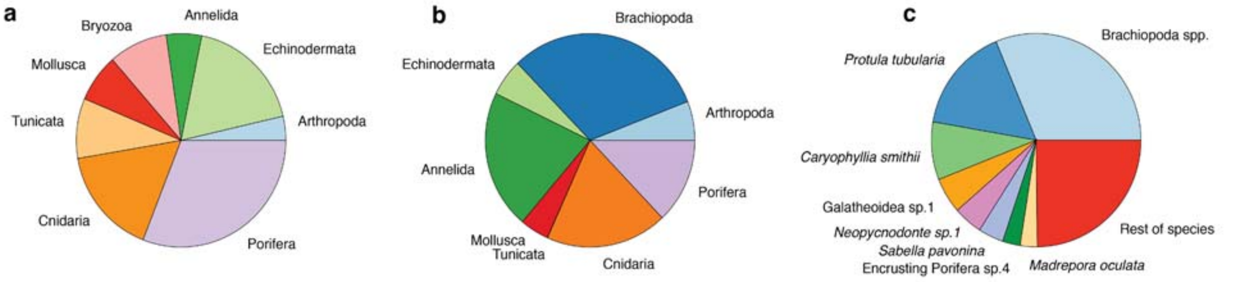


Figure 5.1.12. Summary of the main findings for assemblage F. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

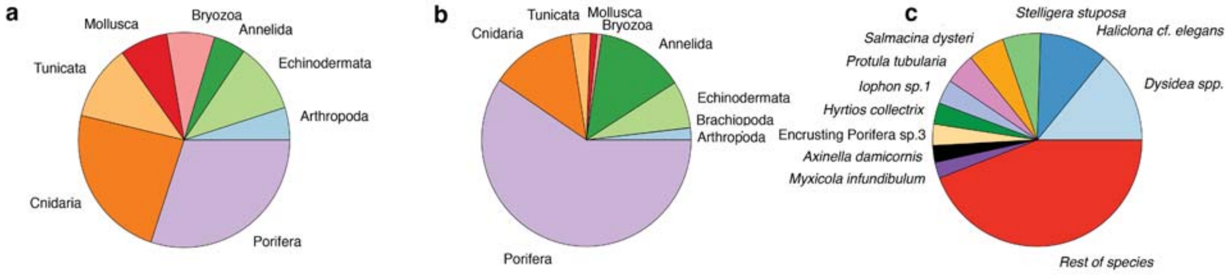


Figure 5.1.13. Summary of the main findings for assemblage G. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

observed, but grouped under the category Galatheoidea spp. Besides the results provided by the IndVal analysis, this assemblage was characterized by the cold-water coral *Madrepora oculata*, which showed rather low abundances in terms of number of colonies, but its biomass was larger than that of any other species. Other accompanying species were the cup coral *Caryophyllia smithii*, the Polychaete *Protula tubularia*, the oyster *Neopycnodonte* cf. *zibrowii* and a set of encrusting sponges of a wide range of colors (Fig. 5.1.12c). *Total species richness.* More than 55 species were reported in this assemblage, some of which still remain to be identified. The depths at which this assemblage was found made sample collection extremely complex, and there was a lack of live samples to complete the identification process. This was mostly the case for erect, but specially encrusting, sponges that covered a large part of the outcropping rocks that are found in the submarine canyon. *Diversity.* There were on average 11 species per sampling unit (maximum values of 25), making this assemblage one of the most diverse in the whole study area (Fig. 5.1.7).

Assemblage G (Sponges assemblage). Representative images in Fig. 5.1.5g. *Frequency.* Well represented assemblage, found in 23 ROV tracks and in 229 sampling units (12% of the total area surveyed). *Characteristic species.* The 6 most representative taxa according to the results of IndVal were all erect Porifera: *Dysidea* spp., *Haliclona* cf. *elegans*, *Hyrtios collectrix*, *Axinella damicornis*, *Stelligera stuposa* and *Iophon* sp. (Table 5.1.3). In general, sponge species were found in a rather regular distribution along some of the ROV transects, although certain species were observed forming dense aggregations. This is the case of *Haliclona* cf. *elegans*, which was found reaching local densities above 10 ind·m^{-2}. Other accompanying species of interest were the polychaetes *Myxicola infundibulum* and *Salmacina dysteri*. This last species was observed forming rather large bioconstructions over soft substrates. *Total species richness.* The total number of species registered in this assemblage was 123, the second highest value among all groups. Species belonging to 8 Phyla were identified, although most of the observations corresponded to sponge species, together with some Cnidaria and a few polychaetes (Fig. 5.1.13c). *Diversity.* This assemblage was one of the

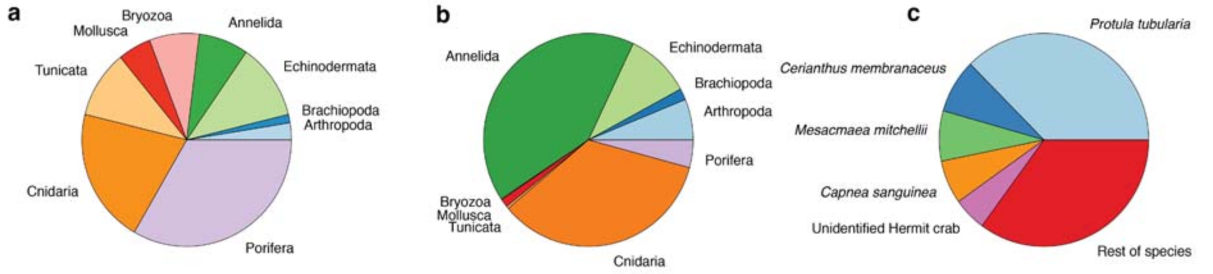


Figure 5.1.14. Summary of the main findings for assemblage H. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

richest and most diverse out of all 9 assemblages, with an average number of species per sampling unit above 9, but reaching values close to 30 in certain areas (Fig. 5.1.7).

Assemblage H (Cerianthid assemblage). Representative images in Fig. 5.1.5h. *Frequency.* Present in 19 ROV dives, occupying almost 230 sampling units (12% of the total area surveyed). *Characteristic species.* IndVal analysis identified the Polychaete *P. tubularia* and the anthozoans *Capnea sanguinea*, *Cerianthus membranaceus* and *Mesacmaea mitchellii* as the most characteristic species (Table 5.1.3). Their abundances were relatively low in all cases, with organisms sparsely distributed along the ROV transects. Holothurians and sea urchins of the species *Echinus acutus* were also present. *Total species richness.* 78 species were identified within this assemblage, most of which were either Polychaetes or Cnidarians. The remaining Phyla were poorly represented (Fig. 5.1.14b). *Diversity.* Sampling units had relatively low numbers of species, with an average value of 4.3 (Fig. 5.1.7).

Assemblage I (Dense brittle star aggregation). Representative images in Fig. 5.1.5i. Assemblage not included in the statistical analyses due to the high densities recorded of the brittle star *Ophiothrix fragilis*. *Frequency.* Very localized assemblage, found in only one of the ROV dives. *Characteristic*

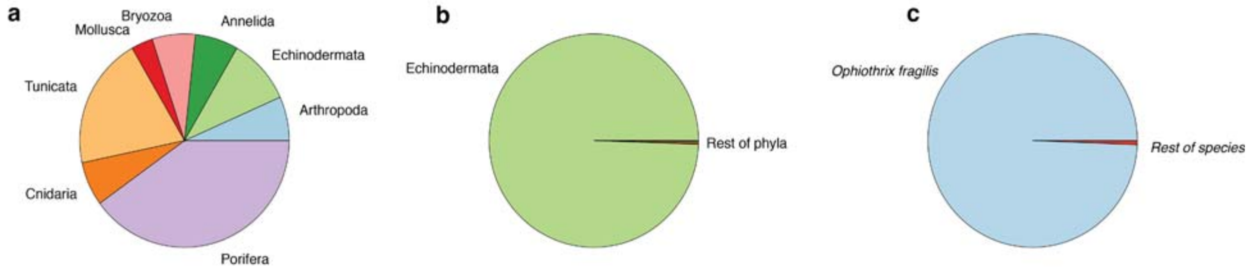


Figure 5.1.15. Summary of the main findings for assemblage I. (a) Percentage of species per phylum. (b) Percentage of organisms per phylum. (c) Percentage of organisms belonging to the 8 most abundant species in this assemblage.

species. *O. fragilis* was observed forming large aggregations hundreds of meters wide, in some areas covering the whole substrate available. Although it was not possible to actually count every single organism due to their constantly moving legs, estimates of local densities gave values that reached 600 ind·m^{-2}. _Total species richness_. A total of 30 species were found within this assemblage, representing 8 different Phyla (Fig. 5.1.15a). Their abundance, compared to that of *O. fragilis*, was almost negligible. _Diversity_. Due to the very high densities of *O. fragilis*, diversity values in this assemblage were some of the lowest recorded.

Box VI

Ancient polychaete formation

The dives performed with the manned submersible JAGO on the southern flank of the submarine canyon during the oceanographic cruise Indemares 2 resulted in an unexpected finding: a large tube-forming polychaete build-up in a fossil or sub-fossil state. Images of its aspect are shown in Fig. B6.1. This biogenic construction, which we believe to be at least 250 m long, was found on the shelf break area furthest away from shore, at 130-140 m depth (approximate location is shown in Fig. B6.2). Making use of the hydraulic grabber of the submarine, we collected a large fragment that gave valuable information about its origin and composition. The primary builder of this "reef" was the Serpulid Polychaete *Spirobranchus triqueter,* which produces white calcareous tubes of a few millimeters long. This species is relatively common in shallow waters of the Atlantic and the Mediterranean Sea, but its depth distribution is still relatively unknown.

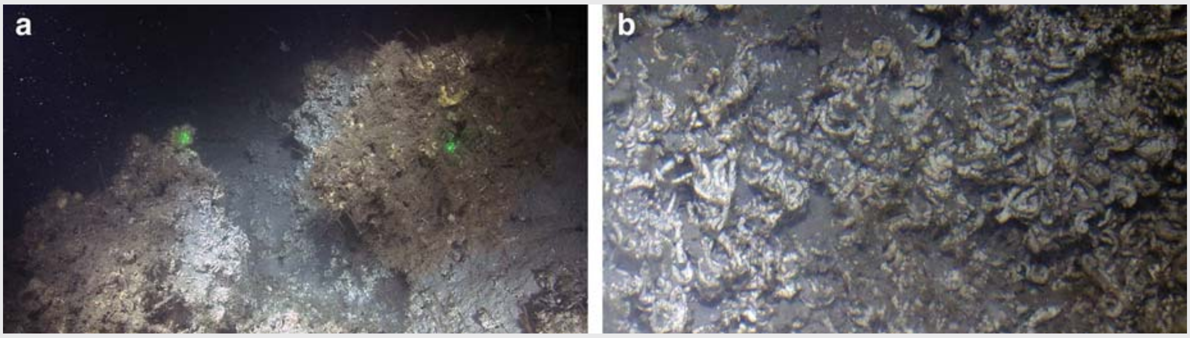


Figure B6.1. Ancient polychaete bioconstruction found on the shelf break of Cap de Creus during a manned submersible dive. (a) General aspect of the biogenic structure. (b) Close up image where the tubes of the polychaete *Spirobranchus triqueter* can be observed. Images by JAGO Team (GEOMAR).

In order to determine whether this formation was recent or produced in past geological eras, some parts of the calcareous tubes were extracted and sent to National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facilities in the USA for carbon-14 dating. Results indicated that this biogenic concretion had an average age of 13,800 years, which dates its formation back to the Last Glacial Maximum (LGM), when sea level was some 100 meters below today's. This implies that this biogenic concretion was formed in a shallow environment and preserved in its original form at 140 m depth for thousands of years.

We can only speculate about the reasons that have kept this polychaete aggregation in the state in which it was found. There are probably two main causes for this: the high current speeds that recurrently hit the area and the low trawling activity that occurs where the concretion is located. Strong bottom currents probably prevent sediments from clogging the polychaete tubes, whilst the lack of fishing activities may have helped towards its conservation.

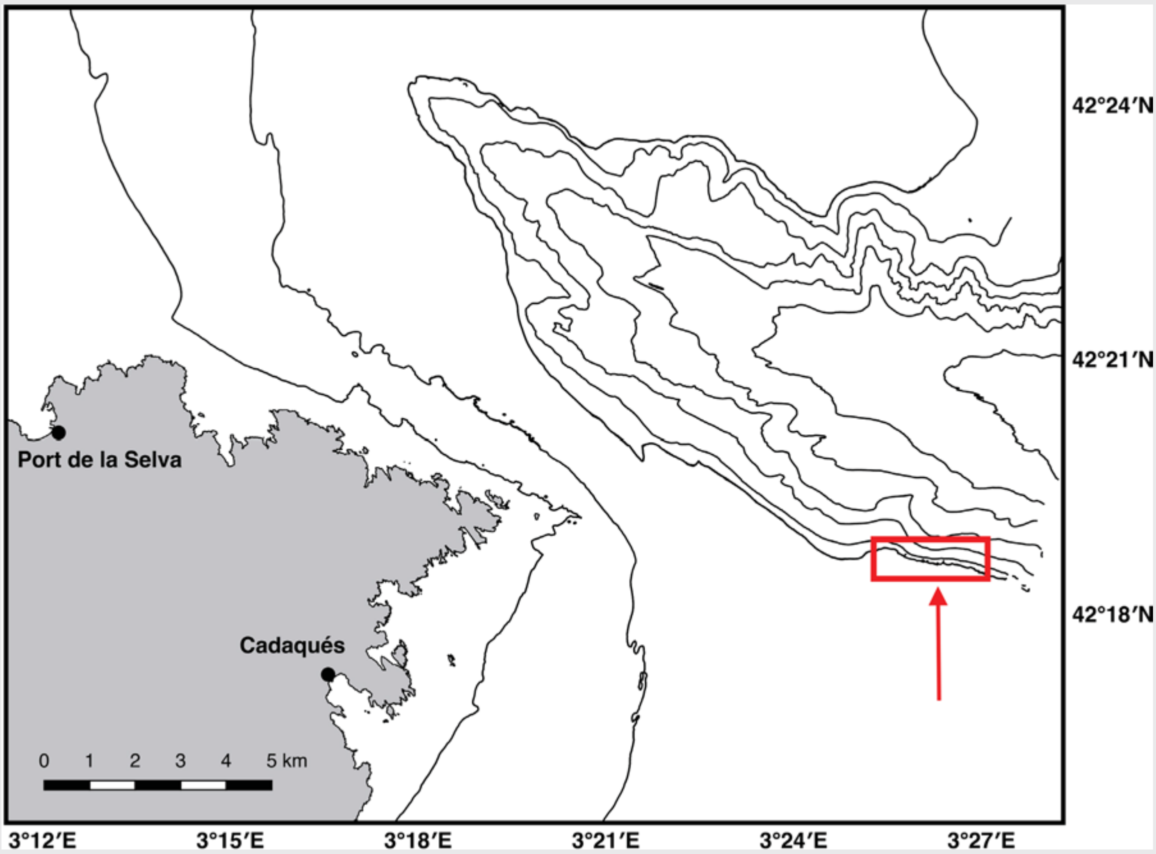


Figure B6.2. Approximate location where the biogenic structure was found in the southern continental shelf off Cap de Creus, at a depth of 130-140 m.

5.2 Effects of environmental parameters on invertebrate fauna

5.2.1 Structure of the environmental data

Having in mind the large topographic heterogeneity of the study area, it seems reasonable to expect strong contrasts in the environmental conditions of the different ROV dives, which ultimately played a role in the spatial distribution of the benthic fauna. At the large scale, two dominant environmental conditions were found along the study area (the continental shelf and the submarine canyon) and a transition area, with a gradual change in the slope (the shelf break). Out of a total of 60 ROV dives, 41 were performed exclusively on the continental shelf (almost 70%) at depths between 80 and 130 m. All remaining dives explored areas located at depths below 130 m, five of which primarily investigated the shelf break (almost 9%) and 14 went deeper into the submarine canyon (23%). Annex V summarizes the available information regarding the main abiotic variables evaluated for each ROV dive. A brief summary follows.

<u>Shelf.</u> Most dives performed on the continental shelf found flat surfaces (steepest slope on the shelf was lower than 12º) dominated by mud, sands and fine gravels. In fact, more than 80% of the area surveyed on the shelf corresponded to soft substrates, and only 5% of the video footage could be assigned to suboutcropping or outcropping rocks. Bottom currents were in general low, although there existed certain variability in transects located on the northern side of the cape. Fishing activity reached its highest values on the shelf, where approximately 10 ROV transects where performed in areas considered of medium or high intensity. On the opposite end, 11 transects were performed in locations with no signs of fishing activity.

<u>Shelf break.</u> This was the least explored area, but it also corresponded to the area with the smallest extent. This thin strip that connects the shelf with the steep slopes of the submarine canyon was characterized by the presence of large accumulations of gravels and pebbles of a biogenic origin. The ROV spent roughly 60% of the time on coarse substrates, and outcropping rocks appeared in less than 5% of the footage. The average slope ranged between 10º and 25º, and bottom currents displayed their highest speeds in this area. Fishing effort was low in general, although one area of the shelf break seemed to be affected by relatively high intensities.

<u>Submarine canyon.</u> The southern wall of the submarine canyon was characterized by steep slopes and the presence of large rocky outcrops, boulders and slabs. In fact, almost 70% of the footage filmed inside the canyon corresponded to areas of hard substrates, with muds and sands observed

in a very small proportion. The topographic and roughness indices reached their highest absolute value in this area, giving an idea of the topographic complexity of the canyon. Bottom currents displayed quite a large variability among transects and bottom trawling was practically non-existing.

The results of the principal components analysis (PCA) performed on the environmental data measured on each sampling unit are shown in Fig. 5.2.1. The two first axes explained around 45% of the variability observed, placing the samples rather well on a bidimensional space. A large set of environmental variables that seem to be very well correlated with each other were placed along the first principal component. On the positive side of the axis we found the samping units characterized by hard substrates (suboutcropping and outcropping rocks) and greater depths, as well as high slopes and high roughness profiles. These samples corresponded to areas inside the submarine canyon, where the pronounced relief favors areas with steep slopes and also where rocks tend to outcrop.

On the opposite end of the axis, we found the samples that corresponded to the flat continental shelf, in most cases characterized by the presence of very soft substrates. The sedimentary nature of the shelf, primarily on the northernmost and southernmost part of the study area, favors the

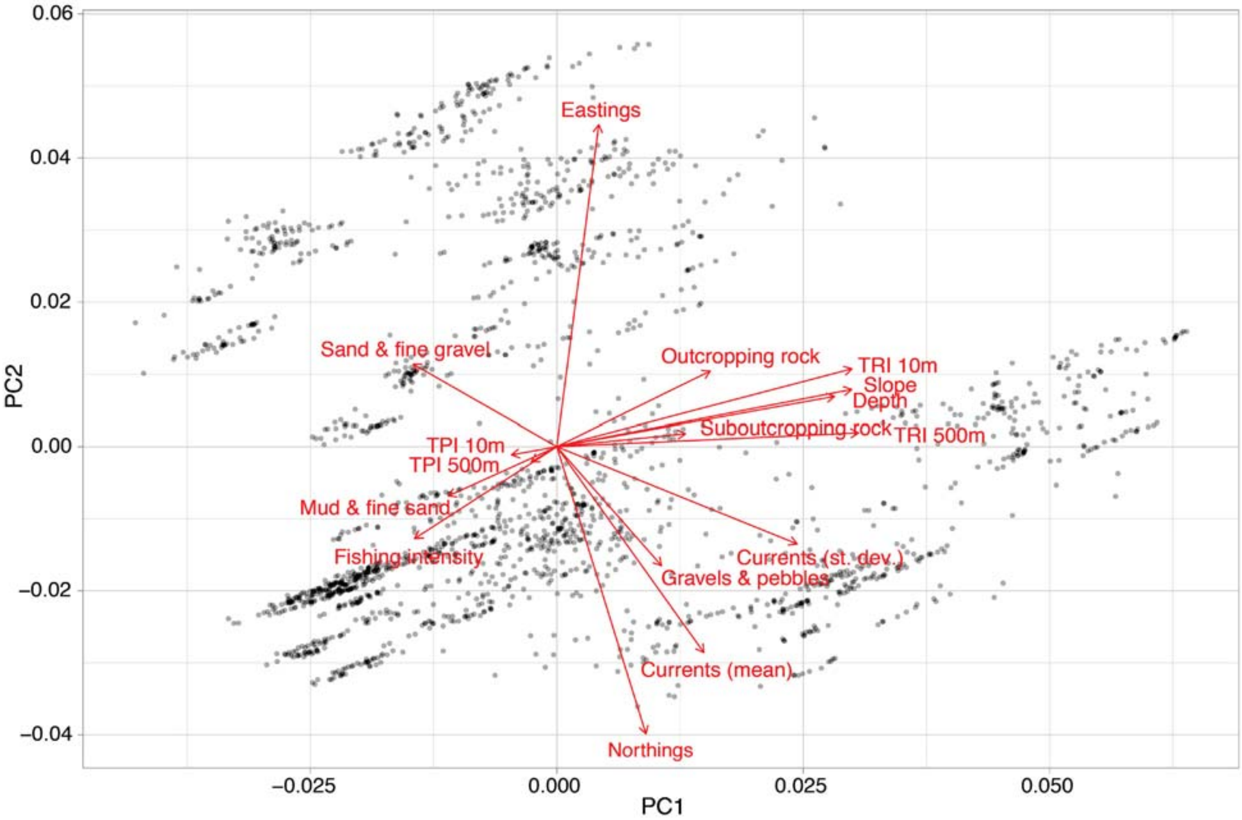


Figure 5.2.1. Two-dimensional PCA ordination of all samples used in this thesis arranged by the different environmental variables measured. PC1 (x-axis) and PC2 (y-axis) together account for 45% of the total sample variability.

deposition of fine particles, mostly muds and sands. High intensities of bottom trawling activities were concentrated in these sampling units, since commercial fishing prefers flat areas characterized by mud or sand, where their mobile gears find fewer obstacles such as large rocks or boulders. Areas with gravels and pebbles as their main substrate type were strongly correlated with high current speeds. In the case of Cap de Creus, these large gravels corresponded to biogenic structures, primarily large mollusk shells (some from ancient eras).

Over the second principal axis we found the factors related with the geographical position of the samples. Sampling units located towards the east are placed in the positive part of the axis, while samples located further north are in the negative side. According to these results, there seems to be a clear ordination alongside a geographical axis, where maximum opposition occurs on the NW to SE axis.

5.2.2 Environmental factors as driving forces

The triplot including species, sampling units and the explanatory variables resulting from the dbRDA analysis is shown in Fig. 5.2.2. The model explained almost 30% of the total variation in the biological matrix (constrained inertia = 29%, unconstrained inertia = 51%). The first 2 axes explained more than 50% of the constrained inertia of the biological data (36% and 18%, respectively). The overall test of significance showed that the canonical relationship between the biological data and the explanatory variables was very significant ($p<0.001$; 999 permutations), as were each of the variables when tested independently ($p<0.001$ for all variables; 999 permutations).

To visualize how the ordination responded to the groups identified in Section 5.1.2, sampling units were colored differently according to the assemblage they belonged to. Similarly to the results of the PCoA (Fig. 5.1.4), the ordination in a reduced space by a dbRDA separates rather well the samples belonging to the different assemblages, although a certain overlap in species composition seems to occur on the edges of the groupings, possibly due to transition areas. Pennatulaceans and alcyonaceans were representative groups in samples located in shallow areas, which are characterized by soft substrates, mainly sands with a percentage of small gravels. In this sense, soft-bottom areas where fishing intensity is low were dominated by species such as *Pteroeides spinosum*, *Alcyonium palmatum*, *Cavernularia pusilla*, *Pennatula rubra* and *Leptometra phalangium*, which mainly belong to assemblages B and D. The tube-forming Polychaete *Lanice conchilega* was well represented in samples with a certain degree of fishing activity, which also related to areas of low current speed and finer sediments, such as muds and fine sands. Locations with strong bottom

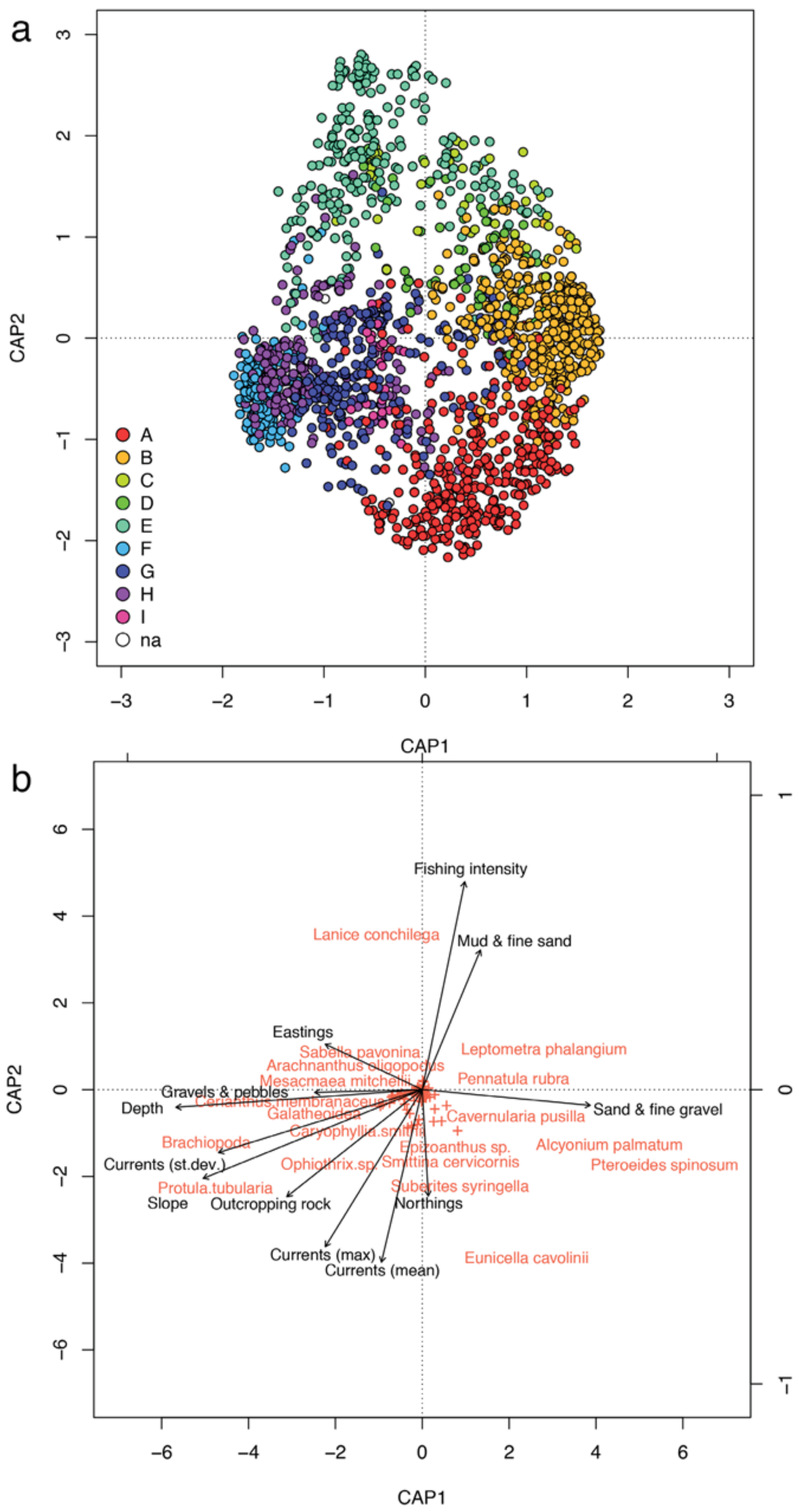


Figure 5.2.2. Constrained ordination (dbRDA based on Bray-Curtis distance) of species data vs environmental data from the continental shelf and submarine canyon off Cap de Creus. (a) Sampling units colored by benthic assemblage. (b) Species scores (red) and environmental variables (black vectors). Due to the high number of species, only those with the highest scores are labeled. Crosses represent the centroids of the remaining species.

currents were characterized by the presence of the gorgonian *Eunicella cavolini*, the sponge *Suberites syringella*, the bryozoan *Smittina cervicornis* and the zoantharid *Epizoanthus arenaceus* (Assemblage A), which seem to be confined to the northern areas off Cap de Creus.

Areas of high slopes and high variability in bottom current speed favoured the presence of rocky outcrops, which were associated with the tube-building polychaete *Protula tubularia*, different species of brachiopods and the solitary cup coral *Caryophyllia smithii* and at least two species of Galathoidea (Assemblage F). Also at such depths, the cerianthid *Cerianthus membranaceus* and the actinia *Mesacmaea mitchelii* (Assemblage H) dominated areas with coarse sediments, where bioclastic shells constitute a large fraction of gravels and pebbles.

In terms of assemblage characterization, the limits of each environmental feature within each assemblage are shown in Fig. 5.2.3. In general terms, this can be summarized as follows:

Assemblage A (*Eunicella cavolini, Suberites syringella*). Located on the flat surfaces of the shallow continental shelf, at depths between 90 and 110 meters, on the northern side of the cape. All samples were found in a mixture of substrate types, from sands with gravels to outcropping rocks. This assemblage was characterized by the presence of high bottom current speeds, on average the highest recorded, together with assemblage H. Fishing activity was relatively low, although some locations with relatively high intensities were also recorded.

Assemblage B (*Pteroeides spinosum*, *Pennatula rubra*, *Alcyonium palmatum*). Mainly located on flat areas of the continental shelf, at depths between 90 and 130 m. A large percentage of the samples were found in soft substrates composed primarily by sand and a small fraction of gravels, although this assemblage was also found in muddy areas (around 20%). Average current speeds were lower than those recorded in assemblage A. Fishing activity was generally of low intensity.

Assemblage C (*Sabella pavonina*, *Andresia parthenopea*). Located in flat areas of the continental shelf, in depths between 80 and 110 m. It was generally observed in muddy/sandy bottoms very influenced by bottom trawling activities. The average bottom current speeds were the lowest recorded.

Assemblage D (*Leptometra phalangium*). Assemblage found on flat areas of the continental shelf, restricted to areas with sand where a small proportion of gravels was also registered. It was generally observed in areas of low fishing intensities.

Assemblage E (*Lanice conchilega*, *Arachnanthus oligopodus*). Assemblage primarily found in areas of the continental shelf, but also reaching parts of the shelf break, especially in front of the cape. Average current speed was very variable, with episodes of strong currents. This possibly determined the substrate composition, which was a mixture between muds (20% of the times), sands with small gravels (35%) and large gravels and pebbles (45%). Fishing activity was very variable, with areas of low/medium intensity and areas with very high intensities.

Assemblage F (Brachiopoda, *Madrepora oculata*). Located in areas inside the canyon, showing the deepest distribution of all assemblages. Most samples were found in areas of steep slopes, which were characterized by the predominant presence of large rocks, boulders and slabs. Current speed was moderate, with areas of high intensity. Fishing activities of bottom trawlers were almost non-existent.

Assemblage G (*Dysidea* spp., *Haliclona* cf. *elegans*, *Hyrtios collectrix*, *Axinella damicornis*, *Stelligera stuposa*). Located primarily on the deepest part of the southern continental shelf, also entering the submarine canyon. Dominant substrates were sands with a fraction of gravels, although there probably was a relative part that corresponded to suboutcropping rocks that remained unnoticed. Certain samples were also found on rocky outcrops. Average current speeds were generally low, although certain episodes of high intensities were registered. Fishing was almost non-existing.

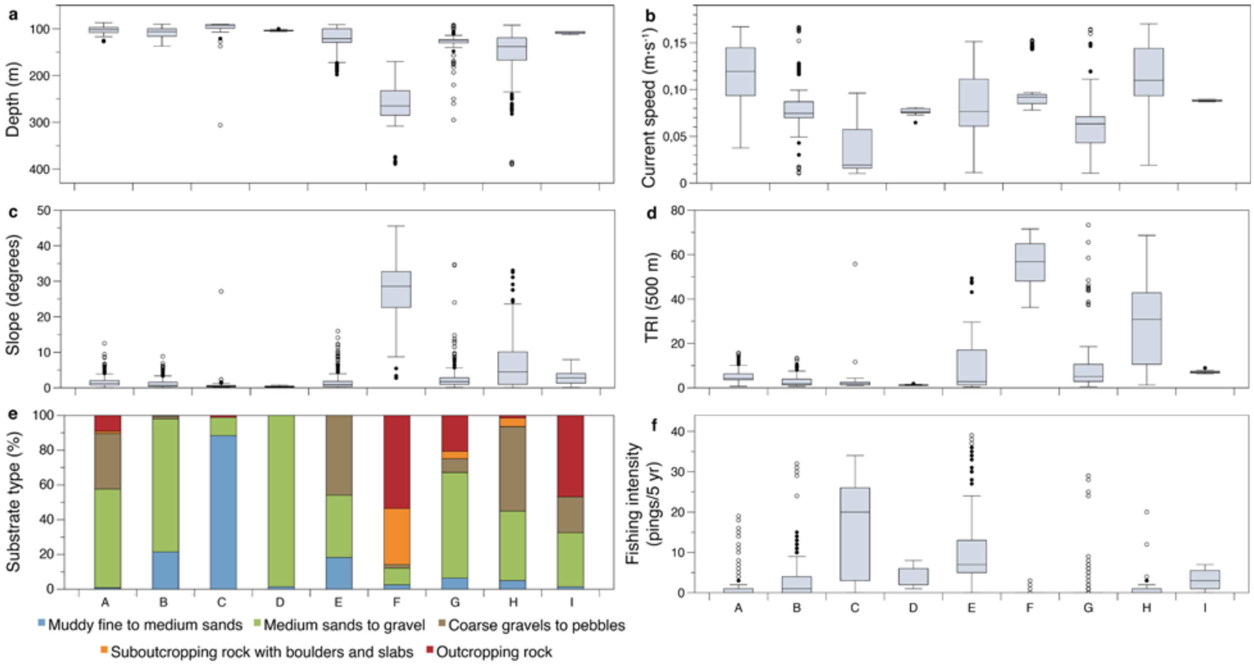


Figure 5.2.3. Boxplots showing characteristics of (a) bathymetry, (b) current speed, (c) slope, (d) TRI, (e) substrate type and (f) fishing intensity of the 8 benthic assemblages identified in the ROV images from Cap de Creus continental shelf and submarine canyon.

Assemblage H (*Cerianthus membranaceus*, *Capnea sanguinea*, *Protula tubularia*). Located on sloping surfaces of the shelf break and inside the submarine canyon, in a depth range of 110 to 250 m. This assemblage dwells in an area characterized by very strong bottom currents that probably clean the seafloor from fine particles. Substrate was primarily composed of gravels and pebbles, with a certain proportion of coarse sands. Barely any fishing activity was registered in this area.

Assemblage I (*Ophiothrix fragilis*). Located in a single spot on the continental shelf, in front of the easternmost part of the cape. It did not seem to show any preference for a substrate, and the brittle star aggregation developed indistinctly on top of sands, gravels, pebbles or outcropping rocks. Not much fishing activity was registered in this area.

5.3 Predictive mapping of diversity and megafauna assemblages

The spatial distribution of species richness and diversity over the continental shelf and submarine canyon off Cap de Creus predicted by the Random Forest algorithm is provided in Fig. 5.3.1. The models produced seemed to perform rather well, explaining 66% of the variability in the case of species richness and 60% for exponential of Shannon diversity. Both maps provided similar images. Areas predicted as very rich in terms of species composition were also the most diverse.

According to the model, a very high diversity area can be found along the 100-meter isobath on the northern part of the cape. This large area, which extends for more than 5 km, corresponds to the gorgonian assemblage dominated by *Eunicella cavolini* and the sponge *Suberites syringella* (Assemblage A). Another rich and diverse area is located close to the shelf break, on the southern part of the study area. It also extends for a few kilometers, following a similar depth range. It corresponds to the sponges aggregation (Assemblage G), one of the most diverse assemblages identified. Another hotspot can also be found on the canyon head, at depths of 200 m. It corresponds to the cold-water coral community (Assemblage F), which also provides habitat for a large number of accompanying species. The least diverse areas were always found on the soft bottoms of the continental shelf, generally at depths between 80 and 100 m. Particularly large is the area predicted in front of Cadaqués, which extends for at least 6 km along the coast with some 3.5 km in width.

According to the percentage increase in mean squared error, the most important factor determining the spatial distribution of species richness and diversity was substrate type (Fig. 5.3.2). Other important factors in both cases were depth and current speed. Fishing intensity had a larger effect on the distribution of biological diversity (60% increase in MSE) than it did on species richness (25% increase in MSE).

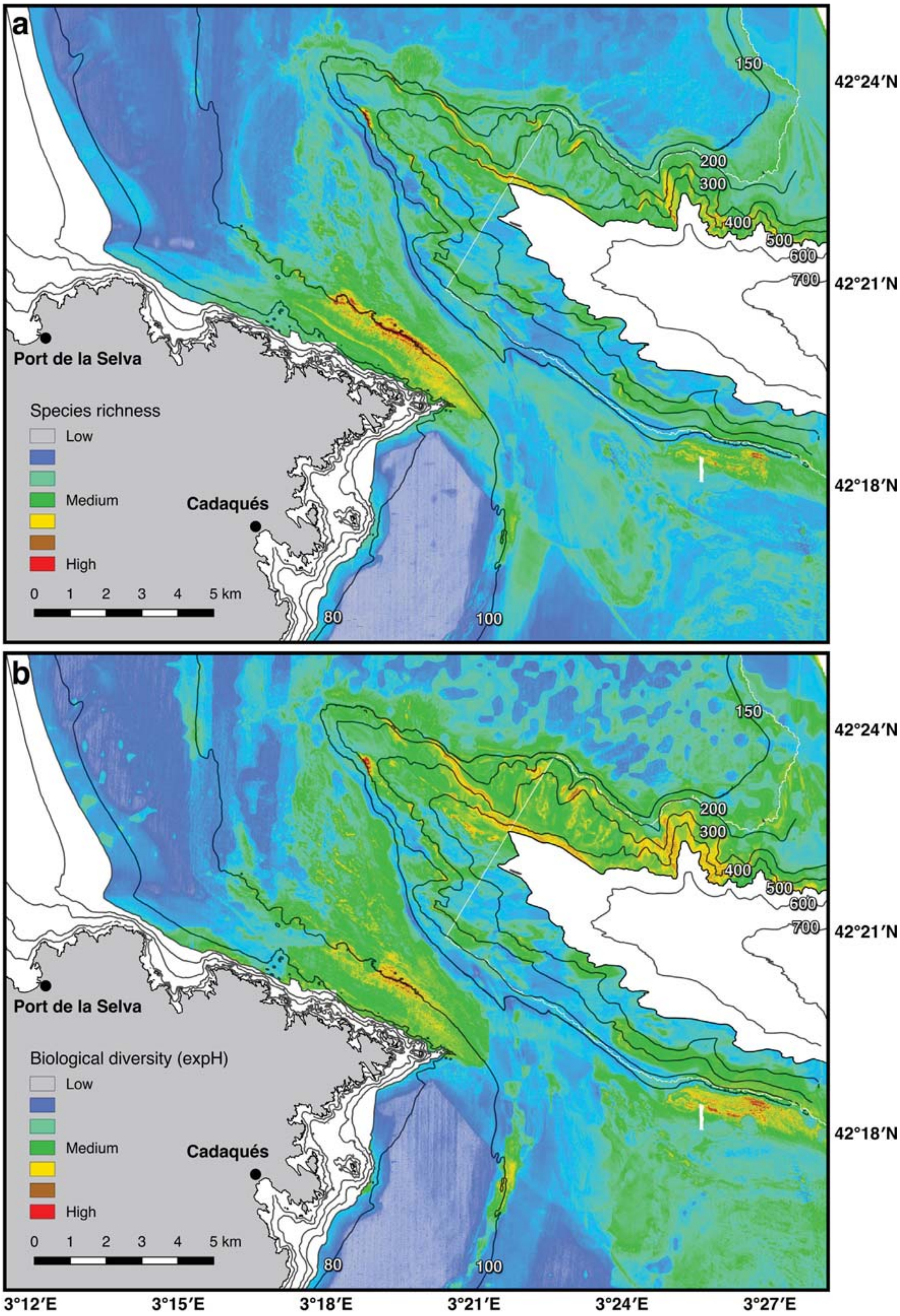


Figure 5.3.1. Predictive distribution maps of (a) megafauna species richness and (b) exponential of Shannon diversity index for the marine area off Cap de Creus based on Random Forest model. Only predictions between 70 and 400 m depth are shown.

In the case of benthic assemblages, the predictive map resulting from the RF model is given in Fig. 5.3.3. The model had an overall accuracy of 81% with an out-of-bag estimated error rate of 15.38% (Kappa = 0.78). The model predicted with highest accuracy the gorgonian (A) and the cold-water coral assemblages (F), with class errors below 10%. Higher rates of incorrect predictions were among those assemblages found in similar areas, as was the case of the pennatulacean assemblage (B), which tended to be confounded with assemblages A or G. A similar situation was observed with Assemblage H, which was sometimes predicted as assemblages F or G. In this sense, highest-class errors were obtained for assemblages G and H (23%) and C (28%).

According to the mean decrease in accuracy, depth was the most important factor determining the spatial distribution of the benthic assemblages, followed by seabed roughness (TRI 500 m), current speed and substrate type (Fig. 5.3.2). The intensity of fishing activities partly explained the observed patterns, but overall it was less important than the environmental parameters listed above. According to the prediction provided by the RF model (Fig. 5.3.3), the pennatulacean assemblage (B) occupies the lower part of the continental shelf, in areas where fishing intensity is low. In similar depths but with high trawling intensities, Assemblage C is dominant. The gorgonian assemblage (A) is predicted on a narrow strip off the northern part of the cape, at depths between 80 and 130 m. Its distribution seems very restricted to areas of the shelf where bottom currents are stronger. The sponges assemblage (G) is predicted on the lowest part of the shelf, in areas relatively far from shore and where fishing intensity is rather low. The shelf break and a large part of the submarine canyon seem to be occupied by Assemblage H (main species *Cerianthus membranaceus*), which

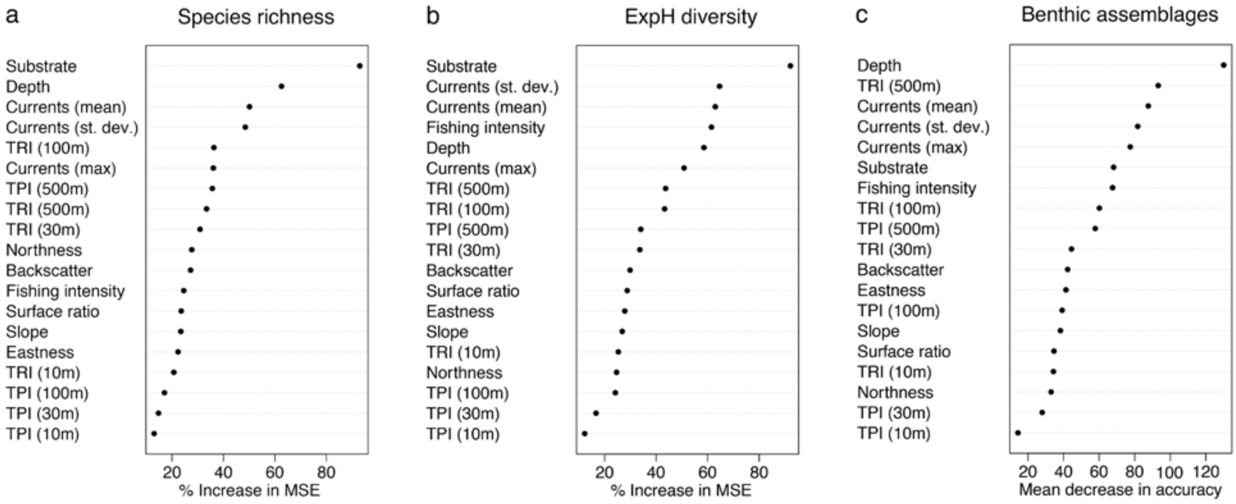


Figure 5.3.2. Variable importance for the random forest models for (a) species richness, (b) exponential of Shannon diversity and (c) megabenthic assemblages. Importance of each variable is reported as percentage increase in mean squared errors in the case of species richness and expH diversity and mean decrease in accuracy in the case of benthic assemblages. TRI: Terrain Ruggedness Index; BPI: Bathymetric Position Index.

shows a preference for areas with coarser substrates. A fine strip between 200 and 300 m depth, sometimes reaching further down to 400 m, is dominated by the cold-water coral assemblage (F), which mostly prefers hard substrates, relatively common features in areas of steep slopes inside the submarine canyon.

The samples corresponding to the ophiuroid assemblage (I) were not included in the predictive model due to its very restricted distribution and the high mobility of its characteristic species, which would make predictions rather complex. Since it was only found in an area of the continental shelf in front of the cape, at around 100 m depth, its presence was highlighted on the predictive map as a white star (Fig. 5.3.3).

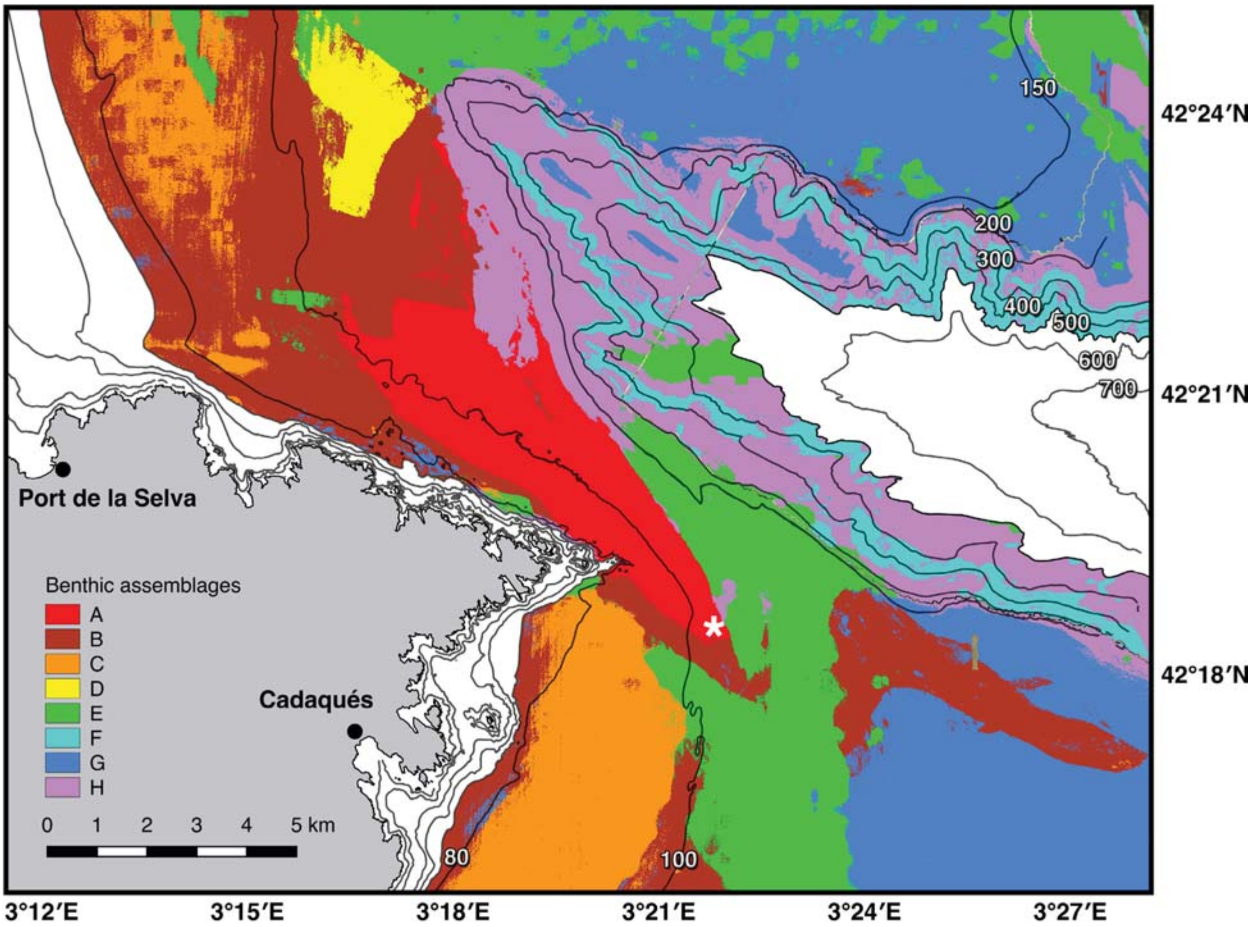


Figure 5.3.3. Predicted distribution of megafaunal assemblages in the continental shelf and submarine canyon off Cap de Creus, based on the Random Forest model. A: Gorgonian assemblage (*Eunicella cavolini*, *Suberites syringella*); B: Pennatulacean assemblage (*Pteroeides spinosum*, *Pennatula rubra*, *Alcyonium palmatum*); C: Impoverished assemblage (*Sabella pavonina*, *Andresia parthenopea*); D: Crinoid assemblage (*Leptometra phalangium*); E: *Lanice* assemblage (*Lanice conchilega*, *Arachnanthus oligopodus*); F: Cold-water coral assemblage (Brachiopoda, *Madrepora oculata*); G: Sponges assemblage (*Dysidea* spp., *Haliclona* cf. *elegans*, *Hyrtios collectrix*, *Axinella damicornis*, *Stelligera stuposa*); H: Ceriantharid assemblage (*Cerianthus membranaceus*, *Capnea sanguinea*, *Protula tubularia*); * *Ophiothrix* assemblage, not added to the prediction due to its restricted distribution and high mobility.

5.4 Effects of bottom trawling over invertebrate megafauna

5.4.1 Spatial distribution of fishing intensity

Fishing data obtained through the vessel monitoring system (VMS) showed that the trawling effort of the commercial fleet does not distribute homogenously along the study area (see Fig. 4.13). There existed two high intensity areas on the shallower part of the continental shelf (depths between 70 and 100 m), both on the northern and southern side of the cape. These two areas are characterized by their flat morphology and a very fine-grained sediment composition. Trawling intensity seemed to decrease as distance from shore increased, a pattern especially evident on the southern shelf. There were almost no records of commercial boats operating at a distance further than 10 km from the coastline on the southern part of the study area.

There was also an area excluded from bottom trawling between the area north off the cape and the submarine canyon, where bottom currents are stronger and rocks tend to outcrop in between sedimentary areas. There were no commercial trawling activities on the canyon head either (150 to 500 m), where rapid changes in slope are a common feature. It was further deep, at depths between 500 and 600 m inside the submarine canyon, where another high intensity hotspot was detected. This area is characterized by relatively flat surfaces covered by very fine sediments.

Overall, 139 marks left by bottom trawlers were spotted on the video images, appearing on both the northern and the southern continental shelf. Trawl marks were observed in 12 of the 60 ROV dives, all of them at depths between 90 and 120 m (exceptionally, one trawl mark was registered at 140 m depth). It was very difficult to determine the limits between different trawling events by merely looking at the marks left by bottom trawlers. This was mostly the case for those situations where the ROV cruised very close to the seabed. But in any case, the number of marks reported along a video transect seemed to be a good indicator of the trawling history in each of the surveyed areas.

In general terms, there was quite a high match between the number of trawl marks observed in each dive (standardized to 100m of seabed) and the fishing intensity determined by the VMS data (Fig. 5.4.1). There was, however, one dive performed on the northern continental shelf, close to the canyon head, which showed trawl marks in an area identified as free from trawling by the VMS records. The highest number of trawl marks in a single dive was 68, which corresponded to an average of 12 marks per 100 meters (ROV dive 58). Four other transects also had relatively high trawl mark densities, with average values above 3 marks per 100 m (ROV dives 16-34-57-59).

All trawl marks were observed on substrates belonging to the categories *Muddy fine to medium sands* and *Medium sands to gravel*, and no marks were identified in coarser sediments. Up to 17 different megafauna species were registered in sampling units where trawl marks were observed. More than 80% of those samples held 3 or less species (22% lacked any species whatsoever), with an average number of species slightly above 1.5.

Almost half of the species identified in areas with trawl marks appeared in very low numbers, being present in less than 5% of the samples. Some others, however, seemed to be relatively frequent. *Sabella pavonina* was the most common species in areas where trawl marks were observed (40% of the samples, maximum density of 1.4 ind·m^{-2}), followed by *Alcyonium palmatum* (28%, 3.2 ind·m^{-2}), *Lanice conchilega* (17%, 10.8 ind·m^{-2}), *Pteroeides spinosum* (17%, 3.2 ind·m^{-2}), *Andresia parthenopea* (17%, 0.8 ind·m^{-2}) and *Pennatula rubra* (8%, 1.2 ind·m^{-2}). It is interesting to point out that the crinoid *Leptometra phalangium* registered a density of 8.6 ind·m^{-2} in a sampling unit where a trawl mark was observed.

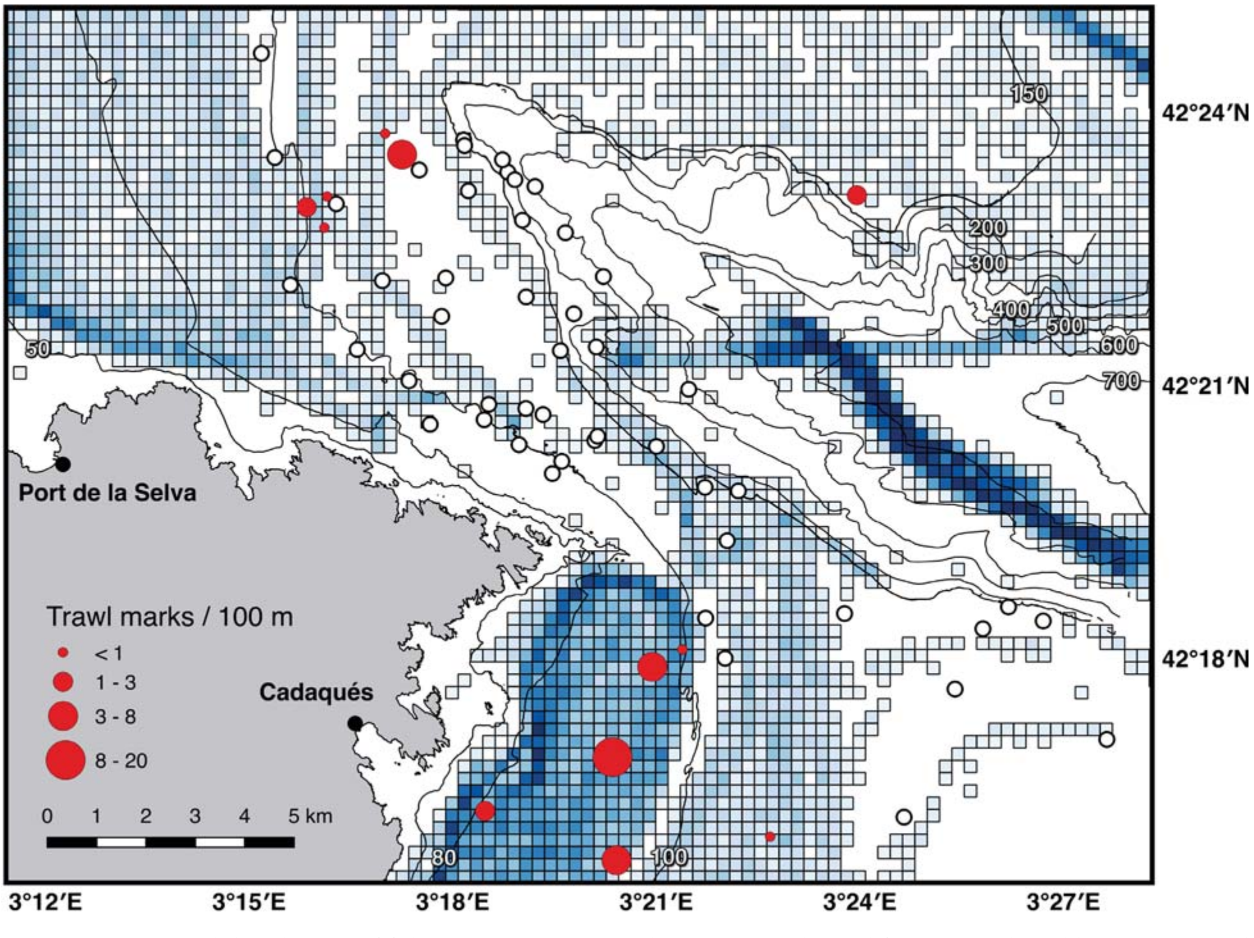


Figure 5.4.1. Spatial distribution of fishing intensity in Cap de Creus area derived from VMS data (blue squares) and number of trawl marks observed in the ROV dives (red circles), standardized to marks per 100 linear meters. White points represent ROV tracks where no trawl marks were detected.

5.4.2 Effects of fishing intensity over invertebrate species

There was a negative and significant decrease in species richness with increasing values of trawling intensity, as modelled by a negative binomial regression fit as a generalized linear model (Analisis of deviance, p-value < 0.001) (Fig. 5.4.2a). In areas where fishing was absent, the variability in the number of species per sampling unit was very large, ranging from 0 to more than 20, with an overall mean of around 5 species per sample (average values displayed as red dots). As fishing intensity increased, the average number of species approached values close to 0, with reduced variability among samples. A similar pattern was observed for biological diversity calculated through the exponential of Shannon index (Fig. 5.4.2b). In this case, the relationship was fit as a generalized linear model with an inverse gamma distribution (Analisis of deviance, p-value < 0.001). Areas with high fishing efforts held diversity values that, on average, were very close to 0.

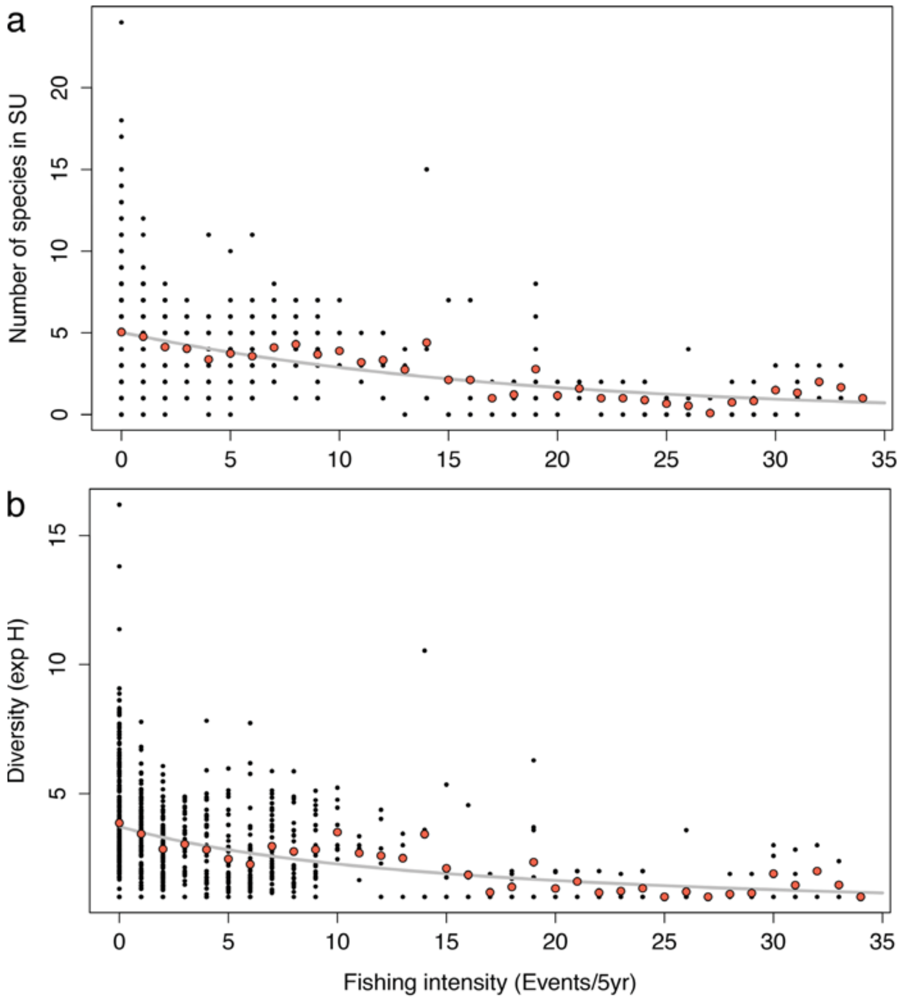


Figure 5.4.2. Scatterplots showing (a) species richness and (b) exponential of Shannon diversity of megafauna in the continental shelf off Cap de Creus against increasing levels of fishing intensity. Red points represent average values. Grey lines show modelled values.

The biplot resulting from the dbRDA analysis relating species composition of shelf samples to the categorical variable "fishing intensity" is shown in Fig. 5.4.3. The ordination plot shows a gradual change in the structure of the community as fishing intensity increases, with centroids progressively further away from the reference values in a scenario of no fishing activity. Samples in areas with no trawling have less dispersion and mostly appear close to each other. As fishing intensity increases, dispersion is larger and samples appeared unstructured along the plot. PERMANOVA analysis indicated that fishing intensity was significant (P-perm < 0.001, table not shown), and all 4 fishing regimes were significantly different from each other with regards to their species composition and abundance according to pair-wise comparisons (P-perm < 0.001 in all cases, table not shown).

A total of 115 megafauna species were reported from the soft continental shelf off Cap de Creus. Species that scored highest in determining the ordination of the samples are displayed in the biplot. According to their substrate preferences, 7 of the species that appeared on the biplot were selected to evaluate their response to different fishing scenarios: the soft coral *Alcyonium palmatum*, the pennatulaceans *Cavernularia pusilla*, *Pennatula rubra* and *Pteroeides spinosum*, the crinoid *Leptometra phalangium* and the polychaetes *Lanice conchilega* and *Sabella pavonina*. The other two species (*Eunicella cavolini* and *Protula tubularia*) were excluded from the analysis due tor their preference for hard surfaces in their adult life stages (shells, stones of bear rock), to which they attach to in order to fully develop.

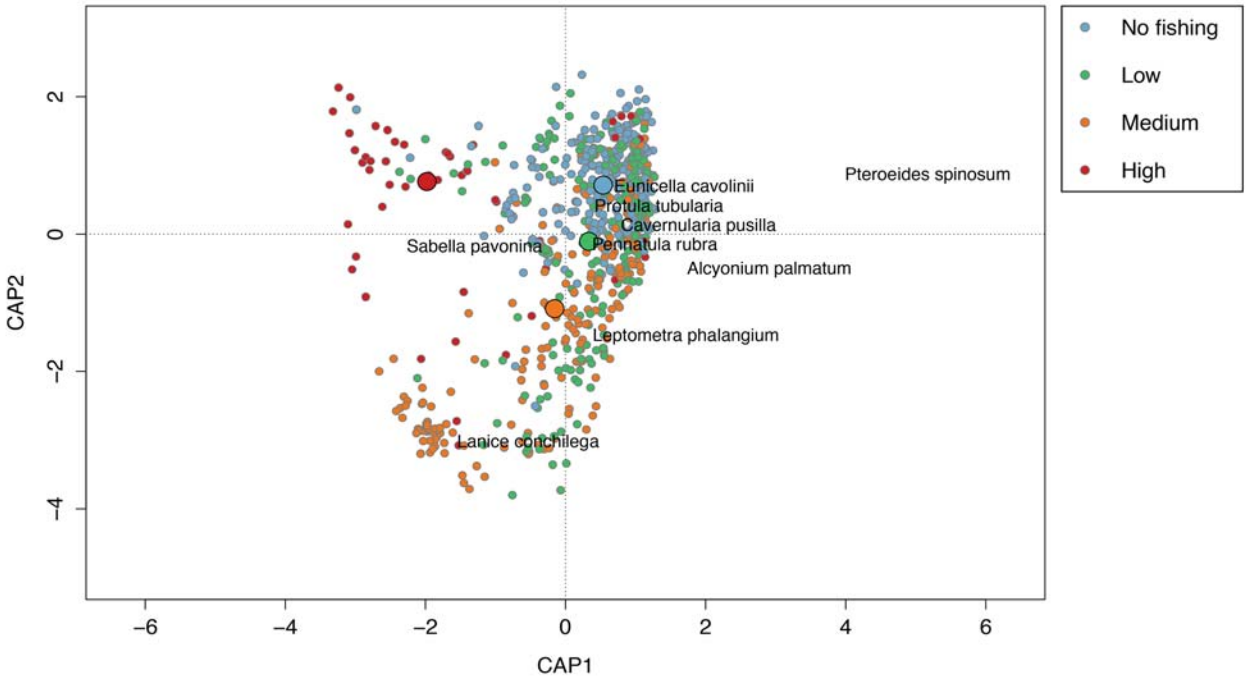


Figure 5.4.3. Distance-based RDA ordination relating fishing intensity to megafaunal data. Analysis was performed on principal coordinate axes obtained from Bray-Curtis dissimilarities of square-root transformed species densities. Colour points in biplot represent sampling units. The most important species in the ordination are superimposed. No fishing: No pings in 5 years; Low intensity: 1-3 pings; Medium intensity: 4-12 pings; High intensity: >12 pings.

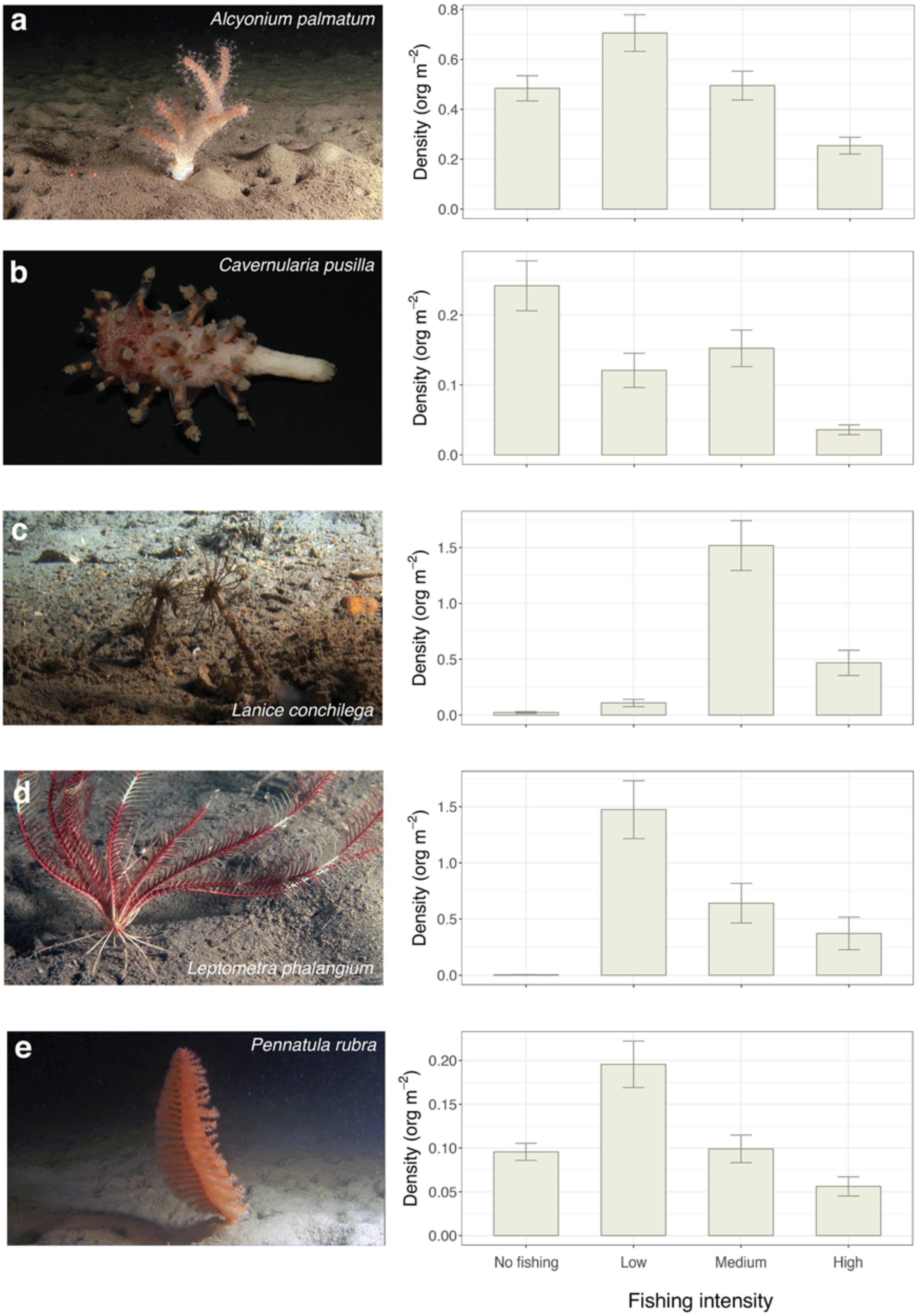


Figure 5.4.4. Barplots with average density values (org·m^2) for the selected species in the soft bottoms continental shelf off Cap de Creus, affected by different levels of bottom trawling intensity. a: *Alcyonium palmatum*; b: *Cavernularia pusilla*; c: *Lanice conchilega*; d: *Leptometra phalangium*; e: *Pennatula rubra*; f: *Pteroeides spinosum*; g: *Sabella pavonina*. Fishing intensity levels: No fishing: No pings in 5 years; Low intensity: 1-3 pings; Medium intensity: 4-12 pings; High intensity: >12 pings. Images a, c, d, e, f and g by Nemo ROV (Gavin Newman) and image b by Pablo J. López-González.

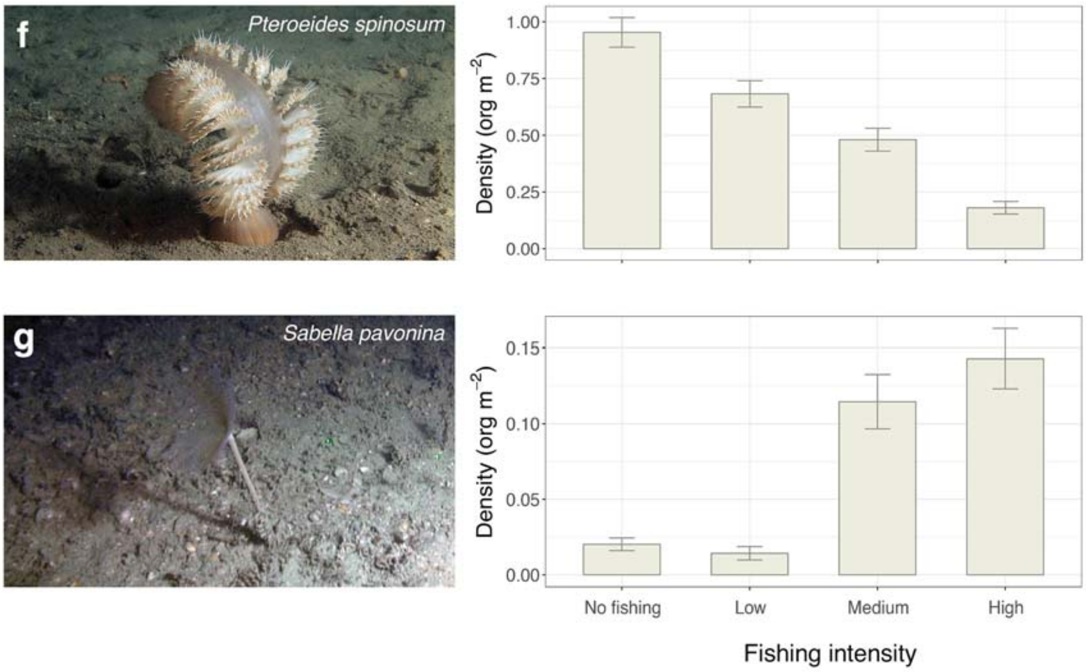


Figure 5.4.4. (Continued)

Mean abundances for the seven selected species in the four fishing scenarios are shown in Fig. 5.4.4. In general terms, two different responses were observed. On the one hand, there were species that had progressively lower density values as fishing intensity increased (clear examples are *P. spinosum* and *C. pusilla*), although this trend was not very strong for *P. rubra* and *A. palmatum*, whose maximum recorded densities appeared in a low intensity scenario. In the case of *P. spinosum*, density values were four times lower in high fishing intensity areas, being reduced from 1 col·m^{-2} to less than 0.25 col·m^{-2}. *L. phalangium* also showed a decrease in mean abundance as fishing effort increased, although no specimens were registered in areas free from trawling. On the other hand, both polychaete species displayed a contrasting behavior, since their highest mean abundances were recorded in medium and high fishing intensity scenarios (Fig. 5.4.4 c-g). Density values for *L. conchilega* were highest in areas with medium trawling effort (*ca.* 1.5 org·m^{-2}), and although density values were generally low for *S. pavonina*, its highest mean densities were registered in areas of the continental shelf with high fishing efforts (0.15 org·m^{-2}).

5.4.3 Selection of indicators

Increasing levels of fishing intensity had a negative effect on species richness (number of species per sampling unit) and diversity (exponential of Shannon index) (Fig. 5.4.2). Since these two indices also show statistic practicability (abundant, representative and easy to quantify) and are socially comprehensible, we considered they should be incorporated in the monitoring proposal. Table 5.5.1 provides the basic environmental requirements of the 7 potential indicator species.

Results

With the exception of *Sabella pavonina*, all species exhibited a clear preference for continental shelf environments. *Cavernularia pusilla* and *Pennatula rubra* had a very restrictive distribution range, being found exclusively in depths between 90 and 130 m. Other species extended their bathymetric distribution into the submarine canyon, but their presence below the shelf break was almost negligible. This was the case of the cnidarians *Alcyonium palmatum* and *Pteroeides spinosum*, as well as the crinoid *Leptometra phalangium*, all of them characteristic species of the Mediterranean continental shelf. Both polychaete species had a wider distribution range, appearing in high numbers in certain areas inside the submarine canyon. In terms of substrate preferences, all 7 species fully developed in soft bottom environments.

All species were found in muddy and sandy habitats in more than 60% of the occasions, with some species displaying a strong preference for these type of substrates. Regarding their representativity, *P. spinosum* and *A. palmatum* were the most common species of the continental shelf, present in over 50% of the samples. These two species showed a very wide spatial distribution, appearing in almost all shelf areas. The rest of the species were not as common, and their occupancies ranged between 15 and 30%. *L. phalangium* had the most restrictive distribution, appearing only in specific areas of the continental shelf (it was only present in 13% of the shelf samples). According to these results, only 2 species fully satisfied the requirements for being good indicator species: the soft coral *A. palmatum* and the sea pen *P. spinosum*. We considered that these two species should be selected due to their negative response to increasing levels of fishing intensity and their presence in all areas of the shelf, regardless of grain size and depth. At the same time, it seemed appropiate to select two species that showed a positive response to increasing levels of trawling activity: *L. conchilega* and *S. pavonina*. These two polychaetes were not as frequent as the other two species, but this was possibly due to the lower number of samples that were recorded in medium and high intensity areas.

Table 5.5.1. Main environmental characteristics of the potential indicator species based on the data gathered in the video images. Occupancy data corresponds to the number of samples in which the species was observed taking into account (*) all sampling units from the continental shelf and (**) those located on soft bottoms areas composed of mud and/or sand between 80 and 125 metres. In the case of substrate type, it corresponds to the percentage of shelf samples of each substrate tipology in which the species has been observed. Substrate types: A = Muddy fine to medium sands; B = Medium sands to fine gravel; C = Coarse gravels to pebbles; D = Suboutcropping rock; E = Outcropping rock.

	Occupancy (%)		**Depth (m)**			**Substrate type (%)**				
Species	**All areas ***	**Shelf ****	**Min**	**Max**	**Avg ± sd**	**A**	**B**	**C**	**D**	**E**
Alcyonium palmatum	42.6	55.4	87	270	107 ± 13	15.8	64.7	14.9	0.8	3.8
Cavernularia pusilla	10.8	21	90	127	104 ± 5	26.1	71.8	0	0.1	1.9
Lanice conchilega	16.3	19.7	91	205	121 ± 23	16	46	37.5	0.2	0.2
Leptometra phalangium	7.6	13.3	97	189	107 ± 11	8.6	77.5	13.2	0.5	0.2
Pennatula rubra	15.7	27.3	92	127	106 ± 9	18	78.6	1.4	0.2	1.9
Pteroeides spinosum	42.5	64.4	88	162	107 ± 9	13.2	75.7	8.3	0.4	2.3
Sabella pavonina	16.1	17.6	90	386	161 ± 79	27	34.3	8.2	11.8	18.7

5.5 Baseline data for a monitoring program

5.5.1 Spatial and statistical design

Regarding the spatial design, proposing a monitoring program before management plans have been developed is a complex task since the spatial component of the management plan is fundamental to effectively design a BACI experiment. For this reason, and based on the recommendations provided by Gili *et al.* (2011) we propose a future (hypothetical) exclusion area inside the limits of the new MPA of Cap de Creus where trawling activities should cease completely in order to guarantee the conservation of its benthic assemblages (dotted line in Fig. 5.5.1). The area proposed encloses a relatively small part of the total surface of the declared SCI, but succeeds at including the most sensitive assemblages identified in Part 1 of this thesis. Furthermore, in order to test the differential effects of the historical FI in the evolution of the indicators, the exclusion perimeter not only includes areas that have historically suffered from very low trawling activity, but also areas of high fishing intensity (located in the southern part of the study area), which should see changes in the values of indicator species as management plans come into practice.

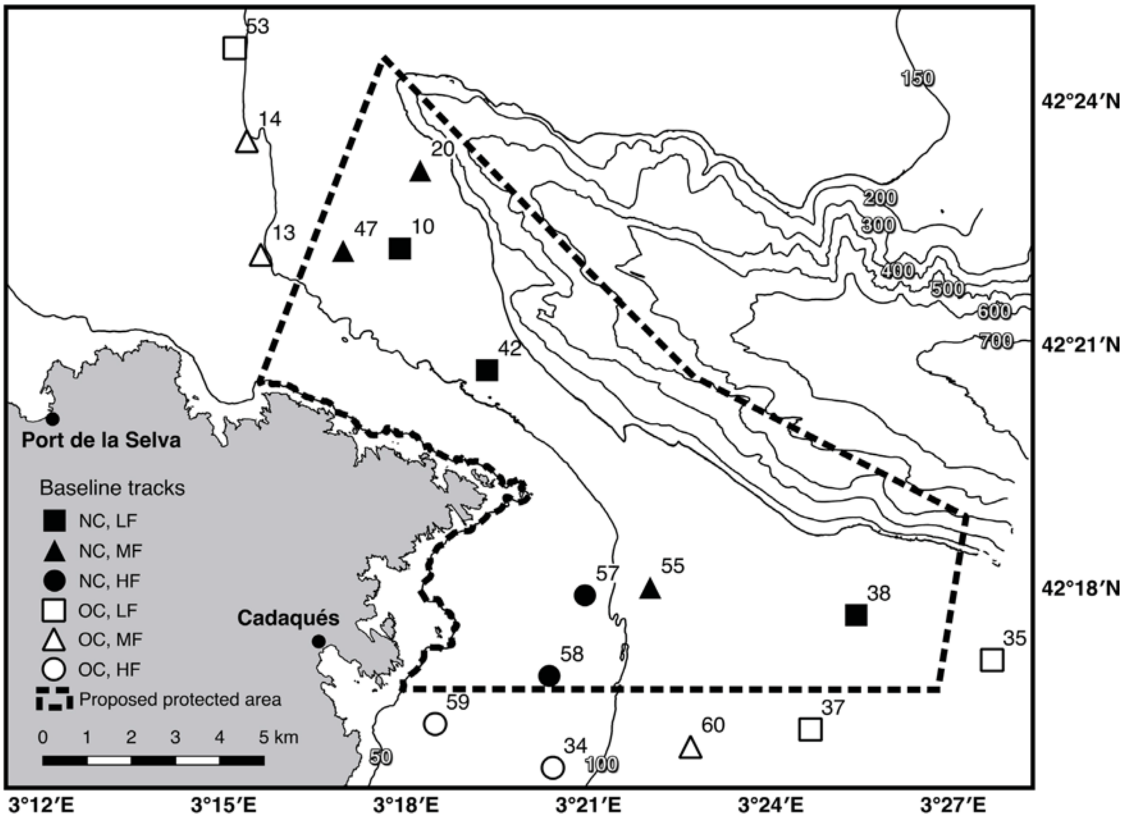


Figure 5.5.1. Location of the monitoring sites inside and outside of the "No fishing" area (dashed line) proposed for the new offshore MPA of Cap de Creus based upon the recommendations given by Gili *et al.* (2011). NC: New closure; OC: Open control. LF: Low fishing intensity. MF: Medium fishing intensity; HF: High fishing intensity. Start and end coordinates for each ROV dive are provided in Annex V.

The experimental design proposed for the future monitoring program to be implemented in Cap de Creus offshore MPA is summarized in Fig. 5.5.1. It includes three factors (Treatment, Time and ROV dive) in three different experimental conditions regarding their fishing intensity (FI): low (LF), medium (MF) and high (HF). Following a Beyond BACI strategy (Underwood, 1994), sampling should be performed several times as part of the monitoring of the MPA, and it is recommended that Time and Treatment be treated as fixed factors. ROV dive is a random factor nested in treatment. Time and Treatment have two levels (Time: Before and After; Treatment: New Closure and Open Control). There exists a little problem in the level of replication in the design proposed, since there are three ROV dives per treatment in low and medium FI, but only two dives for high FI. This is due to a limitation in the available number of tracks that matched all the necessary requirements of substrate type and depth at that level of fishing intensity. Box VII details all the steps required to implement the monitoring program proposed in this thesis in the offshore MPA of Cap de Creus.

5.5.2 Baseline data

Table 5.5.2 shows the baseline data values regarding species richness, diversity and the average density of the 4 indicator species for each ROV transect selected for the monitoring plan.

Table 5.5.2. Baseline data for the selected indicators (± standard deviation) for the continental shelf off Cap de Creus.

					Average density (org·m^{-2})			
ROV dive	**Treatment**	**Fishing intensity**	**Richness (species/su)**	**Diversity (expH')**	***Pteroeides spinosum***	***Alcyonium palmatum***	***Lanice conchilega***	***Sabella pavonina***
35	Control	Low	3.09 ± 2.88	2.77 ± 2.36	0.31 ± 0.30	0.22 ± 0.23	0	0.01 ± 0.06
37	Control	Low	5.57 ± 2.26	4.71 ± 1.63	0.15 ± 0.16	0.04 ± 0.08	0	0.01 ± 0.04
53	Control	Low	4.07 ± 1.87	3.63 ± 1.40	0.30 ± 0.19	0.28 ± 0.29	0.17 ± 0.46	0.13 ± 0.18
10	Impact	Low	5.02 ± 2.21	3.45 ± 1.66	2.21 ± 1.50	0.13 ± 0.18	0.01 ± 0.04	0.01 ± 0.02
38	Impact	Low	4.87 ± 1.77	3.78 ± 1.35	1.04 ± 0.79	0.11 ± 0.21	0.23 ± 0.31	0
42	Impact	Low	9.71 ± 2.87	6.51 ± 2.17	0.18 ± 0.27	2.02 ± 1.76	0	0
13	Control	Medium	3.30 ± 1.28	2.45 ± 1.06	0.50 ± 0.54	0.30 ± 0.36	2.90 ± 4.86	0.01 ± 0.06
14	Control	Medium	3.23± 1.49	3.06 ± 1.35	0.26 ± 0.21	0.23 ± 0.19	0.01± 0.06	0.06 ± 0.14
60	Control	Medium	3.24 ± 1.02	1.82 ± 0.53	0.01 ± 0.03	0.06 ± 0.17	5.04 ± 2.83	0.55 ± 0.37
47	Impact	Medium	3.62 ± 1.28	2.94 ± 1.07	0.89 ± 0.59	0.40 ± 0.67	0.01 ± 0.07	0
20	Impact	Medium	3.75 ± 1.40	3.08 ± 1.07	0.18 ± 0.23	0.03 ± 0.07	0	0.01 ± 0.04
55	Impact	Medium	3.54 ± 2.02	2.19 ± 1.17	2.05 ± 0.85	0.09 ± 0.10	0	0.03 ± 0.08
34	Control	High	1.18 ± 1.83	1.58 ± 1.27	0	0.08 ± 0.17	0	0.09 ± 0.25
59	Control	High	0.79 ± 0.85	1.19 ± 0.46	0	0.05 ± 0.09	0	0.07 ± 0.16
57	Impact	High	1.45 ± 0.72	1.44 ± 0.57	0.01 ± 0.05	0.04 ± 0.11	0.23 ± 0.33	0.25 ± 0.25
58	Impact	High	1.36 ± 1.02	1.49 ± 0.72	0.01 ± 0.04	0.05 ± 0.10	0	0.20 ± 0.32

Box VII

Proposed monitoring protocol

One of the major aims of this PhD thesis is to provide the guidelines to develop a specific monitoring program for benthic megafauna in Cap de Creus using video recordings of the seabed. Being aware of the complexity of carrying out surveys at sea, especially when working past certain depths, and the relatively low implementation of regular ROV surveys in areas of the Mediterranean Sea, this step-by-step guide intends to cover all aspects of (1) the filming of benthic habitats, (2) the analysis of the images, (3) the hypotheses to be tested and (4) the way data should be statistically analyzed, solving all questions that could arise during their implementation.

ROV specifications

There is no need to employ high-end ROVs to evaluate changes in the abundance of megabenthic indicator species. There exist, however, a few requirements that can be considered essential to successfully obtain images for quantitative analyses:

a. The camera mounted on the ROV should record video images at full HD resolution (at least), which will later be of help towards the identification of benthic organisms.
b. The underwater vehicle should be equipped with a precise positioning system, able to record GPS positions at short time intervals (frequencies of one position every 20 seconds or higher can be considered adequate).
c. The vehicle should incorporate two parallel laser beams pointing towards the direction of the camera shot.
d. A minimum distance of 10 cm and a maximum of 50 cm between laser points is recommended.
e. Green lasers provide a better contrast in the images, and their use is advised.

Video surveys

In order to produce video images that can later be quantitatively analyzed, the following indications are recommended:

a. The vehicle should perform linear transects over the seabed, avoiding as much as possible erratic navigations and loops around itself.
b. The ROV must be towed behind the vessel to improve the directionality of the vehicle. Dynamic positioning of the boat will largely contribute to following the same path of the transects provided as baselines when performing the “after” sampling.
c. Distance from the seabed should be kept as small as possible to improve taxonomic resolution, even at the expense of losing field of view. Ideally, the section of the seabed observed at the position of the laser beams should be of approximately 1 meter.
d. It is recommended to cruise at speeds between 0.2 and 0.3 knots, keeping the velocity as constant as possible.
e. The camera should record the full length of the dive, with no stops in the filming at any time during the ROV transect.
f. The time stamped on the video images should be fully synchronous with that of the navigation system. If there is no possibility to have a time stamp, the exact time at which the camera starts recording should be registered. A time stamp can be generated afterwards using video editing software.
g. The length of each dive will always depend on a series of factors that most times are not controlled by the researcher: weather conditions, visibility at depth, presence of abandoned fishing gear, problems with the positioning system, etc. For that reason, we recommend a flexible transect length, but subject to ensuring a minimum of 300 meters of valid footage for each ROV dive to account for the variability in the spatial distribution of the fauna investigated.
h. If possible, a dive log should be produced at the same time video that images are being recorded. This log should incorporate information regarding any issues with the filming, the ROV or the vessel.
i. Once each dive has been completed, image files should be properly labeled. Creating a safe copy of the video images as early as possible is highly recommended.

Video editing

Video images should undergo a series of post-processing steps in the video laboratory before annotating the benthic fauna and identifying the seabed characteristics.

a. Images should be analyzed using a video editing software, either for professional use (e.g. Final Cut, Adobe Premiere or Media Composer), or with a free license (e.g. iMovie, Lightworks or Windows Movie Maker).

b. Each ROV dive should be treated separately, generating a single file for each transect with the editing software selected.
c. After incorporating the files to the editing software, sequences regarding the deployment, immersion and recovery of the ROV should be deleted. Images left should only correspond to the moment the ROV starts cruising at a constant speed over the seabed until the end of the linear transect.
d. Start and end times of each ROV transect after the video has been edited will be used to determine the start and end points of the GPS track.
e. The edited transect should be fully examined in order to identify sequences where the vehicle remains static. Those sequences should also be removed. The end result should only incorporate images with the ROV in motion.
f. The final step of the post-processing consists in determining which parts of the transect will be considered valid for statistical purposes and which parts should not be included. For that reason, bad visibility sequences or images filmed too far away from the seabed will be identified using the time code generated by the video editing software to be later excluded.

GIS positioning

Navigational data obtained from commercial tracking systems might produce a small amount of positional errors, which should be filtered to obtain smooth trajectories of the ROV. This can be manually achieved using GIS software (e.g. ArcGIS or QGIS):

a. Raw navigational data should be incorporated to the GIS software and projected, in the case of Cap de Creus, to the coordinate system WGS84 - UTM31.
b. Those GPS positions that appear too far away from the previous point if ROV speed was kept constant should be considered outliers and removed from the point cloud (Fig. B7.1).
c. Once a smooth trajectory has been achieved (as close as possible to the real path performed by the ROV), points should be joined to obtain the total dive length.
d. If ROV speed was kept fairly constant throughout the whole transect, moving speed can be calculated as the quotient between transect length and the time obtained after

editing the images.

e. If ROV speed showed certain variability in different parts of the dive, the GPS track can be split into segments of a certain length in order to calculate the speed of the ROV in each partition.

f. ROV speed will later be used to identify the position of all species along the transect (as distance from the beginning of the track) using the time code generated by the video editing software.

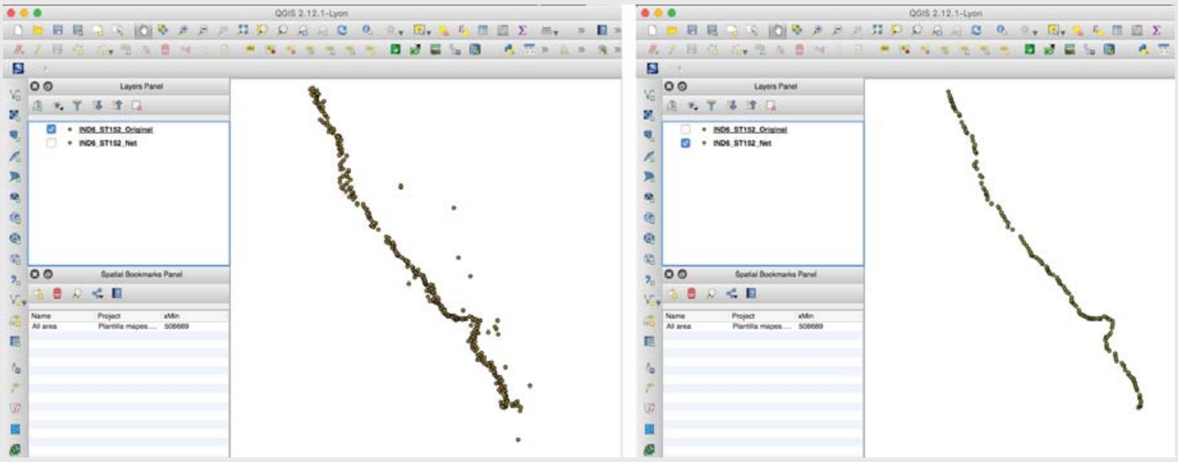


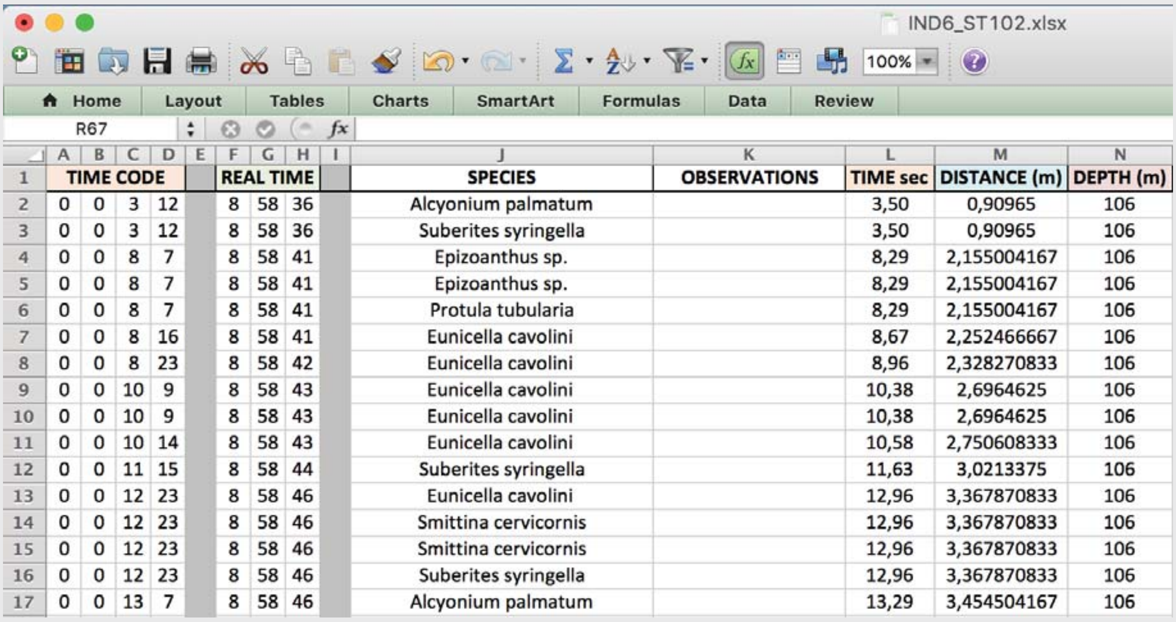
Figure B7.1. Removal of outlier points from raw positional data (left) to leave a smooth trajectory (right) using a GIS software.

Quantitative biological data

The procedure to obtain biological quantitative data still requires the manual input of species identified in the ROV images. Certain aspects should be considered:

a. Density estimates are referred to a rectangular area, which requires a known length and a width to be determined. Selecting the width of the transect to be analyzed largely depends on the distance between the ROV and the seabed, and should be independently evaluated in every situation. Since we recommend ROVs to cruise as close as possible to the sea floor to increase taxonomic resolution, sections across the seabed between 30 and 50 cm have proven very functional.

b. All megafauna organisms should then be identified and assigned the time code generated by the video editing software at the moment they cross the laser beams. Annotation can be done in a simple Excel sheet. We provide an example of how simple annotation can be performed in Fig. B7.2.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	TIME CODE					REAL TIME				SPECIES	OBSERVATIONS	TIME sec	DISTANCE (m)	DEPTH (m)
2	0	0	3	12		8	58	36		Alcyonium palmatum		3,50	0,90965	106
3	0	0	3	12		8	58	36		Suberites syringella		3,50	0,90965	106
4	0	0	8	7		8	58	41		Epizoanthus sp.		8,29	2,155004167	106
5	0	0	8	7		8	58	41		Epizoanthus sp.		8,29	2,155004167	106
6	0	0	8	7		8	58	41		Protula tubularia		8,29	2,155004167	106
7	0	0	8	16		8	58	41		Eunicella cavolini		8,67	2,252466667	106
8	0	0	8	23		8	58	42		Eunicella cavolini		8,96	2,328270833	106
9	0	0	10	9		8	58	43		Eunicella cavolini		10,38	2,6964625	106
10	0	0	10	9		8	58	43		Eunicella cavolini		10,38	2,6964625	106
11	0	0	10	14		8	58	43		Eunicella cavolini		10,58	2,750608333	106
12	0	0	11	15		8	58	44		Suberites syringella		11,63	3,0213375	106
13	0	0	12	23		8	58	46		Eunicella cavolini		12,96	3,367870833	106
14	0	0	12	23		8	58	46		Smittina cervicornis		12,96	3,367870833	106
15	0	0	12	23		8	58	46		Smittina cervicornis		12,96	3,367870833	106
16	0	0	12	23		8	58	46		Suberites syringella		12,96	3,367870833	106
17	0	0	13	7		8	58	46		Alcyonium palmatum		13,29	3,454504167	106

Figure B7.2. Possible layout of an Excel sheet to annotate the position of each organism along an ROV dive. Time code corresponds to the time generated by the video editing software and includes hour, minute, second and frame. Real time corresponds to the actual time when images were recorded, useful for cross-referencing with GIS positioning. It includes hour, minute and second. Distance can be calculated using the average speed of the ROV along the transect by splitting the track into several sections, each several tens of meters long, and calculating the average speed in each section.

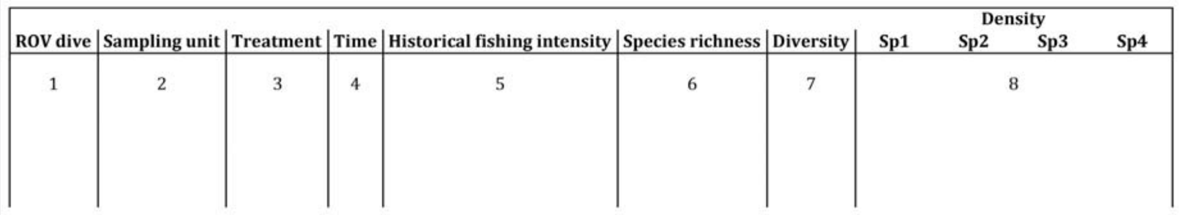
c. Annotation relies on the taxonomic expertise of the researcher in charge of the video analysis. To facilitate the identification process, a visual guide including all megafauna species identified on the continental shelf and submarine canyon off Cap de Creus is provided at the end of this PhD thesis (Annex I). This guide does not intend to cover all the existing variability of shelf and slope environments of the Mediterranean Sea, but shows the results of the work performed in Cap de Creus.

d. It would also be interesting to determine a few key environmental variables once all benthic megafauna has been annotated, such as substrate type, depth or slope, which can later be used to identify drivers of the patterns detected. A simple visual guide with the different substrate types identified on the continental shelf and submarine canyon off Cap de Creus is also given at the end of this document (Annex II).

e. To generate data comparable to that of the baseline provided in this PhD thesis, calculations regarding species richness, diversity and species abundances should be made on sampling units of 5 m^2. These sampling units should be generated as recurring partitionings of the ROV transect. (e.g. 16.6 m long sections if a width of 30 cm has been selected). Calculations made at the transect level will then incorporate not only average values but also a certain measure of the spatial variability.

f. To facilitate the counting of organisms per sampling unit after annotation, a web-based software has been developed to automate the process. Box V summarizes the way data should be acquired so it can be processed using this specific software.

g. The resulting table that should be generated after all organisms have been identified and counted is given in Fig. B7.3.



							Density			
ROV dive	**Sampling unit**	**Treatment**	**Time**	**Historical fishing intensity**	**Species richness**	**Diversity**	**Sp1**	**Sp2**	**Sp3**	**Sp4**
1	2	3	4	5	6	7	8			

Figure B7.3. Table to be filled after annotating all species identified in the ROV transects and calculating their density in each sampling unit of 5 m^2. (1) Code of the ROV dive given in the baseline table. (2) Number of sampling unit in each ROV dive, in ascending order from the begining of the transect. (3) Location of the dive inside the fishing exclusion area (New closure, Impact) or outside the exclusion area (Control). (4) Number of the monitoring event (After 1, After 2, etc). This PhD thesis provides the Before data. (5) Fishing intensity registrered in the area before management measures are applied. It corresponds to the calculations made using the VMS data during 5 consecutive years, between 2007 and 2012. Correspondence between ROV dives and the 3 levels of historical fishing intensity (Low, Medium and High) are provided in the baseline table. (6) Number of species registred per sampling unit (7). Diversity calculated as the Exponential of Shannon index (expH, see Materials and Methods Section). (8) Density values of the 4 species selected as indicator species (Sp1: *Pteroeides spinosum*; Sp2: *Alcyonium palmatum*; Sp3: *Lanice conchilega*; Sp4: *Sabella pavonina*).

Statistical analysis

Hypothesis to be tested: Are the selected descriptors (species richness, diversity and density of megafauna species) responding positively inside the marine protected area due to the management measures applied? This implies that values of species richness, alpha diversity and the density of *P. spinosum* and *A. palmatum* should increase and values of *L. conchilega* and *S. pavonina* should decrease inside the new closure locations in comparison to open control areas.

In practical terms, this question can be answered by applying a simple 2-factor BACI design, with factors Time (Before – After) and Treatment (New Closure – Open Control). Management measures are having the desired effects when (1) the selected descriptors are evolving as expected inside the new closure and (2) the interaction between Time and Treatment is significant. This can be easily tested with a classic ANOVA, although we propose the use of a permutational analysis of variance (PERMANOVA; Anderson 2005) to examine the significance of the F-statistic, a more robust approach than assuming a Fisher distribution for the test statistic. The number of replicates selected for each ROV dive should be as large as possible to improve the robustness of the statistical tests, but we suggest that at least 10 randomly selected sampling units from each dive are included. Finally, the factor Fishing Intensity (FI) is not included in the PERMANOVA design to facilitate the interpretation of the different interactions. For this reason, we propose 3 different PERMANOVA tests, one for each level of FI.

At this point, we provide guidelines on how to perform this test using PRIMER® v.6 (Clarke & Gorley 2006) with the PERMANOVA add-on, one of the most widely used statistical software:

1. Import the Excel table with the biological data (File > Open)
2. Import the Excel table with factors “Time” and “Treatment” (File > Open)
3. Apply a square root or a fourth square transformation to the biological dataset (Analyse > Transform – Select preferred transformation)
4. Generate a dissimilarity matrix using the Euclidean distance (Analyse > Resemblance – Select Euclidean distance)
5. Select a PERMANOVA design with three factors (PERMANOVA+ > Create PERMANOVA design...)
    a. Select “Treatment” as a fixed factor
    b. Select “Time” as a fixed factor
    c. Select “ROV dive” as random factor nested in treatment
5. Execute the PERMANOVA design (PERMANOVA+ > Test design...)
    a. Select “Type III (partial)” Sums of Squares
    b. Select “Fixed effects sum to zero”

Data interpretation

Checking the significance of the factor treatment is not sufficient to demonstrate that the measures applied in the marine protected area are having the expected effects over the recovery of marine fauna: not in all cases when the factor “Treatment” has a significant effect are we demonstrating the positive effects of the creation of a MPA nor can we conclude the MPA is not functioning well just because the “Treatment” effect is not significant (Underwood 1992). To test if the applied management measures are playing a role in the observed recovery (and not other uncontrolled factor), we have to check the existence of statistically significant interactions between the factors “Time” and “Treatment”.

In the graphical representation of a BACI design (Fig. B7.4), this interaction can be depicted as a significant difference in the slope of the temporal evolution of the biological descriptor between "Open control" and "Impact" (New closure) treatments. And this can happen regardless of the initial or final state of the selected indicator. Since we provide data for 3 different situations regarding their FI, the interpretation of the results has to be formulated for each of these situations. Fig. B7.4 provides theoretical examples of how the different indicators may respond after the fishing practices have ceased (a-c-e), although we also provide a set of results that might seem counterintuitive at first, but should be regarded as plausible when management measures are put into practice (b-d-f).

Areas of low fishing intensity

In the case of indicators that should respond positively after fishing practices have stopped, we could expect no change in their values after management measures have been put into practice, both inside and outside of the "New closure" (Fig. B7.4a). In this case, the interaction between the factors Treatment and Time should not be significant. There exists the possibility, however, that the limitation of bottom trawling inside the "New closure" could prompt an increase of the fishing intensity in areas that have historically not suffered from trawling. This could lead to a decrease in the value of the indicator outside of the fishing closure that, depending on the magnitude of the fishing effort, could be observed as a significant interaction between the factors Time and Treatment (Fig. B7.4b).

Areas of medium fishing intensity

We expect this situation to show the most dramatic change in the value of the indicator, shown as an increase of its value inside the "New Closure" area and also as a significant interaction between the factors Time and Treatment (Fig. B7.4c). This result would provide evidence that the management measures are having the desired effect in the conservation of the benthic fauna. It should be noted that if fishing effort increases in areas just outside of the "New closure", the value of the indicator could diminish, increasing the significance of the interaction between Time and Treatment (Fig. B7.4d).

Areas of high fishing intensity

In the same way as the previous situation, we could expect an increase in the value of the indicator inside the protection area after management measures have been put into practice (Fig. B7.4e), which could be detected as a significant interaction between Time and Treatment. This situation would indicate that management measures are having the desired effect.

However, areas that have been adversely affected by fishing practices could show a slow rate of recovery, especially if adjacent areas are also heavily affected. This situation could translate into a null increment in the values of the indicator for some time after the management measures have been applied (Fig. B7.4e). In this case, the lack of significance in the interaction would not be indicating that management measures are not being effective, but simply that we need more time to detect changes in the value of the indicator.

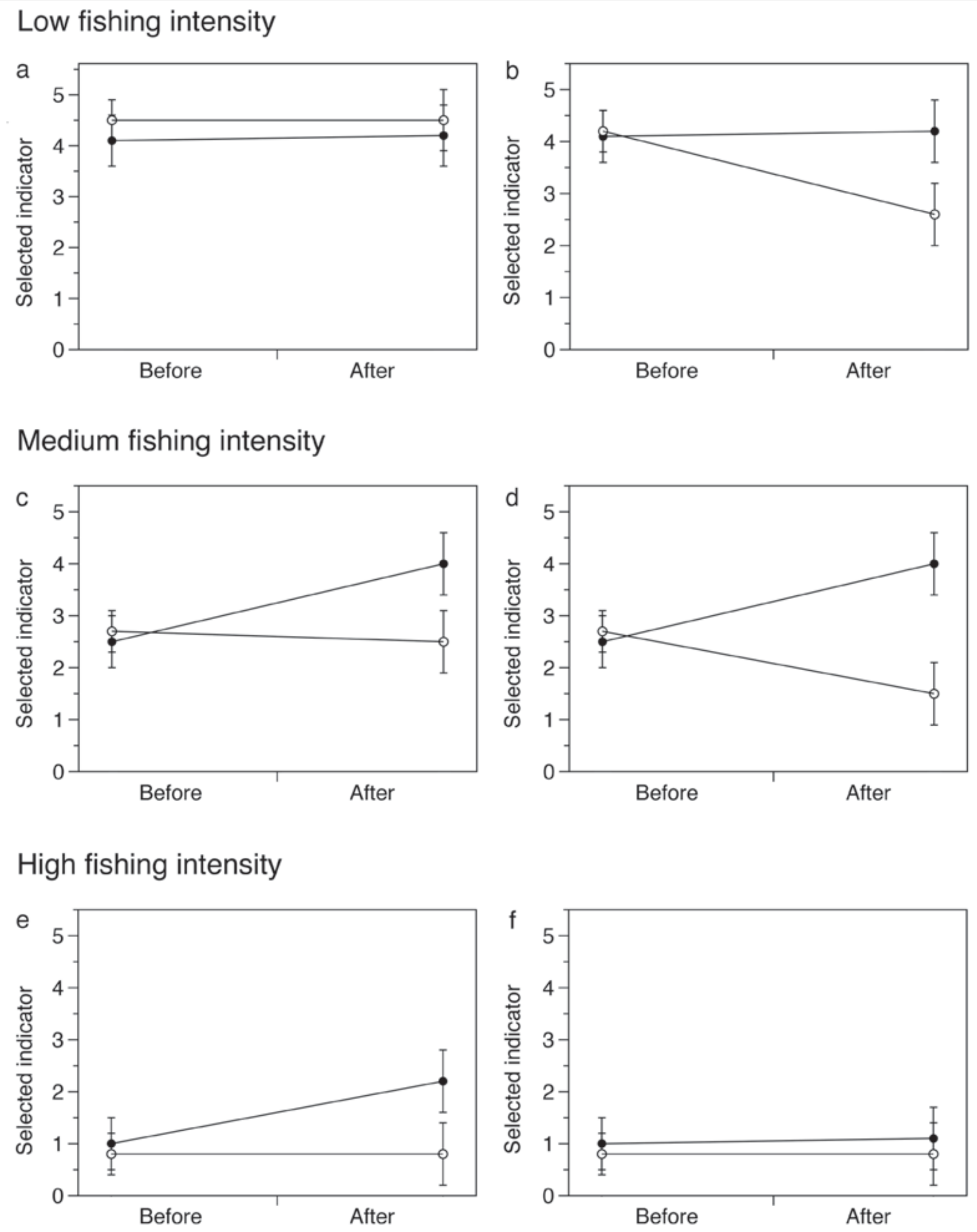


Figure B7.4. Theoretical examples of how the selected indicators could respond under different levels of fishing intensity (FI). Explanations of each case are given in the text. Black circles represent the “New closure” and open circles the “Control” sites.

5.6 Characterization of fish populations

5.6.1 General results

Not considering the small and highly mobile species impossible to count, a total of 1371 fishes were identified and catalogued in the ROV dives performed on the continental shelf and submarine canyon off Cap de Creus. A large percentage of individuals had to be assigned to genus level (787, more than 55% of the fishes) due to the difficulties of differentiating certain species of the same genus in all situations. In particular, four 4 different genera could not be identified to species level when fish were moving fast or at a certain distance from the ROV: *Trachurus trachurus* vs. *T. mediterraneus* (577 individuals), *Trisopterus luscus* vs. *T. minutus* (103), *Scorpaena scrofa* vs. *S. notata* and *S. porcus* (75) and *Lophius piscatorius* vs. *L. budegassa* (4). There were also 2 more genera for which species identification was very complicated: *Callyonimus* (24 individuals) and *Trachinus* (4). The remaining 584 fishes were all identified down to species level.

Overall, 41 different species and 6 different genera made up the list of fish taxa (see Table. 5.6.1). Their abundance was very irregular throughout the different ROV dives, with 3 taxa particularly common: the horse mackerel *Trachurus* spp. (present in more than 60% of the dives), the pout *Trisopterus* spp. (53%) and the scorpionfish *Scorpaena* spp. (51%). Other species were also relatively frequent, appearing in more than 25% of the ROV tracks: the blackbelly rosefish *Helicolenus dactylopterus* (49%), the catshark *Scyliorhinus canicula* (34%), the boarfish *Capros aper* (32%), the hake *Merluccius merluccius* (28%), the comber *Serranus cabrilla* (26%) and the European conger *Conger conger* (26%). Some images of the most abundant or frequent species observed in the video footage are given in Fig. 5.6.1. There were species that only appeared once in the whole footage: *Chelidonichthys lucerna*, *Chelidonichthys obscurus* and *Trigla lyra* in the Triglidae family, the flatfishes *Dicologlossa hexophthalma* and *Scophthalmus rhombus*, the serpent eel *Ophisurus serpens,* the sparids *Pagrus pagrus*, the labrids *Lappanella fasciata* and John Dory *Zeus faber* (Table. 5.6.1). The number of rare species was, in fact, very high, with 34% of the species appearing less than 5 times and up to 55% of them being reported less than 10 times.

There were species that showed a very strong preference for a particular type of substrate, whilst others can be though of as more generalists in terms of habitat selection (Table. 5.6.1). Species strongly associated to hard substrates were *Anthias anthias*, *Conger conger*, *Lepidopus caudatus* and *Phycis phycis*, which were observed around rocky outcrops in more than 80% of the situations. On the opposite end, a series of species were mostly associated with soft substrates, from sandy

Table 5.6.1. Number of individuals, occupancy, depth distribution and substrate preferences for the fish taxa identified in the ROV video images recorded on the continental shelf and submarine canyon off Cap de Creus. Substrate types: A = Muddy fine to medium sands; B = Medium sands to fine gravel; C = Coarse gravels to pebbles; D = Suboutcropping rock; E = Outcropping rock.

			Depth (m)		Substrate type (% of observations)				
Species	**nº ind.**	**% dives**	**Min**	**Max**	**A**	**B**	**C**	**D**	**E**
Acantholabrus palloni	8	9.30	116	200		12.50			87.50
Anthias anthias	16	18.60	94	270	7.14				92.86
Argentina sphyraena	15	9.30	101	217		6.67	93.33		
Ariosoma balearicum	7	9.30	100	107		100			
Boops boops	2	2.33	100	100		100			
Callionymus sp.	24	16.28	95	126	75	25			
Capros aper	29	32.56	116	245	6.90	24.14	31.03	6.90	31.03
Carapus acus	62	13.95	98	176		95.16	1.61	3.23	
Chelidonichthys cuculus	29	23.26	93	126	3.45	82.76	13.79		
Chelidonichthys lucerna	1	2.33	105	105		100			
Chelidonichthys obscurus	1	2.33	107	107			100		
Coelorinchus caelorhinchus	16	11.63	95	390	56.25	12.50	6.25		25
Conger conger	24	25.58	108	306		4.17	12.50		83.33
Coris julis	2	2.33	108	108					100
Dicologlossa hexophthalma	1	2.33	126	126		100			
Diplodus vulgaris	2	4.65	99	106		50			50
Echiichthys vipera	6	2.33	94	96	100				
Epigonus telescopus	5	6.98	265	390	60				40
Eutrigla gurnardus	11	9.30	95	141	18.18	81.82			
Gadiculus argenteus	51	16.28	124	265	47.06	11.76	33.33	7.84	
Gymnammodytes cicerelus	5	4.65	164	385					100
Helicolenus dactylopterus	83	48.84	103	295	15.66	21.69	19.28	3.61	39.76
Lappanella fasciata	1	2.33	125	125					100
Lepidopus caudatus	42	23.26	166	390	7.14		9.52		83.33
Lepidorhombus boscii	5	6.98	96	128	40	60			
Lophius sp.	4	6.98	93	130	25	75			
Macroramphosus scolopax	10	9.30	123	161	10	50	10		30
Merluccius merluccius	26	27.91	100	235	7.69	73.08	19.23		
Mullus barbatus	8	18.60	95	160	12.50	62.50	12.50		12.50
Mullus surmuletus	3	4.65	94	102			100		
Ophisurus serpens	1	2.33	107	107		100			
Pagellus acarne	6	9.30	97	270		66.67	16.67		16.67
Pagellus erythrinus	4	6.98	96	243		75			25
Pagrus pagrus	1	2.33	150	150			100		
Phycis blennoides	11	16.28	98	308	27.27	18.18	27.27		27.27
Phycis phycis	13	18.60	97	385		9.09			90.91
Scophthalmus rhombus	1	2.33	95	95					100
Scorpaena spp.	75	51.16	95	159	1.33	38.67	49.33	2.67	8

Table 5.6.1. (Continued)

			Depth (m)		Substrate type (% of observations)				
Species	**nº ind.**	**% dives**	**Min**	**Max**	**A**	**B**	**C**	**D**	**E**
Scyliorhinus canicula	25	34.88	96	183	24	52	20		4
Serranus cabrilla	36	25.58	92	128	11.11	22.22	25	8.33	33.33
Spicara smaris	3	4.65	108	126		33.33	66.67		
Trachinus sp.	4	6.98	95	117	50	25	25		
Trachurus spp.	577	62.79	98	285	2.77	50.09	8.49	7.11	31.54
Trigla lyra	2	4.65	97	168	50		50		
Trigloporus lastoviza	9	13.95	96	170	11.11	66.67	22.22		
Trisopterus sp.	103	53.49	95	167	13.13	69.70	10.10	1.01	6.06
Zeus faber	1	2.33	130	130					100

muds to sands with gravels. Characteristic species of mobile substrates were *Ariosoma balearicum*, *Carapus acus*, *Chelidonichthys cuculus*, *Eutrigla gurnardus*, *Merluccius merluccius*, *Coelorinchus caelorhinchus* and the species in the genus *Pagellus* and *Mullus*. Finally, there were a large number of species that were reported in all types of substrates, not showing a clear preference for one or another: *Capros aper*, *Gadiculus argenteus*, *Helicolenus dactylopterus*, *Phycis blennoides*, *Serranus cabrilla* and *Trachurus* spp., which appeared in similar proportions on sands, gravels and rocky outcrops.

In terms of behavior, the vast majority of species were reported as solitary individuals. Most species did not seem to respond to the presence of the ROV or the manned submersible, either positively or negatively, unless the vehicle approached them from a very close distance. Strictly benthic fishes, such as those belonging to the families Triglidae (including the genera *Chelidonichthys*, *Trigla* and *Eutrigla*), Scorpaenidae (*Scorpaena* and *Helicolenus*), *Merluccius merluccius* and all flatfish species (*Lophius, Lepidorhombus*, *Dicologlossa*, etc.) were observed laying over the substrate completely static when the ROV moved past them. Some other demersal species simply kept moving in front of the vehicle, always maintaining a minimum distance. This was the case of *Trisopterus* spp., *C. acus*, *A. balearicum*, the two Phycidae species, *M. scolopax* or *A. anthias*, among others

Only 2 fish species were observed forming large schools: *Trachurus* spp. and *Lepidopus caudatus*. These schools were not very abundant in all cases, but there were records of at least 65 individuals swimming together in the case of *Trachurus* spp., and more than 40 in the case of *L. caudatus*. Both species were recognized as very active swimmers and showed a positive reaction towards the presence of the underwater vehicle.

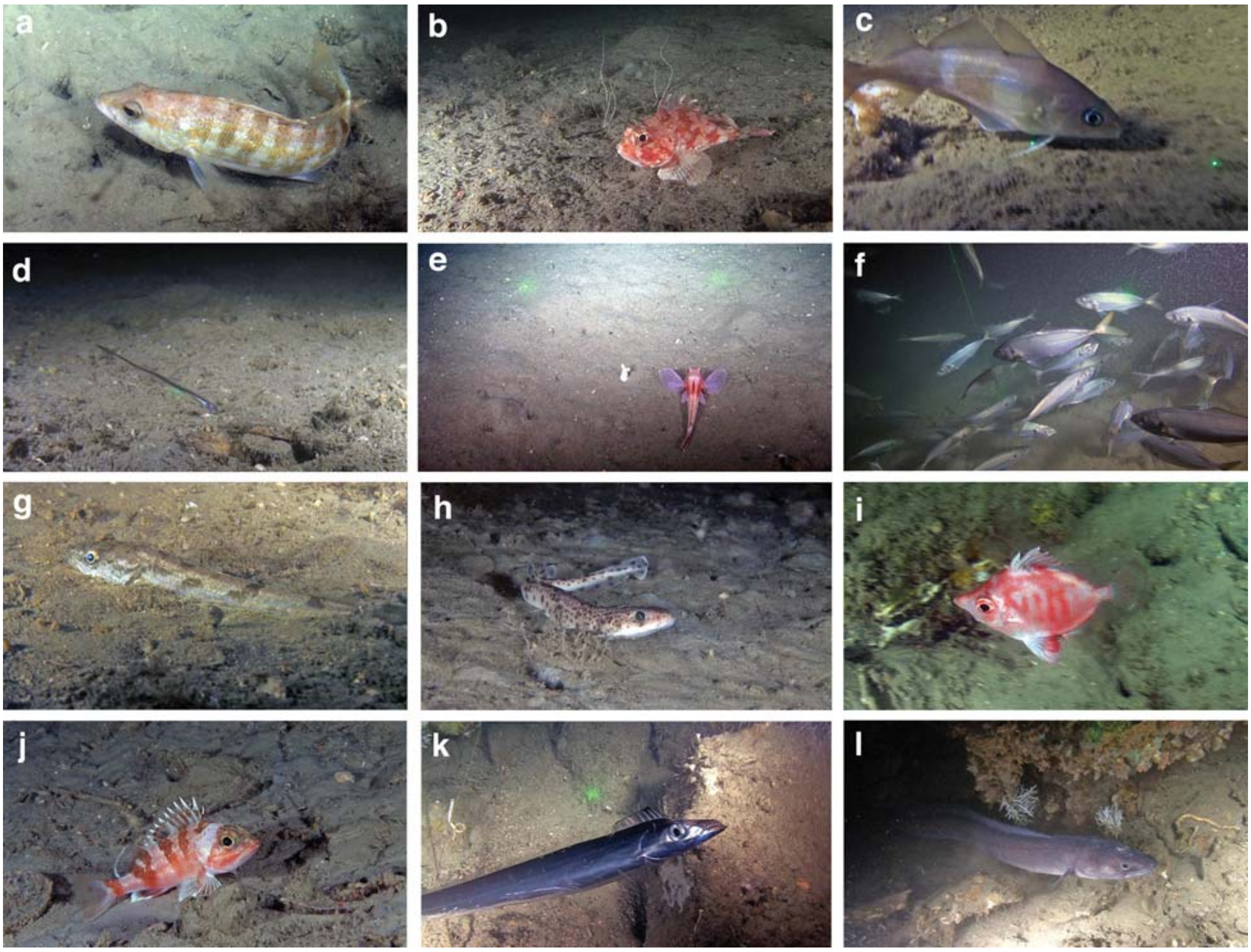


Figure 5.6.1. Images of some of the most abundant or frequent fish species observed in the ROV footage of Cap de Creus, ordered by preferred depth, from shallow to deep: (a) *Serranus cabrilla*, found on all types of substrates on the continental shelf, at depths of 90-120 m; (b) *Scorpaena* sp., mostly observed on sands and gravels of the shelf, at an average depth of 110-115 m; (c) solitary *Trisopterus* sp. found on soft substrates of the shelf at depths of 110-120 m; (d) *Carapus acus*, primarily observed on sandy bottoms of the shelf; (e) *Chelidonichthys cuculus*, a Triglidae species with preference for sandy/gravelly bottoms between 90 and 120 m; (f) a school of *Trachurus* sp., very active mackerels found both on the shelf and inside the submarine canyon; (g) *Merluccius merluccius*, this commercial hake species has been observed laying on sands between 100 and 240 m; (h) *Scyliorhinus canicula*, a common catshark of sandy bottoms down to 200 m; (i) *Capros aper*, a very cosmopolitan species found on all substrates of the shelf break and submarine canyon; (j) *Helicolenus dactylopterus*, a rosefish very common of rocky habitats inside the submarine canyon, (k) *Lepidopus caudatus*, strictly canyon species often observed forming schools around hard substrates; and (l) *Conger conger* very commonly observed hiding between rocky outcrops inside the canyon, at depths of 200-300 m. Images a, e, f, i, k, and l by JAGO Team (GEOMAR) and b, c, d, g, h and j by Nemo ROV (Gavin Newman).

5.6.2 Spatial and bathymetric distribution

The vertical distribution of all reported taxa is shown in Fig. 5.6.2. Certain species exhibited a very restricted distribution in terms of depth preference, and were mostly found on the flat areas of the continental shelf. In this sense, Triglidae species (*C. cuculus*, *C. lucerna*, *C. obscurus*, *E. gurnardus* and *T. lyra*) were primarily reported in depths of 90 to 150 meters, as it was also the case of all flatfish species (*D. hexophthalma, Lepidorhombus boscii*, *S. rhombus* and *Lophius* spp.).

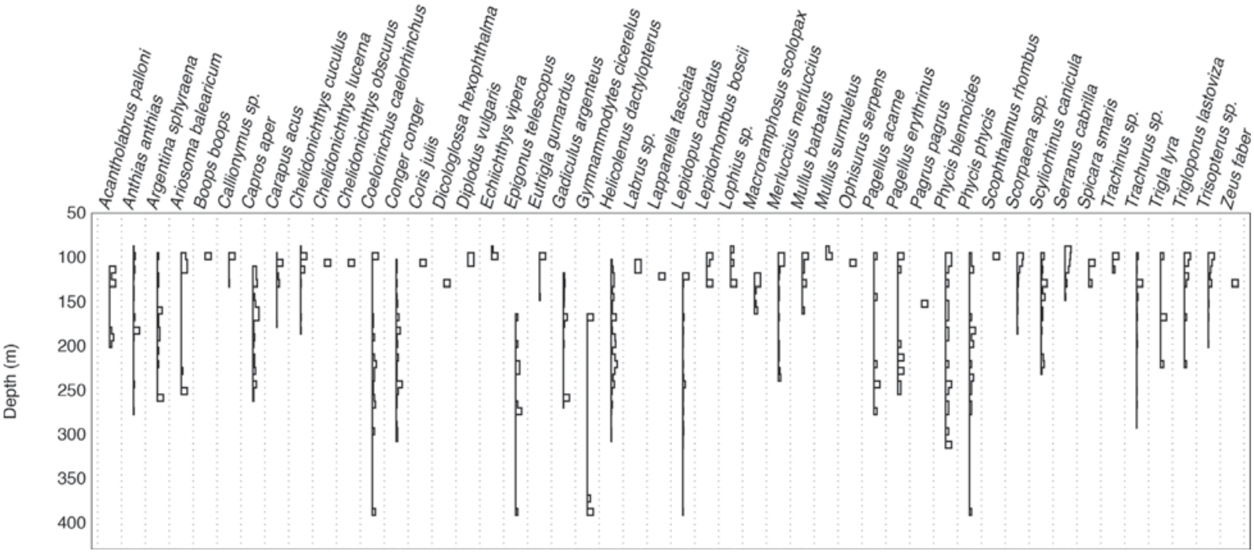


Figure. 5.6.2. Depth distribution of all fish taxa identified in the video footage, displaying minimum and maximum recorded depths. Percentage of appearances in each depth is displayed in the form of histograms. Taxa are ordered alphabetically.

Other species that showed a shallow distribution were mostly small fishes, such as *Boops boops*, *Callyoniums* sp., *Diplodus vulgaris*, *Labrus* sp., *Mullus* spp., *O. serpens*, *S. cabrilla*, *Spicara smaris* and *Trachinus* sp. On the opposite end of the distribution, only 2 species were reported exclusively in canyon environments: *Epigonus telescopus* and *Gymnammodytes cicerelus*.

The rest of the species exhibited a wider range in their bathymetric distribution, which included the shelf, the shelf break and the submarine canyon. Some species were particularly abundant both in shelf and canyon environments, such as *C. aper*, *C. conger*, *H. dactylopterus*, *P. blennoides* and *S. canicula*. Three species had a particularly wide distribution, with a depth range that included the shelf and the deepest part of the canyon that was explored, at almost 400 meters depth: *Coelorinchus caelorhinchus*, *L. caudatus* and *P. phycis*.

The spatial distribution of the 12 most characteristic species is shown in Fig. 5.6.3. Shelf species were observed both off the northern and southern sides of the cape, and no species showed preference for any area in particular. *Trisopterus* spp. and *Scorpaena* spp. had a wider distribution range than any other shelf species, occupying a vast area of the continental shelf. Only *L. caudatus* exhibited a distribution restricted to the canyon area, with most of the observations accumulated on the canyon head. *Trachurus* sp. was not only the most abundant species, but also the species with the wider distribution range, which included the northern and the southern continental shelf as well as the shelf break and the submarine canyon.

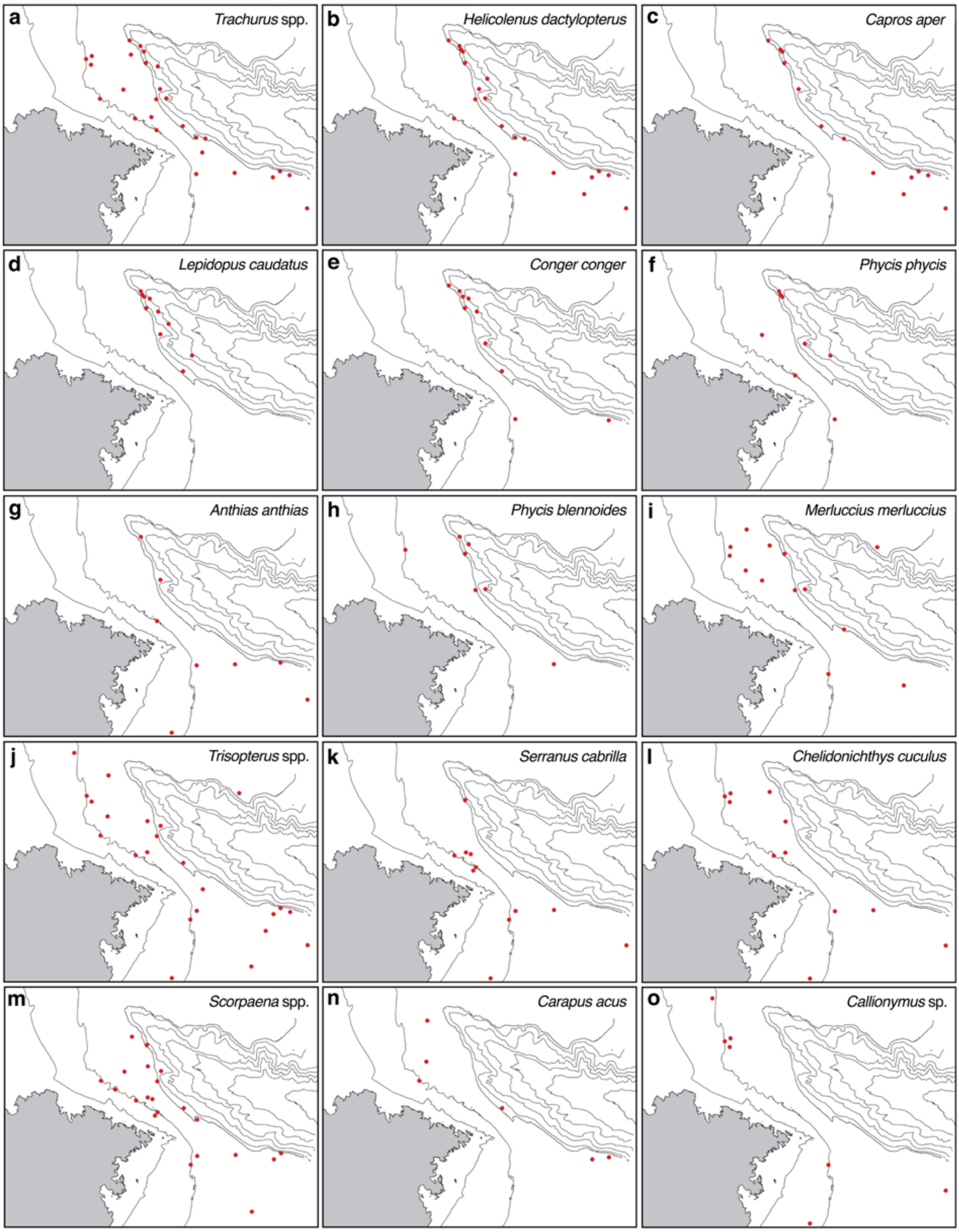


Figure 5.6.3. Maps displaying fish occurrences on the continental shelf and submarine canyon of Cap de Creus. Maps a-i show the most representative species of Assemblage 1 (according to IndVal results), maps j-r show the most representative species of Assemblage 2. (a) *Trachurus* spp., (b) *Helicolenus dactylopterus*, (c) *Capros aper*, (d) *Lepidopus caudatus*, (e) *Conger conger*, (f) *Phycis phycis*, (g) *Anthias anthias*, (h) *Phycis blennoides*, (i) *Merluccius merluccius*, (j) *Trisopterus* spp., (k) *Serranus cabrilla*, (l) *Chelidonichthys cuculus*, (m) *Scorpaena* spp., (n) *Carapus acus*, (o) *Callionymus* sp., (p) *Trigloporus lastoviza*, (q) *Ariosoma balearicum* and (r) *Mullus barbatus*.

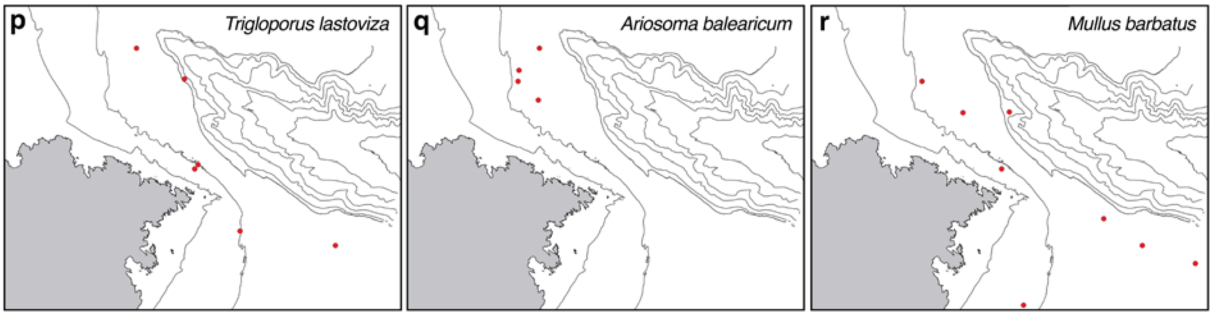


Figure 5.6.3. (Continued)

5.6.3 Community analyses

The optimal number of compact, well-segregated fish assemblages was determined after comparing the overall average *silhouettes* of 10 different cluster solutions. In this case, generating 2 different groups seemed to be the best solution to divide the biological dataset (Fig. 5.6.4a). The hierarchical dendrogram constructed using Ward's algorithm to classify the 56 sampling units in 2 different assemblages is given in Fig. 5.6.4b. According to the results of the IndVal analysis, Assemblage 1 was mainly characterized by the presence of *Trachurus* spp., *Helicolenus dactylopterus*, *Capros aper, Lepidopus caudatus*, *Conger conger*, *Phycis phycis* and *Anthias anthias* (Table. 5.6.2). More than 40% of the organisms identified within this assemblage corresponded to *Trachurus* spp., which showed very high local densities in some areas. It had an average of 7 individuals per sampling unit of 200 m, with a maximum registered value of 66 individuals in one single sample. *L. caudatus* was also relatively abundant, with an average density of 2.4 individuals per sampling unit, followed by *H. dactylopterus*, (average of 1.4 individuals per sample, maximum value of 7) and *Conger conger* (0.92 on average, local maximum of 8).

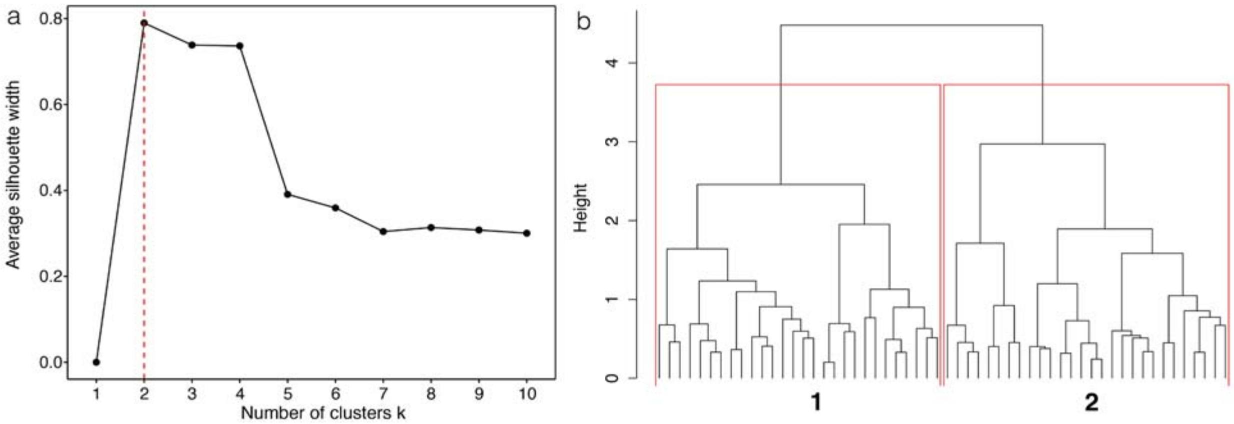


Figure 5.6.4. Cluster analysis of fish fauna. (a) Overall average silhouette for each cluster solution. The optimal number of clusters is 2, indicated by a dashed line. (b) Ward's hierarchical dendrogram of species composition constructed using Bray-Curtis dissimilarities from square-root transformed data. Clusters are indicated by the numbers 1 and 2.

Table 5.6.2. Indicator Value (IndVal) for the 10 most important species of each fish assemblage identified in the video images recorded on the continental shelf and submarine canyon off Cap de Creus.

Group	Species	IndVal	Group	Species	IndVal
1	*Trachurus* spp.	0.617	2	*Trisopterus* sp.	0.673
	Helicolenus dactylopterus	0.441		*Serranus cabrilla*	0.250
	Capros aper	0.255		*Chelidonichthys cuculus*	0.214
	Lepidopus caudatus	0.250		*Scorpaena* spp.	0.214
	Conger conger	0,.250		*Carapus acus*	0.198
	Phycis phycis	0.250		*Callionymus* sp.	0.193
	Anthias anthias	0.214		*Trigloporus lastoviza*	0.143
	Phycis blennoides	0.179		*Ariosoma balearicum*	0.119
	Merluccius merluccius	0.163		*Mullus barbatus*	0.114
	Argentina sphyraena	0.160		*Merluccius merluccius*	0.107

Assemblage 2 was characterized by the presence of relatively small-sized fishes like *Trisopterus* spp., *Serranus cabrilla*, *Chelidonichthys cuculus*, *Scorpaena* spp. and *Carapus acus*. Abundance values for those species were considerably lower than those registered for the most distinctive species of Assemblage 1. *Trisopterus* spp. was by far the most abundant taxa, representing almost 25% of all reported fishes in Assemblage 1. It had an average density of over 2 individuals per sampling unit, with a maximum of 9. The second most abundant species was *C. acus*, with 12% of the individuals identified belonging to this species. It had an average density of 1 individual per sampling unit, (maximum of 9). All other species had average densities below 0.5 individuals per sampling unit.

The ordination of the samples in a reduced space through a non-metric multidimensional scaling (nMDS) is shown in Fig. 5.6.5. There seemed to be a clear influence of depth on the structuring of the samples. Although a limited number of samples belonging to Assemblage 1 were found on shelf and shelf break environments, the vast majority of the sampling units appeared in deeper areas, in depths of 150 to 300 m. Conversely, all Assemblage 2 samples were restricted to shelf environments, at depths between 90 and 130 m. Substrate type did not seem to play such an important role in the structuring of the samples. Assemblage 1 was mostly found in coarse and hard substrates, with 43% of the samples located in areas dominated by coarse gravels and pebbles and 36% in rocky outcrops. On the other hand, Assemblage 2 was primarily found on soft substrates, with 60% of the observations appearing in areas with medium sands and fine gravels. Since the distribution of benthic assemblages was also very dependent on depth, there existed certain differences in the composition of the invertebrate fauna for each of the fish assemblages. Assemblage 1 was mostly found in areas characterized by the presence of cold-water corals (invertebrate Assemblage F) and cerianthid species (H), while Assemblage 2 was found in areas dominated by pennatulaceans (B), gorgonians (A) and crinoids (D).

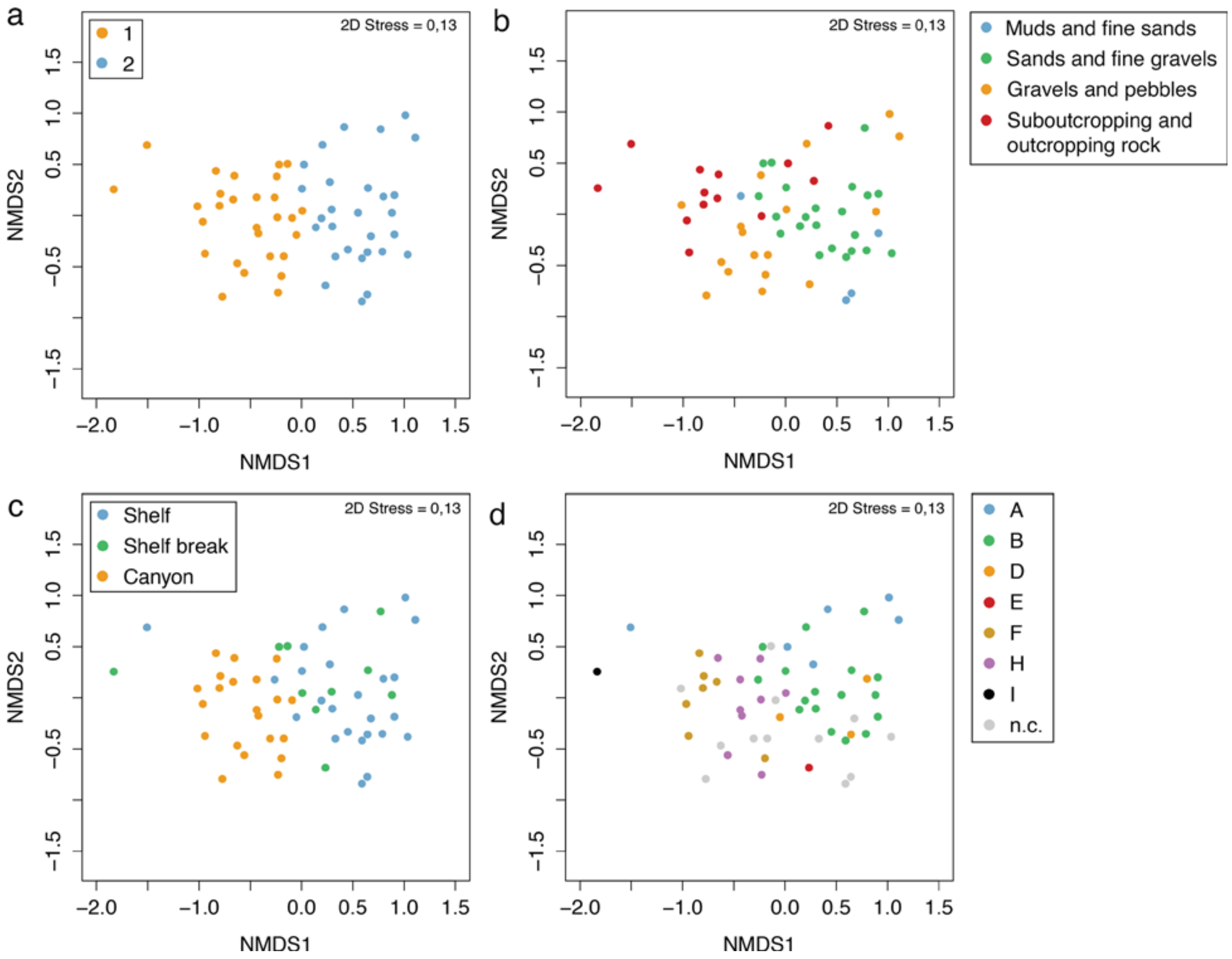


Figure 5.6.5. Non-metric multidimensional scaling (nMDS) of the 56 sampling units used to characterize the fish assemblages in the continental shelf and submarine canyon off Cap de Creus. Different parameters were superimposed to identify potential drivers of the observed patterns: (a) groups derived from Ward's hierarchical classification, (b) dominant substrate types throughout the sampling units, (c) spatial location of the samples and (d) characteristic invertebrate assemblage.

5.7 Presence of marine litter

5.7.1 Domestic and fishing-related items

A total of 421 items of a human origin were identified in the video images from the continental shelf and submarine canyon off Cap de Creus. Although the typology of the items reported was very diverse, most objects derived from fishing practices, both bottom trawling and artisanal fishing (longlines and trammel nets). More than 90% of the reported items corresponded to abandoned, lost or otherwise discarded fishing gears (ALDFG), among which longlines were by far the most abundant item (368). In a smaller proportion, trammel nets were also observed (12), mostly entangled around rocks or lying flat over the seabed. One large trammel, however, net was found fully expanded and ghost fishing. Three bottom trawl nets and a few cables used to drag these types of nets were also observed. The abundance of domestic litter was very low if compared to the number of ALDFG. A total of 28 items were reported in almost 10,000 m^2 of area explored, which corresponds to an overall density of 28 items per ha. Their composition was very diverse, and items of different origins could be identified: car tires (3), plastic bags (1), plastic bottles (3), tin cans (4),

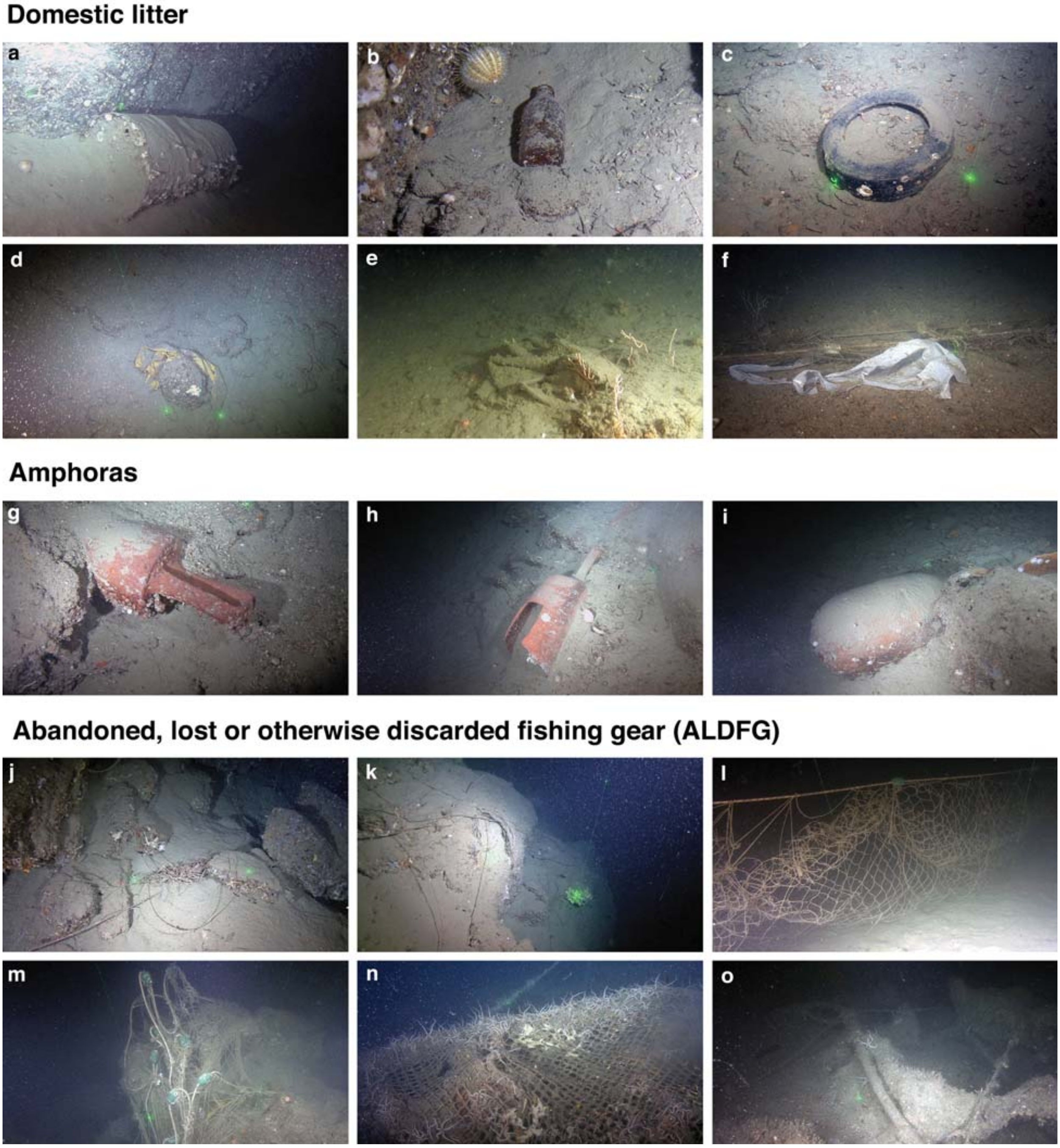


Figure 5.7.1. Images of the most representative litter items observed on the video footage from Cap de Creus continental shelf and submarine canyon. General waste items, which include multiple origins (a-f), some of the best preserved amphoras identified in the images (g-i) and the most common fishing gears abandoned over the sea floor, which include long-lines (j-k), trammel nets, both entangled over hard substrates or ghost fishing (l-m) and bottom trawl nets (n) and the cables used to tow them (o). All images by JAGO Team (GEOMAR).

Table 5.7.1. Classification of all litter items identified in the video images recorded on the continental shelf and submarine canyon off Cap de Creus. Items are organized by origin and area where they were found. * items per 100 m. ** items per ha.

		All areas		Northern shelf		Southern shelf		Submarine canyon	
Type of debris		**N**	**De***	**N**	**De***	**N**	**De***	**N**	**De***
Fishing gear	Bottom-trawl net	3	0.01	2	0.01	1	0.01	0	0
	Bottom-trawl cable	10	0.03	1	0.01	9	0.08	0	0
	Longline	368	1.20	33	0.21	46	0.43	289	7.69
	Trammel net	12	0.04	0	0	12	0.11	0	0
		N	**De****	**N**	**De****	**N**	**De****	**N**	**De****
Debris	Amphora	6	5.74	2	4.17	0	0	4	24.61
	Clothing	3	2.87	2	4.17	0	0	1	6.15
	Metal	5	4.78	0	0	1	2.48	4	24.61
	Plastic	9	8.60	3	6.25	2	4.96	4	24.61
	Glass	5	4.78	2	4.17	2	4.96	1	6.15

glass bottles (5) and clothes (3). The largest identified item was a metallic oil drum, found inside the canyon at a depth of 250 m. There were also 6 amphorae laying over the soft substrates of the shelf and submarine canyon, the origin of which could not be determined. Some images of the ALDFG found on the shelf and canyon off Cap de Creus and some examples of the types of domestic litter and amphorae observed in the images are given in Fig. 5.7.1.

5.7.2 Spatial distribution of marine litter

Domestic litter items appeared in 20% of the ROV dives. There was not a clear trend in terms of their distribution along the whole study area, since they appeared both on the shelf and inside the submarine canyon. Mean densities were higher in deeper areas (Table 5.7.1), although differences were not significant. The most commonly found objects on the continental shelf were those that derived from plastic or had plastic components, although inside the canyon their proportion was similar to those made of metal (Fig. 5.7.2).

Fishing related items appeared in just over 50% of the ROV tracks. Longlines were the most abundant gear both on the shelf (76 items) and inside the submarine canyon (289 items) (Table 5.7.1). In the latter case, although an average density of almost 8 long-lines per 100 meters was estimated for the entire upper canyon, there were dives in which local densisties reached almost 30 items per 100 m (ROV transects 1 and 9). Trammel nets and bottom trawl nets were only observed on the continental shelf, mostly on the southern side of the cape (Fig. 5.7.2).

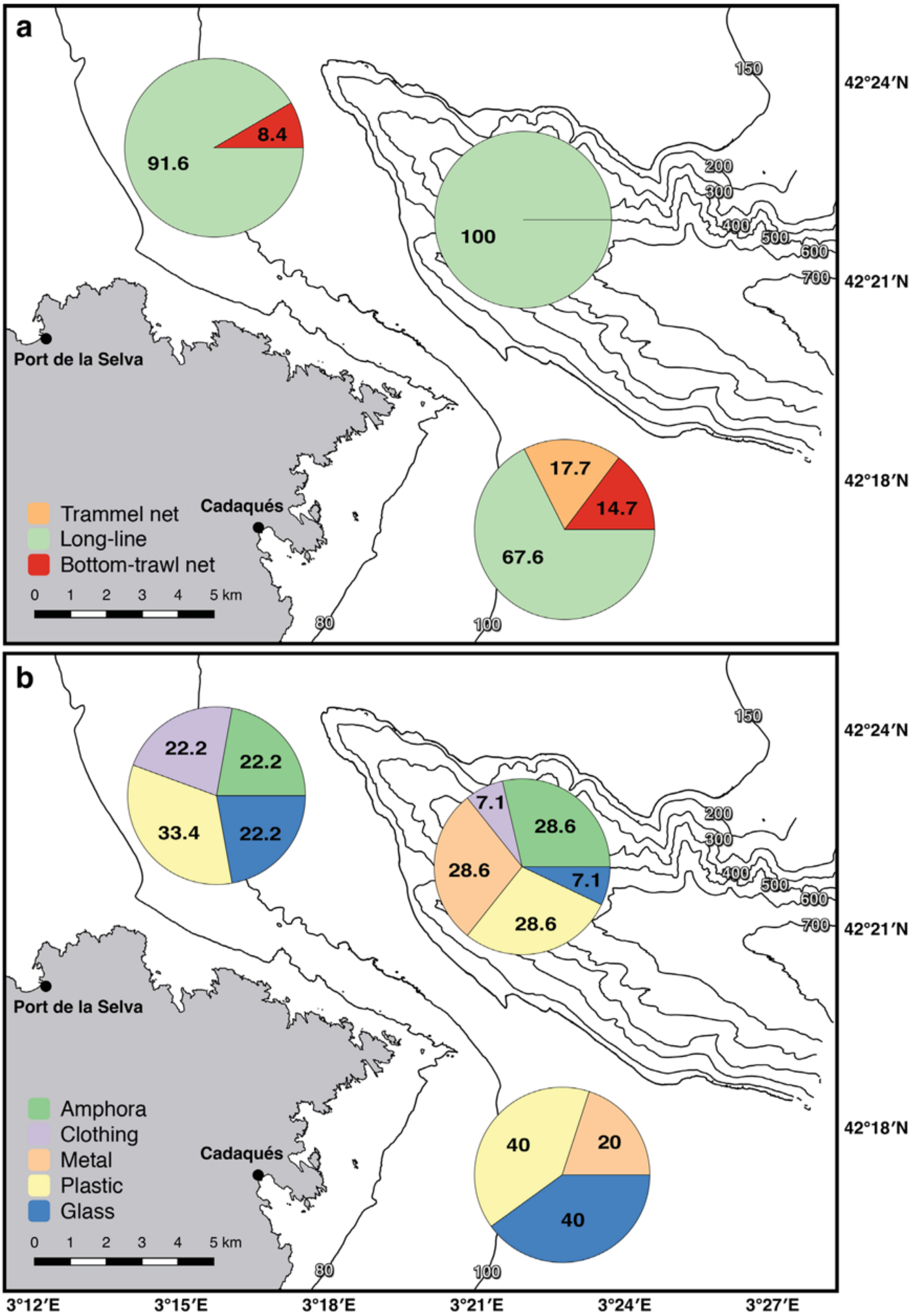


Figure 5.7.2. Spatial distribution of the different litter items observed in the ROV images separating between (a) abandoned, lost or otherwise discarded fishing gears, and (b) domestic items. The proportion of each category is given for the continental shelf (80-150 m), both the northern and the southern part, and also for the submarine canyon (150-400 m).

Table 5.7.2. Total number of cold-water coral colonies identified in the video images (N) belonging to the three most important species dwelling in Cap de Creus submarine canyon (*Madrepora oculata*, *Lophelia pertusa* and *Dendrophyllia cornigera*) and the percentage of colonies that were observed entangled in abandoned longlines.

ROV	Length	Depth range	Long-line density	*M. oculata*		*L. pertusa*		*D. cornigera*	
dive	(m)	(m)	(items / 100m)	N	%	N	%	N	%
1	72	215-186	25	43	9.3				
2	203	386-377	5.42	7	0	3	0		
3	554	199-173	7.76	58	6.9	2	50	15	0
4	98	302-302	7.14	4	0	1	0		
5	258	316-302	5.81	37	2.7			1	0
7	472	234-236	6.57	5	0			1	0
8	188	293-264	17.55	3	33.3	1	100	6	16.7
9	485	282-165	24.54	145	15.2			3	66.7

5.7.3 Interaction of litter with benthic organisms

A total of 335 colonies of cold-water coral species were identified in the video transects performed inside the canyon head (302 *Madrepora oculata*, 7 *Lophelia pertusa* and 26 *Dendrophyllia cornigera*). On average, more than 10% of those colonies had long-lines entangled around them, although there existed a certain degree of variability at the transect level (Table 5.7.2). In general terms, coral colonies seemed to remain alive with the nylon lines twisted around them, but their distance from the camera did not allow for a closer examination to determine the percentage of polyps affected. It was also impossible to determine to what extent colonies had some parts broken due to the interaction with longlines. Fig. 5.7.3 shows the aspect of two colonies of *Madrepora oculata* with longlines entangled around their skeleton.

At the same time, some of the thickest longlines acted as substrate for other species to live on. It was certainly difficult to identify most of the species that appeared growing as epibionts, but most of them were bushy hydrozoan and bryozoan species, together with the very abundant polychaete *Sabella pavonina*. Besides the effects produced by longlines on the canyon head, other fishing gears were observed to be causing a certain degree of impact over the fauna. A trammel net was found ghost fishing over the soft bottom of the continental shelf in transect 34 (see Fig. 5.7.1i). Three large lobsters were found caught in it afighting to free themselves, together with other fishes impossible to identify. Also on the shelf, a large bottom trawl net was found laying over a relatively wide extension of sand and small outcropping rocks (see Fig. 5.7.1). It was covered by a large amount of small organisms, in which to include sponges, bryozoans, hydrozoans and some brittle stars from the species *Ophiothrix fragilis*.

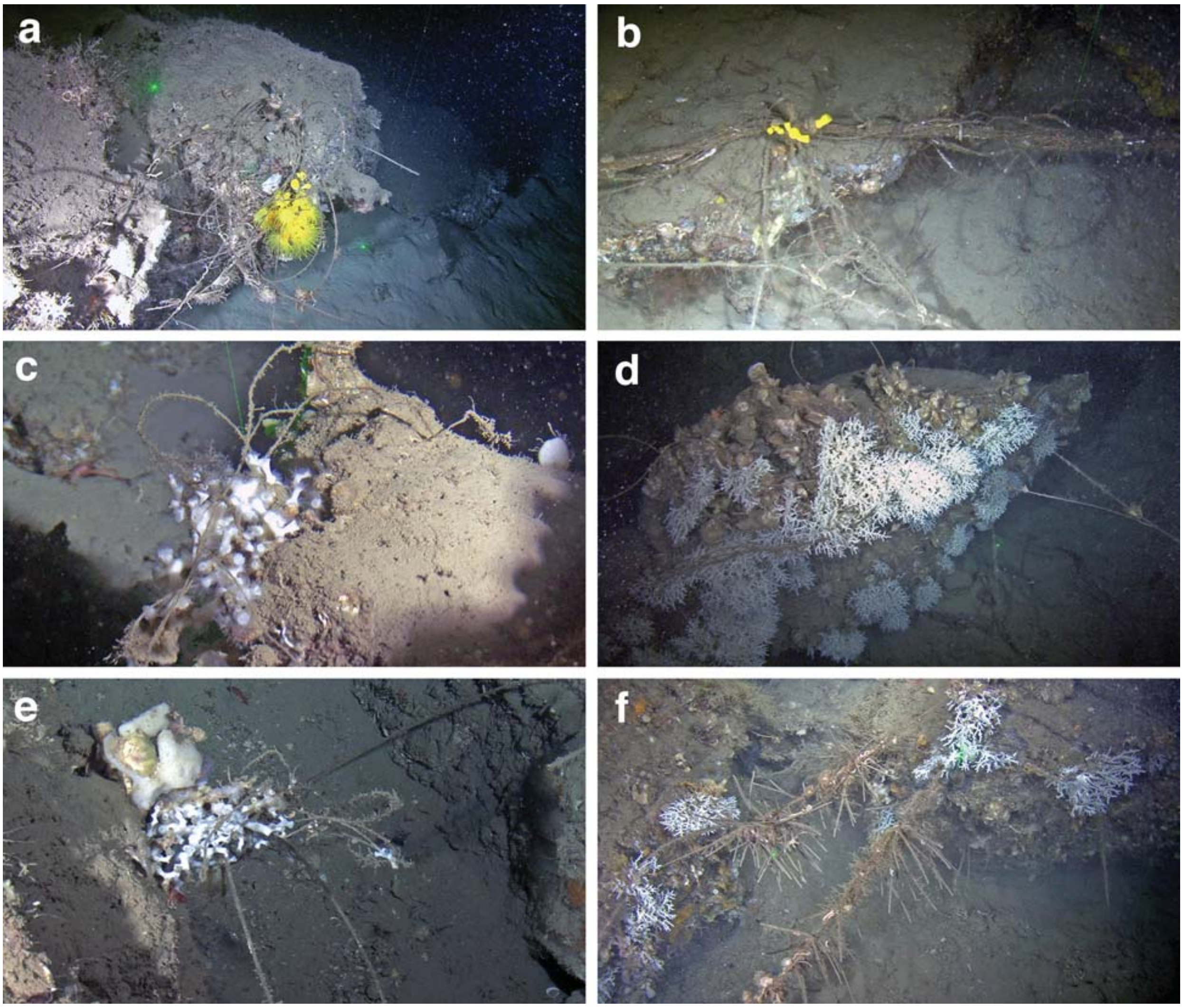
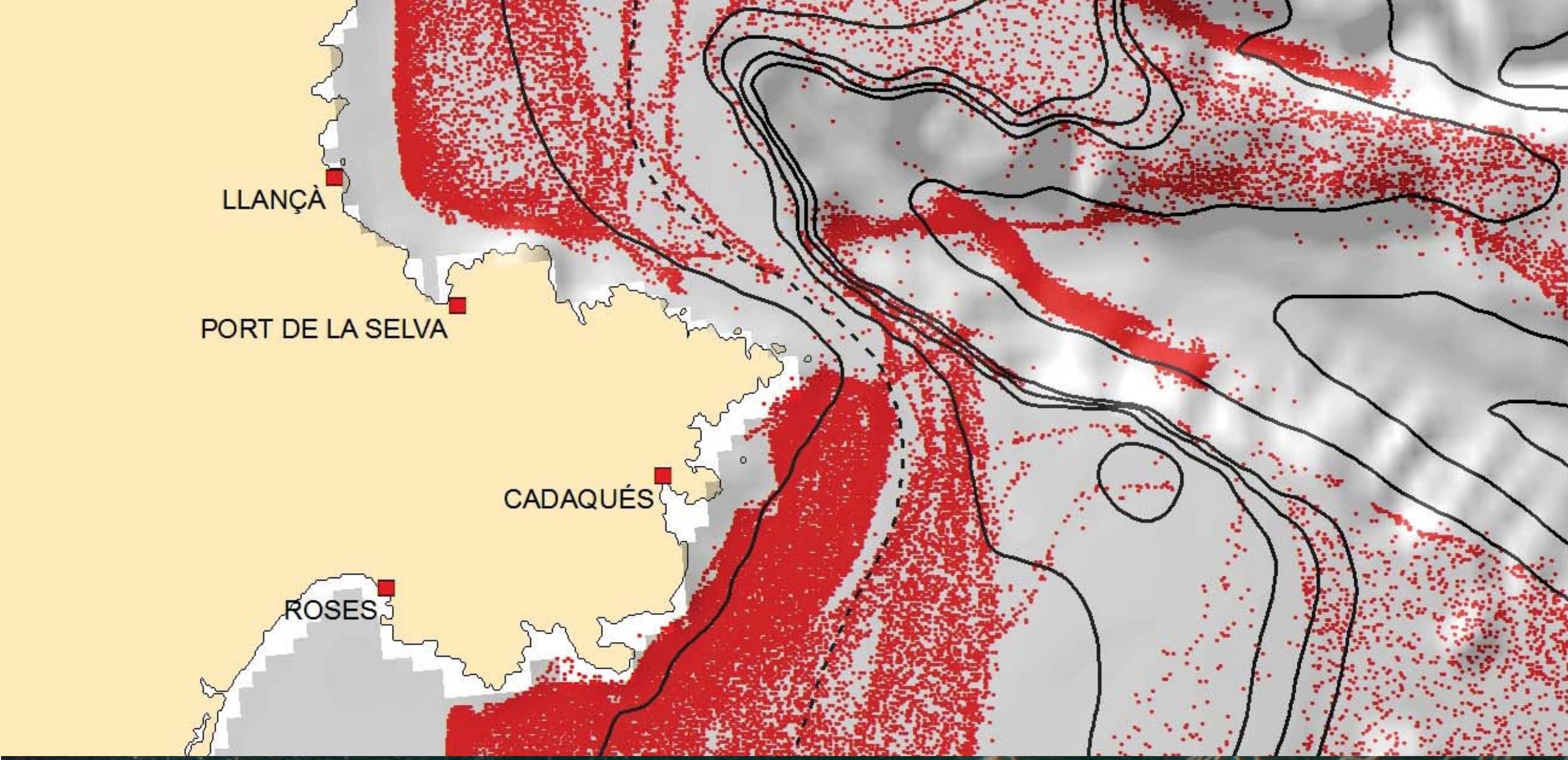


Figure 5.7.3. Selected images of entangled longlines around different cold-water coral species inside Cap de Creus submarine canyon. (a-b) *Dendrophyllia cornigera*; (c) *Lophelia pertusa*; (d-e-f) *Madrepora oculata*. All images by JAGO Team (GEOMAR).



Discussion

Cover images by Susana Requena (ICM-CSIC) and JAGO Team (GEOMAR)

6. Discussion

The publication of the Marine Strategy Framework Directive (MSFD) and its transposition to the Spanish law represented a series of scientific challenges that should be (urgently) addressed. One of the most critical aspects corresponded to finding a set of techniques and descriptors sufficiently sensitive, precise and statistically robust to support the continuous monitoring demanded by the adaptive management, one of the pillars of the MSFD.

Designing a monitoring plan requires previous information not always available: (1) a quantitative description of the natural heritage, (2) an evaluation of how much human activities are affecting the natural resources and (3) a selection of indicators sensitive to such impacts to ultimately quantify changes that may occur through time. Those requirements become more complex when dealing with great depths, such as continental shelves, where direct access (i.e. through SCUBA diving) is not possible. At present, ROV-supported video images seem the only available cost-effective technique to overcome these limitations. Surprisingly enough, the potential of ROVs as quantitative experimental tools has just become to be explored in other MPAs worldwide (see the example of Sheehan, Stevens & Attrill 2010).

The final objective of this PhD thesis was to fulfill all these tree requirements. First, we were interested in characterizing the benthic assemblages found within the limits of the future offshore MPA of Cap de Creus, later using that information to evaluate the adequacy of video surveys to monitor the evolution of benthic megafauna through time and also to generate distribution maps required for management purposes. Second, we intended to quantify the effects of the most important source of human disturbance in the area (bottom trawling) and also identify the most sensitive elements to consider them as indicators of fishing activity. Third, in the case of a positive response, we wanted to provide stakeholders and managers the necessary tools to initiate a monitoring plan following the demands for an adaptive management, which would ultimately allow for a robust quantification of how the benthic fauna found on the continental shelf of this future marine protected area evolves in time.

Since the results obtained in each of the steps taken to reach this ultimate goal have become sufficiently large to be treated as separate entities, they are independently discussed in the following sections.

6.1 Composition and structure of invertebrate communities

6.1.1 Methodological remarks

A. Adequacy of ROVs to characterize and monitor benthic fauna

Describing how the structure and function of benthic communities evolves through time can become a tedious and relatively complex task, especially below SCUBA depths. Several remote techniques have historically been designed to sample shelf and deep-sea environments, each of them providing valuable information about certain aspects of the benthic ecosystem (Jamieson, Boorman & Jones 2013). Ideally, a thorough ecological evaluation of the benthic fauna inside the limits of an MPA would require a sampling device capable of:

(1) evaluating a relatively large area,
(2) working efficiently regardless of substrate type,
(3) generating samples at a very low cost to improve replicability,
(4) generating sufficient abundance data for statistical analyses,
(5) providing information about high taxa with different life strategies,
(6) reaching a very high taxonomic resolution to avoid confounding similar species and
(7) generating the smallest impact on the benthic organisms as possible.

There is currently no sampling gear or methodology that can perform well in all those aspects, but a clear shift from extractive techniques towards the use of visual and less destructive sampling devices can be observed (Mallet & Pelletier 2014). Even though we are fully aware that ROVs are nowadays very popular tools to evaluate the ecology of benthic organisms, assemblages and habitats, we still wanted to assess the pros and cons of using these vehicles to characterize benthic fauna in Mediterranean shelf and slope environments, to finally determine their potential use in a monitoring program as part of the management plan designed for a Mediterranean offshore MPA. If ROVs perform well in most of the aspects listed above, the capacity of these vehicles to revisit the same locations through time with a high degree of accuracy would make them the perfect candidates to sustain long-term monitoring programs.

Spatial coverage and cost. Classic works that studied the ecology of deep benthic fauna relied on samples collected using extractive techniques (mainly grabs and epibenthic sleds), which are limited in their spatial coverage and do not perform well in areas of hard substrates (Eleftheriou

2013). In addition, samples taken on board using these methods require trained taxonomists to sort and identify infauna or small macrofauna species, a time consuming, specialist-dependent and relatively expensive process that makes these devices unsuitable for regular monitoring studies. The use of ROVs has given us the possibility to operate continuously with very little time restrictions, and therefore are capable of exploring much larger areas than with other extracting techniques, which generally have to work with sampling units of a limited size that has to be set before their deployment. In this PhD thesis, almost 1 hectare of seabed was comprehensively examined after analyzing 60 ROV dives, reaching approximately 33 km in length. Samples covered all substrate types (with more than 15% of the footage recorded on top of rocky outcrops and boulders) and the whole bathymetry range of the study area (80-400 m). Hence, the locations explored seem representative enough of the benthic habitats that can be found in Cap de Creus shelf and submarine canyon. Van Veen grabs, which sample a surface area of 0.1-0.2 m^2 and for some decades have been the standard sampler for quantitative benthic studies in shelf environments (Eleftheriou & Moore 2013), would not have permitted such a comprehensive evaluation in that many contrasting environments.

Numbers for a robust statistical evaluation. Classic extracting techniques that provide true quantitative data are those based on the coring principle (e.g. box corers), while trawls, sledges, dredges and grabs result in various degrees of semi-quantitative sampling only (Blomqvist 1991). Beam, Agassiz and otter trawls can standardize the duration of the tow for comparative studies among different locations, but the exact area swept cannot be easily estimated and abundance results should be handled with care (Eleftheriou & Moore 2013). Underwater images can provide an advantage in this respect, since the size of the sampling unit can be easily determined under certain circumstances (presence of parallel lasers on the image, accurate positioning data and known distance/angle from the seabed in the case of still pictures).

In our survey, the number of organisms identified in the video images in almost 1 hectare of seabed exceeded 90,000 individuals. Excluding the large aggregation of brittle stars, which accounted for more than 45,000 organisms and displayed local densities above 600 ind·m^{-2}, an average of almost 30 megafauna organisms per sampling unit of 5 m^2 was reported, with local values of almost 300 individuals per sample in areas of high biomass. Such values provided not only accurate density estimates at a very small scale, but also a good testing ground to perform robust community analyses using univariate and multivariate statistical techniques. The large variability in abundances between rich and poor sites also provided a solid background for statistical comparisons.

Fraction detected. No existing technique is capable of providing accurate information about all species that make up the whole benthic community. Grabs and corers are very efficient in sampling organisms that live inside the first layers of the sediment, while trawls and dredges sample the animals lying on the surface of the seabed and some burrowing species (Eleftheriou & Moore 2013). ROVs possibly display a very low discriminative capacity in comparison to these other classic methods, and this can become a major drawback when analyzing certain ecological patterns. As we stand now, only the larger fraction of the epibenthic fauna can be detected through video images, a portion defined as megafauna. Sampling devices that extract the upper part of the sediment will always be able to detect species that remain unnoticed to ROVs, in the same way that samples collected using sleds and examined under the stereoscope will uncover species not visible to imaging methods.

Community studies performed in shelf environments of the Mediterranean Sea using Van Veen grabs have historically identified a large number of species, in excess of 500 in the Gulf of Lions (Desbruyères, Guille & Ramos 1972) and Crete (Karakassis & Eleftheriou 1997). Most of the organisms captured with this technique are burrowing polychaetes, nematodes and crustaceans that generally live buried or half buried inside the sediment. In our study, around 165 different morphospecies were identified in the video footage, a number that although lower to classic studies, seems adequate to characterize epibenthic assemblages in deep Mediterranean environments. Most organisms that could be detected in our study were sponges, anthozoans and echinoderms, groups very different to those characterizing grab or sled samples.

Taxonomic accuracy. Besides the discriminative capacity of ROVs, identifying organisms down to species level directly from video images still involves a certain degree of complexity. While taxonomists have reduced their unwillingness to identify species from video footage in the past few years, mostly thanks to improvements in image quality and a wider accessibility to images filmed in a large number of locations, some abundant Phyla still remain a challenge. In the case of Cap de Creus continental shelf and submarine canyon, more than half of the reported taxa could unquestionably be assigned to species level, with a relatively small part classified under higher taxonomic levels (genus or family in most cases). This was mostly due to the difficulty of differentiating between congeneric organisms that belonged to taxonomically similar species, for example certain crustaceans (e.g. *Munida rugosa* vs *Munida intermedia*) or sea cucumbers (e.g. *Holothuria forskalii* vs *Holothuria tubulosa*). Inevitably, there was a set of organisms (around 35% of the total number of taxa) that had to be assigned to what we considered separate species but for which identification below Phyla level was not possible. In these situations, specific categories of morphospecies were artificially created to facilitate the subsequent statistical analyses to characterize the benthic ecosystem. This is a common practice in underwater video surveys, especially for areas currently under exploration (see for example Davies *et al.* 2014 or Duffy *et al.* 2014).

In our case, not all Phyla exhibited the same degree of taxonomic complexity in their identifications. Anthozoan and echinoderm species reported very few problems to specialists, mainly due to their relatively large size and the reduced number of cryptic species. Conversely, identifying Porifera species directly from video images became more problematic than previously expected. From the 55 different sponge taxa that were recognized from the video footage, only 34 could be assigned to species or genus level (60%). The limited number of biological samples collected on deep bottoms and/or over hard substrates (where encrusting Porifera were common) was the primary cause of this low taxonomic resolution. Porifera required live samples to be collected, treated with nitric acid and examined under the microscope for accurate identifications to be carried out. For this reason, we strongly encourage future surveys to allocate larger efforts in the collection of specimens for which direct identification from still images can become a difficult task. Furthermore, selectively sampling those species that have not yet been identified using the grabber of the ROV or the manned submersible greatly improves the correspondence between images and their identification, since life samples can change in shape or colour when brought to surface.

Overall, it seems like ROV-operated video samplings perform well in most aspects necessary to develop robust community analyses, and hence they should be strongly considered as potential candidates for the future monitoring programs, not only for Cap de Creus, but also in other offshore marine protected areas of the Spanish territory and even of the Mediterranean Sea. The success story of the shallow MPA of Lyme Bay (Stevens *et al.* 2014), which has now been monitored using a towed camera system for almost 10 years, provides hope that a similar strategy could be implemented in Cap de Creus, where deeper areas of the continental shelf could be routinely examined in application of the MSFD.

B. Size of sampling unit

Studies on shallow-water ecosystems have recommended the use of samples with a minimum area of 2 m^2 for community analyses of epibenthic fauna (Weinberg 1978a) or 2.5 m^2 for monitoring purposes in rocky or coralligenous substrates (Garrabou *et al.* 2015). There are not many studies that have dealt with the problem of scale at greater depths (but see Gonzalez-Mirelis, Bergström & Lindegarth 2011). The methodology developed by the Benthic Suspension Feeders group at the Institute of Marine Sciences (ICM-CSIC) to analyze video footage allows data to be collected as a continuum throughout the whole ROV transect. This standard methodology allows for robust comparisons between samples of different sizes, giving us the possibility of selecting the size of the sampling unit that is most convenient for the statistical analyses to be performed.

The results of the species/area curve indicated that 5 m^2 (16.67 x 0.3 m) was a very suitable area to perform community analyses with the data obtained from the continental shelf and submarine canyon off Cap de Creus. This sampling unit contained, on average, 60% of the species that would have been found in the area if a very large sampling unit had been used. This corresponded to an average of more than 6 different species per sample, with a local maximum of 32. If we had followed the recommendations given by Weinberg (1978a) for shallow communities, the average number of species would have been around 3.5, with a local maximum of 22. In terms of abundance, differences are even larger, with an average of 46 individuals in 5 m^2 samples, with a marked variability between biomass rich and poor areas, in contrast to the 20 individuals that on average can be found in 2 m^2 sampling units (see Section 4.6.1).

Another positive aspect of selecting 5 m^2 sampling units was the relatively low number of samples that showed no distinguishable fauna, compared to samples of smaller sizes. As sampling size was increased, the percentage of zeros was reduced rapidly. For instance, up to 22% of the sampling units of a size of 1 m^2 did not register a single organism, 12% in the case of 2 m^2, and that fraction was reduced to only 4% in the case of 5 m^2 sampling units. The increased number of samples that at least had one organism simplified the data treatment, since most multivariate analyses cannot deal with samples that do not hold a single organism.

C. Statistical analyses

Defining benthic communities cannot be considered a straightforward task, since species aggregations vary from loose assemblages to well-knit, coevolved and rather stable sets of organisms that interact among them and with the physical environment (Mills 1969). In general, the concept of species association refers to a group of species that are 'significantly' found together regardless of their interactions, an idea that is not always easily translated into a series of well-defined analytical steps that would lead to a conclusive result (Legendre & Legendre 2012). Although exploratory in nature, multivariate statistical analyses, and cluster analyses in particular, are capable of discriminating groups at different levels, each of them with varying degrees of crispness. Hierarchical cluster analysis is particularly adept at showing that biological groupings are rarely clear-cut, and hence, the determination of how many assemblages can be derived from a biological matrix can become a subjective call. In fact, this partitioning could be considered a classification that we impose onto our data in order to facilitate the interpretation of nature and has profound implications for its management.

Results of cluster analyses are dependent on the measure of dissimilarity selected and the algorithm used. We decided to employ the Bray-Curtis dissimilarity index (reasons for this discussed at length in Section 4.6.4) and we then applied Ward's algorithm to partition the data. This last method minimizes the same "squared error" criterion used in multivariate analysis of variance, producing compact groupings of reasonably similar size (Legendre & Legendre 2012). Once data was clustered, we aimed to objectively determine the number of species aggregations that could be derived from the video data with the objective of reducing the potential bias that an arbitrary cut in the dendrogram would entail.

Some scientific studies that aim to determine how marine species are grouped with each other to form 'well-defined benthic communities' use arbitrary levels of similarity to partition the biological data set, always trying to represent the naturalist's perception of what's out there. In our case, we decided to use a measure of group compactness to find the best cluster solution, where samples within each cluster were more similar to one another than to samples in the remaining clusters. This methodology led to the definition of 9 distinct megabenthic assemblages, providing a sound classification of the data into realistic and interpretable "habitats" (sensu MSFD). Although it is true that some overlap exists between certain groups (especially those that share the same type of substrate and a similar depth range), our results are consistent with the analyses of species-environment relationships (see Section 5.2) and provide a coherent classification of species occurrences for prediction, mapping and managing purposes.

6.1.2 Description of the natural heritage of Cap de Creus continental shelf and submarine canyon

A. Fauna composition and diversity

The ROV footage recorded on the continental shelf and submarine canyon off Cap de Creus revealed the existence of at least 170 megabenthic species, excluding fish and highly mobile species, such as cephalopods. Although comparisons should be handled with care, we could look at studies performed in other areas of similar depths using ROVs as their main sampling device to provide a rough idea of the diversity found on the shelf and submarine canyon off Cap de Creus. Table 6.1 provides a reduced list with some examples of ROV explorations from different areas of the Mediterranean, Atlantic and Pacific Ocean carried out during the last 5 years. In all cases, the number of taxa identified in the images was lower, being the study performed on the South West Approaches of the Atlantic the only one providing similar results (Davies *et al.* 2014).

Table 6.1. Number of taxa identified in recent ROV surveys performed in areas of the Mediterranean, Atlantic and Pacific Ocean.

Location	**Geographic area**	**Depth range (m)**	**ROV dives**	**Num. taxa**	**Reference**
South Tyrrhenian	Mediterranean	70-130	34	118	Bo *et al.* 2012
Gioia canyon	Mediterranean	40-450	14	41	Pierdomenico *et al.* 2016
South West Approaches	NE Atlantic	138-1165	44	161	Davies *et al.* 2014
Rockall Bank	NE Atlantic	250-450	5	81	Robert *et al.* 2014
Ormonde Peak, Gorringe Bank	NE Atlantic	30-230	5	59	Ramos *et al.* 2016
Southern California submarine canyons	NE Pacific	300-800	25	35	Duffy *et al.* 2014
Cobb Seamount	NE Pacific	35-1155	16	74	Du Preez *et al.* 2016

Besides the large sampling effort performed for this PhD thesis (which could partly explain the large differences with other studies), we can only speculate about the reasons why the number of megafauna species detected in a relatively small area was so high. It is very likely that the complex environmental setting found in Cap de Creus plays a key role in the maintenance of such high diversity rates. The topographic complexity of the marine area of Cap de Creus, with a very narrow continental shelf and a submarine canyon almost reaching the coastline (Lastras *et al.* 2007), generates a large number of ecological niches, which can be colonized by a wide variety of benthic organisms. The area is considered very rich in terms of nutrient and phytoplankton concentration, much higher than the adjacent open Mediterranean Sea, mostly due to the large discharge of the Rhone river in the northern part of the Gulf of Lions (Cruzado & Velasquez 1990).

Shelf waters from the gulf are mostly funnelled through the submarine canyon off Cap de Creus after being cooled down by the cold northern winds, a process that generates intense events of dense water movement from the shelf to the deep sea (Canals *et al.* 2006). Such a large flux of water provokes strong near-bottom currents that mobilize large amounts of suspended particles through the shelf towards the submarine canyon (Palanques *et al.* 2006), favoring the development of a large number of suspension and filter feeders. Furthermore, some of these species are capable of generating large three-dimensional structures that exert a positive influence on the species richness at the local scale, since they serve as substrate for attachment, shelter, feeding or parasitism for many other accompanying organisms (Buhl-Mortensen *et al.* 2010). In fact, the environmental conditions seem to be very appropriate for organisms belonging to the Phyla Porifera and Cnidaria, which represented more than 50% of the total number of species identified.

Aiming to provide a thorough idea of the diversity and relevance of the megafauna observed in the video images, including their distribution and the conservational status, the occurrences of the most relevant species from each Phylum are discussed in the subsequent sections (remember that the complete list of species with their representativeness and maximum densities determined from the video images is provided in Annex III). None of the species identified in the video footage off Cap de Creus is currently included in the IUCN Red List of Threatened Species (www.iucnredlist.org). A few species, however, are currently considered under threat due to their vulnerability to human disturbances:

- The sponges *Axinella polypodes*, *Sarcotragus foetidus* and *Tethya* sp. and the reef forming corals *Lophelia pertusa* and *Madrepora oculata* are included in the List of endangered or threatened species (Annex II) of the 'Protocol concerning Specially Protected areas and Biological Diversity in the Mediterranean' (UNEP-MAP-RAC/SPA 2013).
- The red coral *Corallium rubrum* and the spiny lobster *Palinurus elephas* are included in the List of species whose exploitation is regulated (Annex III) of the 'Protocol concerning Specially Protected areas and Biological Diversity in the Mediterranean' (UNEP-MAP-RAC/SPA 2013).
- The red coral *Corallium rubrum* is included in the List of species of community interest whose taking in the wild and exploitation may be subject to management measures of the Habitats directive (Annex V) (Council of the European Union 1992) and of the Spanish law on Natural Heritage and Biodiversity (Annex VI) (BOE 2007).

Porifera. Sponges were the most diverse group of the megafauna, with more than 30% of the total number of species belonging to this Phylum. Such diversity of species was mostly related to the high number of sponges observed in the deepest areas of the southern continental shelf, in those ROV dives performed furthest away from shore. Outcropping and suboutcropping rocks were partially colonized by a large variety of erect and encrusting sponges, for which only a fraction has currently been identified to species level. Large and abundant organisms were successfully classified thanks to the live samples collected during the Indemares surveys using epibenthic sleds and the hydraulic grabber of the manned submersible. Sponge diversity in the area is so high that we are currently developing an extensive identification process to generate a detailed species catalogue, which will complement the existing information provided in this PhD with new samples collected in sleds and trammel nets of artisanal fishermen. The most abundant sponges of the deep continental shelf were *Dysidea tupha*, *D. avara, Stelligera stuposa* and *Haliclona* cf. *elegans,* the latter covering some rocky outcrops with local densities of up to 14 ind·m^{-2}. Also relevant were the less frequent but very large *Poecillastra compressa*, *Axinella polypoides* and *Desmacidon fruticosum*, species already identified in the same area over 100 years ago (Pruvot 1895).

In shallower depths, the yellow erect-reptant *Suberites syringella* was the dominant sponge, observed with densities of 5-8 ind·m^{-2} in depths of 100 m on the northern shelf. This sponge has historically been documented as a very common species in shelf environments of the western (Bertolino *et al.* 2013; Pansini & Musso 1991) and eastern Mediterranean Sea (Voultsiadou-Koukoura & Van Soest 1993), generally appearing as solitary individuals or gathering in close groups with lower density values than those recorded in Cap de Creus. Moving further deep into the submarine canyon, large rocky outcrops were mostly dominated by a wide variety of encrusting sponges, sometimes accompanied by the laminar-shaped sponge *Pachastrella monilifera*. Due to the lack of samples from the submarine canyon, a large percentage of the diversity provided by the encrusting species still remains unidentified, and further surveys will be required to uncover the whole sponge diversity of the study area.

Cnidaria. A large number of Cnidaria species were identified in the shelf and slope habitats off Cap de Creus. Pennatulacean diversity was surprisingly high, with representatives of 6 of the 11 species that have historically been reported in the muddy and sandy bottoms of the Mediterranean Sea: *Pteroeides spinosum*, *Pennatula rubra*, *Cavernularia pusilla*, *Funiculina quadrangularis, Virgularia mirabilis* and *Veretillum cynomorum* (Bo, Numa & Otero 2017). It is very likely that *Kophobelemnon stelliferum* is also present, but its restricted distribution inside the submarine canyon at depths below 300 m (Reyss 1971) might have kept this species away from the sight of the ROV. Interestingly, Cap de Creus alone holds all the pennatulacean species that have previously been identified along the whole Catalan coast (Gili & Pagès 1987), with the exception of *Pennatula phosphorea*, which might have been confounded in the past with *P. rubra*.

In terms of abundance, *P. spinosum* and *C. pusilla* showed the highest densities, always in areas not affected by fishing practices. Both species displayed lower values than those recorded in other areas of the Mediterranean (Porporato *et al.* 2014) or the Atlantic (Altuna, Aguirrezabalaga & Martinez 2008), where exceptional densities of 70 col·m^{-2} have been reported for *C. pusilla*. Unexpectedly, *Funiculina quadrangularis* was observed in only two occasions, despite being the most common pennatulacean species of the Cantabrian Atlantic (Ruiz-Pico *et al.* 2017) and central Mediterranean Sea (Terribile *et al.* 2015). Even though Reyss (1971) reported an average of only 1 colony per 100 m^{2} in areas inside the submarine canyon, we are unsure whether this species was more common in the continental shelf before commercial fishing practices began to operate in the area.

Several alcyonacean species were also identified on the continental shelf, being *Alcyonium palmatum* and *Paralcyonium spinulosum* the most frequent species, with densities of 6 to 9 col·m$^{-2.}$

In the case of gorgonians, from the 6 different species identified between the shelf and submarine canyon, only *Eunicella cavolini* formed large aggregations. It is distributed along a narrow strip of land that stretches for more than 10 km north of the cape, following the 100 m isobath. The highest recorded densities were of 25 col·m^{-2}, similar to those reported in other areas of the Mediterranean Sea, where it also forms dense patches at depths of 70-100 m (Bo *et al.* 2011b).

In areas of the shelf break and the upper part of the slope, where substrate is composed by a mixture of coarse bioclastic gravels and fine/medium sands, two species of cerianthids were relatively common: *Cerianthus membranaceus,* with a wider distribution and relatively low densities, and *Arachnanthus oligopodus*, with a more restricted distribution but with local densities of more than 25 ind·m^{-2}. Extensive fields of this species have already been documented in other areas of the Mediterranean (Bo *et al.* 2017), and it would seem interesting to further investigate aspects of its ecology and distribution. Inside the submarine canyon, the cold-water coral species *Madrepora oculata*, *Lophelia pertusa* and *Dendrophylia cornigera* were the most iconic species. The presence of cold-water corals off Cap de Creus has been known for more than 100 years (Pruvot 1895), and the number of studies that have provided information about their ecology and distribution began in the 1970s (Reyss 1971) and have continued until the present days (Orejas *et al.* 2009). As expected, *M. oculata* was the dominant cold-water coral species, with patches consisting of more than 8 col·m^{-2}, with a total biomass larger than any other benthic invertebrate identified inside the submarine canyon.

Echinodermata. Overall, one out of every two organisms identified in the video images were echinoderms. This is not surprising if we consider the large aggregation of brittle stars of the species *Ophiothrix fragilis* that was recorded on the central continental shelf. Five dense patches were filmed during one single submarine dive at depths of 105-110 m, just in front of the westernmost part of the cape. Maximum densities were estimated to be around 600 individuals per m^2, values far lower than the almost 2000 ind·m^{-2} that have been recorded in several areas of the British Isles (Warner 1971). Brittle star aggregations are not a rare phenomenon in Mediterranean environments, and besides the aggregations already documented in Cap de Creus (Guille 1965), there is knowledge of dense patches on shallower areas of the shelf along the Rousillon coast (Guille 1964) and the Adriatic Sea (Fedra *et al.* 1976). Besides this ophiuroid species, echinoderms were very common throughout the whole shelf and the submarine canyon, present both in soft and hard substrates.

The highest number of echinoderm species were registered on the soft bottoms of the continental shelf, where sea stars (e.g. *Anseropoda placenta*), sea lillies (e.g. *Leptometra phalangium*), sea urchins (e.g. *Echinus acutus*, *Spatangus purpureus*, *Cidaris cidaris*) and sea cucumbers (e.g. *Holothuria* spp., *Cucumaria* sp., *Parastichopus regalis*) coexisted in similar environments. With the exception of the crinoid *Leptometra phalangium*, their abundances were in general very low, with most species observed as solitary individuals. Guille (1965) identified the sea urchin *Echinus acutus* as one of the most frequent and abundant species of the continental shelf off Cap de Creus. Even though it is still present at all depths and over all substrates, we are unsure whether this pattern still remains in present days.

Polychaeta. Although this is probably the richest and most diverse group of the macrofauna in soft-bottom areas of the continental shelf, only 12 polychaete species were identified in the ROV images. Most burrowing organisms could not be detected using this technique, and only species provided with large external structures used for protection against predators or to improve access to suspended particles could be identified, suggesting that polychaete species richness is largely underestimated in our study. The most abundant polychaete was the widely distributed *Lanice conchilega*, found in densities of 10-20 ind·m^{-2} in areas of the shelf and the shelf break (90-200 m). Although such densities might seem relatively high, aggregations of this species in intertidal areas of the North Sea and the English Channel reach several thousand individuals per square meter, with maximum densities ever recorded of 7000 ind·m^{-2} in the Bay of Seine (Ropert & Dauvin 2000). It is a very pervasive species in the Mediterranean Sea, documented all the way from the Northwestern basin (Ramirez-Llodra *et al.* 2009) to the Turkish coasts (Mutlu, Çinar & Ergev 2010). Not many works, however, report their depth distribution or its abundance. It is very likely, however, that shelf and slope areas of the Mediterranean do not provide the environmental requirements for *Lanice conchilega* to reach densities similar to those recorded in shallow sedimentary bottoms of the Atlantic. Also relatively abundant in certain soft-bottom areas of the shelf was the terebellid *Lanicides* sp., with local densities of up to 25 ind·m^{-2}. Densities were only calculated in those videos where it was easily distinguishable from the seabed, which could only be achieved when the ROV moved very close to the bottom. Its cryptic aspect made its identification very complex, so it had to be removed from the statistical analyses to avoid a very large bias in its spatial distribution and abundance.

In areas characterized by the presence of coarser sediments and rocky outcrops, both in the deeper part of the shelf and inside the submarine canyon, the serpulid *Protula tubularia* became the most common polychaete species, with local densities of almost 15 ind·m^{-2}. This species has a very wide distribution along the Mediterranean Sea, present in all basins from superficial waters to more than 1000 m depth, although it has a preferred depth distribution between 70 and 150 m (Ben-

Eliahu & Fiege 2009). Other relevant polychaete species were the tube forming *Sabella pavonina,* present on the muddy bottoms of the continental shelf and also on the rocky outcrops inside the submarine canyon, the serpulid *Salmacina dysteri*, which made relatively large aggregations on the soft bottoms of the shelf, and *Myxicola infundibulum*, found as solitary individuals in all areas of the shelf and the shelf break (95-180 m depth).

Other Phyla. Among remaining Phyla, brachiopods were the most abundant group. Overall, approximately 3500 brachiopod individuals were identified in the video images, primarily inside the submarine canyon (130-400 m depth), with densities of 15-25 ind·m^{-2} in certain rocky outcrops, where a local maximum density of 48 ind·m^{-2} was recorded. Two species of brachiopods are very common in bathyal depths of Western Mediterranean Sea (*Gryphus vitreus* and *Terebratulina retusa*), both capable of attaching to large rocks and gravels using their pedicles (Logan *et al.* 2004). These two species had already been identified by Reyss (1971) in the submarine canyon off Cap de Creus, but the definition of the video recordings made it impossible for us to differentiate between both species directly from the images. In the end, they had to be grouped under the same category, so the dominant brachiopod species in the submarine canyon off Cap de Creus remains unknown. A similar situation occurred with the giant oysters observed on the vertical walls of the canyon, which could be either *Neopycnodonte cochlear* or *N. zibrowii,* both species already identified in other submarine canyons of the Gulf of Lions (Fabri *et al.* 2014).

Not many crustacean species were identified in the images. This is possibly due to the lack of resolution of the video images, which may have generated an underrepresentation of this group in the study area. The galatheids *Munida* spp. (most likely to be *M. intermedia* in most cases, but *M. rugosa* was also observed) were very characteristic on the shelf and the submarine canyon, usually hidden under rocks or large shells. These species are very common in deep areas of the Mediterranean shelf and slope, with a distribution that extends to the 800 m isobath (Abello, Valladares & Castellón 2009). Hermit crabs were also common in all surveyed areas, sometimes displaying an aggregative behavior, especially in certain areas of the continental shelf. It was very complicated to determine the species in all situations, but we believe at least the genus *Pagurus* and *Dardanus* are present in bathyal areas of Cap de Creus. Although *Munida* spp. and Hermit crabs appeared to be the most common crustaceans in the video footage, the soft bottoms of the Catalan shelf and slope are generally dominated by other crustacean species, including *Liocarcinus depurator, Sergestes arcticus* and *Nephrops norvergicus* (Abello *et al.* 2009), none of which were observed in our images. It is very likely that large crustaceans can avoid the ROV either by actively swimming away or by hiding inside burrows or crevices, as it could be the case of *N. norvergicus.* This would imply that ROVs only register a fraction of crustacean megafauna, underestimating their abundance and providing a certain bias to the results.

In the case of Bryozoans, only the species *Smittina cervicornis* seemed relatively abundant in the video images, mostly appearing on coarse areas of the central continental shelf. It was mainly observed as an accessory species, with maximum densities of up to 4 col·m^{-2}. This species has previously been identified as one of the characterizing bryozoans of the coarse sand and gravel bottoms of the continental shelf off Cap de Creus (Madurell *et al.* 2013). None of the other characterizing species besides *Reteporella* spp., however, could be identified form the video footage. This is most likely caused by the reduced size of most species, which makes them cryptic to the video techniques. We are sure that the ROV footage largely underestimated Bryozoan species richness, a group best studied using extracting techniques due to their small size.

B. Benthic assemblages: structure and representativeness

The benthic megafauna of Cap de Creus continental shelf and submarine canyon displayed a very discernible community structure, with 9 contrasting species associations. In the Mediterranean Sea, there exists an important transition area between 150-170 m depth, which coincides with the end of the continental shelf and the beginning of the slope (Pérès 1967). Soft-bottom areas of deep circalittoral and bathyal environments of the Mediterranean Sea are generally seen as very poor in terms of species richness and benthic assemblage diversity in comparison to littoral habitats, especially due to the low light availability and the homogeneity of the landscape (Pérès 1967). This does not seem to be the case of Cap de Creus, whose continental shelf is characterized by a great variety of habitats that range from high-depositional fine-grained areas to large rocky outcrops. The availability of many ecological niches has favored the presence of at least 7 different benthic assemblages that coexists in a relatively small continental shelf. Conversely, areas of the shelf break and submarine canyon were only characterized by the presence of 2 major assemblages, one of which was found exclusively below the 170 m depth range.

It is important to point out that sampling effort was greatly unbalanced, with most ROV dives performed on the continental shelf (approximately 80% of the samples were filmed between 80 and 150 m depth), leaving the submarine canyon under-represented. This asymmetry has probably affected the resulting image, with a very fine characterization of the species associations of the continental shelf and a broader result in the case of the submarine canyon. It is possible that a larger sampling effort in deeper areas would have unveiled a more complex community structure, providing a more clear pattern than the one detected.

(A) Gorgonian assemblage.

This was the richest and most diverse assemblage of Cap de Creus continental shelf, with almost 130 megafauna species identified in areas dominated by the sea fan *Eunicella cavolini*. Gorgonians have long been regarded as engineering species that provide shelter and refuge for a large number of associated organisms, both in shallow (Ponti *et al.* 2016) and deep-sea benthic ecosystems (De Clippele, Mortensen & Buhl-Mortensen 2015). In our case, a large number of species from different Phyla were observed living directly attached to sea fans, most possibly in the search of better feeding conditions due to the capacity of gorgonian gardens to alter the current regime and sedimentation rates (Valisano *et al.* 2016). Epibiont species living on top of *E. cavolini* included ascidians (e.g. *Distomus variolosus*), cnidarians (e.g. *Alcyonium coralloides*), bryozoans (e.g. *Turbicellepora avicularis*), molluscs (e.g. *Pteria hirundo*), echinoderms (e.g. *Ophiohrix fragilis*) and crustaceans (e.g. *Inachus* sp.), among others. Gorgonians not only favored the presence of epibionts, *E. cavolini* also seemed to generate favorable conditions for a large number of sessile and mobile organisms to coexist in the same area. The most common species that appeared in association with *E. cavolini* were the sponge *Suberites syringella*, the soft corals *Alcyonium palmatum* and *Paralcyonium spinulosum* and the bryozoan *Smittina cervicornis*.

Habitat equivalences. We have included the gorgonian assemblage under the habitat 'Circalittoral communities of rocky bottoms dominated by sessile filter feeders with a dominance of *E. cavolini*' from the IEHEM list (Templado *et al.* 2013; corresponding code in Table 6.2), although, when looking to its composition, the IEHEM assemblage might make reference to a more littoral community. It would be interesting to update the definition to include deeper shelf populations. A similar situation occurs with the EUNIS and RAC/SPA habitat lists, in which assemblages dominated by *E. cavolini* most possibly make reference to shallow benthic communities ('Coralligenous biocenosis, facies with *Eunicella cavolini*'; corresponding codes in Table 6.2).

Conservation status. There is no specific protection for gorgonian dominated assemblages in the Mediterranean Sea beyond the regulation given to the red coral *Corallium rubrum* in certain areas due to its exploitation as an economically valuable resource. Certain gorgonian species (e.g. *Callogorgia verticillata* or *Ellisella paraplexauroides*) are currently included in the list of endangered or threatened species under the Barcelona Convention (UNEP-MAP-RAC/SPA 2013), but specific actions should be taken to promote the conservation of shelf dwelling gorgonians whose viability could be severely affected by commercial fishing practices. In the Atlantic region, although aggregations of gorgonians are not subject to directed national or international protection regimes,

Table 6.2. Equivalences of the 9 invertebrate assemblages identified in the video images recorded in Cap de Creus continental shelf and submarine canyon with biocenosis included in the 3 most representative lists of habitats: EUNIS; RAC/SPA and IEHEM. See Box II for further details on these habitat lists.

Assemblage	Indicator species (IndVal)	EUNIS	RAC / SPA	IEHEM
A	*Eunicella cavolinii, Smittina cervicornis, Suberites syringella, Epizoanthus* sp.	A4.269	IV. 3. 1. 10	0302022307
B	*Pteroeides spinosum, Cavernularia pusilla, Pennatula rubra, Alcyonium palmatum*	A5.392 A5.393 (?)	IV. 1. 1. 2 IV. 1. 1. 3 (?)	0304051401 0304051403
C	*Sabella pavonina, Andresia partenopea*	N/A	N/A	0304051303 03004051404
D	*Leptometra phalangium, Pteroeides spinosum, Pennatula rubra*	A5.472	IV. 2. 3. 2	04020403
E	*Lanice conchilega, Arachnanthus oligopodus*	N/A	N/A	0304051405 0402031103
F	*Brachiopoda, Galathea spp., Caryophyllia smithii, Incrusting sponges, Madrepora oculata*	A6.61	V. 3. 1	04010112 04030301
G	*Dysidea* spp., *Haliclona elegans, Hyrtios collectrix, Axinella damicornis, Stelligera stuposa*	A4.12 (?)	IV. 3. 3	0302022402 04010208
H	*Protula tubularia, Capnea sanguinea, Cerianthus membranaceus,* Hermit crabs, *Mesacmaea mitchelli*	N/A	N/A	04020207 0402031104
I	*Ophiothrix fragilis*	A5.381	IV. 2. 1. 1	0304051504

they are now classified under the category of "Coral gardens", which are considered 'Threatened and/or declining' due to their sensitivity to demersal trawling and longlining, temperature change and acidification in the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR). Internationally, habitat-forming gorgonians found in the high-seas are considered Vulnerable Marine Ecosystems (VME) by FAO (2009), with the objective of encouraging governing bodies to develop specific fishery management plans to avoid adverse impacts on these fragile and slow-growing communities.

(B) Pennatulacean assemblage.

Sea pens are characteristic species of the soft-bottom areas of the Mediterranean and Atlantic continental shelf, inhabiting both muddy and sandy substrates (Gili & Pagès 1987; Ruiz-Pico *et al.* 2017). Although sea pens are generally found as isolated colonies or in very low densities along vast extensions of the continental shelf, monospecific aggregations displaying high abundances have been reported both in Mediterranean (Porporato *et al.* 2014) and Atlantic shelf habitats (Altuna *et al.* 2008). In the case of Cap de Creus, the pennatulacean association was the most widespread assemblage of the continental shelf, appearing in a total of 28 ROV dives, both north and south of the cape. The dominant species were the sea pens *Pteroeides spinosum*, *Cavernularia pusilla* and *Pennatula rubra*, which appeared in association with the soft coral *Alcyonium palmatum*.

In terms of diversity, a total of 65 megafauna species (excluding highly mobile invertebrates) and more than 30 fish species were identified associated to the pennatulacean assemblage in the ROV images. A high number of the fish species observed were of commercial value, in which we can include the Mediterranean hake *Merluccius merluccius*, the phycid hakes *Phycis phycis* and *P. blennoides*, the red mullet *Mullus barbatus*, the flatfishes *Dicologlossa hexophthalma* and *Lepidorhombus boscii,* the monkfish *Lophius* sp., the poor cod *Trisopterus* sp., the gurnards *Chelidonichthys cuculus*, *C. obscurus* and *Eutrigla gurnardus*, the catfish *Scyliorhinus canicula*, and the seabrams *Pagellus acarne* and *P. erythrinus*, among others. Such a high number of accompanying species makes this assemblage the richest and more diverse association of the soft-bottom areas of the continental shelf off Cap de Creus. Only recently, pennatulaceans have started to be considered key components of the mud and sand circalittoral and bathyal ecosystems, where their tridimensional structures generate new ecological niches in a very homogeneous landscape, playing a fundamental role in the maintenance of the biological diversity (Buhl-Mortensen *et al.* 2010). Pennatulaceans act as nurseries for mobile species (e.g. fish and crustaceans), which use them as shelter during their early life stages (Baillon, Hamel & Mercier 2014).

<u>Habitat equivalences.</u> This combination of species has already been identified by the IEHEM as a common assemblage in deep circalittoral muds and muddy sands in the Atlantic Ocean and also as part of the detritic circalittotal bottoms dominated by invertebrates in the Mediterranean Sea (Templado *et al.* 2013; corresponding codes in Table 6.2). The correspondence with the RAC/SPA and the EUNIS list of habitats is not as straightforward as originally supposed, since the characterizing species of this assemblage can only be included in the Mediterranean biocenosis of coastal terrigenous muds, whose dominant species are the sea pens *Virgularia mirabilis* and *Pennatula phosphorea* (RAC/SPA: IV.1.1.2; EUNIS: A5.392) or the soft coral *Alcyonium palmatum* (RAC/SPA: IV.1.1.3; EUNIS: A5.393). It would be interesting for both catalogues to extend the categories included in the sublittoral muds and mixed sediment habitats to incorporate the specificities of the pennatulacean assemblage described off Cap de Creus.

<u>Conservation status.</u> Due to the sensitivity of pennatulaceans to bottom trawling activities and their role in diversity maintenance, OSPAR has recently included this group of invertebrates in the ‘List of threatened and/or declining species and habitats in the Northeastern Atlantic’ (Curd 2010), aiming to provide a general framework to improve their conservation status. No specific protection measures, however, exist for sea pens in the Mediterranean region. Information that improves our current knowledge about their distribution and abundance is therefore of key interest towards the implementation of future conservation measures in shelf environments of the Mediterranean Sea.

(C) Impoverished assemblage.

This is the poorest assemblage in terms of species richness and diversity, with only 24 megafauna species identified in the video images. Densities were extremely low in all cases, being *Sabella pavonina*, *Alcyonium palmatum* and *Andresia partenopea* the only species that appeared with certain regularity. Only *S. pavonina* registered maximum densities above 1 ind·m^{-2}, giving an indication of how low the abundances of those species were in this assemblage.

Habitat equivalences. It is very complicated to relate this assemblage to the international habitats lists (Table 6.2.), and we could only find certain resemblance to the circalittoral muddy detritic bottoms dominated by sabellid species included in the IEHEM list. No other correspondences could be made.

Conservation status. Given its impoverishment, this assemblage does not seem to have any special conservation interest.

(D) *Leptometra phalangium* assemblage.

The crinoid *Leptometra phalangium* has long been considered one of the most characteristic species of the detritic bottoms of the Mediterranean continental shelf (Pérès & Picard 1964). Early exploratory works identified aggregations of this species in several shelf-break areas of the NW Mediterranean (120-170 m), characterized by a mixture of gravel, sand and muds with strong bottom currents as a common feature (Pérès 1967). Since then, studies in central (Colloca *et al.* 2003) and eastern Mediterranean regions (Kallianiotis *et al.* 2000) have also reported dense aggregations of this species, which generally display average densities of around 5 ind·m^{-2} and local maximum values of 12-15 ind·m^{-2}; (Colloca *et al.* 2004). In the case of Cap de Creus, *L. phalangium* displayed a more shallow distribution to what would have been expected, mostly concentrated in specific areas of the middle shelf, at depths of 100-110 m. Average densities were slightly higher than those recorded in other areas, with maximum densities above 20 ind·m^{-2}. Such differences can possibly be explained by the particular oceanographic setting of Cap de Creus, where strong bottom currents regularly transport large quantities of particulated suspended matter through the continental shelf as part of the down-canyon sediment dynamics (Palanques *et al.* 2006), which could favor the development of dense populations of this benthic suspension feeders. Surprisingly, areas of the shelf break (*ca.* 140-150 m depth) were not colonized by *L. phalangium*. This could possibly be related to the very high current speeds that hit these areas during strong cascading events in Cap de Creus, where bottom currents can reach speeds of up to 50 cm/s (Puig *et al.* 2008).

The association of *L. phalangium* to several commercial fish species, including the Mediterranean hake and the red mullet, have highlighted its role as a nursery ground for recruits and juveniles of several fish species (Colloca *et al.* 2004). In our case, only 13 fish species were identified in the images filmed within this assemblage, including the Mediterranean hake *Merluccius merluccius*, the poor cod *Trisopterus* sp., the catshark *Scyliorhinus canicula* and many gurnard species such as *Chelidonichthys cuculus*, *C. lucerna*, *Eutrigla gurnardus* and *Trigloporus lastoviza*. Such a limited species list might be the result of the low number of ROV dives performed in *L. phalangium* assemblage, and it is possible that a larger sample size would have unveiled a larger number of fish species.

Habitat equivalences. The bottoms with the crinoid *Leptometra phalangium* have historically been included in the shelf-edge detritic assemblage ('Biocenose des Fonds Detritiques du Large'), first described by Pérès & Picard (1964). The wide distribution of this assemblage in many areas of the Mediterranean shelf has favored its inclusion in most habitat lists that deal with Mediterranean Sea environments. In all cases, *L. phalangium* is always placed in bathyal habitats of the shelf break (corresponding codes in Table 6.2).

Conservation status. Studies on commercial catches have revealed the dominance of this species in the by-catch produced by the bottom trawling fleet in the southern Gulf of Lions, unveiling its vulnerability to bottom trawling activities (Mallol 2005). This reason, together with its capacity to provide feeding, refuge or reproduction for commercial species, has prompted its consideration as a Sensitive Habitat (SH) relevant for fisheries, making any area with *L. phalangium* beds a potential candidate site to become a Specially Protected Area of Mediterranean Importance (SPAMIs) (UNEP-MAP-RAC/SPA 2010).

(E) ***Lanice conchilega*** assemblage.

This assemblage was primarily characterized by the presence of the polychaete *L. conchilega*, with accompanying species displaying very low values in the IndVal analysis. Aggregations of this polychaete in subtidal areas of the English Channel have reported a positive influence on macrofaunal density, species richness and community composition when the bio-engineered habitat is well defined (Rabaut *et al.* 2007). In fact, *L. conchilega* patches seem to increased habitat quality in areas of a uniform habitat, resulting in a higher survival rate of the associated benthic species (Van Hoey *et al.* 2008). In the case of Cap de Creus, a total of 50 megafauna species were observed in this assemblage, most of them with generally low abundances. It is very likely that the structuring capacity of *L. conchilega* requires a minimum density to be reached to significantly improve species richness and the biomass of the surrounding fauna. In the English Channel, where *L. conchilega* can reach densities of up 1500 ind·m^{-2}, a strong positive relationship has been observed between the steadily increasing densities of *L. conchilega* and those of the macrobenthic fauna (Rabaut *et al.* 2007). We are unsure how dense *L. conchilega* aggregations could be in areas of the continental shelf and shelf break in Mediterranean environments, and scarce data can be found to compare with our results.

Habitat equivalences. The EUNIS list of habitats only considers *L. conchilega* dominated assemblages for sublittoral areas of the Atlantic and there is no direct correspondence for Mediterranean shelf/slope aggregations. A similar situation occurs with the list of biocenoses developed under the Barcelona Convention. In both cases, this assemblage can only be related to the 'Mediterranean communities of shelf-edge detritic bottoms' based on the location where it was found. The IEHEM list includes *L. conchilega* dominated assemblages both in circalittoral and bathyal detritic bottoms (corresponding codes in Table 6.2). It would be interesting to highlight the presence of relatively high densities of the cerianthid *Arachnanthus oligopodus* within this assemblage, with local densities that reached values of 26 ind·m^{-2}. These high abundances could possibly indicate the presence of a facies of this cerianthid species within this assemblage, already considered in the IEHEM list as 'Detritic bathyal bottoms with cerianthids (*Arachnanthus*)'.

Conservation status. *L. conchilega* forms dense beds in sublittoral areas of the Atlantic that have a very high functional value, favoring the associated biological diversity and providing feeding grounds for birds and fishes, ultimately displaying a relatively high socioeconomic value (Godet *et al.* 2008). No information exists on how beneficial this species could be to promote fish or megafauna diversity in circalittoral and bathyal areas of the Mediterranean, and it would seem interesting to evaluate their role in ecosystem functioning below certain depths. Nevertheless, there is no legal protection in Europe for this species despite their high heritage value in areas where it is found forming dense aggregations.

(F) Cold-water coral assemblage.

This is one of the best-studied assemblages of the deep Mediterranean Sea, whose ecology has been evaluated in areas of the Gulf of Lions since the 1970s (e.g. Best 1970; Reyss 1971). Recent surveys have provided evidence of its widespread distribution along the whole Mediterranean Sea, with sightings not only in NW Mediterranean, but also from the Adriatic (Freiwald *et al.* 2009), the Ionian (Mastrototaro *et al.* 2010) and the Aegean Seas (Vafidis, Koukouras & Voultsiadou-

Koukoura 1997). Most of these cold-water coral assemblages are dominated by the scleractinian coral *Madrepora oculata* (Taviani, Freiwald & Zibrowius 2005), a contrasting situation to that found in the Northeastern Atlantic, where higher abundances of *Lophelia pertusa* are generally reported, especially at depths of 200-400 m (Buhl-Mortensen *et al.* 2015b). In the submarine canyon off Cap de Creus, as it was expected, the larger percentage of coral colonies observed belonged to the species *M. oculata* (90%), and only 2% corresponded to *L. pertusa* colonies (the remaining 8% were colonies of the yellow coral *Dendrophyllia cornigera*). One of the first attempts to describe the Mediterranean 'Biocenosis of deep-sea corals' was made by Pérès (1967), although no sufficient samples were collected at the time to provide a general definition. A few years later, Reyss (1971) provided a more detailed description of the 'Biocenose des coraux blancs' from Cap de Creus and Lacaze-Duthiers submarine canyons, with most of the megafauna matching the observations made for this PhD thesis.

In the Mediterranean Sea, cold-water coral reefs are known to play a key role in promoting the species richness and abundance of the benthic fauna, both in the case of invertebrate (Mastrototaro *et al.* 2010) and fish species (D'Onghia *et al.* 2010). Bearing in mind the small sampling effort performed on the cold-water coral assemblage of Cap de Creus (only 8% of the samples), it is interesting to note that more than 50 different species were identified in the images associated to this assemblage. Especially significant was the abundance of brachiopods and oysters in certain rocky areas, with maximum densities of more than 9 ind·m^{-2} in both cases. A wide variety of encrusting sponges were also reported in the images, for which further studies will be needed to determine the species to which they belong. In the case of fish fauna, although only 12 species were identified, especially significant was the abundance of two commercially exploited species: *Conger conger* and *Phycis phycis*.

Habitat equivalences. The large number of studies made on cold-water coral reefs have favored the inclusion of this assemblage in all available habitat lists (see corresponding codes in Table 6.2).

Conservation status. The capacity of cold-water coral reefs to generate tridimensional structures that serve as refuge for many organisms, especially larvae and juvenile fish species (Baillon *et al.* 2012), and their fragility to extracting techniques, such as bottom trawling (Althaus *et al.* 2009) and long-lining (Sampaio *et al.* 2012), has led to their inclusion in the list of Sensitive Habitats in the Mediterranean high seas by UNEP (UNEP-MAP-RAC/SPA 2010) and their declaration as Vulnerable Marine Ecosystems by FAO in the International Guidelines for the Management of Deep-sea Fisheries in the High Seas (FAO 2009).

(G) Sponges assemblage.

This is one of the richest assemblages of the continental shelf, with a total of 123 different megafauna species identified in the video images. Although species belonging to 8 different Phyla were reported, the most characteristic megabenthic species belonged to the Phylum Porifera. We did not expect to find sponge aggregations in such a good conservation status in shelf areas off Cap de Creus, which have historically suffered from a relatively high fishing effort (Gómez *et al.* 2006). Although this assemblage appeared in a total 23 ROV dives, the best-preserved locations were observed during the last two cruises, primarily in 5 dives performed in the deepest part of the continental shelf, on the southern side of the cape. In these areas, large rocky outcrops were mostly colonized by the sponge *Haliclona* cf. *elegans*, which completely covered certain rocks with densities of almost 15 ind·m^{-2}. These high abundances might be explained by the availability of hard substrates and by the strong bottom currents that recurrently hit the continental shelf, which remove sediments and enhance food particle fluxes and thus favoring the development of suspension and filter feeders (Genin, Paull & Dillon 1992). Accompanying species, such as the sponges *Iophon* sp., *Dysidea* spp. and *Desmacidon fruticosum* appeared in relatively low densities when *H. elegans* was dominant. Conversely, mixed sedimentary areas were more species rich but with lower abundances overall. In those cases, the dominant sponges were *Dysidea* spp., *Stelligera stuposa* and *Axinella damicornis*, accompanied by solitary individuals of large dimensions of the species *Poecillastra compressa* and *Axinella polypoides*.

This association of species was first described by Pérès (1967) as the offshore rocky bottom assemblage ('Roche du large'). Although the match between both species lists is very high, some of the dominant species identified by Pérès (1967) are slightly different to those reported in the video images. At the same time, there also exist some species defined by the author as characteristic of this assemblage that were not found on the continental shelf off Cap de Creus, not even as solitary individuals. This is the case of *Rhizaxinella pyrifera*, *Phakellia ventilabrum* and *Petrosia ficiformis*. Differences in species composition could possibly be explained by environmental or regional differences in the area surveyed by the author, or even historical changes that have occurred during the past 50 years. Besides the sponge fauna, the remaining groups identified in the video images had a high degree of correspondence with the species listed by Pérès (1967). Some examples include *Reteporella* sp., *Bonellia viridis*, *Munida* sp., *Holothuria forskalii*, *Cidaris cidaris*, *Echinus melo* and serpulid species, among others, which are also present in the descriptions provided by Pérès (1967).

Sponge grounds of the Atlantic generate the environmental conditions to promote an increase of the diversity and abundance of the local epibenthic megafauna community in comparison to non-sponge grounds, both in littoral (Klitgaard 1995) and deep-sea habitats (Beazley *et al.* 2013). These type of facilitation has only started to be examined in Mediterranean sponge-dominated assemblages (Bo *et al.* 2011a), and further studies are needed to determine the role played by dense sponge grounds in enhancing benthic diversity in Mediterranean shelf and slope areas. In our case, the Sponges assemblage was the most diverse (average *expH'* of 6,82) and the second most species-rich assemblage off Cap de Creus, with almost 10 species identified on average per sampling unit and an absolute maximum of 28. The structural habitat generated by sponge grounds also seems to provide shelter for several fish species, to the extent that they should be considered important nursery areas for juvenile fishes (Hogg *et al.* 2010). In our case, even though only 10% of the samples belong to this assemblage, up to 19 different fish species were reported in the images, some of them with high commercial value (e.g. *Conger conger*, *Phycis blennoides*, *Trisopterus* sp. and *Lepidorhombus boscii*).

Habitat equivalences. Even though this assemblage has been reported in Mediterranean waters for decades, there is not a clear correspondence with the EUNIS habitat classification system, and the most similar sponge ground listed only relates to Atlantic fauna (A4.12: 'Sponge communities on deep circalittoral rock'). There exists a high degree of correspondence at the level of genera, but it would be very interesting if EUNIS updated this category to include the Mediterranean specificities. In the case of the regional catalogues, correspondences are more straightforward. The list of habitats included in the Barcelona Convention derives from the work developed by Pérès, so this assemblage is easily included under the category 'Biocenosis of shelf-edge rock'. The IEHEM list, in an attempt to report all the existing sponges associations, has several habitats that make reference to sponge grounds, both in circalittoral and bathyal environments. In the end, we selected two potential candidates as reference habitat for this assemblage, one for each depth range (corresponding codes in Table 6.2).

Conservation status. The role played by sponge grounds in enhancing biodiversity and their sensitivity to commercial fisheries has favored their consideration as a Sensitive Habitat relevant for fisheries in the Mediterranean high seas by UNEP (UNEP-MAP-RAC/SPA 2010) and as a Vulnerable Marine Ecosystem by FAO in the International Guidelines for the Management of Deep-sea Fisheries in the High Seas (FAO 2009). Regarding specific regulations for the Mediterranean Sea, two taxa observed in the sponge assemblage (*Axinella polypoides* and *Tethya* spp.) are currently included as threatened species in Annex II of the Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (UNEP-MAP-RAC/SPA 2013).

(H) Cerianthid assemblage.

This was the most common assemblage of the mixed sedimentary areas of the shelf break and the submarine canyon off Cap de Creus, primarily characterized by the presence of a large number of sea anemones and echinoderms. The most conspicuous species was the tube-dwelling anemone *Cerianthus membranaceus*, found accompanied by other anthozoan species, such as *Capnea sanguinea*, *Mesacmaea mitchellii* and *Sagartia elegans*, all of them always found as solitary individuals. Detritivore species were also relatively common, especially the sea urchin *Echinus acutus* and sea cucumbers of the genus *Holothuria*. This assemblage dwells in very unstable substrates, largely affected by strong bottom currents, which reach very high intensities during cascading events. The mobility of the large bioclastic gravels and pebbles that make up the sediment due to intensity of the currents, together with the lack of consolidated substrates, has contributed to a reduction in the number of sessile species, which might find it very difficult to survive attached to moving pebbles and large shells.

Habitat equivalences. There does not exist a direct correspondence between this assemblage and anyone of those listed in the EUNIS catalogue of European habitats, besides its Atlantic counterpart: '[*Cerianthus lloydii*] and other burrowing anemones in circalittoral muddy mixed sediment'. A similar situation occurs with the RAC/SPA biocenosis list, which does not include bathyal mixed sedimentary assemblages. We encourage representatives of UNEP and EUNIS to extend the habitats described for the Mediterranean bathyal environment, which are largely underrepresented in both lists. The Spanish catalogue IEHEM, on the other hand, includes the cerianthid assemblage both for bathyal muds and mixed sediments: 'Bathyal muds with dominance of cerianthids' and 'Bathyal detritic bottoms with cerainthids (*Arachnanthus*, *Cerianthus, Pachycerianthus*)'. See corresponding codes in Table 6.2.

Conservation status. There is no reference in the literature about the vulnerability of this assemblage to human impacts, such as bottom trawling. There is no current regulation or conservation strategy to protect the components that make up this assemblage.

(I) Brittle-star aggregation.

Massive aggregations of the species *Ophiothrix fragilis* have been reported for decades in different areas of the Atlantic (Warner 1971) and the Mediterranean Sea (Guille 1964). This assemblage was already included in the early work of Pérès & Picard (1964) as a distinctive facies of the muddy detritic biocenosis ('Biocoenose des fonds detritiques envases, DE') due to the extraordinary abundance of this brittle star, which was observed covering more than 90% of the available substrate. Both its wide distribution and its distinctiveness have favored its inclusion as a separate assemblage in most habitat lists, making it easy to find its corresponding habitat (codes for each reference list in Table 6.2). In areas of dense aggregations of brittle stars, epibenthic diversity tends to be relatively low as a result of the dominance of this brittle-star species (Sanvicente-Añorve, Leprêtre & Davoult 2002). A similar pattern was observed in the few dense patches recorded during one single dive on the southern continental shelf off Cap de Creus. In fact, this assemblage provided the lowest diversity values overall, with an average *expH'* of 1.09 and a maximum registered value of 1.69. Only a few scattered gorgonian and alcyonarian colonies as well as some cerianthids could be observed in areas of high brittle star densities. Due to the potentially high mobility of this assemblage (Holme 2009), which can relocate in search of better environmental conditions (i.e. food availability), it would seem desirable to repeat the same ROV dive to detect whether the observed population is temporally stable or its distribution fluctuates through time.

(*) Facies of large hydroids.

The hydroid *Lytocarpia myriophyllum*, a soft-bottom species that can reach heights of over 1 meter, did not appear as a separate assemblage in the cluster analysis due to its low number of records, but deserves mentioning due to its importance as a habitat forming species. *L. myriophyllum* was mostly observed as scattered colonies throughout the whole continental shelf, found in patches of almost 4 col·m^{-2} in two separate areas of the soft sedimentary bottoms of the shelf. The presence of this species in areas of homogeneous substrates increases the quality of organic matter, the abundance and richness of meiofauna and serves as substrate for other taxa (Cerrano *et al.* 2014).

<u>Habitat equivalences.</u> Its role as an ecosystem engineer that supports an important epifauna diversity (Di Camillo *et al.* 2013) was already identified by Pérès (1967), who included the aggregations made by this species as a specific facies of the shelf-edge detritic, named 'The facies of large hydroids'. This facies was not included in the EUNIS catalogue neither in the classification of benthic marine habitat types that derived from the Barcelona Convention, and surprisingly, there is no direct correspondence to any of the IEHEM habitats.

<u>Conservation status.</u> Distributional data about this species in the Mediterranean Sea is generally scarce. The low number of colonies identified in the video footage might be indicating the high sensitivity of this assemblage to commercial fishing practices, a situation that has already been

suggested by Di Camillo *et al.* (2013). The capacity of *L. myriophyllum* to increase spatial complexity and enhance interspecific interactions, together with its apparent low abundance in soft bottom areas of the Mediterranean continental shelf should encourage local authorities to introduce specific measures to preserve this ecosystem engineering species.

6.1.3 Temporal changes occurred during the last 40 years

Although it would seem desirable to compare our results to those obtained in classic works performed in the same study area, the possibility of doing so might be rather limited. The complexity of comparing new data generated using ROVs with that obtained in early exploratory works that used extracting devices limits our capacity to understand the magnitude of the changes that may have occurred to the invertebrate benthic assemblages in the last decades. Evaluating temporal changes in the structure of benthic communities requires the same methods to be used and the same sites to be revisited (or at least as closely as possible). Unfortunately, the three most comprehensive studies performed in Cap de Creus until now were made using sampling methodologies different to the one selected for this PhD thesis: a Van Veen grab (Desbruyères *et al.* 1972), simple annotations from a manned submersible (Guille 1965) and a combination of epibenthic sleds and qualitative data from visual observations obtained from submarine dives (Reyss 1971) (see Box III for further details).

There exists the possibility to perform a qualitative comparison with the works of Guille (1965) and Reyss (1971) thanks to the annotations derived from their visual explorations (no quantitative data was provided) but, unfortunately, the thorough evaluation of the continental shelf performed by Desbruyères *et al.* (1972) does not provide much information about megafauna species to assess the possible changes occurred to the benthic ecosystem during the last 40-45 years. The fraction of the biota detected using Van Veen grabs is completely different to that identified by ROVs, and also provides a very limited spatial representativity due to the small area surveyed in each deployment (Eleftheriou & Moore 2013). In the case of the work of Desbruyères *et al.* (1972), most samples were characterized by small-sized species that live buried inside the sediment (polychaetes, crustaceans, molluscs and nematodes), which are (almost) impossible to detect via imaging methods. The evaluation of 17 grab samples collected inside the limits of our study area led to the identification one major benthic assemblage (defined as 'Muddy bottoms with *Amphiura filiformis*') that included three different sub-communities. It is very difficult to establish a link between any of the assemblages identified by Desbruyères *et al.* (1972) and the 6 groups that derived from our video analysis, although we could attempt to draw some relationships:

- 'Sub-community of muddy detritic with *Venus ovata*', which displayed a very similar spatial distribution to the *Eunicella cavolini* assemblage identified in our surveys (DE in Fig. B3.1). The only species in common in both inventories was the anthozoan *Epizoanthus arenaceus*. The remaining species found in the grab samples were primarily small molluscs, burrowing polychaetes and brittle stars.
- 'Sub-community of muds with *Nucula sulcata*', found in areas with very fine-grained sediments (VC and VL in Fig. B3.1). This community was distributed in an area where 3 of the assemblages identified in the video images are present: the sabellid, the crinoid and the pennatulacean assemblages. There is no shared species, however, between the 3 assemblages identified in the video images and the most abundant species reported by Desbruyères *et al.* (1972).
- 'Sub-community of offshore detritic with *Auchenoplax crinita*', found furthest away from shore in areas of sands and gravels down to 160 m (DL in Fig. B3.1). The most common species was also *Epizoanthus arenaceus*, accompanied by small molluscs and crustaceans. Three assemblages derived from the video images share the area allocated by Desbruyères *et al.* (1972) to this assemblage (pennatulacean, sponge and cerianthid assemblages), although no species are shared by any of the inventories.

In the case of the observations made by Guille (1965) during two dives performed in the southern continental shelf, the lack of quantitative data limits the potential comparisons that could be made between the community structure existing in 1964 and today's assemblages. The species listed by the author, however, do provide a fantastic opportunity to detect changes in the species composition of the continental shelf. It is very interesting to see that most species identified 40 years ago also appeared in the video images analyzed for this PhD thesis, suggesting that no considerable changes have occurred to the species catalogue of Cap de Creus continental shelf. The author accurately described the species observed in both dives, clearly indicating how fauna organized itself according to the dominant substrate types:

- Flat soft-bottom areas at depths of 120 m were characterized by several species also observed in today's mud/sand assemblages: the soft coral *Alcyonium palmatum*, the sea pens *Veretillum cynomorium* and *Pteroeides spinosum*, the anemone *Andresia partenopea*, the sea stars *Anseropoda placenta*, *Astropecten irregularis* and *Luidia clliaris* and the sea cucumber *Parastichopus regalis*. It is interesting to mention the high abundances detected by Guille (1965) of the sea urchin *Echinus acutus*, which is now a rare species at those depths, but rather abundant on shelf-break environments and in some areas of the submarine canyon. The author also described the presence of a massive aggregation of brittle stars of the species *Ophiothrix quinquemaculata* (*O. fragilis*), with densities above 200 ind·m^{-2}. Such aggregation also appeared in our survey, only a few kilometers north of their diving locations. The author states that the presence of such aggregation over the detritic substrates of the shelf was unexpected, since until then it had always been reported in littoral (muddy) detritic bottoms. It might be a complex task, but it would seem interesting to evaluate the capacity of this aggregation to relocate to newer areas in search of more suitable environmental conditions and trace its potential movement through time, as it has been done for other Atlantic populations (Blanchet-Aurigny *et al.* 2012).

- Sand and gravel areas at depths of 130 m characterized by the presence of echinoderm species also common in our video images: *Echinus acutus*, *Holothuria forskalii*, *Parastichopus regalis* and *Cidaris cidaris*. This species composition resembles that of Assemblage H.
- Outcropping rocks at depths of 120-130 m, characterized by a series of sponge species that the author relates to the 'Roche du large' assemblage described by Pérès & Picard (1964). Some sponge species identified by Guille (1965) are also characteristic of our Assemblage G (*Axinella damicornis*, *Poecillastra compressa*, *Suberites syringella*), but there are several mismatches, since we identifed *Dysidea* spp. and *Haliclona* cf. *elegans* as the most important species to define this assemblage, and the author listed *Geodia* sp. and *Calyx nicaeensis* as common species, which were not identified in our video images. It is interesting to mention the presence of the gorgonian *Eunicella stricta* as identified by the author (possibly *Eunicella cavolini*), which is rather rare in the ROV dives performed on the southern continental shelf.

Finally, changes in the faunistic composition of the submarine canyon can be evaluated, to a certain extent, thanks to (1) the observations provided by Reyss & Soyer (1965) from several submarine dives and (2) the extensive study published a few years later by Reyss (1971) with all the information available for macrofauna species that were collected in benthic sleds made down to 1000 m. From the 8 assemblages identified by Reyss in soft-bottom environments, 4 share some resemblance to the clusters derived from our ROV image analysis. Two of those assemblages, however, have not been observed at the same depths found by Reyss. The *Leptometra* assemblage, for instance, was only recorded on shelf depths (ca. 100 m), when Reyss found it dwelling on the canyon flanks, at 200-300 m depth. A similar situation occurs with the distribution of the brittle star *Ophiothrix fragilis*, which was found in areas of 300-350 m depth inside the canyon when we only observed it in a very particular spot of the continental shelf. The other two assemblages that can be linked to what was found in the ROV images are the association with *Brissingela coronata* and the bottoms with *Microcosmus* and *Echinus*, which are most possibly related to Assemblage H, mainly due to the presence of a large number of cerianthids and the sea urchins *Cidaris cidaris* and *Echinus acutus*. The remaining soft-bottom assemblages identified by Reyss cannot be linked to the groups found in our surveys, and this could possibly respond to a series of factors:

- Bottoms with *Kophobelemnon* and *Funiculina*. According to Reyss, this assemblage had its upper distribution at *ca.* 350 m depth, very close to our deepest sampling locations. It is very likely that this species can still be found inside the submarine canyon, but our sampling design did not cover enough area in the deepest part of the slope to detect it.
- Bottoms with *Caryophyllia clavus*. We also found specimens belonging to the genus *Caryophyllia* inside the submarine canyon, but their numbers were not large enough to constitute a distinctive assemblage. *Neopycnodonte* sp., an accompanying species according to Reyss, was also found in relatively high numbers in the ROV footage.
- Bottoms with *Salmacina dysteri*. There were a few locations where *S. dysteri* was observed forming relatively large aggregations, not sufficient to be detected by the multivariate analysis. It is very likely that this fragile organism has suffered from the fishing activities of the last 40 years, now possibly displaying lower abundances.
- Bottoms with *Hyalinoecia tubicola* and *Venus casina*. Both species are very small to be detected via imaging methods, limiting the capacity of ROVs to identify this assemblage.

In the case of hard substrates, Reyss & Soyer (1965) differentiated between morphological aspects of the mother rock and related them to specific fauna compositions. According to the authors, the terraces located on the southern flank of the submarine canyon held invertebrate fauna that resembles the cold-water coral assemblage, not only with *Madrepora oculata* and *Lophelia pertusa* in common, but also with individuals of the genus *Caryophyllia*, *Desmophyllum*, *Corallium*, *Dendrophyllia* and *Phakellia*. Reyss & Soyer (1965) identified the Sponges assemblage inside the submarine canyon, but also identified certain accompanying species that we have not observed in our video footage, mainly the black coral *Anthipates fragilis* and gorgonians of the genus *Primnoa*, together with some other sponge species for which we have no record.

Overall, it seems that there have not been many changes in the fauna composition of the shelf and canyon environments off Cap de Creus, at least at the level of the fauna catalogue. We cannot be sure how much human activities have modified the distribution of benthic assemblages, but it seems that they have not been transformed completely.

6.2 Environmental-vs-fishing effects on assemblage distribution

6.2.1 Factors explaining species distribution

The area surveyed is characterized by a very complex topography, including a submarine canyon that lays only 6 kilometres away from the coastline. The continental shelf is dramatically narrowed by the incision of the canyon in front of the promontory, widening towards the northern and southern sides. This particular composition has generated a wide array of benthic environments in a very restricted area, ranging from the flat muddy bottoms of the continental shelf all the way to the large rocky outcrops found in the vertical walls of the submarine canyon. The high spatial heterogeneity found in this area could largely explain the high number of megafauna species identified in the video images, since complex terrains can offer a large number of niches for different species to coexist in the same region, a relationship historically reported for terrestrial (Tews *et al.* 2004) and aquatic systems (Buhl-Mortensen *et al.* 2012).

At the scale of our study, the spatial structures displayed by the invertebrate megafauna mainly responded to different combinations of depth, substrate type and bottom current speed, as observed in the results of the dbRDA (Fig. 5.2.2). Depth can be considered the main environmental factor determining the distribution of the benthic fauna in Cap de Creus continental shelf and submarine canyon. Although it should not be considered a factor as such but an axis along which other environmental parameters organize themselves (e.g. temperature, water pressure, light availability, etc.), it has historically been regarded as the main structuring factor in littoral (Chappuis *et al.* 2014), sublittoral (Garrabou, Ballesteros & Zabala 2002) and deep Mediterranean ecosystems (Cartes *et al.* 2009). In our case, a clear zonation pattern could be observed at the level of the shelf break, with six assemblages found almost exclusively on the continental shelf, two spread between the lower shelf and the upper slope and one assemblage located exclusively inside the submarine canyon.

Substrate type, which was not completely independent from the dominant bottom current regime, also played an important role in the structuring of the benthic fauna. In fact, local values of alpha diversity (*expH'*) were mostly determined by substrate composition, with highest numbers of megafauna species found in areas where outcropping or suboutcropping rocks were dominant. In particular, maximum values of *expH'* were as high as 20 in rocky bottoms, close to 17 in mud/sand environments and they remained as low as 14 in coarser sedimentary areas dominated by large gravels and pebbles. Furthermore, not only diversity responded to changes in seafloor characteristics, but also certain benthic assemblages showed a marked preference for specific substrate types. This was the case of the Pennatulacean and the Crinoid assemblages, which were mostly found in mixed sedimentary areas (mud, sand and small gravels) with a high sand component. On the other end, the cold-water coral assemblage was exclusively observed over large rocks that outcrop from the steep walls of the southern lip of the submarine canyon. In this case, suspension and filter feeders (cold-water corals, brachiopods, oysters and encrusting sponges) that live attached to hard surfaces benefit from the high availability of food particles that results from the funneling of shelf waters towards the deep-sea through the submarine canyon (Pasqual *et al.* 2010).

In this regard, the assembly and maintenance of epifauna diversity in benthic communities has historically been linked to water flow dynamics, with communities exposed to higher current speeds generally displaying higher species richness and higher abundances (Palardy & Witman 2014). Increased water flows contribute to the removal of sediments and generate a greater flux of food particles (Genin *et al.* 1992), which favor the growth of suspension feeders due to increases in their capture rates (Best 1988). Not only for the cold-water coral assemblage, this also seems to be the case of the gorgonian *Eunicella cavolini*, which fully develops in areas of the shelf exposed to strong bottom currents, displaying local densities of up to 25 col·m^{-2}. The tridimensionality produced by the branches of this octocoral coupled with the great food availability provided by the bottom currents seems to be sustaining a very diverse assemblage in the flat areas of the continental shelf, which would otherwise be less species rich in absence of such ecosystem engineers.

Nevertheless, our results suggest that megafauna diversity does not increase lineary with bottom current speed. In fact, the very strong bottom currents that recurrently hit the shelf break area might be limiting particle deposition and favor the accumulation of large gravels and pebbles, mostly of a biological origin. The dynamic nature of this substrate type could be limiting the settlement of long-lived sessile species, which cannot attach to stable surfaces over which develop to large sizes. This results in an impoverished assemblage largely dominated by mobile fauna, mostly detritivores (sea urchins and holothurians) and burrowing anthozoans, such as small actinians and cerianthids.

6.2.2 Environmental vs fishing effects

Considering the survey area as a whole, fishing intensity was not identified as a very important factor in the distribution of the benthic fauna, certainly having less influence than depth and substrate type. This unexpected result can possibly be explained by the restrictive set of conditions required by the fishing fleet to develop their activities, in most cases in soft sedimentary areas of a flat nature. At the regional scale, the commercial fleet tends to accumulate their activity in patches of very high fishing intensity and generally leave unsuitable areas unfished (Stelzenmüller & Rogers 2008). This pattern was very clear in the case of Cap de Creus, where its complex geological setting (including a steep submarine canyon incising a topographically heterogeneous continental shelf) limits the activity of commercial trawlers to specific areas of the shelf (Fig. 4.13). For that reason, it is understandable that the effects of fishing practices in the diversity of species and assemblages must be low when considering the study area as a whole. However, we can extract a clear conservation issue from here: even though fishing is not the most important factor in the structuring of benthic assemblages, we can expect fishing intensity to be strikingly important in those areas where fishing occurs at very high frequencies.

Furthermore, there is room to expect that such areas might experiment strong changes in species composition and diversity after limiting bottom-trawling activities within the limits of the new MPA. At the same time, large areas of the shelf or inside the submarine canyon that are currently not affected by the activity of bottom trawlers should not reveal apparent changes after the implementation of such management measures. These aspects will be further discussed in Part 4, after considering the effects of fishing intensity on the structure and diversity of benthic assemblages dwelling in shelf environments.

6.3 Predictive mapping of megafauna diversity and benthic assemblages

The MSFD demands member states to develop specific actions in their territorial waters to prevent a further deterioration of their marine habitats, implementing an ecosystem approach to management that incorporates information about the biological components and the human activities that threaten their sustainability in the long run (Council of the European Union 2008). Although an ecosystem approach to management is not necessarily place-based, ecosystems are located in fixed areas and hence the spatial component is of critical importance when developing conservation strategies (Katsanevakis *et al.* 2011). In the specific case of Cap de Creus, the design and implementation of management plans will largely benefit from the maps generated in this PhD thesis, which provide detailed information about the distribution of the different benthic assemblages and bottom trawling activity, its most important human threat to date.

The application of management measures within the limits of the MPA of Cap de Creus will generate conflicts between users and policy makers. Hence, the predictive maps developed as part of this thesis should help managers to set conservation priorities, with the objective of protecting vulnerable marine ecosystems and achieving a sustainable use of the biological resources. Maps displaying the distribution of benthic communities, although very informative and easy to interpret, only provide categorical information and fail to provide evidence of the conservation status of each biological assemblage along its distribution area (Cogan *et al.* 2009). For this reason, this thesis provides a combination of benthic community and megafauna diversity maps, which offer complementary information that should help local authorities to define conservation actions aiming to preserve the biological diversity in those areas that require immediate attention.

6.3.1 Methodological remarks

The Random Forest (RF) algorithm was chosen to map the spatial distribution of species richness, diversity and all megabenthic assemblages identified in the video images. RF has gained popularity in the past decade as one of the most powerful tools to map shallow and deep-sea patterns of species richness and diversity (e.g. Robert *et al.* 2014) and also benthic communities (e.g. Gonzalez-Mirelis & Lindegarth 2012; Preez, Curtis & Clarke 2016). Beyond its very high classification accuracy, one of the advantages of predicting with RF is that it can be used to model continuous variables and categorical data (Cutler *et al.* 2007), which gave us the possibility to use the same algorithm to map all three biological parameters. The explanatory variables selected, together with the large amount of biological data that was extracted from the ROV images, seemed to provide a good training set for the 3 models to generate robust spatial predictions.

In the case of species richness and diversity, the models explained around 60% of the variability in both cases, generating relatively similar outcomes in which we can place a very high degree of confidence. In the case of benthic communities, the RF algorithm also performed well, with an overall accuracy of 81% (out-of-bag estimated error rate of 15.38%). Model errors differed depending on the assemblage predicted. In this regard, the gorgonian and cold-water coral assemblages were better classified than the rest, with an error rate of just 9% in both cases. This high accuracy most possibly relates to the restricted spatial distribution of both assemblages, which require very specific environmental conditions to fully develop. In the case of the Gorgonian assemblage, it was primarily found in shelf areas (*ca.* 100 m depth) where bottom currents are very strong all year round, with a characteristic mixture of sediments (sand, gravels and small rocks). The cold-water coral assemblage, on the other hand, was mostly observed in steep walls or large rocky outcrops inside the submarine canyon, preferentially at depths of 200-300 m.

Three other benthic assemblages, on the contrary, were misclassified in more than 20% of the situations. The Impoverished assemblage (Assemblage C) showed the highest classification error rate, being confounded by the Pennatulacean assemblage in almost 25% of the cases. This situation might be explained by the difficulty of distinguishing both assemblages in areas exposed to intermediate fishing intensities. In these cases, pennatulaceans might display very low-density values, making it very difficult for the model to discriminate between both assemblages. In the same way, the Sponges assemblage was confounded with the Pennatulacean assemblage in 15% of the situations. This is most possibly explained by the complexity of characterizing transition areas found in the boundaries of these two assemblages, in which classifying into separate groups can be a rather complex task.

Overall, we believe that some methodological cues helped towards achieving the good performance displayed by the 3 models. These cues are summarized as follows:

1. Spatial design

It is common practice to use a two-step survey methodology to obtain data that will later be used to map broad-scale areas of the marine benthos. The first step usually includes the creation of a physical map based on acoustic data (e.g. multibeam or side-scan sonar), followed by a second step aimed to survey the biological diversity, whose sampling strategy will be determined by the features revealed from the remote sensing data (Diaz, Solan & Valente 2004). In our case, a detailed bathymetric map was already available before the beginning of the research cruises, with only small areas of the submarine canyon and the outer continental shelf that required to be completed. This situation allowed us to plan the position of all ROV and manned submersible dives in advanced, prior to the surveys, contributing to better test the hypotheses set for this thesis.

Most limitations in the quality of predictive models are generally caused by a poor spatial accuracy or a low number of biological data points (Guisan *et al.* 2006). There is evidence that predictions based on few records perform worse than those undertaken on a large dataset, and that is because, with decreasing sample sizes, uncertainty increases, outliers gain importance and samples are insufficient to accurately describe highly complex ecological niches (Reiss *et al.* 2015). At the same time, to efficiently map the spatial distribution of the benthic fauna, the biological sampling not only requires a good geographic coverage, but it should also consider the existing topographic variation of the area under study, the different sediment types and the gradients between the major geological features (Buhl-Mortensen *et al.* 2015a).

To our surprise, the continental shelf off Cap de Creus is not as homogenous as continental shelves from other Mediterranean regions in terms of its faunistic composition: new species and assemblages were being identified as new dives were being performed. Since predictive maps rely on a strong relationship between the biological features and the environmental parameters, and since soft-sediment assemblages are known to occur in more than one soft-bottom substrate type (Hewitt *et al.* 2004), the presence of such high diversity of assemblages required a sampling strategy that covered as much area of the continental shelf as possible. In the end, a total of 60 ROV and submersible dives performed in a relatively small area were used in the modeling, which provided a full coverage of all 'depth-by-substrate' situations, ranging from the muddy flat bottoms of the shelf to the large rocky outcrops of the canyon. Consequently, the large sampling size generated was not only suitable to thoroughly characterize the whole set of biological assemblages, but also sufficiently spread along the shelf and the submarine canyon to efficiently model their spatial distribution.

It should be noted that some specific locations of the southern continental shelf still remain to be explored. This was caused by the intense activity of artisanal fishermen in these areas during summer months, when most of the surveys took place. The amount of time that long-lines and trammel nets were set made it impossible for the ROV to be submerged with a full guarantee of not getting entangled. Future cruises should aim to survey such areas in order to corroborate the capacity of our models to successfully predict in areas where no data is currently available.

2. Modeling approach: assemble first, predict later

One aim of this PhD thesis is to identify patterns in species associations (Part 1) and then relate them to abiotic and human factors to model their spatial distribution (Parts 2-3). This two-stage approach, defined by Ferrier & Guisan (2006) as 'assemble first, predict later' is one of the most widespread methodologies used to map community types and seabed habitats worldwide (Brown *et al.* 2011). Its application with our dataset incorporated two main advantages with respect to top-down approaches, in which acoustically-derived habitat groups are first produced and then related to community composition. On the one hand, it can be applied regardless of differences in data resolution (Hewitt *et al.* 2004), which is very convenient when incorporating oceanographic and human factors into the model. On the other, because it does not rely on a previous classification of the acoustic data into habitats, reducing the uncertainty produced by the lack of fidelity of certain biological communities to distinct acoustic habitat groups (Hewitt *et al.* 2004). This is especially evident in the case of sedimentary environments, like those found on the continental shelf off Cap de Creus, where several community types were found across similar sedimentary habitats.

3. Size of sampling unit and map accuracy

The technique used to analyze the ROV images (i.e. splitting the continuous record into discrete sampling units of an equal area) gave us the possibility to determine fine-scale relationships between the observed fauna and the different environmental parameters, which complemented the large-scale picture provided by the distribution of the 60 ROV dives. Indeed, the set of images generated almost 2000 different 5m^2-sampling units, which provided a large testing ground to determine most of the assemblage-environment relationships required to efficiently map the distribution of species and assemblages. The string of contiguous samples, however, makes data not independent, which could influence the performance of the models. Even though we were aware of the problems that spatial autocorrelation can generate when performing statistical inference, we did not specifically account for the effects of autocorrelation in our models. It is very difficult to determine how much this might have affected the predictive accuracy of the models, but it is possible that the inclusion of side-by-side sampling units may have artificially increased the strength of the relationships between the biological variables and the abiotic factors (Legendre 1993). It should be noted that, as argued by Gonzalez-Mirelis *et al.* (2009), the problem of autocorrelation should not be of much concern when generating predictions to map biotope distributions since predictive mapping uses the mean and does not look at the variance of the distributions, which is less affected by autocorrelation. Given the difficulty of sampling at certain depths and the cost derived from ROV surveys on board of research vessels, we decided to use all available data to run the RF algorithm, since a selection of samples to reduce autocorrelation could have also reduced the predictive capacity of the model for less represented assemblages.

4. Environmental layers

The results of Part 2 showed that geological parameters alone proved insufficient to adequately determine the spatial distribution of the benthic fauna in our study area. We were convinced that the incorporation of other layers into the models, including oceanographic and human factors, would largely improve their predictive ability. In our case, the predictive mapping of megabenthic diversity and its organization in discrete assemblages was mostly based on a set of geomorphological layers that derived from the swath bathymetry data, including slope, orientation, relative position (topographic position index) and terrain variability (terrain ruggedness index). All those layers were directly calculated from the bathymetry raster at a resolution of 10x10 meters. The models also included a seabed backscatter map, which was constructed using the strength of the echoeing signal that derive from the swath bathymetry, and a map displaying the distribution of the different sediment types provided by TRAGSA.

We believed that incorporating oceanographic data to the models would contribute to better predict biological parameters. This approach is relatively common in benthic habitat mapping, and there are studies that include wave action, water currents or tidal stress in their models, both for shallow (Bekkby, Rinde & Erikstad 2008; Robinson *et al.* 2011a) and deep-sea environments (Davies *et al.* 2008; Leverette & Metaxas 2005). In our case, it seemed reasonable to take into account the strength of the dominant bottom currents, which recurrently hit the southern part of the Gulf of Lions, reaching considerable speeds in autumn and winter months (Petrenko, Dufau & Estournel 2008). Bottom currents resuspend sediments and enhance the transport of food particles from the shelf towards the submarine canyon (Palanques *et al.* 2009) and could ultimately favor the development of dense assemblages of benthic suspension feeders (Genin *et al.* 1992), becoming a very important factor in the distribution of benthic fauna.

The inclusion of bottom current data in our models was not a straightforward task due to the spatial and temporal variability displayed by bottom current speeds in Cap de Creus and the horizontal resolution of the oceanographic layer provided by the SYMPHONIE model (Marsaleix *et al.* 2008), which has a minimum resolution of 700 m in areas adjacent to Cap de Creus. It is common that oceanographic datasets display coarser resolutions than geological or depth data, with patterns defined at scales larger than hundreds of meters (Kenny *et al.* 2003). In practical terms, and to overcome this situation, the spatial resolution of the bottom current raster layer was resampled to meet the 10x10 m grid provided by the bathymetry layer, which proved to be a better solution than resampling the bathymetry data up to the grid size provided by the bottom currents. The resampling was performed to 3 different current layers to account for the temporal variability displayed by this oceanographic variable in Cap de Creus: average current speed (calculated for a 4-year period), maximum current speed recorded in a single month and standard deviation of monthly averages. Such layers generated a good overview of how bottom currents spatially distribute in the study area during different years, not only showing what areas are subject to stronger or weaker currents, but also providing information about the scale of the fluctuations that occur between different months (see Fig. 4.11).

The availability of data derived from the Vessel Monitoring System (VMS) also seemed to be a good opportunity to incorporate in the models the long-term effects of bottom trawling, a very destructive human activity that has possibly altered the distribution of benthic fauna in many areas of the world (Watling & Norse 1998). We believed that using the distribution of bottom trawling intensity as a raster layer could improve the prediction accuracy of the models, especially in those areas that have historically suffered from high fishing intensities, where differences in species richness or abundance could not possibly be fully explained by environmental factors. Estimates of fishing effort derived from VMS data are increasingly common in studies that aim to determine the effects of bottom trawling, but we have failed to find other studies that incorporate this information in predictive models of faunal distribution. Since VMS data is becoming more accessible to scientific organizations and marine managers (Lee, South & Jennings 2010), it seems reasonable that this information should feed predictive maps of benthic species and assemblages, especially for those areas currently exposed to these type of fishing practices.

6.3.2 Distribution of megafauna diversity and benthic assemblages in Cap de Creus

Megafauna diversity

This PhD thesis provides the first attempt to model the distribution of benthic megafauna diversity in the offshore marine area of Cap de Creus, which soon will become a new MPA. Results of the predictive model show that diversity does not distribute homogenously along the study area, as it could have been expected. The RF model predicted three main areas that can be considered hotspots of megabenthic diversity, both in terms of species richness and Exponential of Shannon diversity (Fig. 5.3.1). They corresponded to three different megafauna assemblages: shelf gorgonians, sponge grounds and cold-water corals. All of these areas still display a considerably good conservation status, and hence, should be included as priority areas for conservation if biodiversity is to be maintained within the limits of the future MPA.

<u>Gorgonian assemblage.</u> Areas of high diversity associated to *E. cavolini* were predicted along a narrow strip of around 5 km in length on the northern side of the cape, following the 100 m isobath. This area possibly offers the best conditions for this assemblage to fully develop, since it is characterized by a mixture of substrates, strong bottom currents all year round and low fishing intensity. The presence of small rocky outcrops in between sands and coarse biogenic gravels are possibly of key importance in promoting the recruitment of gorgonian larvae that will later develop into highly dense aggregations (Weinbauer & Velimirov 1996). The increased growth rates displayed by *E. cavolini* in areas exposed to strong bottom currents (Velimirov 1975) might have favored the development of stronger branches with more polyps per surface area (Velimirov 1976), which may secondarily promote the settlement of a rich associated community that benefits from their tridimensional structures (Ponti *et al.* 2016). The video images showed a very rich associated community in terms of taxonomic groups and growth forms, with fauna developing both attached to the gorgonian branches and as free-living organisms. The number of organisms hiding or directly

growing on the branches was very high, and comprised organisms from a wide variety of taxa, including anthozoans, sponges, ascidians, crustaceans, mollusks and echinoderms.

Sponges assemblage. A similar situation was found in areas at the end of the continental shelf, in the southern side of the submarine canyon, where the Sponges assemblage developed some very diverse hotspots. In this case, diversity values mostly corresponded to the large variety of sponge species that could be identified, some of which reached relatively large sizes. The good conservation status of this assemblage seems to be the consequence of the low degree of anthropogenic pressure that affects this part of the continental shelf, where disturbance by bottom trawling is almost negligible. The large number of rocky outcrops that emerge in the area possibly limits the capacity of bottom trawlers to operate in the area, providing a natural barrier that protects these fragile assemblages from their most important threat to date (Maldonado *et al.* 2016).

Cold-water corals. The third biodiversity hotspot was predicted in a narrow band along the 200-300 m depth isobath inside the submarine canyon. It is in these areas where the cold-water coral assemblage possibly finds the most suitable areas to develop. The model predicted this assemblage both on the northern and the southern wall of the submarine canyon, although there is only data available for southern side. It would be very interesting to perform new surveys to determine the real distribution of this assemblage in the northern wall, validating the predictions made by the model for areas that have not been yet surveyed.

As expected, substrate type was the most important factor determining the distribution of species richness and megafauna diversity in Cap de Creus shelf and canyon areas, followed by depth and average current velocity. Maximum diversity values were registered in samples characterized by the presence of suboutcropping and outcropping rocks that are subject to moderate bottom current conditions. This very much responds to (1) the possibility of sessile species to attach to stable surfaces, which gives them the possibility to fully develop and generate the conditions to act as nursery areas, (2) the higher food availability that bottom currents provide to these shelf- or slope-dwelling species and (3) the lower likelihood that hard substrates can be affected by commercial fishing activities.

Interestingly, fishing intensity did not have an equivalent effect on species richness as it did on diversity, being a more important factor in the modeling of the exponential of Shannon diversity. This result could indicate that fishing practices may not drastically reduce the number of species present in soft sedimentary areas, but might generate a change in species composition. It is possible that species more resistant to the swipe of trawl nets could be favored by intermediate levels of fishing intensity, which could contribute to a shift in their abundance, favoring their dominance in areas that were previously characterized by more sensitive species. These aspects will be further discussed in Part 4.

The three assemblages described above rely on the tridimensional structures created by very slow growing organisms. From a management point of view, it is important to recognize that such long-lived species are very vulnerable to human activities, especially bottom trawling (Clark *et al.* 2016). Since their recovery would require tens of years, if it ever happens (Althaus *et al.* 2009; Williams *et al.* 2010), knowledge about their exact distribution should encourage local managers to take priority actions before any further damage is caused.

Benthic assemblages

Contrary to benthic diversity, there have been attempts to determine the spatial distribution of benthic assemblages in Cap de Creus marine area. The early works of Desbruyères *et al.* (1972) and Reyss (1971) focused on the continental shelf and submarine canyon respectively, and more recently Sardá *et al.* (2012) provided the spatial distribution of the benthic communities inside the limits of the littoral MPA, in shallow bottoms down to 60 m depth. This thesis updates the distribution maps provided almost 40 years ago using new samples and modern modeling techniques, but perhaps most importantly, it also provides the opportunity to expand the current littoral map to include areas that will soon be included in the offshore MPA of Cap de Creus (BOE 2014a).

The resulting distribution map bears little similarity with those provided by Desbruyères *et al.* (1972) and Reyss (1971). In both cases, however, the differences detected must be primarily attributed to methodological aspects and it would be very adventurous to infer changes in the distribution of the benthic fauna since the 70s merely using the data available. The fraction of the biota detected in historical surveys and the approach employed to map its distribution make comparisons very difficult. The case of Desbruyères *et al.* (1972) is particularly evident, since a top-down approach was used to determine the different benthic assemblages, in which distinct sediment types were first identified and then related to the dominant fauna, collected in their case using Van Veen grabs. In fact, Desbruyères *et al.* (1972) identified three different communities on the continental shelf off Cap de Creus (Fig. B3.1), whose spatial limits are coincident with changes in the sediment type that are still detectable today. The community structure identified in the ROV video images analyzed in this PhD thesis is relatively more complex than that of Desbruyères *et al.* (1972), with more assemblages than those previously identified.

A clear zonation pattern can still be observed as depth increases. Such pattern relates to the physical gradients produced by depth, which have long been identified as a strong structuring factor in continental margins worldwide, where assemblages tend to occupy restricted depth bands (Carney 2005). In fact, the predictive model identifies depth as the most important factor determining the spatial distribution of the benthic assemblages in Cap de Creus area: the Pennatulacean and the Impoverished assemblage distribute along the shallow continental shelf, the *Lanice conchilega* assemblage mostly occupies the middle shelf and the sponge assemblage is restricted to the deepest part of the shelf and upper slope. Moving further deep, the cerianthid assemblage can be found along the shelf edge and expands to the lowest part of the submarine canyon, mostly in flat or slopping areas. Finally, the cold-water coral assemblage mostly distributes along a strip between 200 and 300 m depth, although it can potentially appear in deeper areas if substrates are suitable.

The new map showing the distribution of the main benthic assemblages will be of great help for stakeholders when defining the management plan to be implemented in the future MPA of Cap de Creus. Furthermore, the distribution shown here should be used as a baseline to detect future changes in the distribution of benthic assemblages once management measures are put into practice.

6.4 Fishing impact on benthic megafauna

The Marine Strategy Framework Directive (MSFD) not only demands Member States to identify the essential biological features and characteristics of its marine waters (Parts 1-2-3-6 of this PhD thesis), it also requests a thorough analysis of the predominant pressures and impacts that can threaten their sustainability in the long run (Article 8, Council of the European Union 2008). Bottom fishing using mobile gears has been regarded for decades as one of the most important threats to the marine benthic realm (Watling & Norse 1998). This activity is currently practiced in most continental shelves of the European continent, with the majority of the fishing effort taking place below 200 m (Eigaard *et al.* 2016). Cap de Creus is not an exception, and bottom trawling could potentially be considered one of the most important sources of physical impact to its continental shelf.

The detrimental effects of bottom trawling activities over the marine seabed are numerous and have historically been evaluated at many different levels. In general terms, it has been reported that commercial fishing practices using mobile gear produce changes in seabed morphology (Puig *et al.* 2012), sediment dynamics and water turbidity (Martín *et al.* 2014a), as well as a severe reduction (or even depletion) of commercial fish stocks (Myers & Worm 2003) and a direct removal of benthic sessile species (Clark *et al.* 2016; Hiddink *et al.* 2017). It is very likely that shelf areas in front of Cap de Creus, where bottom-trawling intensities are highest, may have experienced changes in the composition and texture of the seabed over the years, as it has historically been documented for many other areas of the world (Jones 1992). The constant plowing of the seabed by the trawl nets reduces the structure above and below the sediment surface producing a severe habitat homogenization (Thrush & Dayton 2002), as it has been reported for deeper areas of the Mediterranean Sea, where intense trawling activity has smoothed the morphology of the sea floor to the extent of reducing its original complexity (Puig *et al.* 2012).

Knowing that the negative effects of bottom trawling on the marine benthic fauna have been proven in numerous studies (see Hiddink *et al.* 2017 for an extensive review), this part of the thesis aimed to determine the specific relationship between the fishing activity of the local fleet and the structure, abundance and diversity of the megafauna dwelling in Cap de Creus continental shelf, using both underwater video images and the intensity of the trawling activities based on the Vessel Monitoring System (VMS).

6.4.1 Methodological remarks

Bottom trawling intensity in Cap de Creus area was calculated following the speed-based approach described by Lee *et al.* (2010), a point summation method that produces a relatively high (spatial) resolution of the fishing effort when data from many vessels can be accumulated over a long period of time, whether a whole fishing season or even several consecutive years. This was the case of our study, in which VMS data was analyzed for a 5-year period (2007 to 2012) from a fleet composed of 44 vessels. Such standardized methodology is currently being used in many other areas of the Mediterranean (Demestre *et al.* 2015; Maina *et al.* 2016) and Atlantic Ocean (Eigaard *et al.* 2016), and was selected due to the low impact that data filtering has on the calculated distribution and intensity of the fishing effort (Jennings & Lee 2012). It is true that determining fishing grounds based on vessel speed could generate certain errors in the final outcome due to misclassification. Nevertheless, the use of observer data has provided estimates of classification accuracy around 80 to 90% in the case of towed gears (Skaar *et al.* 2011), suggesting that a high degree of confidence can be placed in this type of analysis.

The lack of observer data to validate our results was compensated with a simple correlation between the number of trawl marks per 100 m that were observed in the video images and the estimated fishing effort. Although trawl marks might remain visible to sonographs for at least a year after trawling occurs (Palanques, Guillén & Puig 2001), the number of marks identified in the video images was relatively small in relation to the amount of area explored, limiting our capacity to establish a robust statistical analysis between the fishing intensity derived from VMS data and the marks observed. Instead, we simply superimposed the observed marks over a georeferenced map of the fishing effort (see Fig. 5.4.1). Such image showed that most trawl marks were identified in areas considered of high fishing intensity, reassuring our confidence in the VMS results.

All biological surveys used to evaluate the impacts of bottom trawling on benthic megafauna (ROV image acquisition) were performed during the same time interval for which VMS data was analyzed, with the aim of maximizing the temporal match of all data included in the statistical analyses. Furthermore, not only this temporal match seemed necessary, but also the spatial resolution at which the fishing effort was evaluated. We believed that fishing intensity had to be given in a scale as close as possible to that of the image analysis in order to reduce possible artifacts in the statistical outcomes. Studies that intend to determine fishing grounds at national and regional levels generally use grid sizes of tens of kilometers (e.g. 21 km for UK waters; Jennings & Lee 2012), but the relatively small size of our study area implied that the limits of the fishing grounds had to be determined with upmost accuracy. Although it has been reasoned that VMS data should be evaluated in a grid size larger than the maximum distance that a vessel can travel between 2 consecutive VMS points (Gerritsen, Minto & Lordan 2013), such an extensive grid size would incorporate a very large bias in our study, especially when determining the limits between areas of different fishing intensity. For this reason, and taking into account that data was gathered over a period of 5 years, a second filtering was performed over the VMS data, leaving only one position per vessel per day. With this second filtering we ensured that the estimated fishing effort could not be affected by the time elapsed between signals, and areas of high fishing intensity could be equally detected. The resulting map must be regarded as a relative measure of fishing intensity rather than absolute values, since with the available VMS data it is very complex to determine the average number of times per year that trawl nets sweep the seabed (Gerritsen *et al.* 2013).

The final grid size used to calculate fishing intensity was selected after some trials using different grid sizes, from tens of meters to more than a kilometer. Large grid sizes incorporated a proportionally higher number of vessel detections per pixel, hence reducing the potential error made in areas of low fishing intensity. But their use incorporated some uncertainty when aiming to identify the boundaries between areas of high and low fishing intensity, something that could potentially generate an even larger bias in the successive statistical analyses. Very small grid sizes, on the other hand, generated a large number of artifacts inside areas of high fishing effort (identified as grids with no data), suggesting small no-fishing areas surrounded by high fishing intensity grids. This error was most possibly caused by the type of filtering applied to the data and the low position accuracy of VMS points: vessel positions only incorporated up to 3 decimal values which, in our study area, corresponded to a minimum distance between points of *ca*. 65 meters. In the end, we determined that a grid of 250x250 meters produced the most accurate delimitation of the trawling grounds in Cap de Creus with the final idea of determining the potential effects of varying levels of fishing intensity on the distribution and diversity of the benthic fauna.

6.4.2 Spatial distribution of the fishing effort

In the case of Cap de Creus, the results of the VMS data analyzed for the years 2007 to 2012 (Fig. 4.12) indicate that fishing practices were mostly restricted to soft bottom areas between 80 and 125 meters, although a very high intensity area exists inside the submarine canyon (*ca.* 500-700 m). This area is most possibly related to the catch of the red shrimp *Aristeus antennatus* (Martín *et al.* 2014b), a commercial species with a very high economic value in the Catalan market. As expected, the set of environmental parameters that favor the commercial activity of trawlers off Cap de Creus corresponds to large flat areas dominated by mud and sand, which in our study area are mostly located on the shallow and middle continental shelf. In particular, it seems that fishermen have historically learnt to avoid areas where the seabed displays high rugosity or where the presence of rocky outcrops could potentially cause the loss of nets due to entanglement. Indeed, the local fleet seems to behave in a similar manner to that of other regions in the Mediterranean Sea, where the highest efforts are generally recorded in muddy-sand environments (Demestre *et al.* 2015).

6.4.3 Effects of varying levels of fishing intensity over benthic megafauna

Ideally, long-term modifications of the ecosystem produced by bottom trawling should be experimentally tested following a Before/After and Control/Impact experimental design, which requires biological data to be collected before and after bottom trawlers begin to operate in an area. There is limited availability of potential situations where such an experimental design could be implemented since, in most coastal zones, few areas are currently free from trawling to act as control sites (Gray *et al.* 2006; although see the example of a BACI design to test the effects of trawling on the Scotian Shelf in Kenchington *et al.* 2006). Furthermore, quantitative information about megafauna species before the trawling fleet began to operate is generally unavailable, as it is the case for Cap de Creus. Hence, we can only infer the effects of bottom trawling activities by comparing areas with different fishing regimes, something that limits the robustness of the results obtained. Consequently, the experimental design of Part 4 had to be planned and implemented following a correlational approach, in which conclusions are drawn from comparisons between areas with different fishing intensities, as identified from the VMS data.

Effects of bottom trawling activity on megafauna species richness and diversity

There seems to exist a rather clear pattern of decline in the number of species dwelling on the soft bottoms of Cap de Creus continental shelf as fishing intensity increases, a response that is similar to that of the biological diversity (see Fig. 5.4.2). This expected result is in consonance with most studies that have assessed the effects of bottom trawling activities on benthic megafauna, in most cases reaching a similar outcome (e.g. Veale *et al.* 2000; Buhl-Mortensen *et al.* 2016; Yesson *et al.* 2016). In Cap de Creus, areas classified as high-fishing intensity held very few species per sampling unit (or none at all), indicating that high fishing efforts could be directly related to a severe impoverishment of the soft-bottom benthic community. Such a reduction in the average number of species could be the result of the homogenization of the seabed produced by bottom trawlers (Dayton *et al.* 1995), which could be limiting the habitat complexity of the continental shelf. At the same time, the removal of structure-forming species might generate a secondary effect, further reducing species richness due to their capacity to provide shelter and surface for attachment to a relatively diverse associated fauna (De Clippele *et al.* 2015).

It is interesting to point out that some very low diversity values were also recorded in areas that, according to our analysis, had not been affected by commercial fishing during the 5-year period that was analyzed. Such differences could either be explained by (1) the possible inability of our analysis to detect with high accuracy areas of low fishing intensity due to the filtering applied to the data, or (2) to the natural variability existing in the composition of shelf-dwelling megafauna assemblages in undisturbed areas of Cap de Creus. We cannot be completely sure that other factors that were not considered in our analysis, besides depth and substrate type, could also be playing a role in the structuring of the benthic fauna. Nevertheless, the overall trend leads towards a reduction in the average number of species and its associated diversity as fishing intensity increases.

It should be mentioned that one of the major drawbacks of our analysis relates to the relative measure of fishing effort that could be derived from the VMS data, which limits our capacity to predict how severe the effects of trawling can be depending on the number of days an area is being trawled. It would be very interesting to reconstruct the tracks performed by the vessels to obtain a more precise idea of how much effort benthic assemblages are supporting, but interpolating vessel tracks using the current 2-hour time interval between VMS records could lead to very inaccurate results (Gerritsen *et al.* 2013).

Effects of bottom trawling activity on the structure of the megafauna community

Beyond the observed reduction in the number of species present, the dbRDA analysis showed that fishing intensity could also be directly controlling the species composition of the megafauna community, since a clear substitution of the dominant fauna can be observed as fishing effort increases. This change in the dominance of certain taxa might be directly related with the capacity of species to recover from trawling disturbance: vulnerable species possibly tend to reduce in numbers or even disappear as trawling intensity increases and more resilient or robust taxa might be favored by such activities (Buhl-Mortensen *et al.* 2016). Specifically, soft-bottom areas of the shallow continental shelf off Cap de Creus catalogued as low fishing intensity were characterized by different species of pennatulaceans and alcyonaceans (i.e. *Pteroeides spinosum*, *Cavernularia pusilla*, *Pennatula rubra* and *Alcyonium palmatum*), which locally reached relatively high densities. Although data on the longevity and life-history traits of these species is scarce, their erect position above the sediment (which makes them very catchable; Jørgensen, Planque & Thangstad 2016) and their year-round reproductive cycle (which may limit their recovery capacity; Edwards & Moore 2008) suggest that their resistance to the constant passing of trawl nets might be limited.

Most experimental studies have shown that bottom trawling has favored assemblages dominated by small benthic organisms with short life spans to the detriment of large long-lived species (Kaiser 1998). This change in the composition of species could also lead to a change in the functional structure of the community, with filter-feeding and sessile organisms being relatively more abundant in areas less trawled in comparison to areas more exposed to bottom fishing, which are dominated by mobile animals, infauna species and scavenging invertebrates (Tillin *et al.* 2006). Such changes could be explained by two main reasons: first, because small short-lived species might be able to withstand the mortality caused by the nets and second, because they could end up benefiting from a reduced competition from other larger species that become depleted (Jennings *et al.* 2001). In the case of Cap de Creus, as fishing intensity increases, the contribution of anthozoans to the community composition gets drastically reduced, being replaced by other species, most of which are polychaetes. Indeed, the two dominant polychaete species observed in areas of medium and high fishing intensity (*Lanice conchilega* and *Sabella pavonina*) could benefit from moderate trawling disturbances due to their capacity to survive the swipe of the nets by burying themselves in the sediment (Hutchings 1998).

Effects of bottom trawling activity on key structural species

With a view to finding indicator species sensitive to bottom trawling activities, we evaluated the abundance of key soft-bottom megafauna organisms to varying levels of fishing intensity. We initially selected those species that ranked highest in the ordination used to test the effects of trawling on the structure of the benthic community. From this pool of species, only those that completed their whole life cycle in fine-grained sediments were chosen, aiming to avoid potential biases due to sediment characteristics. The gorgonian *Eunicella cavolini* and the serpulid *Protula tubularia* were removed from the list since their larvae requires a hard surface for attachment to further develop, whether an exposed rock, a small stone or even a broken shell. A total of 7 species finally remained, from which two clear patterns could be observed. As expected, a set of species showed a negative response to increasing levels of fishing intensity (i.e. lower average density values as trawling effort increases), but rather unexpectedly, two other species showed higher abundances in areas of medium and high fishing activity.

<u>Negative response to trawling activity.</u> Among the species that showed a decline in their density as fishing intensity increased, the clearest pattern was displayed by two pennatulaceans (*Pteroeides spinosum* and *Cavernularia pusilla*), which had average densities four times lower in highly disturbed areas in comparison to *no-fishing* situations. Two other anthozoans (*Alcyonium palmatum* and *Pennatula rubra*) also showed a similar trend, with their lowest densities recorded in areas of high fishing activity. In the latter case, however, maximum densities were not registered in unfished locations, but in areas of low fishing intensity. Although pennatulaceans are broadly found at low densities on continental shelves, they have the capacity to form dense aggregations when undisturbed (Ruiz-Pico *et al.* 2017), which could partly explain this result. Such an aggregative behavior could also be behind the strange absence of the crinoid *Leptometra phalangium* in unfished areas. At the same time, there also exists the possibility that some areas registered as untrawled might have been affected by some isolated trawling activity prior to sampling, which could have gone unnoticed in the VMS analysis. In any case, all groups that showed a negative response to fishing have previously been classified as taxa with a very high risk of being caught by trawl nets due to their size and erect position (Jørgensen *et al.* 2016). Furthermore, with the exception of *Cavernularia pusilla*, all other species have been reported as trawling by-catch in the continental shelf of the Gulf of Lions, some of which being relatively frequent and abundant, such as *L. phalangium*, *P. spinosum* and *A. palmatum* (Mallol 2005).

<u>Positive response to trawling activity.</u> In contrast, two polychaete species showed a clear increase in their abundance with fishing intensity. This unexpected result highlights the capacity of certain

species to succeed in disturbed environments, where they would otherwise appear as infrequent or rare. This is not the first time that these species have shown resistance to bottom trawling activity. *Lanice conchilega*, for instance, has proven its capacity to resist the physical disturbance produced by trawl nets in intertidal flats, with their population not collapsing until a continued high frequency of fishing pressure is reached (Rabaut 2009). It is very likely that intermediate levels of disturbance favor the development of this species, which can hide under the sediment when nets swipe the sea floor, finding their competitors removed after the disturbance. Further increases in the level of trawling, however, could generate a very unstable environment for this species to survive, shown as a decrease in their density in areas of highest fishing activity. In the case of *Sabella pavonina*, its highest densities were displayed in areas with maximum levels of fishing intensity. Such a result might be explained, not only by the capacity of this species to bury under the sediment, but also by their ability to replace lost parts through the regeneration of body segments (Berrill 1931), which could be an efficient strategy to withstand such high levels of disturbance.

It would be particularly interesting to take this analysis one step further and compare the average size of these 7 organisms and compare it for each level of fishing intensity. Such analysis could help determine whether trawling activities, beyond the effects described above, are favoring smaller species or limiting the growth of larger species, as predicted by the ecological theory (Kaiser 1998). Furthermore, if body dimensions could be used to estimate species wet weight (as proposed by Durden *et al.* 2016), this analysis could also be used to estimate average species biomass at different levels of fishing intensity, providing an idea of how much biomass is lost due to bottom trawling along the whole study area. This approach might not be feasible at the level of the community, but could potentially provide reference for a certain number of species, and maybe allow for comparisons between other areas explored with ROVs.

6.4.4 Selection of descriptors

Several ecological indicators have historically been employed to assess the effects of fishing activities on commercial stocks to provide tools to improve fisheries management. Some of these indicators have included species richness of the fish community, abundance of economically valuable species or the average length of fish populations (see Rochet & Trenkel 2003 for an extensive review). The incorporation of ecosystem-based management principles in the MSFD implied that assessments of the ecosystem's condition made under such regulation should also consider the benthic ecosystem (Borja *et al.* 2010). Although we are aware that there is no biological indicator capable of providing a complete image of how the benthos changes due to human influence, it seems reasonable that indicators at the community level of organization are probably the most reliable to detect the effects of fishing activities, or their recovery from them (Fulton, Smith & Punt 2005). However, due to the complexity of providing raw community data as a whole, we opted for a set of indicators that are, not only sensitive to different levels of trawling intensity, but also simple to identify, count, measure and interpret, as well as easier to communicate to political or social agents, following the recommendations given by Dale & Beyeler (2001). The indicators selected for the monitoring program proposed in this PhD thesis can be grouped into two distinct typologies: (1) those that provide information at the community level and (2) those that provide information for key structural species.

In the first group we decided to include both species richness and a measure of biological diversity, in this case the exponential of Shannon entropy. Both species richness and diversity have historically shown strong sensitivity to increasing levels of fishing intensity (Veale *et al.* 2000; Hiddink *et al.* 2006), and their values are easily interpretable. Furthermore, their use in a monitoring program does not require trained taxonomists, since there is no need to identify all organisms observed in the video images down to species level. In practical terms, the observer should only need to determine the number of species present, regardless of the taxa they belong to. This intends to make all video analyses simpler and faster.

In the second group, the selection was made following the considerations suggested by Jones & Kaly (1996), who defined the most important characteristics that organisms used in a biomonitoring program should exhibit. At this point, we made a clear difference between organisms that displayed a negative and a positive response to increasing levels of trawling intensity. Two species were selected between the pool of organisms that showed a reduction in their density values with fishing: *Pteroeides spinosum* and *Alcyonium palmatum*. These two species are easily identifiable in underwater images, are relatively abundant in continental shelf environments of the Gulf of Lions, display very low mobility, have populations that remain relatively stable through time in absence of disturbance, are relatively long-lived and are ecologically important due to their size and tridimensional structure. On the other hand, since only two species showed an increase in their abundance with trawling (*Lanice conchilega* and *Sabella pavonina*), their selection was relatively simple. It is true that these two species might not display all the characteristics required for an indicator species as defined by Jones & Kaly (1996), but they can be regarded as indicators of trawling activity, and an increase in their numbers in areas closed to fishing could be indicative of a potential activity not rigorously regulated.

6.5 Monitoring program for Cap de Creus offshore MPA

To accomplish the objectives set by the adaptive management described in the MSFD, continuous monitoring programs must be launched in the different MPAs in order to detect temporal changes in the elements that constitute the marine ecosystem (Council of the European Union 2008). The launching of a monitoring program, however, is not a straightforward task. First of all, because marine systems are complex entities where multiple interactions occur, not only among organisms but also between them and the environment, including human activities. We should not expect that all taxa identified on the video images from Cap de Creus will fluctuate in the same manner after management measures are put into practice, and hence, the evolution of the community cannot be reduced to the monitoring of one simple quantitative indicator. Secondly, because bathyal and deep-sea benthic ecosystems are very stable, so we should not expect sharp changes to occur in short periods of time. And thirdly, because the factors that drive such changes are multiple, and separating the effects produced by management measures from the remaining environmental factors is not a simple assignment and requires complex experimental designs and sophisticated statistical techniques.

In the case of Cap de Creus, it could seem rather disappointing that the monitoring protocol presented in this PhD thesis is merely based on two synthetic indexes (species richness and exponential of Shannon diversity) and the abundance of 4 indicator species. And even more so after having analyzed over 30 km of seabed and identified almost 100,000 individuals that belong to 170 morphospecies. Undoubtedly, many other changes will occur to the composition and abundance of the benthic fauna of Cap de Creus once the MPA is established. But the complexity of the responses might hinder the hypotheses that can be formulated, which should be based on a realistic experimental design that allows changes in the benthic ecosystem to be quantitatively detected. Bearing that in mind, we believe that one of the strongest points of our proposal is not only that it is based on specific hypotheses that can be statistically tested, but also the simplicity and replicability of its experimental design, fulfilling one of the most important requirements of an effective monitoring program: its viability.

To understand the difficulties that a BACI experiment based on data collected by ROV video recordings must face and to recognize the simplicity of the experimental design proposed, we should look at the demanding methodological filters that were incorporated at all levels of the protocol. We will discuss them separately in the following sections.

6.5.1 Spatial design

The lack of a clear spatial delimitation of what will be the future no-take zone inside the boundaries of Cap de Creus MPA implies that our spatial design had to be based on the spatial zonation suggested by Gili *et al.* (2011). We have not implemented their design, but further improved their proposal by incorporating the results obtained in this PhD thesis. The limits of the no-take zone proposed in this document (dotted line in Fig. 5.5.1) are based on a set of assumptions, summarized as follows:

- A representation of all invertebrate assemblages identified in the video images was incorporated inside the no-take zone, both for the continental shelf and the submarine canyon.
- The shape of the polygon was kept as simple as possible, maximizing the compactness of the assemblages to be protected and making them as coalescent as possible.
- Extensive areas of the continental shelf susceptible of being fished were left outside the no-take zone, with the twofold aim of causing little damage to the fishing industry and also to have a good "Control" condition.
- The no-take zone proposed also protects an area that has historically been targeted by the bottom trawling fleet, which can (and should) be used in the future as an "Impact" area to evaluate the recovery capacity of the benthic assemblages of the continental shelf in absence of bottom fishing.

Being aware that a complex experimental design would incorporate a high degree of uncertainty when implemented, the monitoring plan proposed in this PhD thesis has been kept as simple as possible, with a sampling strategy that should facilitate its long-term application. With the boundaries of no-take zone in mind, we developed an experimental design based on 3 levels of fishing intensity, defined for simplicity as low, medium and high. Furthermore, the ROV dives that should be repeated through time are located in a depth range (80 to 120 m) and a substrate type (mud, sand and small gravels) that makes its acquisition relatively easy, avoiding areas of high slopes, complex topographies and large rocks. This way, the design tries to favor areas where the ROV has low chances of getting entangled, securing the success of the forthcoming surveys. Also, the categorization of the experimental treatment (fishing intensity) in 3 levels aims to generate a scenario of minimum complexity. To make the comparisons between surveys statistically robust, three spatial replicates were selected for each level of fishing intensity, which constitutes the minimum number of replicates necessary for a sound statistical analysis.

6.5.2 Benthic descriptors selected for the monitoring protocol

Community descriptors. Figs. 5.4.2 and 5.4.3 show the clear response displayed by the benthic community of Cap de Creus continental shelf against increasing values of fishing pressure, both in terms of its structure and its diversity. With these results in hand, it seems that species richness and megafauna diversity could be considered the most appropriate indices to monitor the evolution of the benthic ecosystem once management plans are put into practice. Furthermore, the smaller variability displayed between replicates of these two indices when compared to the density of megafauna species suggests that they can be selected as the preferred descriptors of the temporal evolution of benthic system under study. Their use in the future monitoring program of Cap de Creus, however, embraces a potential drawback. Both these indices are highly observer-dependent, more especially so in poorly studied habitats, such as the Mediterranean continental shelf. The use of the species inventory provided as an Annex at the end of this document should facilitate the work of all observers involved in the future monitoring program, which will not need specific training in invertebrate taxonomy, further reassuring that new results will be comparable to the baseline data provided. Furthermore, if the identification of certain species is unclear, they could be left as morphospecies and still be used to calculate species richness and the Exponential of Shannon diversity.

Indicator species. As described by Jones & Kaly (1996), a set of requirements must be fulfilled by indicator species to be suitable to monitor the evolution of marine benthic ecosystems. In the specific case of the continental shelf, the species selected must meet the following requirements: (1) abundance and widespread distribution in soft-bottom habitats, (2) limited or very reduced mobility, (3) ease of identification and, most importantly in our case, (4) sensitivity to varying levels of fishing effort (see Section 1.4.2 for further details). Such requirements have proven to be highly demanding for the megafauna dwelling on the continental shelf off Cap de Creus, to the point that important high taxa had to be turned down, as it was the case of certain sponges and echinoderms. In the end, only some cnidarians and a crinoid (negative response) and some polychaetes (positive response) fulfilled all the criteria imposed. And even within those species, some displayed very large differences in density among samples located in areas of the same fishing intensity. Such differences are probably related to natural demographic fluctuations that occur in species of a moderate longevity, which potentially limits their use in long-term monitoring programs that seek to be robust and reliable. A very clear example of this situation was found in the sea lily *Leptometra phalangium*, which has historically been reported as a very common and abundant species on the continental shelf of the Gulf of Lions (Mallol 2005) and was initially regarded as a potentially good indicator species. Its very patchy distribution, however, produced unexpected results, with minimum average densities recorded in areas not subjected to any kind of fishing pressure.

A possible way to overcome the natural fluctuations displayed by populations of single species with a patchy distribution in undisturbed environments could be through the formulation of a combined index, which would incorporate density values not only from one, but from a set of sensitive species. By opposing the densities of species reacting positively and negatively to fishing practices (in the form of a quotient), such index could increase the differences between levels of fishing effort and provide stability to the average values recorded in each experimental situation. This option was not looked into detail during this PhD thesis, but efforts should be made in the future to identify a robust index based on megafauna species for Western Mediterranean continental shelves.

6.5.3 Monitoring protocol

The need for coordinated monitoring programs in the context of the MSFD requires that the selected experimental designs are consistent across European regions, so results are comparable between different MPAs (Article 11; Council of the European Union 2008). In this regard, methods employed should be standardized across environmentally similar areas, formulating consistent sampling strategies, selecting equivalent ecological indicators and proposing robust experimental designs. There have been attempts at the national level to produce *best-practice* guidelines that describe with much detail how to efficiently monitor the benthic ecosystem through time (see for example the “Monitoring guidance for marine benthic habitats” recently published by the JNCC; Noble-James, Jesus & McBreen 2017). Nevertheless, if the monitoring of marine ecosystems has to move from theoretical concepts to specific hypothesis testing, the details of each step of the process become very relevant.

A large number of European MPAs have now implemented monitoring programs to assess the effectiveness of the management measures being applied (see a review of cases in Álvarez-Fernández *et al.* 2017). There exists a wide array of sampling methodologies that are used to evaluate biological diversity in these MPAs, from fishing nets to SCUBA diving (see Murphy & Jenkins 2010), with numerous ecological indicators currently in use (Gallacher *et al.* 2016). Such a large diversity of methods has prompted the development of standardized procedures, aiming to facilitate comparisons across areas and geographical regions. Most of the guidelines proposed are SCUBA-based (e.g. Garrabou *et al.* 2015), and little information is currently available on how to successfully sample the seabed with ROVs in order to implement a robust monitoring plan. This is mostly due to the limited number of shallow-water MPAs that make use of this type of technology to evaluate the effectiveness of the protection measures applied. One of the most iconic cases can be found in Lyme Bay, where a well-replicated video survey was implemented to monitor changes in the benthic ecosystem after an area of 206 km^2 was closed to bottom towed fishing (Stevens *et al.* 2014). The experimental design and its execution proved satisfactory for detecting temporal changes in the invertebrate benthic community, with evidence of recovery for at least three indicator species of different taxonomic groups and also for the overall species richness (Sheehan *et al.* 2013).

These successful experiences make us very optimistic about the outcomes of our proposal to monitor the benthic megafauna dwelling on the continental shelf off Cap de Creus using an ROV. We believe that the step-by-step guide provided should be sufficiently detailed to reduce all potential problems that could arise during its execution, and at the same time, sufficiently easy to be applied by any research institution or stakeholder. Furthermore, it is also a relatively cheap methodology, which should make it a good candidate to become a routinely procedure in the forthcoming years as part of the management plans of the MPA. In this regard, the methodology described does not require expensive ROVs to perform the data collection, since all sampling areas are located in trawable grounds that lack complex topographies that could compromise the integrity of the device being used.

The current lack of protocols that describe the processes and techniques required to monitor deep, offshore areas using ROVs possibly responds to the recent implementation of the MSFD and the limited number of offshore MPAs that are located on the continental shelf. We believe that the monitoring protocol described in this PhD thesis might be also used to monitor other offshore MPAs in the Mediterranean region created in the frame of the MSFD. It is very possible, however, that certain aspects of the methodology will require a certain modification to meet the specificities of the local topography and megafauna. Indeed, differences in species composition and density should be expected, which might limit a direct transposal of the protocol, even for areas that show similar environmental characteristics. It is very likely that the 4 indicator species selected for Cap de Creus appear as rare (or even nonexistent) in other Mediterranean regions, and for that reason, exploratory surveys should be performed prior to implementing the protocol described here.

6.5.4 Baseline data

Only those who face the challenge of producing concluding results from a BACI experiment would fully appreciate the value of having baseline data collected in a repeatable, standardized way. Indeed, one of the most common difficulties that arise when implementing an adaptive approach to management based on a continuous monitoring program is the lack of baseline data to which the observations made inside the boundaries of the newly created MPA can be compared. Not accidentally, the schedule of the MSFD clearly demands member states to begin their monitoring programs before management measures are put into practice (Council of the European Union 2008), prompting managers and stakeholders to evaluate the ecological state of the system before implementing management plans and fostering the collection of robust baseline data.

In the specific case of Mediterranean continental shelves, the lack of quantitative information collected for monitoring purposes is substantial, even more so if we disregard those studies that make use of intrusive sampling methodologies, such as experimental beam trawls. This thesis has made some progress in this regard. The baseline data provided, however, is limited to a simple table with density data for a small number of species and the value of two indices (Table 5.5.2). It might seem that the values provided are poor and disproportionate if compared to the bulk of information that has been processed and analyzed throughout this thesis, which is made of 60 ROV dives with a total of 33 km of video transects, nine benthic assemblages and up to 170 morphospecies. The idea of this section of the PhD was to carry out a very long process of sieving from the original dataset with the final objective of providing manageable descriptors of the system under study, not only sensible to fishing, but also fulfilling the three basic requirements for being useful in a monitoring program: abundant, easy to recognize and with limited mobility. In this respect, the table provided, when added to the operating protocol, constitutes for the very first time, the baseline information required to immediately implement a monitoring program on a Mediterranean continental shelf using video images recorded with a ROV. Furthermore, those values can be used to automatically generate results after management plans are implemented and monitoring surveys are carried out.

6.5.5 Hypothesis testing and future outcomes

It is widely accepted that no-take MPAs are effective ways to protect marine benthic diversity, at the same time that favor an increase in abundance and size of some sessile invertebrates (Sheehan *et al.* 2013) and/or fish species (Edgar *et al.* 2014). However, it is still very complex to predict the way benthic communities will evolve after a change in management measures is applied. Even in the case of monitoring programs that implement a very simple experimental design, like the one proposed here, the potential responses displayed by the benthic system could lead to very complex outcomes, and hence to disparate hypothesis proposals. Furthermore, the time it takes for benthic communities to recover after an area is protected from human activities cannot be predicted through the marine ecological theory and should be based on case-specific studies. There are experiments suggesting that the recovery, in terms of abundance, of benthic species varies across functional groups, ranging between less than a year to over 10 years (Lambert *et al.* 2014). For deep-sea coral species, the recovery from human disturbance can be very limited and prolonged in time, taking even centuries after fishing practices have ceased (Clark *et al.* 2016).

The consequences of a continuous ploughing of the seafloor by trawl nets might be far more detrimental for the benthic community than the mere removal of organisms, with areas historically exposed to high levels of trawling intensity potentially showing a more limited capacity of recovery (Dernie *et al.* 2003). In the specific case of Cap de Creus, we initially considered the possibility of including differences in fishing effort as an independent factor in the PERMANOVA design, as it has been suggested by Anderson, Gorley & Clarke (2008) for this type of analyses. However, we decided to evaluate the capacity of recovery of the benthic megafauna separately for areas that have suffered different intensities in bottom trawling. This responds to the differences that we should expect in the recovery patterns of the community across varying levels of fishing effort. A single model that included FI as a factor would result in significant interactions among factors that could potentially obscure the actual patterns observed for each level of fishing intensity.

In this regard, the different outcomes that could be initially expected to occur to the benthic descriptors selected are summarized in Fig. B7.4 and associated text. Once the spatial distribution of the fishing effort changes substantially due to management measures, areas that have suffered from high fishing intensities should display the slowest recovery rates, even if they will probably undergo the most striking ecological succession process of all, from a very impoverished assemblage (observed at present) to a complex shelf community enriched with a wide variety of suspension feeders. If protection measures are long-lasting and restrict the access of bottom fishing within the limits of the no-take zone, the results of the monitoring program could provide fundamental clues on how much the benthic ecosystem of the Mediterranean continental shelf has been altered due to these type of fishing practices, that is if it ever recovers to its pristine state.

6.6 Characterization of fish assemblages

6.6.1 Faunistic composition

It is widely accepted that no sampling device is capable of providing perfect information about the distribution and abundance of the entire fish community (Stoner *et al.* 2008). Hence, we cannot conclude that the fraction detected in our video footage and described in this PhD thesis is representative enough of the fish community that inhabits Cap de Creus continental shelf and submarine canyon. It is also true, however, that ROVs should be considered suitable tools to sample structurally complex habitats and very useful to evaluate behavioral patterns, complementing the information provided by classic methodologies (Cappo, Harvey & Malcolm 2003). In this sense, the results provided in this thesis must be regarded as an opportunity to improve our knowledge about the diversity of the fish fauna in an area that has traditionally been sampled using bottom trawls. Considering all the video footage analyzed, around 50 different fish species were identified in Cap de Creus shelf and submarine canyon. This corresponds to a small fraction of the 352 species that have historically been reported for the whole Gulf of Lions (Quignard & Tomasini 2000), and still a relatively small number if compared to the 133 species identified by Mallol (2005) in the catches of commercial bottom trawlers that operate in the southern part of the gulf.

Regarding this last case, the higher number of species detected by Mallol (2005) could be attributed to the large area that can be swept by trawl nets on a daily basis, a sampling methodology that always provides a large number of rare and less frequent species. Furthermore, the possibility of identifying the fishes using life samples contributes to the reporting of small-sized and criptic species that probably go unnoticed to ROVs. What seems surprising, however, is the high number of species that were exclusively identified in our study (Table 6.2). In fact, the degree of affinity between our inventory and that of Mallol (2005) was rather low (Jaccard index of 0.23), with only 35 species in common. Discrepancies are most likely related to the catchability of the fishing gear employed, since most species not observed in the catches of bottom trawlers have a deeper distribution range (*Epigonus telescopus*), prefer hard substrates (*Phycis phycis*, *Scorpaena scrofa* and *Acantholabrus palloni*) or can bury under the sediment and maybe avoid being fished (*Ariosoma balearicum* and *Gymnanodytes* sp.). Yet, there are several species thet have only been observed in the video images for which an explanation has not been found: *Chelidonichthys cuculus*, *C. obscurus*, *Coris julis*, *Dicologlossa hexophthalma*, *Diplodus vulgaris*, *Echiichthys vipera*, *Lappanella fasciata* and *Pagrus pagrus*.

Fabri *et al.* (2014) identified a total of 61 fish taxa (50 of which down to species or genus level) in 17 submarine canyons of the Gulf of Lions (in which Cap de Creus is not included) after evaluating the images recorded in an extensive ROV survey. Although our dataset only shares 22 species with that of Fabri *et al.* (2014) (Table 6.2), the list of most abundant fishes identified in both works is very similar (e.g. *Anthias anthias*, *Capros aper*, *Coelorinchus caelorhincus*, *Gadiculus argenteus, Helicolenus dactylopterus*, *Phycis blennoides* and *Trachurus* sp.). The dissimilarities between both inventories can probably be explained by the differences in the bathymetrical range explored, since

Table 6.3. List of fish species identified in the ROV images from Cap de Creus evaluated for this PhD thesis, in the hauls of bottom trawlers that operate of the southern part of the Gulf of Lions analyzed by Mallol (2005) and in the ROV footage from the French submarine canyons evaluated by Fabri *et al.* (2014). Marked with a * those fishes left at the level of genus by Fabri *et al.* (2014).

Species	**This PhD**	**Mallol 2005**	**Fabri *et al.* 2014**	**Species**	**This PhD**	**Mallol 2005**	**Fabri *et al.* 2014**
Acantholabrus palloni	x		x	*Conger conger*	x	x	x
Alosa alosa		x		*Coris julis*	x		
Alosa fallax		x		*Dalatias licha*		x	
Anthias anthias	x	x	x	*Deltentosteus quadrimaculatus*		x	
Antonogadus megalokynodon		x		*Dicentrarchus labrax*		x	
Argentina sphyraena	x	x	x	*Dicologlossa hexophthalma*	x		
Argyropelecus hemigymnus		x		*Diplodus vulgaris*	x		
Arnoglossus imperialis		x		*Echelus myrus*		x	
Arnoglossus laterna		x		*Echiichthys vipera*	x		
Arnoglossus rueppelli		x		*Echiodon dentatus*		x	x
Arnoglossus thori		x		*Electrona rissoi*		x	
Ariosoma balearicum	x			*Engraulis encrasicolus*		x	
Aspitrigla cuculus		x		*Epigonus constanciae*		x	
Aspitrigla obscura		x		*Epigonus denticulatus*		x	x
Benthocometes robustus			x	*Epigonus telescopus*	x		x
Benthosema glaciale		x		*Etmopterus spinax*		x	x
Blennius ocellaris		x		*Eutrigla gurnardus*	x	x	
Boops boops	x	x		*Gadella maraldi*		x	
Borostomias antarcticus		x		*Gadiculus argenteus*	x	x	x
Callionymus maculatus	x	x		*Galeus melastomus*		x	x
Capros aper	x	x	x	*Glossanodon leioglossus*		x	
Carapus acus	x	x		*Gnathophis mystax*		x	
Cataetyx alleni		x		*Gobius niger*		x	
Cataetyx laticeps		x		*Gymnammodytes cicerelus*	x		
Centrolophus niger		x		*Helicolenus dactylopterus*	x	x	x
Cepola rubescens		x		*Hexanchus griseus*			x
Ceratoscopelus maderensis		x		*Hoplostethus mediterraneus*		x	x
Chauliodus sloani		x	x	*Hygophum benoiti*		x	
Chelidonichthys cuculus	x			*Hymenocephalus italicus*		x	x
Chelidonichthys lucerna	x	x		*Labrus* sp.	x		
Chelidonichthys obscurus	x			*Lampanyctus crocodilus*		x	
Chimaera monstrosa		x	x	*Lappanella fasciata*	x		
Chlorophthalmus agassizi		x	x	*Lepidion lepidion*		x	x
Citharus linguatula		x		*Lepidopus caudatus*	x	x	
Coelorhynchus coelorhynchus	x	x	x	*Lepidorhombus boscii*	x	x	x

Table 6.3. (Continued)

Species	This PhD	Mallol, 2005	Fabri et al, 2014	Species	This PhD	Mallol, 2005	Fabri et al, 2014
Lepidorhombus whiffiagonis		x	x	*Phycis blennoides*	x	x	x
Lepidotrigla cavillone		x		*Phycis phycis*	x		x
Lepidotrigla dieuzeidei		x		*Polyprion americanus*			x
Lestidiops sphyrenoides		x		*Pomatoschistus marmoratus*		x	
Lesueurigobius friesii		x		*Pomatoschistus microps*		x	
Liza ramada		x		*Pomatoschistus norvegicus*		x	
Lophius budegassa	x	x		*Raja asterias*		x	*
Lophius piscatorius	x	x	x	*Raja clavata*		x	*
Macroramphosus scolopax	x	x	x	*Sarda sarda*		x	
Maurolicus muelleri		x		*Sardina pilchardus*		x	
Melanostigma atlanticum		x		*Sardinella aurita*		x	
Merluccius merluccius	x	x	x	*Scomber scombrus*		x	
Microchirus variegatus		x		*Scophthalmus rhombus*		x	
Micromesistius poutassou		x	x	*Scorpaena elongata*		x	
Molva dipterygia macrophthalma		x	x	*Scorpaena notata*	x	x	
Monochirus hispidus		x		*Scorpaena scrofa*	x		x
Mora moro		x		*Scyliorhinus canicula*	x	x	x
Mullus barbatus	x	x	x	*Serranus cabrilla*	x	x	
Mullus surmuletus	x	x		*Serranus hepatus*		x	
Myctophum punctatum		x		*Serranus scriba*		x	
Nemichthys scolopaceus		x		*Solea vulgaris*		x	
Nezumia aequalis		x	x	*Sparus aurata*		x	
Nezumia sclerorhynchus		x		*Sphyraena sphyraena*		x	
Notacanthus bonapartei		x	x	Spicara flexuosa		x	
Notolepis rissoi		x		Spicara maena		x	
Notoscopelus elongatus		x		Spicara smaris	x	x	
Ophichthus rufus		x		*Sprattus sprattus*		x	
Ophidion barbatum		x		*Squalus acanthias*		x	
Ophisurus serpens	x	x		*Stomias boa*		x	x
Oxynotus centrina			x	*Symbolophorus veranyi*		x	
Pagellus acarne	x	x		*Symphurus nigrescens*		x	
Pagellus bogaraveo		x	*	*Synchiropus phaeton*		x	x
Pagellus erythrinus	x	x	*	*Syngnathus acus*		x	
Pagrus pagrus	x			*Synodus saurus*			x
Paralepis coregonoides		x		*Torpedo marmorata*		x	
Paraliparis murieli		x		*Trachinus draco*	x	x	
Peristedion cataphractum		x	x	*Trachurus mediterraneus*	x	x	*

Table 6.3. (Continued)

Species	This PhD	Mallol, 2005	Fabri et al, 2014	Species	This PhD	Mallol, 2005	Fabri et al, 2014
Trachurus picturatus		x	*	*Trigloporus lastoviza*	x	x	
Trachurus trachurus	x	x	*	*Trisopterus minutus*	x	x	
Trachyrincus scabrus			x	*Uranoscopus scaber*		x	
Trachyrhynchus trachyrhynchus		x		*Vinciguerria attenuata*		x	
Trigla lyra	x	x	x	*Zeus faber*	x	x	x

this PhD thesis placed most of the effort in evaluating areas of the continental shelf, while Fabri *et al.* (2014) located all ROV dives inside the submarine canyons, at depths between 180 and 700 m. In fact, there was a large number of species identified in Cap de Creus that showed a clear preference for soft-bottom areas of the continental shelf, showing a very restrictive bathymetric distribution. This was the case of all Triglidae and flatfish species and some small-sized fishes (e.g. *Serranus cabrilla*, *Boops boops* and *Mullus* spp.).

The results of the multivariate analyses (cluster and nMDS) identified two very characteristic fish assemblages inside the limits of the offshore MPA off Cap de Creus: a shelf/shelf-break assemblage and a submarine canyon assemblage. Experimental trawls performed in the Catalan shelf and slope by Demestre, Sánchez & Abelló (2000) determined the presence of 5 different assemblages, which somehow can be linked to our findings. On the one side, the canyon assemblage found off Cap de Creus seems to be linked to their cluster B, characterized by a set of species that show a deeper distribution range, such as *Lepidopus caudatus*, *Phycis* spp. or *Coelorinchus caelorhincus*. On the other, our shelf assemblage seems to be a mixture of some of the other clusters identified by Demestre *et al.* (2000), all of them found at different depths on the shelf, which included species like Trisopterus minutus, different Trigliidae species, *Mullus barbatus* and *Merluccius merluccius*. The distribution of the two assemblages identified in Cap de Creus was largely dominated by depth, an environmental parameter also identified as the main driving factor in the structuring of fish communities in the Catalan shelf and upper slope (Demestre *et al.* 2000), and also in other areas of the Mediterranean (Moranta *et al.* 1998) or the Atlantic (Porteiro *et al.* 2013; Ross, Rhode & Quattrini 2015).

6.6.2 Use of fish species in a video-based monitoring program

The possibility of monitoring the evolution of fish species in the offshore MPA of Cap de Creus using extracting techniques, such as experimental trawls, would not be socially accepted. The use of ROV images to monitor fish abundance has already been suggested as an alternative tool to bottom trawling (Cappo, Harvey & Shortis 2006), and there exist MPAs that have started monitoring programs using this technique (Karpov, Bergen & Geibel 2012; Haggarty, Shurin & Yamanaka 2016). The potential bias produced by the mobility of fishes and their reactive behavior to an underwater vehicle, however, could be limiting the reliability of the obtained results (Stoner *et al.* 2008). In our case, the analysis of the video footage provided very discouraging results regarding their use as indicator species in a potential ROV-based monitoring program. After exploring approximately 30 kilometers of shelf and canyon environments, only 1371 fishes were observed, almost half of which corresponded to the genus Trachurus. This equals to an average of less than 50 fishes per linear kilometer when including all species. In fact, the number of fishes reported per ROV dive was in general so limited that the size of the sampling unit used to characterize the invertebrate megafauna proved completely inadequate to detect spatial structures in the fish fauna and had to be extended. In the end, 200-meter long sampling units were selected to obtain relative measures of fish abundances that could be used in multivariate analyses. The use of such a large strip of seabed implied that the total size of the surface sampled that could be used was reduced to approximately 11 km. This was mostly caused by the difficulties encountered when looking for areas that were characterized by a homogenous substrate, a similar depth range and no visibility issues throughout.

The numbers obtained when working with these larger sampling units initially encouraged the idea of using fish species for monitoring purposes. On average, the number of fishes detected in the 200-meter long sampling units was around 13, with a mean number of species per sample of 4.5. These values provide a numerical ground that seemed good enough for community analyses. But when data was examined species by species, spatial occupancy (percentage of samples where each species is present) dropped to very low numbers in most cases: more than 90% of the species appeared in less than 20% of the samples. Only *Trisopterus* spp., *Helicolenus dactylopterus*, *Merluccius merluccius* and *Trachurus* spp. displayed certain regularity throughout the whole study area, with occupancies that ranged between 25 and 40% of the samples. Not only occupancies were mostly low, the abundance of each species per sampling unit was in general very little too. Only 3 species had an average density above 1 fish per sample (*Trisopterus* spp., *Lepidopus caudatus* and *Trachurus* spp.) and only 9 species registered more than 5 individuals in at least one sampling unit, three of which have certain commercial value: *Trachurus* spp., *Trisopterus* spp. and *Conger conger.*

Discussion

We are unsure whether the registered abundances represent true fish densities for most species or our results are largely biased due to differences in fish behavior. Certain big fishes showed a marked attraction towards the ROV or the submarine, the prime examples being *Trachurus* spp. and *Lepidopus caudatus*. Both species were observed forming large schools that moved around and followed the underwater vehicle as it cruised along the seabed. On the opposite end, most demersal or strictly benthic species were observed as solitary individuals relatively unaffected by the presence of an underwater vehicle. Examples of fishes not responding to the presence of the ROV can be found in the Trigliidae and the Scorpaenidae families, as well as most flatfish species, which remained immobile as the vehicle cruised by. The largest bias generally occurs by attraction or avoidance occurring outside the field of the camera (Stoner *et al.* 2008), so we cannot be sure that all individuals belonging to the same species displayed an identical behavior. Furthermore, the way our study was designed makes it impossible for us to evaluate to what extent the presence of the ROV could be affecting the fish densities recorded off Cap de Creus.

It is also possible that images were not recorded in the best possible way to evaluate fish abundances. Since our primary goal was to characterize the invertebrate megafauna, the ROV was kept as close as possible to the seabed and the camera mostly pointed towards the substrate. For this reason, the field of view was restricted to a portion of the sea floor, and some fish species might have remained unnoticed if they moved at a certain distance form the seabed. It would be very interesting to test whether camera angle or distance from the seabed can affect recorded fish abundances, and those results could be very useful in the planning of future surveys. An alternative would be to use an ROV equipped with a forward-facing camera to evaluate fish densities and a downwards-facing camera to evaluate the diversity of the invertebrate assemblages.

With the results in hand, the selection of fish species as ecological indicators in a potential monitoring program seemed rather unrealistic. Species that did not show a negative reaction towards the ROV were present in very low numbers and showed relatively low occupancies. Selecting any of those species could make statistical comparisons between years very noisy, with stochastic movements of the animals strongly affecting the results. In terms of abundance, only *Trachurus* spp. provided sufficiently large numbers to generate statistically robust analyses (577 of a total of 1371 individuals). Its condition of pelagic species and its positive response to the presence of an underwater vehicle, however, might limit its capacity to prove the ‘reserve effect’ produced by the creation of a no-take zone in the MPA through an evaluation of its abundance inside and outside of such fishing exclusion area.

6.7 Presence of marine litter

The increase in the use of disposable and non-degradable materials in human societies has began to set off alarms at many levels, specially within the scientific community, due to the negative effects that their disposal has over marine fauna (Kühn, Bravo Rebolledo & van Franeker 2015). The number of studies that, either specifically designed for that purpose or not, identify human-derived objects lying over the seabed has not ceased to increase in the past decades, most of them showing that marine litter can now be found in most marine areas of the world, and this also includes continental margins and the deep sea (Pham *et al.* 2014b; Woodall *et al.* 2014). For this reason, the European Union has put marine litter into focus, with initiatives that aim to reduce its presence along the different marine areas of the continent. The MSFD already identified marine litter as one of the 11 indicators to be considered when evaluating the conservation status of marine habitats, so Member States are enforced to carry out assessments to identify the quantity of litter present in their littoral waters, especially those that will become part of the Natura 2000 network of MPAs of the European continent (Council of the European Union 2008).

This situation also applies to the marine area of Cap de Creus. This PhD thesis has used video images recorded on its continental shelf and submarine canyon to characterize the benthic assemblages, but it seems mandatory to use those same images to provide information on the amount of marine litter that can be currently found within the limits of the future MPA. Knowledge about the typologies that make up the human-derived objects observed over the seabed can be useful to determine the type of activity that originates them (Pham *et al.* 2014b), which will be ultimately indispensible to put forward specific policies in Cap de Creus to reduce litter input in the forthcoming years. Furthermore, the results of this thesis must serve the competent authorities to design effective management measures to tackle the serious threat imposed by the presence of litter on the seabed, either through direct actions to remove the items already identified or through the reduction of the inputs via a strict control of the land sources. Finally, the data provided must be regarded as baseline information to put in practice a monitoring plan that evaluates the quantity and typology of marine litter that reaches the seabed off Cap de Creus.

6.7.1 Lost or abandoned fishing gears

One of the major sources of non-degradable plastics that pollute the oceans derive from the activity of fishing vessels, which sometimes lose or abandon their gears after getting entangled in rocks or other hard surfaces (Macfadyen, Huntington & Cappell 2009). Due to the demands of the commercial fishing industry, fishing gears have become very resistant and durable (Gregory 2009), representing a long-time threat to benthic fauna when discarded at sea. In the case of Cap de Creus, an area located relatively far away from densely populated cities, lost or abandoned fishing gears (ALDFG) represented the highest percentage of marine litter items in the video images. In fact, more than 90% of the human-derived objects reported had a fishing-related origin, with longlines being, by far, the most abundant item (386 fishing cables from a total of 421 human-derived items). Such high abundance must be attributed to the historical importance of the bottom long-line fishery in the area, which distributes all along the continental shelf and the submarine canyon (Purroy, Requena & Gili 2014), representing one of the most important fisheries below 100 m depth. Its presence, however, has suffered from the strong sociocultural changes occurred in the last decades, leading to a progressive decline of their activities in Cap de Creus (Gómez *et al.* 2006).

Longlines have become a very common feature in seamounts and submarine canyons worldwide due to their likelihood of getting entangled in hard grounds (Macfadyen *et al.* 2009). Lost cables can currently be observed in almost all canyons of the European continent, especially in Gulf of Lions (Fabri *et al.* 2014), the Bay of Biscay (van den Beld *et al.* 2016) and the coast of Portugal (Oliveira *et al.* 2015). The presence of longlines in Cap de Creus was substantial, especially on the canyon head, at depths between 180 and 300 m, where an average of 8 longlines every 100 linear meters was registered (Table 5.7.1). They were particularly abundant in two dives, with local densities reaching values of around 25 cables per 100 m. Such density of cables is possibly one of the highest ever recorded in slope habitats of the European continent, comparable to areas that register very important fishing pressures (e.g. Angiolillo *et al.* 2015). It is to be noted that, due to the different methodologies employed by other authors to evaluate marine litter density (trawl surveys, continuous video and still images), comparison across areas can sometimes be relatively difficult. Although marine litter data is generally provided as items per square kilometer, we decided that the best way to estimate ALDFG density in Cap de Creus was as items per 100 linear meters, since fishing gears generally appeared as large cables or nets lying over the seabed, across the image. In our case, the use of a more standard methodology to measure ALDFG density would have contributed to an overestimation of their real density, since longlines and trammel nets can extend for several hundreds of meters.

Long-line fishing has a far smaller impact on vulnerable marine ecosystems than commercial bottom trawling (Pham *et al.* 2014a), but its effects over the benthic fauna should not be overlooked. We visually evaluated the impact of longlines on the three main cold-water coral species present in Cap de Creus submarine canyon, with the idea of using them as indicators of the level of impact to the whole community. Although the number of corals affected varied across dives, the highest percentage of corals entangled occurred in areas with a higher density of longlines. Overall, an average of 10% of all cold-water colonies identified in the images had cables entangled around them, a situation that could eventually lead to suffocation and provoke the death of the colony (Sheavly & Register 2007). This value might appear relatively low, especially considering the high number of longlines detected and also when compared to other Mediterranean areas, which have reported up to three times more corals affected by longlines (Angiolillo *et al.* 2015; Bo *et al.* 2014). It is very likely that our results only provide a partial image of the real impact caused by longlines, and we would need further information to better estimate of the full extent of their effects. First, we currently have no data about the percentage of corals that were partially broken or fully removed from their natural habitat due to fishing practices. Second, there is not by-catch data from logbooks that would provide valuable information on how many colonies are being collected by fishermen every year. And third, lost gears that are currently not affecting corals will likely generate an impact in the future. Cap de Creus is an area of strong bottom currents, which reach maximum densities at certain times of the year (i.e. dense-shelf water cascading, Canals *et al.* 2006). Such high-intensity events could potentially move the longline cables, generating further entanglements and potentially breaking up colonies that are currently not affected.

Other types of fishing gears were also observed in the ROV images, but their number was almost negligible compared to longlines. Their presence was reduced to a few trammel nets and a large bottom trawling net, primarily in soft-bottom areas of the continental shelf. Although accidental entanglement is significantly reduced in such environments, trammel fishing and bottom trawling also contribute to the overall input of ALDFG that reaches the seabed (Macfadyen *et al.* 2009). As well as longlines, lost nets also cause damage to marine benthic fauna due to their capacity of continue fishing once abandoned (ghost fishing; Gilman *et al.* 2016). The impacts to benthic fauna related to ghost fishing can last several decades, and the removal of these types of gears in soft-bottom environments could be a relatively simple solution to reduce their numbers (Gilardi *et al.* 2010). Since our ROV images are accurately georeferenced, it would be highly advisable to attempt net recoveries in the soft bottom grounds of Cap de Creus to reduce ghost fishing once the MPA is declared. The methodology to retrieve nets is relatively simple, and consists of a “creeper” towed by a vessel in the area where nets have been observed with the idea of hooking the net to bring it to surface (see further details of different methods in Graham *et al.* 2009). Early attempts have shown the feasibility of these practices to remove gillnets in deep-sea areas of Norway, Rockall and Porcupine (see results in Large *et al.* 2009), and it could seem interesting to develop pioneering surveys with local fishermen to put experiences that have proven successful in other areas of Europe to work in Cap de Creus.

6.7.2 Domestic items

The density of domestic items was much lower than that of ALDFG. In fact, only 28 items were observed in over 30 km of seabed explored. About 30% of litter items had a plastic origin, a fraction relatively lower than that registered in other areas of the Mediterranean (Galgani *et al.* 2000; Tubau *et al.* 2015) and Atlantic basins (Urban-Malinga *et al.* 2018). Even though the number of items observed was not very large, the variety of items was considerable and included plastic bags, glass bottles, car tires, oil drums and even ancient amphorae.

The low number of items reported on the video images possibly relates to three main reasons. First, Cap de Creus is a rather unpopulated area due to its inclusion as a terrestrial Natural Park in 1983. Its two main villages, Cadaqués and Port de la Selva, are relatively isolated inside the limits of the park, with poor road connections to larger cities. Second, it is very likely that the small items that reach the continental shelf get removed by the commercial trawling fleet, which disposes them on land after their daily activities. Third, the strong bottom currents that recurrently hit the area are possibly moving the small and lighter items towards the deepest part of the submarine canyon, which becomes an accumulation area, as it was previously observed by Tubau *et al.* (2015). Although the number of litter objects in Cap de Creus might still remain low, efforts should be made by the competent authorities to reduce the quantity of litter that reaches the sea from land, together with outreach campaigns targeting recreational boating to raise awareness of the problems generated by the dumping of litter while at sea.

Conclusions

7. Conclusions

Use of ROVs to characterize epibenthic assemblages and the use of megafauna in monitoring programs

1. The discriminating capacity of the video images resulted in 167 invertebrate morphospecies identified, which validate the use of ROVs to characterize epibenthic assemblages in deep areas of the Mediterranean Sea, inaccessible to conventional SCUBA diving.

2. The use of ROVs to identify invertebrate assemblages based on megafauna species avoids the detrimental consequences for the benthic ecosystem of using extracting techniques, such as bottom trawls or epibenthic sleds.

3. The high densities recorded for certain invertebrate species and their habitual presence in wide areas of the continental shelf makes us optimist about the use of sessile epifauna as indicator species in future monitoring programs using ROVs.

4. The number of fishes identified in the video images was relatively low, possibly due to a negative response in front of a motorized vehicle or maybe due to real low densities. In any case, to accumulate substantial abundances to perform multivariate analyses, the size of the sampling unit had to be made relatively large, which makes us pessimistic in the use of fishes as indicator species in a future monitoring program.

Natural heritage in Cap de Creus continental shelf and submarine canyon

5. The set of ROV dives performed over the continental shelf and submarine canyon off Cap de Creus revealed the existence of a very diverse benthic ecosystem where most of the marine high taxa were represented. A total of 167 different morphospecies belonging to the sessile invertebrate megafauna and almost 60 fish species were identified in the video footage. Such high richness in the number of benthic species revealed the extraordinary ecological value of the new MPA of Cap de Creus.

6. The quantitative analysis of the video images led to the identification of 9 different invertebrate assemblages: (A) gorgonian shelf assemblage dominated by the species *Eunicella cavolini*; (B) soft-bottom shelf assemblage with pennatulaceans and alcyonaceans, where *Pteroeides spinosum*, *Alcyonium palmatum* and *Pennatula rubra* are characteristic species; (C) highly-trawled low-diverse soft-bottom assemblage with *Sabella pavonina*; (D) sandy bottom shelf assemblage with the crinoid *Leptometra phalangium*; (E) deep shelf and shelf break assemblage

with the polychaete *Lanice conchilega*; (F) cold-water coral assemblage with *Madrepora oculata* in hard substrates of the submarine canyon; (G) 'roche du large' assemblage with high diversity of sponges; (H) cobbles and pebbles of the shelf break and submarine canyon with cerianthids and sea urchins; and (I) massive aggregation on the continental shelf of the brittle star *Ophiothrix fragilis*.

7. Two fish assemblages were identified on the video footage, which corresponded to the shelf and shelf break, on one side, and the submarine canyon, on the other. The shelf assemblage was characterized by highly-mobile small-sized fishes, such as *Trisopterus* spp., *Serranus cabrilla*, the triglidae *Chelidonichthys cuculus* and *Trigloporus lastoviza*, and different species of the genus *Scorpaena*. The submarine canyon assemblage presented more mobile and larger fishes, in which to include *Trachurus* spp., *Helycolenus dactylopterus*, *Lepidopus caudatus*, *Conger conger* or *Phycis phycis*.

Spatial distribution of benthic fauna

8. The distribution of invertebrate megafauna over the study area is strongly determined by a combination of certain environmental parameters, being depth, current speed and substrate type the most influencing. When the analysis is limited to locations susceptible of being fished (soft-bottom areas of the continental shelf), fishing intensity plays a predominant role (see below).

9. The good correlation between the environmental parameters and the megafauna allowed us to generate predictive maps that performed well, both for species richness and diversity (*ca*. 65% accuracy) and megabenthic assemblages (*ca*. 80%). These results give us enough confidence on the maps generated, representing a reliable tool that can be used for management purposes.

10. Three diversity hotspots have been identified: (1) a longitudinal band over the 100 m depth isoline on the northern side of the cape, where the gorgonian assemblage fully develops, (2) an area close to the shelf break, around 150 m depth, where a very well preserved sponge assemblage has been discovered, and (3) some areas on the canyon head at *ca*. 200 m depth with a well established cold-water coral assemblage is found.

Impact of commercial fishing activities over benthic fauna

11. An increase of the commercial activity of bottom trawlers not only has a negative effect on species richness and diversity of soft-bottom assemblages of the continental shelf, it also alters the structure of the community, producing a change in the dominant species.

12. When looking at the relationship between fishing intensity and the most common species of the continental shelf, two patterns were observed: on the one hand, some species showed a decrease in their mean abundance as fishing intensity increases, and on the other, two polychaete species showed a positive response to increasing levels in the fishing effort.

13. The relationship between fishing intensity and benthic megafauna has given us the possibility to select a set of indicator species of the level of fishing effort in areas of the continental shelf.

Monitoring of benthic fauna dwelling on the continental shelf

14. Four species (*Alcyonium palmatum*, *Pteroeides spinosum*, *Lanice conchilega*, *Sabella pavonina*), species richness and Exponential of Shannon diversity index were selected to become the basis of a site-specific monitoring programme for the new offshore MPA of Cap de Creus.

15. The monitoring plan proposed to evaluate through time the efficacy of the management measures implemented is based on a statistically robust BACI design, spatially explicit and properly replicated: 3 experimental conditions (low/medium/high fishing intensity), 2 areas (inside/outside the no-take zone) and 3 replicates per situation.

16. For the first time in continental shelves of the Spanish State, the monitoring protocol together with the baseline data provided will help towards an immediate implementation of a monitoring program that can be statistically tested as soon as management measures are put into practise.

Marine litter

17. The quantity of domestic litter items observed on the continental shelf and submarine canyon off Cap de Creus was relatively low, with only 28 items observed in 1 ha explored. The origin of such items was diverse, with components made of plastic, metal, clothing and glass. 6 amphorae were observed throughout the study area.

18. More than 90% of the reported items corresponded to abandoned, lost or otherwise discarded fishing gears (ALDFG). The density of ALDFG was rather high, particularly in areas of the canyon head, at depths of 200-400 m. Longlines were the most common fishing item both on the shelf and the submarine canyon. On average, 8 longlines were registered every 100 linear meters on the canyon head, with density peaks of 25 lines per 100 m in two ROV dives.

19. Approximately 10% of cold-water coral colonies had longlines entangled around them, giving an idea of the damaging effects of abandoned fishing gears over structuring species in deep environments.

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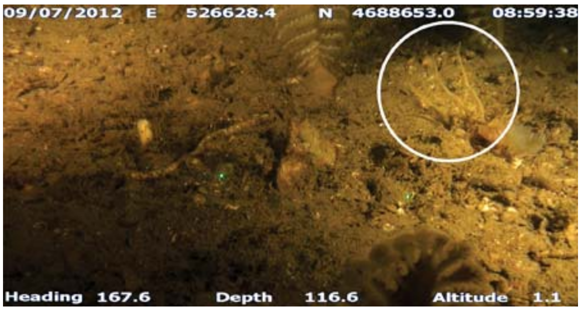
Annexes

ANNEX I

INVENTORY OF MEGAFAUNA SPECIES

All images shown here were obtained during the Life+ Indemares cruises in Cap de Creus by means of the vehicles Bleeper ROV, Nemo ROV (Gavin Newman) and JAGO submarine (GEOMAR) except the images of *Dendrophyllia cirsoide*s and *Raspaillia* (*Parasyringella*) *humilis*, which were provided by Sandra Mallol and *Weberella bursa*, provided by Pablo J. López-González.

There are still species for which identification to species level has not yet been possible. It is expected that new samples collected in the area or surroundings will help towards the completion of this catalogue.

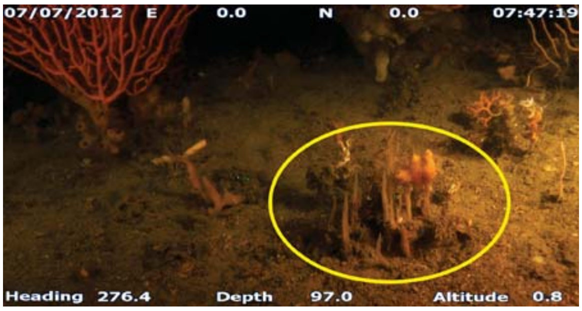


Amphilectus fucorum

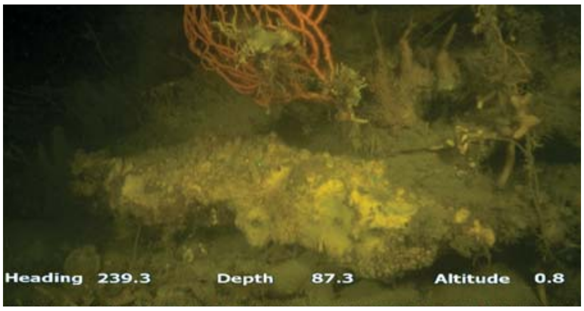
Axinella damicornis

Axinella polypoides

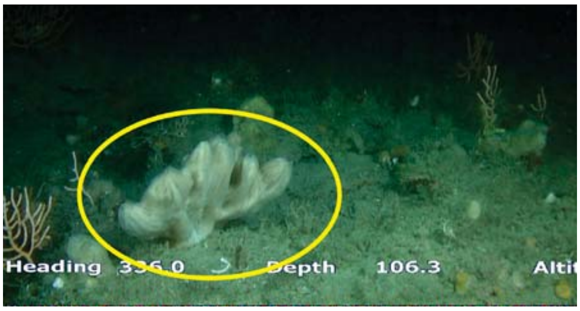
Axinella verrucosa



Ciocalyptra penicillus



Cliona cellata



Crella sp.1

Crella sp.2

Dendrilla cirsioides

Dendroxea lenis


Desmacidon fruticosum


Dysidea spp.


Haliclona (Halichoclona) sp.1

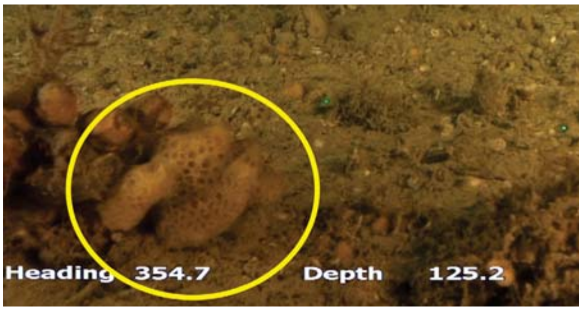

Haliclona (Halichoclona) sp.2


Haliclona (Reniera) sp.1


Haliclona cf. elegans

Haliclona sp.1


Hemimycale collumella

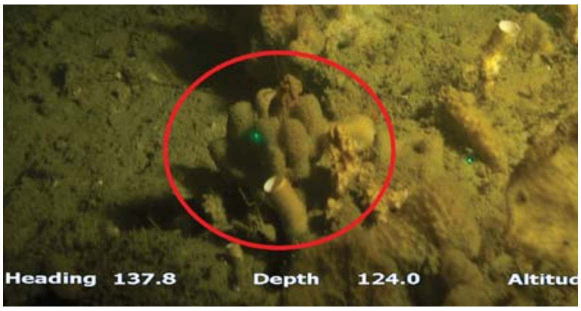


Hemimycale sp.1


Hexadella sp.1

Hexadella sp.2

Hymedesmia sp.



Hyrtios collectrix

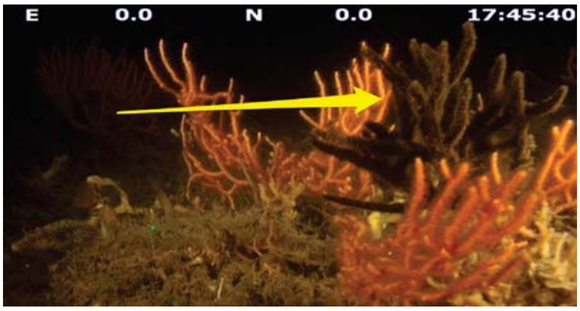
Iophon sp.1

Pachastrella monilifera

Poecillastra compressa

Polymastia spp.

Raspailia (Parasyringella) humilis



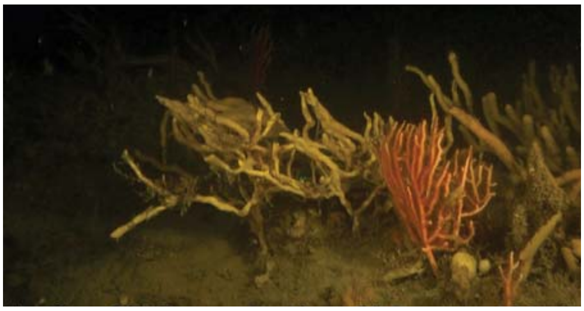
Raspailia (Raspailia) viminalis

Rhizaxinella sp.

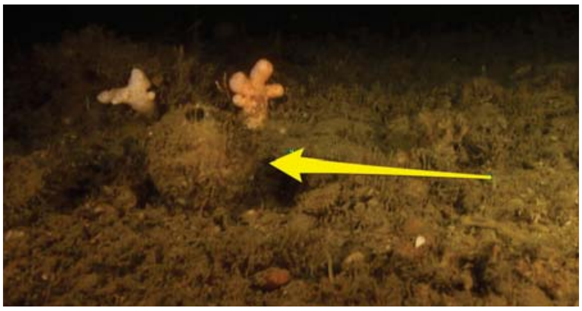
Sarcotragus foetidus

Stelligera stuposa

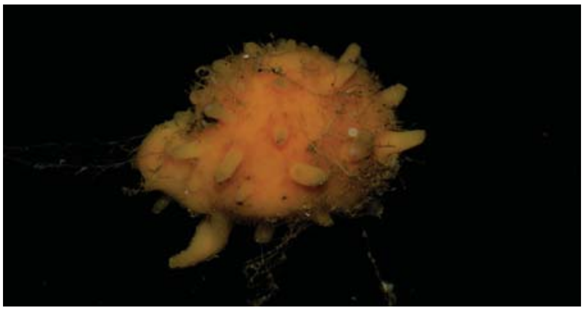
Suberites domuncula



Suberites syringella



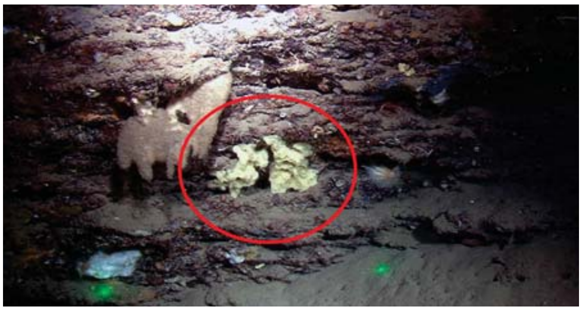
Tethya aurantium



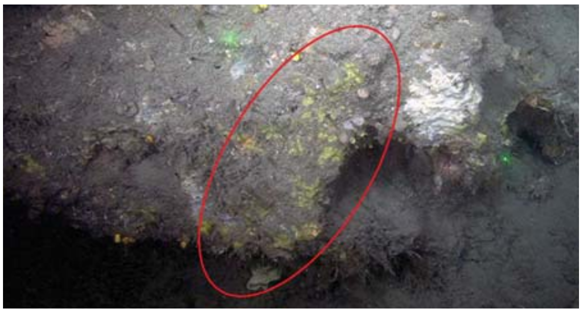
Weberella bursa

Unidentified encrusting Porifera sp.1

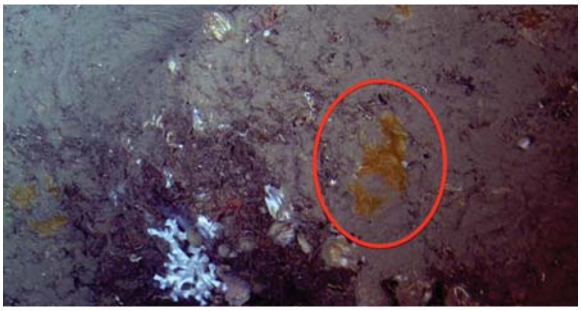
Unidentified encrusting Porifera sp.2



Unidentified encrusting Porifera sp.4

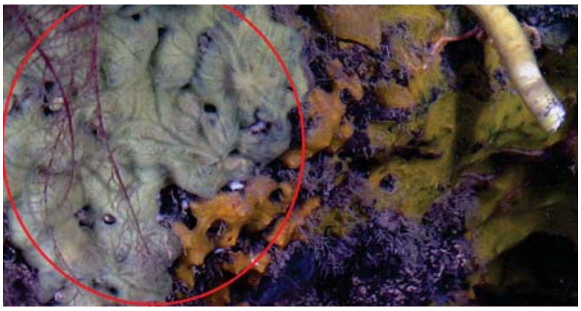


Unidentified encrusting Porifera sp.5



Unidentified encrusting Porifera sp.6

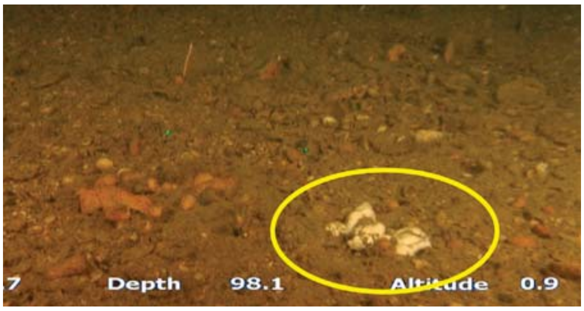
Unidentified encrusting Porifera sp.7



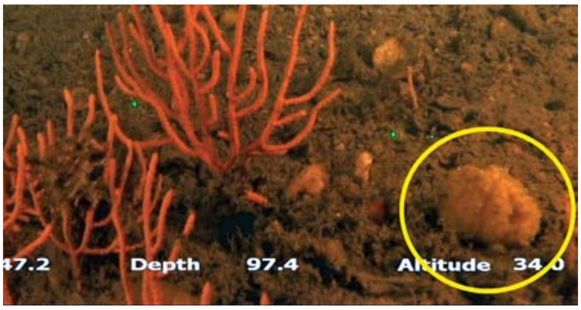
Unidentified encrusting Porifera sp.9

Unidentified encrusting Porifera sp.10

Unidentified erect Porifera sp.1

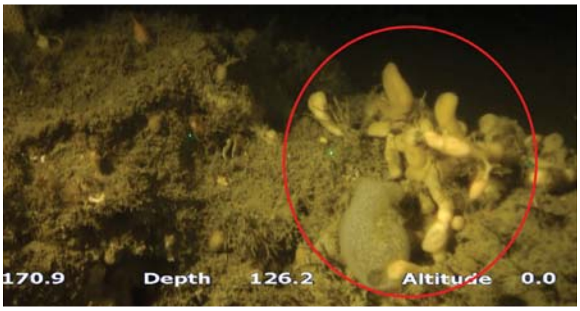


Unidentified erect Porifera sp.2



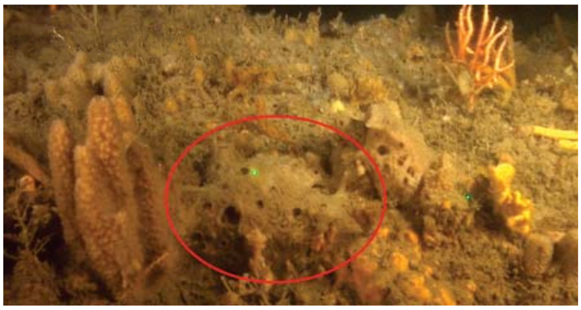
Unidentified erect Porifera sp.3

Unidentified erect Porifera sp.4



Unidentified erect Porifera sp.5

Unidentified erect Porifera sp.7



Unidentified erect Porifera sp.8

Unidentified erect Porifera sp.9

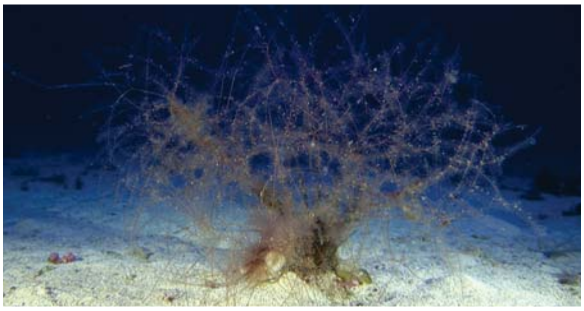
Unidentified erect Porifera sp.10

Unidentified erect Porifera sp.11

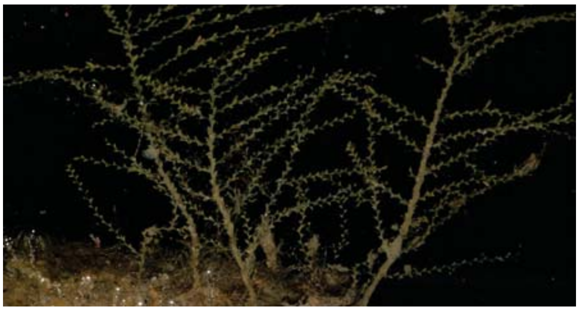
Unidentified erect Porifera sp.12

CNIDARIA HYDROZOA

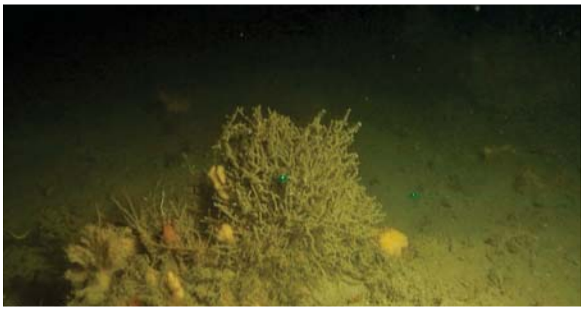
Lytocarpia myriophyllum



Nemertesia ramosa



Sertularella gayi



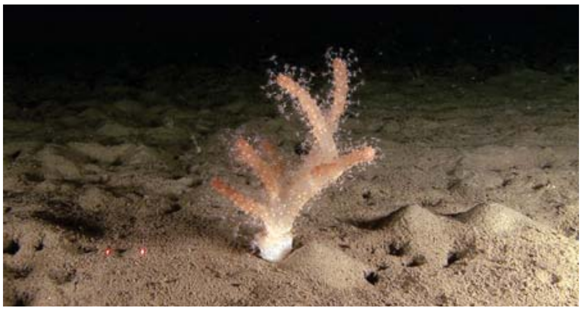
Unidentified Hydrozoa sp.1

Unidentified Hydrozoa sp.2

CNIDARIA ANTHOZOA

Alcyonium coralloides

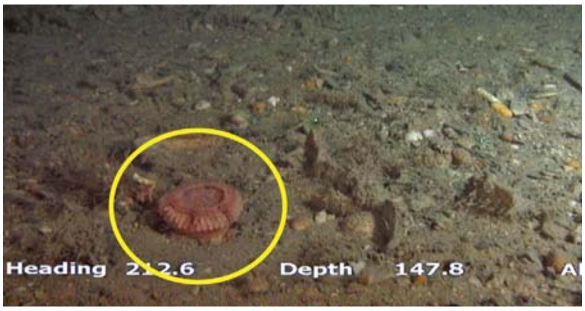
Alcyonium glomeratum



Alcyonium palmatum

Andresia partenopea

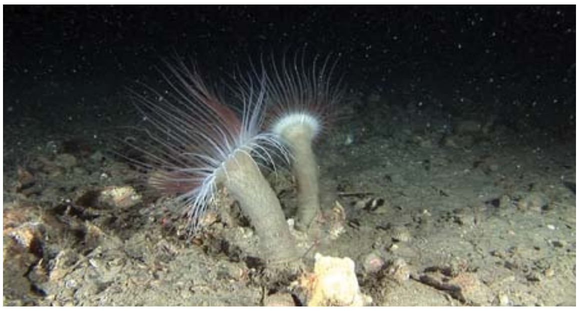
Arachnathus oligopodus



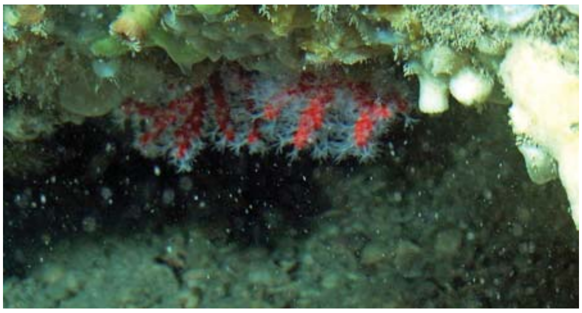
Capnea sanguinea

Caryophyllia smithii

Cavernularia pusilla

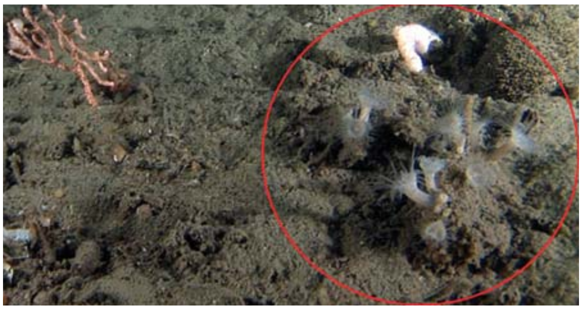


Cerianthus membranaceus

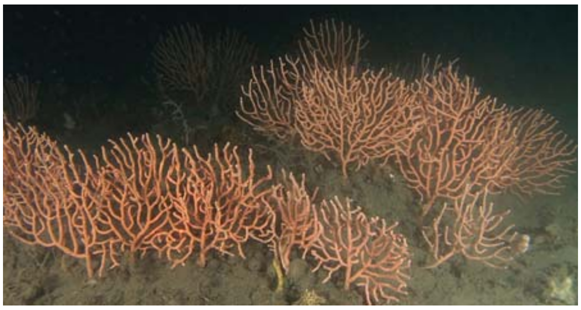


Corallium rubrum

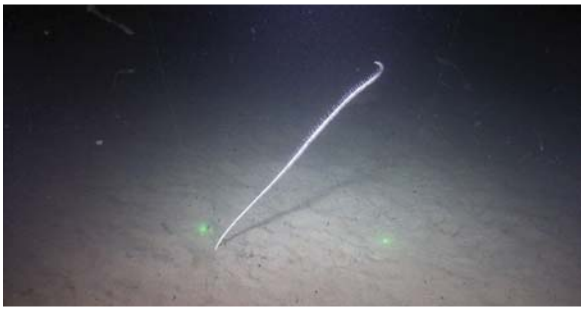
Dendrophyllia cornigera



Epizoanthus sp.1



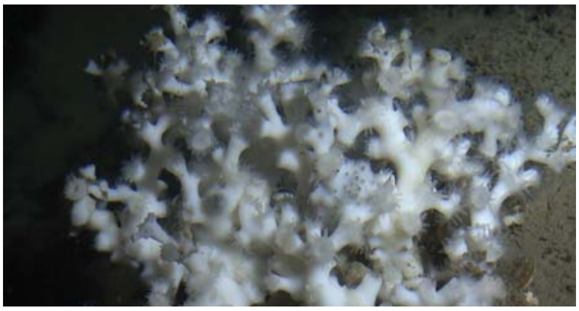
Eunicella cavolini



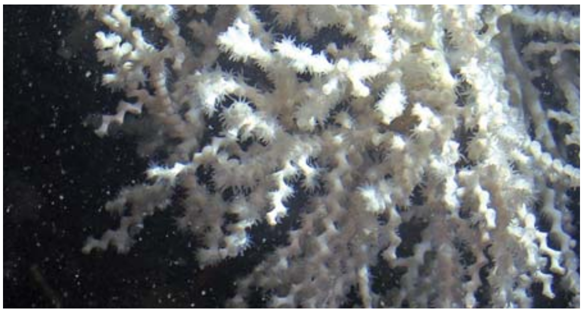
Funiculina quadrangularis

Leptogorgia sarmentosa

Leptosammia pruvoti



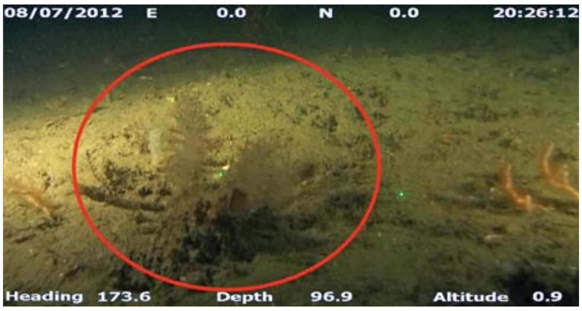
Lophelia pertusa



Madrepora oculata

Mesacmaea mitchellii

Cf. *Muriceides* sp. / Cf. *Villogorgia* sp.



Paralcyonium spinulosum

Paramuricea clavata

Parazoanthus axinellae

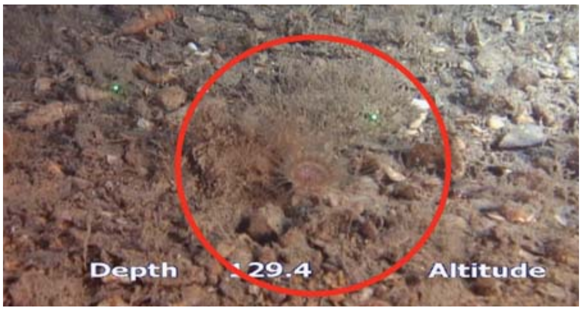
Pennatula rubra


Pteroeides spinosum

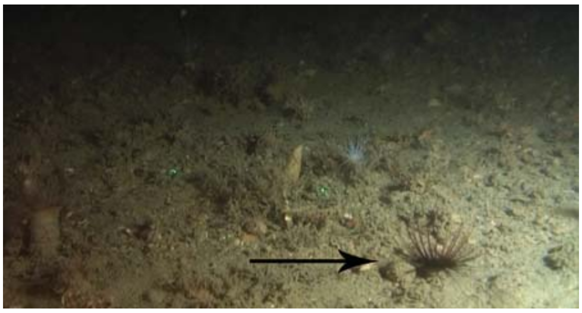

Sagartia elegans


Veretillum cynomorum

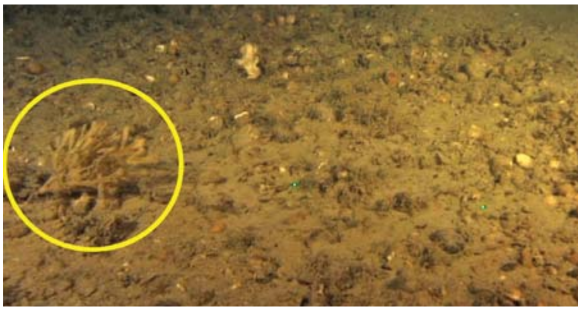

Virgularia mirabilis



Unidentified *Anthozoa* sp.1


Unidentified *Anthozoa* sp.2


Unidentified *Anthozoa* sp.3

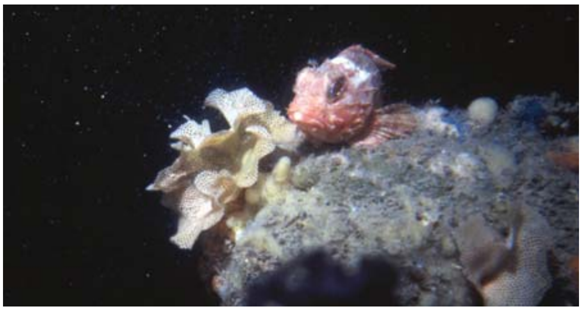


Chartella tenella / Hincksinoflustra octodon

Frondipora verrucosa

Myriapora truncata

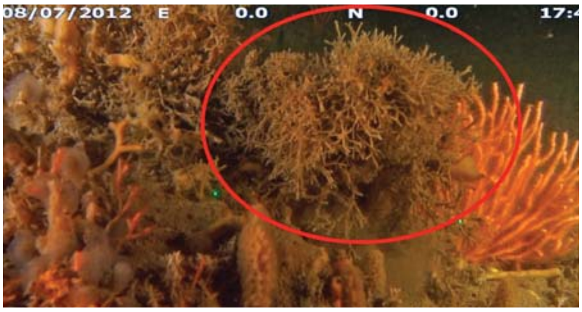
Omalosecosa ramulosa



Reteporella spp.

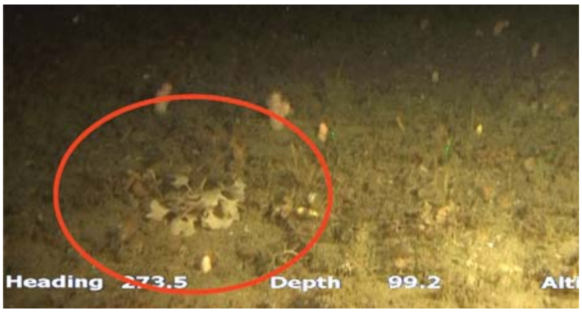
Smittina cervicornis / Adeonella calveti

Turbicellepora avicularis



Unidentified Bryozoa sp.1

Unidentified Bryozoa sp.2



Unidentified Bryozoa sp.3

Bonellia viridis

ANNELIDA POLYCHAETA

Aphrodita aculeata

Lanice conchilega

Lanicides sp.1

Myxicola infundibulum

Protula tubularia

Sabella pavonina

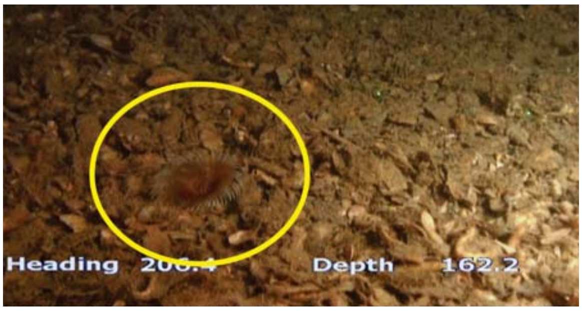
ANNELIDA POLYCHAETA


Salmacina dysteri


Unidentified Polychaete sp.1

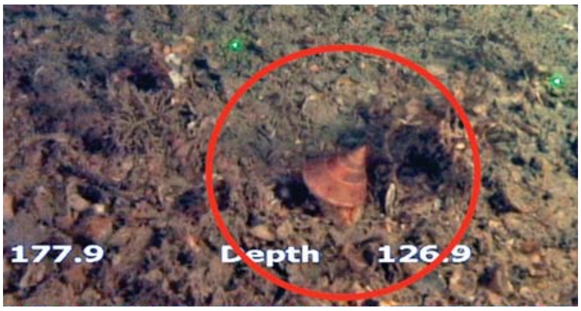

Unidentified Polychaete sp.2


Unidentified Sabellidae sp.1



Unidentified Sabellidae sp.2

MOLLUSCA GASTEROPODA



Calliostoma zizyphinum


Fellimare sp.1

MOLLUSCA GASTEROPODA

Unidentified Gasteropoda sp.1

Unidentified Gasteropoda sp.2

Unidentified Gasteropoda sp.3

MOLLUSCA BIVALVIA

Chlamys sp.1

Neopycnodonte sp.1

Pteria hirundo

Unidentified Bivalvia sp.1

MOLLUSCA BIVALVIA

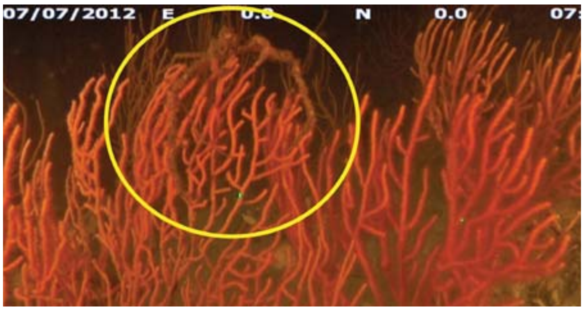
Unidentified Bivalvia sp.2

BRACHIOPODA

Unidentified Brachiopoda spp.

ARTHROPODA CRUSTACEA

Dardanus arrossor



Inachus sp.

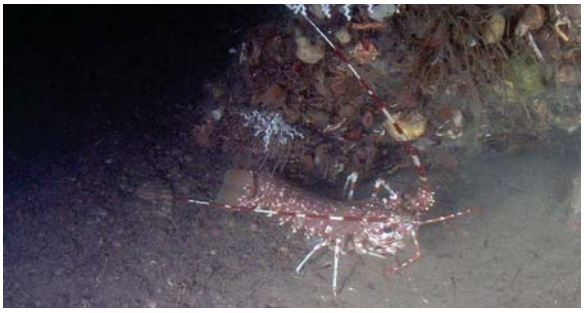
Munida intermedia

Munida rugosa

Nephrops norvegicus

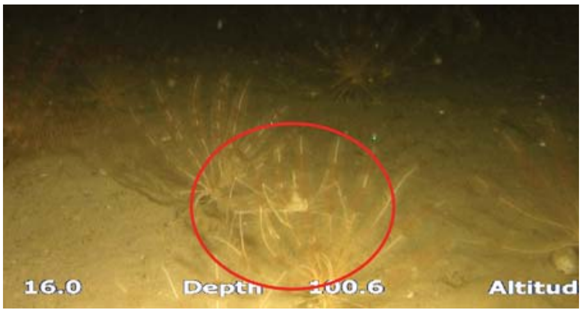
Paromola cuvieri

Pagurus prideauxi



Palinurus spp.

Unidentified *Cirripedia* sp.1



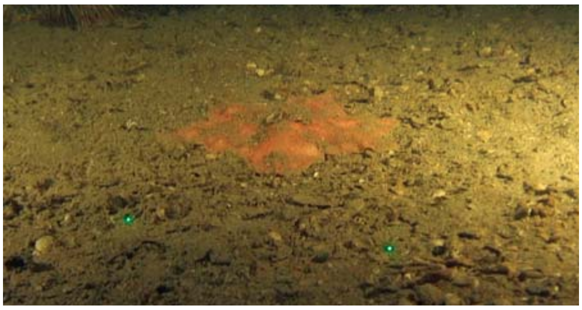
Unidentified *Crustacea* sp.1

Unidentified *Crustacea* sp.2

Unidentified *Decapoda* sp.1

Unidentified Galatheoidea spp.

ECHINODERMATA



Anseropoda placenta

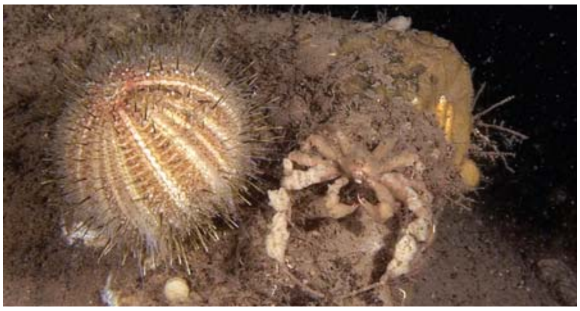
Antedon mediterranea

Astropecten sp.1

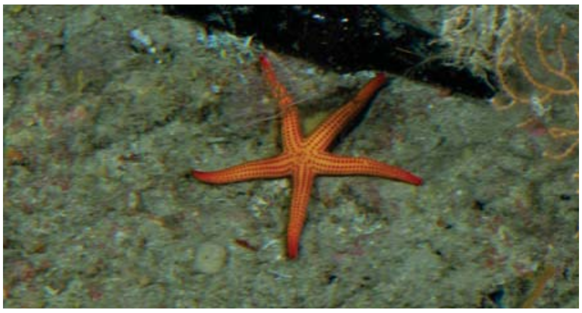
Cidaris cidaris

Echinaster (Echinaster) sepositus

Echinus acutus



Echinus melo

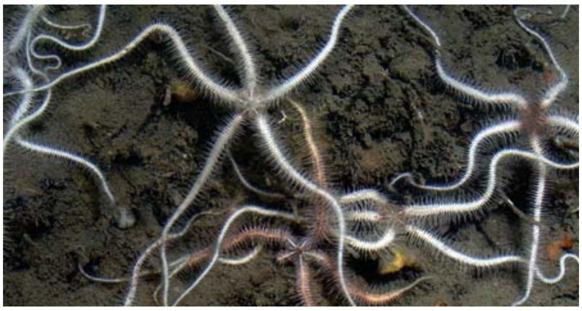


Hacelia attenuata

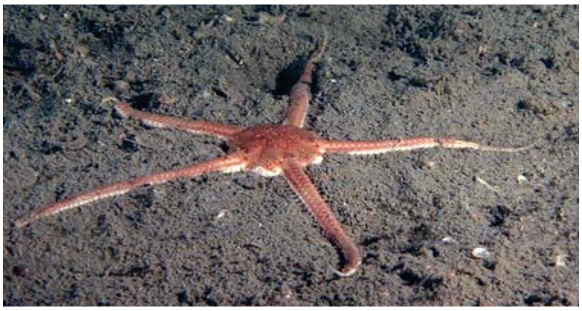
Holothuria spp.

Leptometra phalangium

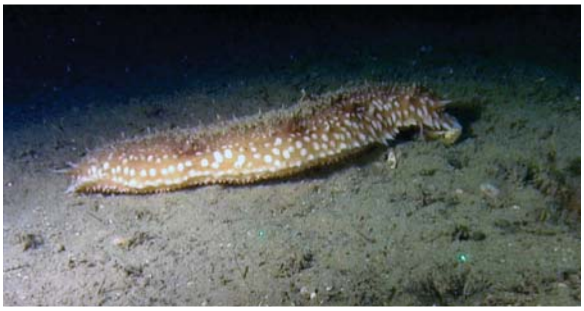
Luida ciliaris



Ophiothrix fragilis

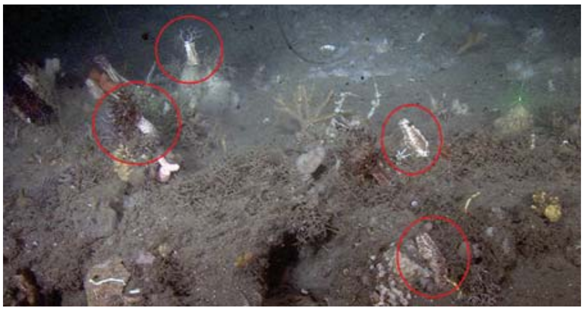


Ophiura ophiura



Parastichopus regalis

Spatangus purpureus

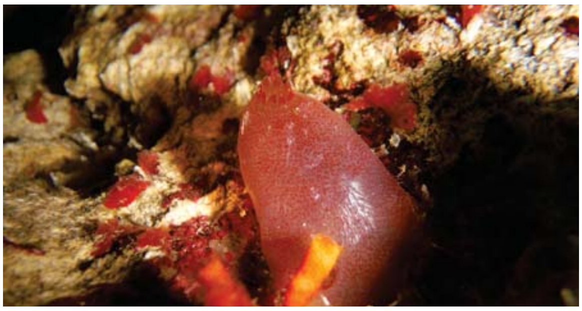


Unidentified *Cucumariidae* sp.1

Unidentified Holothuroidea sp.1

Unidentified Ophiuroidea sp.1

TUNICATA



Ascidia mentula

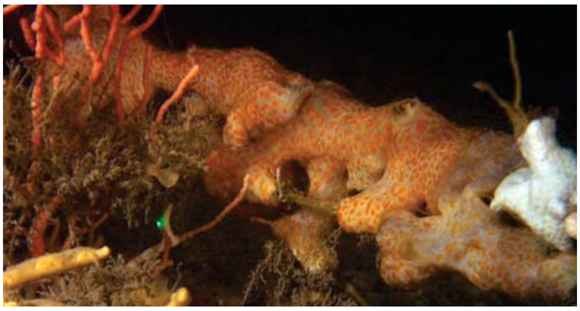
Didemnum cf. coriaceum

Distomus variolosus

Halocynthia papillosa

Microcosmus sp.

Unidentified Ascidia sp.1



Unidentified Ascidia sp.2

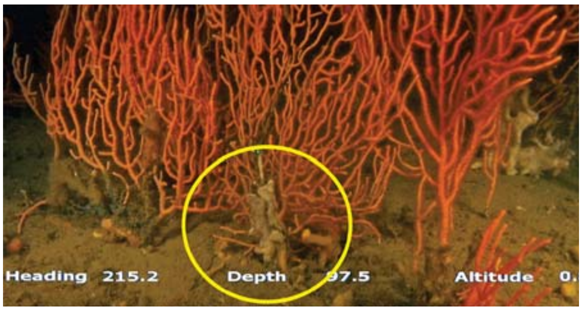
Unidentified Ascidia sp.3

Unidentified Ascidia sp.4

Unidentified Ascidia sp.5

Unidentified Ascidia sp.6

Unidentified Didemnidae sp.1



Unidentified Didemnidae sp.2

Unidentified Didemnidae sp.3

Unidentified Didemnidae sp.4

Unidentified Polyclinidae sp.1

ANNEX II

SUSBTRATE TYPES

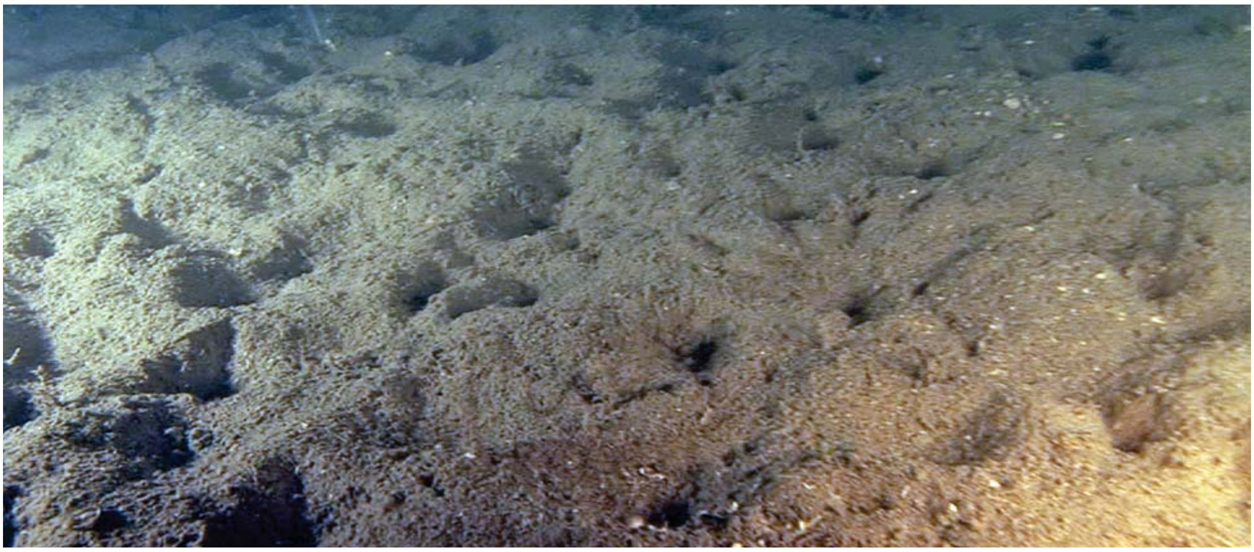
All images shown here were obtained during the Life+ Indemares cruises in Cap de Creus by means of JAGO submarine (GEOMAR)

Muddy fine to medium sands

Muddy fine sands

Fine to medium sands

Medium sands to gravel



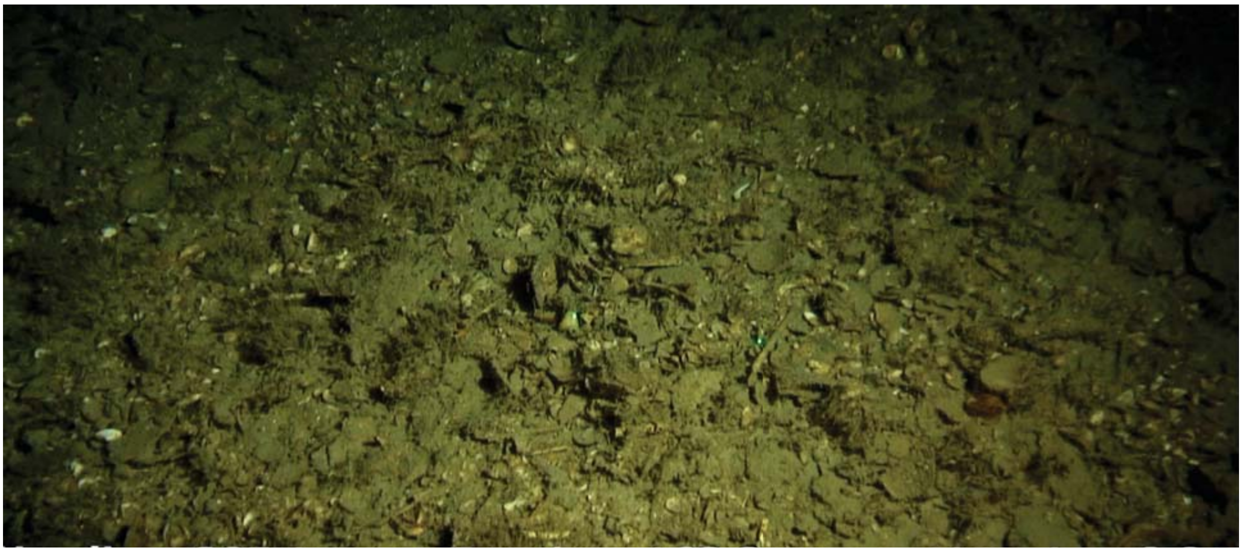
Medium to coarse sands

Medium sands to fine gravel

Coarse sand to coarse gravel

Coarse gravels to pebbles

Gravel to very coarse gravel

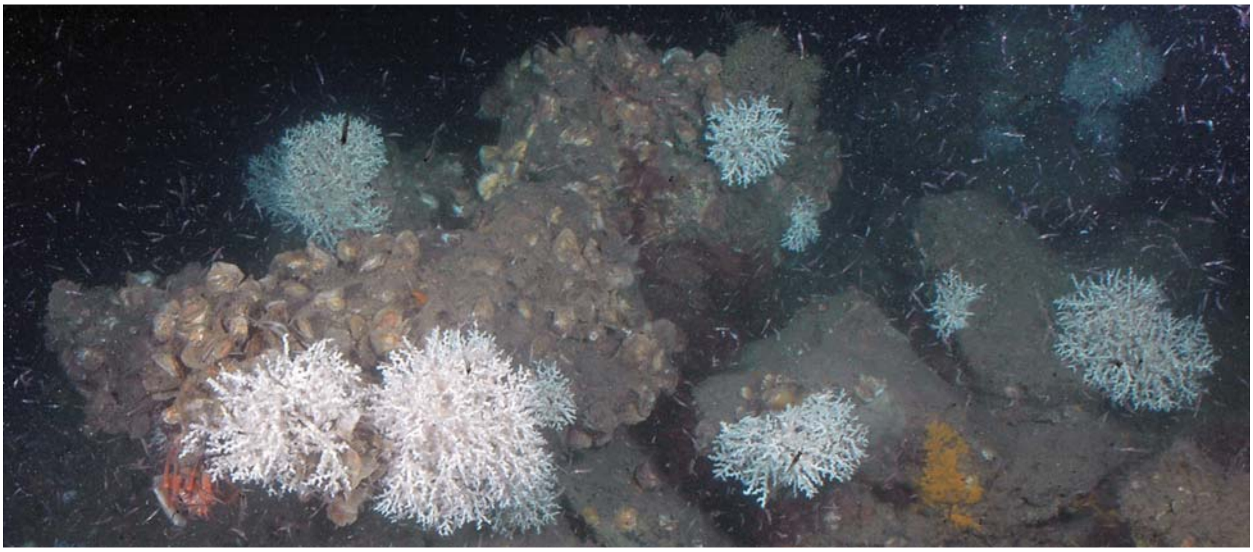


Coarse gravels and pebbles

Suboutcropping and outcropping rock

Suboutcropping rock

Suboutcropping rock with boulders and slabs



Outcropping rock

ANNEX III

List of species with density values and occupancy data

Phylum	Species	Total nº of organisms	Max. density (ind·m^{-2})	% of tracks	% of sampling units
Porifera	*Amphilectus fucorum*	41	1.20	18.33	1.41
	Axinella damicornis	180	2	21.67	4.07
	Axinella polypoides	26	0.80	11.67	0.70
	Axinella verrucosa	44	1.20	13.33	1.61
	Ciocalypta penicillus	8	0.20	10	0.45
	Cliona cellata	9	0.40	15	0.50
	Crella sp.1	9	0.40	8.33	0.45
	Crella sp.2	19	0.40	8.33	0.85
	Dendrilla cirsioides	1	0.20	1.67	0.10
	Dendroxea lenis	367	2.40	28.33	7.58
	Desmacidon fruticosum	72	1.20	20	2.06
	Dysidea spp.	1083	5.20	33.33	11.50
	Haliclona (Halichoclona) sp.1	25	0.80	13.33	0.80
	Haliclona (Halichoclona) sp.2	32	0.40	10	1.41
	Haliclona (Reniera) sp.1	13	0.40	10	0.55
	Haliclona cf. *elegans*	781	14	31.67	6.53
	Haliclona sp.1	127	1.20	30	3.16
	Hemimycale collumella	16	0.80	8.33	0.65
	Hemimycale sp.1	6	0.40	8.33	0.25
	Hexadella sp.1	28	1.4	3.33	0.55
	Hexadella sp.2	88	2.4	8.33	1.46
	Hymedesmia sp.	120	1.8	16.67	3.26
	Hyrtios collectrix	193	3.60	18.33	4.07
	Iophon sp.1	209	5.40	18.33	3.47
	Pachastrella monilifera	33	0.60	6.67	0.90
	Poecillastra compressa	68	1	10	2.41
	Polymastia spp.	98	1.60	28.33	3.47
	Raspailia (Parasyringella) humilis	132	1.20	33.33	4.32
	Raspailia (Raspailia) viminalis	12	0.40	10	0.60
	Rhizaxinella sp.	3	0.40	3.33	0.15
	Sarcotragus foetidus	88	1.60	21.67	2.96
	Stelligera stuposa	604	7.40	35	7.28
	Suberites domuncula	5	0.20	8.33	0.30
	Suberites syringella	1999	14.40	45	14.57
	Tethya aurantium	41	0.80	23.33	1.81
	Weberella bursa	4	0.20	3.33	0.25
	Unidentified encrusting Porifera sp.1	2	0.20	1.67	0.15
	Unidentified encrusting Porifera sp.2	248	2.80	21.67	4.62
	Unidentified encrusting Porifera sp.4	456	6.60	31.67	7.63
	Unidentified encrusting Porifera sp.5	8	0.40	1.67	0.35
	Unidentified encrusting Porifera sp.6	138	1.80	15	3.37
	Unidentified encrusting Porifera sp.7	177	1.60	31.67	4.62
	Unidentified encrusting Porifera sp.9	51	1.40	6.67	1.26
	Unidentified encrusting Porifera sp.10	257	2.20	16.67	4.32
	Unidentified erect Porifera sp.1	37	1	6.67	1.21
	Unidentified erect Porifera sp.2	29	0.80	18.33	1.10
	Unidentified erect Porifera sp.3	56	0.80	16.67	1.81
	Unidentified erect Porifera sp.4	25	0.60	6.67	1.05

Phylum		Species	Total nº of organisms	Max. density (ind·m^{-2})	% of tracks	% of sampling units
Porifera		Unidentified erect Porifera sp.5	7	0.40	6.67	0.30
		Unidentified erect Porifera sp.7	7	0.40	5	0.35
		Unidentified erect Porifera sp.8	3	0.20	1.67	0.15
		Unidentified erect Porifera sp.9	11	0.40	1.67	0.50
		Unidentified erect Porifera sp.10	57	1.20	20	2.21
		Unidentified erect Porifera sp.11	5	0.20	5	0.30
		Unidentified erect Porifera sp.12	78	3	11.67	1.71
Cnidaria	Hydrozoa	*Lytocarpia myriophyllum*	314	4	31.67	5.32
		Nemertesia ramosa	3	0.40	3.33	0.15
		Sertularella gayi	446	4.80	55	9.69
		Unidentified Hydrozoa sp.1	189	2.20	26.67	4.77
		Unidentified Hydrozoa sp.2	52	9.00	6.67	0.30
Cnidaria	Anthozoa	*Alcyonium coralloides*	361	5.20	25	5.32
		Alcyonium glomeratum	260	17.60	20	2.36
		Alcyonium palmatum	3905	9	78.33	42.64
		Andresia partenopea	39	0.80	11.67	1.31
		Arachnanthus oligopodus	1638	26.60	20	5.63
		Capnea sanguinea	334	1.60	38.33	9.64
		Caryophyllia smithii	1432	11.40	56.67	16.27
		Cavernularia pusilla	748	4.20	36.67	10.85
		Cerianthus membranaceus	521	1.80	58.33	12.51
		Corallium rubrum	40	1.60	8.33	0.90
		Dendrophyllia cornigera	31	0.40	13.33	1
		Epizoanthus sp.1	1076	8.80	25	8.64
		Eunicella cavolinii	4979	25.60	45	18.68
		Eunicella singularis	4	0.40	5	0.20
		Funiculina quadrangularis	2	0.20	1.67	0.15
		Leptogorgia sarmentosa	11	0.40	15	0.60
		Leptopsammia pruvoti	23	1.80	1.67	0.30
		Lophelia pertusa	7	0.40	6.67	0.30
		Madrepora oculata	291	8.60	13.33	3.06
		Mesacmaea mitchellii	791	1.80	63.33	19.29
		Cf. *Muriceides*/ Cf. *Villogorgia*	1	0.20	1.67	0.10
		Paralcyonium spinulosum	663	6.40	38.33	8.09
		Paramuricea clavata	5	0.40	6.67	0.30
		Parazoanthus axinellae	37	1.80	11.67	0.75
		Pennatula rubra	590	2.00	40	15.72
		Pteroeides spinosum	3861	6.60	65	42.54
		Sagartia elegans	64	0.80	40	2.41
		Veretillum cynomorum	7	0.20	8.33	0.40
		Virgularia mirabilis	19	0.60	10	0.70
		Unidentified Anthozoa sp.1	48	0.80	20	1.66
		Unidentified Anthozoa sp.2	34	0.80	8.33	1.21
		Unidentified Anthozoa sp.3	8	0.20	5	0.45
Bryozoa		*Chartella tenella / Hincksinoflustra octodon*	4	0.20	3.33	0.25
		Frondipora verrucosa	40	1	13.33	1.46
		Myriapora truncata / Diporula verrucosa	33	0.80	13.33	1.16
		Omalosecosa ramulosa	24	1	5	0.60

Phylum		Species	Total nº of organisms	Max. density (ind·m⁻²)	% of tracks	% of sampling units
Bryozoa		*Reteporella* spp.	87	0.60	38.33	3.16
		Smittina cervicornis / Adeonella calveti	873	4.20	28.33	12.05
		Turbicellepora avicularis	34	1.20	8.33	1.10
		Unidentified Bryozoa sp.1	39	1.40	8.33	1.10
		Unidentified Bryozoa sp.2	52	1.20	15	1.71
		Unidentified Bryozoa sp.3	17	0.80	8.33	0.55
Annelida	Echiurida	*Bonellia viridis*	52	1.60	23.33	1.56
	Polychaeta	*Aphrodita aculeata*	1	0.20	1.67	0.10
		Lanice conchilega	5908	28.20	45	16.32
		Myxicola infundibulum	260	1	48.33	8.84
		Protula tubularia	3770	14.80	78.33	33.50
		Sabella pavonina	884	9.40	71.67	16.12
		Salmacina dysteri	527	7.20	38.33	9.59
		Unidentified Polychaete sp.1	128	3.60	13.33	2.51
		Unidentified Polychaete sp.2	2	0.20	1.67	0.15
		Unidentified Sabellidae sp.1	4	0.20	6.67	0.25
		Unidentified Sabellidae sp.2	83	0.80	25	3.01
Mollusca	Gasteropoda	*Calliostoma zizyphinum*	57	0.60	33.33	2.41
		Felimare sp.1	27	0.80	10	1
		Unidentified Gasteropoda sp.1	5	0.80	3.33	0.15
		Unidentified Gasteropoda sp.2	11	0.20	15	0.65
		Unidentified Gasteropoda sp.3	1	0.20	1.67	0.10
	Bivalvia	*Chlamys* sp.	3	0.20	5	0.10
		Neopycnodonte sp.1	699	9.40	16.67	3.16
		Pteria hirundo	40	0.80	13.33	1.26
		Unidentified Bivalvia sp.1	1	0.20	1.67	0.30
		Unidentified Bivalvia sp.2	2	0.20	3.33	0.10
Brachiopoda		Unidentified Brachiopoda spp.	3455	48	16.67	8.49
Arthropoda	Crustacea	*Inachus* sp.1	10	0.20	8.33	0.55
		Nephrops norvegicus	1	0.20	1.67	0.10
		Munida ssp.	632	4.20	35	8.29
		Palinurus sp.1	5	0.20	6.67	0.30
		Paromola cuvieri	4	0.20	3.33	0.25
		Unidentified Cirripedia sp.1	1	0.20	1.67	0.10
		Unidentified Crustacea sp.1	1	0.20	1.67	0.10
		Unidentified Crustacea sp.2	50	0.60	25	2.06
		Unidentified Decapoda sp.1	26	0.60	6.67	0.90
		Unidentified Hermit crab	531	1	68.33	15.77
Echinodermata	Asteroidea	*Anseropoda placenta*	38	0.40	20	1.76
		Astropecten sp.	5	0.20	6.67	0.15
		Echinaster (Echinaster) sepositus	4	0.40	5	0.20
		Hacelia attenuata	1	0.20	1.67	0.10
		Luidia ciliaris	8	0.80	6.67	0.40
	Crinoidea	*Antedon mediterranea*	334	6.80	16.67	2.16
		Leptometra phalangium	3155	21.40	30	7.63
	Echinoidea	*Cidaris cidaris*	118	0.80	25	3.67
		Echinus acutus	287	3.60	46.67	6.78
		Echinus melo	79	0.80	20	2.81

Phylum		**Species**	**Total nº of organisms**	**Max. density (ind·m^{-2})**	**% of tracks**	**% of sampling units**
Echinodermata		*Spatangus purpureus*	5	0.80	5	0.30
	Holothuroidea	*Holothuria* spp.	227	1.20	26.67	5.78
		Parastichopus regalis	23	0.40	25	1.16
		Unidentified Cucumariidae sp.1	100	1.40	21.67	2.66
	Ophiuroidea	*Ophiothrix fragilis*	45711	ca. 600	33.33	6.03
		Ophiura ophiura	50	0.80	21.67	2.06
		Unidentified Ophiuroidea sp.1	8	1	1.67	0.15
Tunicata		*Ascidia mentula*	13	0.60	8.33	0.55
		Didemnum cf. *coriaceum*	2	0.20	1.67	0.15
		Distomus variolosus	213	4.80	18.33	3.26
		Halocynthia papillosa	2	0.40	3.33	0.15
		Microcosmus sp.	80	0.80	26.67	2.96
		Unidentified Ascidia sp.1	16	0.60	6.67	0.70
		Unidentified Ascidia sp.2	54	1	16.67	1.66
		Unidentified Ascidia sp.3	1	0.20	1.67	0.05
		Unidentified Ascidia sp.4	2	0.20	3.33	0.15
		Unidentified Ascidia sp.5	52	2.20	16.67	0.95
		Unidentified Ascidia sp.6	53	0.80	16.67	2.01
		Unidentified Didemnidae sp.1	157	5.40	16.67	2.41
		Unidentified Didemnidae sp.2	12	0.40	6.67	0.45
		Unidentified Didemnidae sp.3	40	0.60	11.67	1.46
		Unidentified Didemnidae sp.4	9	0.60	5	0.40
		Unidentified Polyclinidae sp.1	394	6.80	18.33	5.07

ANNEX IV

Megafauna diversity in the ROV transects

Annex IV MEGAFAUNA DIVERSITY IN THE ROV TRANSECTS

ROV transect	Number of species	Average number of sp. per SU	Max number of sp. in SU	Average expH diversity per SU
1	24	13.83 ± 2.04	16	8.10 ± 1
2	16	4.61 ± 2.22	10	3.53 ± 1.6
3	40	10 ± 5.16	19	6.46 ± 2.8
4	24	11.5 ± 3.78	16	6.02 ± 1.9
5	29	8.87 ± 4.95	18	4.65 ± 2.1
6	10	4.13 ± 1.25	6	3.52 ± 1.1
7	28	7.07 ± 3.51	16	4.89 ± 2.3
8	29	11.88 ± 3.81	18	6.78 ± 2.8
9	52	12.34 ± 6.11	25	8.08 ± 3.2
10	43	5.3 ± 2.44	13	3.66 ± 1.8
11	24	11.5 ± 0.7	12	7.08 ± 1.7
12	51	12.12 ± 3.11	21	6.83 ± 1.5
13	15	3.3 ± 1.28	6	2.45 ± 1.1
14	13	3.23 ± 1.49	6	3.06 ± 1.4
15	17	4.75 ± 1.35	8	2.22 ± 0.8
16	8	3.33 ± 0.86	5	2.38 ± 0.4
17	26	4.42 ± 2.37	11	3.55 ± 1.5
18	28	8.08 ± 2.12	12	6.01 ± 1.6
19	21	4.96 ± 1.25	8	3.83 ± 1
20	21	3.66 ± 1.42	7	3.02 ± 1.1
21	11	3.38 ± 1.32	6	2.43 ± 1.1
22	51	11.82 ± 3.71	19	8.37 ± 2.8
23	9	5.75 ± 0.5	6	3.61 ± 0.6
24	23	6.54 ± 2.61	13	4.38 ± 1.6
25	23	5.75 ± 1.86	9	4.62 ± 1.5
26	64	14.25 ± 4.11	23	6.82 ± 1.8
27	12	3.61 ± 1.44	7	1.96 ± 0.7
28	14	3.34 ± 0.89	5	2.87 ± 0.7
29	17	3.95 ± 1.43	6	2.57 ± 0.8
30	9	3 ± 0.1	3	2.81 ± 0.3
31	4	1.6 ± 0.89	3	1.57 ± 0.8
32	71	5.36 ± 6.04	27	4.47 ± 4.5
33	44	4.34 ± 3.39	14	3.88 ± 2.5
34	30	1.56 ± 2.92	18	1.83 ± 2
35	63	4.42 ± 4.51	17	3.55 ± 3.3
36	48	3.94 ± 2.59	11	2.35 ± 1.7
37	39	5.63 ± 2.4	14	4.82 ± 1.8
38	20	4.85 ± 2.01	11	3.8 ± 1.6
39	69	11.74 ± 7.44	28	8.84 ± 5
40	35	6.26 ± 2.41	11	3.23 ± 1.8
41	69	21.05 ± 7.23	32	9.72 ± 3.7
42	36	9.66 ± 2.65	15	6.63 ± 2
43	43	11.6 ± 5.14	21	5.07 ± 2
44	23	8.2 ± 2.53	12	6.02 ± 2.1
45	71	11.38 ± 4.52	22	7.05 ± 2.5
46	52	7.74 ± 3.07	18	4.89 ± 1.6
47	17	3.62 ± 1.28	6	2.94 ± 1.1
48	22	4.13 ± 1.48	7	2.35 ± 1.1

MEGAFAUNA DIVERSITY IN THE ROV TRANSECTS

ROV transect	Number of species	Average number of sp. per SU	Max number of sp. in SU	Average expH diversity per SU
49	28	5.38 ± 1.95	10	2.83 ± 1
50	60	6.82 ± 3.94	23	5.05 ± 2.4
51	29	8.29 ± 2.84	14	5.75 ± 1.3
52	18	2.62 ± 1.67	7	2.42 ± 1.3
53	20	4.06 ± 1.87	9	3.64 ± 1.4
54	65	12.17 ± 5.27	25	8.55 ± 3.3
55	14	3.54 ± 2.02	8	2.19 ± 1.2
56	27	5.93 ± 2.23	11	2.67 ± 1.1
57	7	1.44 ± 0.72	4	1.44 ± 0.6
58	7	1.36 ± 1.02	4	1.49 ± 0.7
59	5	0.78 ± 0.85	3	1.18 ± 0.5
60	17	3.24 ± 1.02	5	1.83 ± 0.5

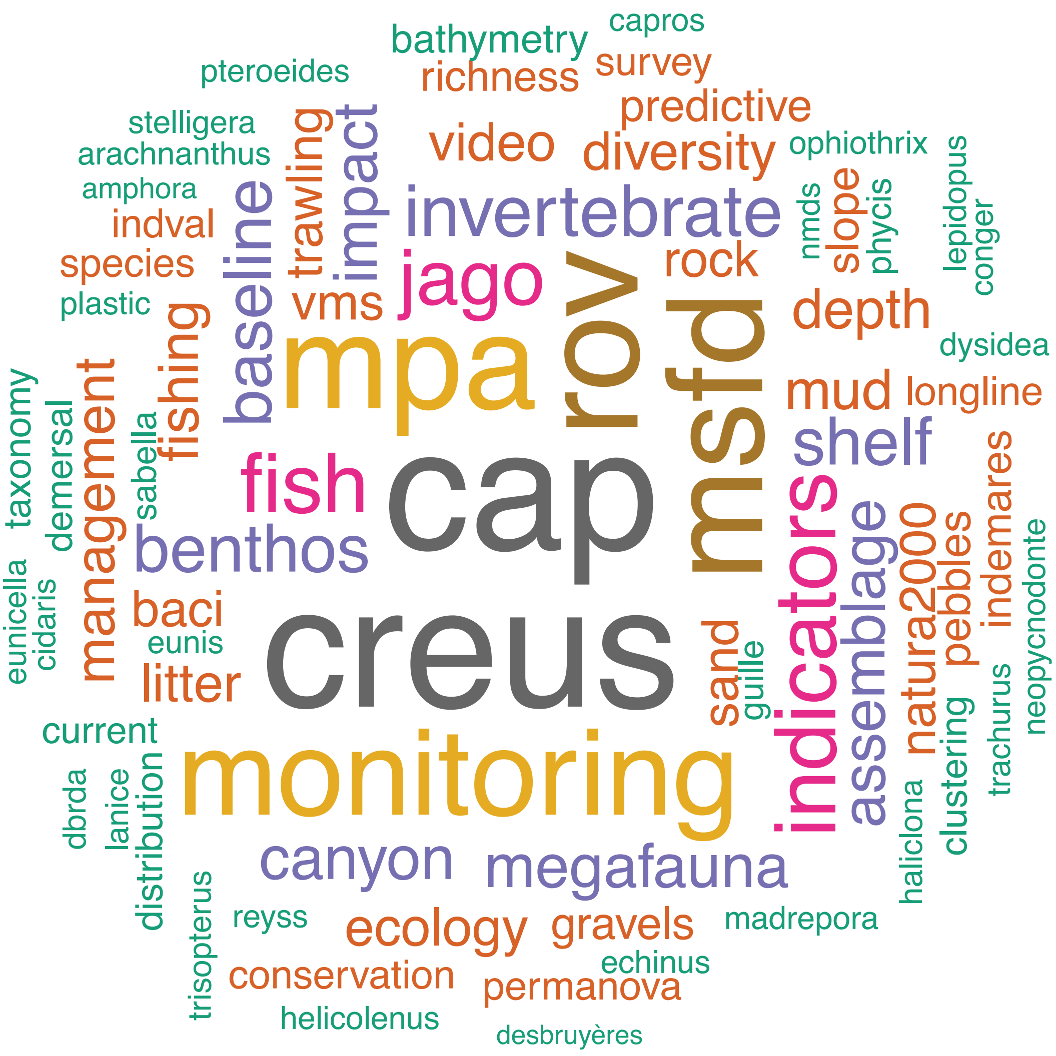
ANNEX V

Environmental features of the ROV transects

Annex IV ENVIRONMENTAL FEATURES OF THE ROV TRANSECTS

ROV	Depth (m)			Substrate type (%)					Slope (deg)		Current vel. (m/s)		Surface ratio		TPI (500m)		TRI (500m)		Fishing (n/5yr)	
dive	Min	Max	Location	Mud/Sand	Sand/Gravel	Gravel/Pebbles	Sub. rock	Rock	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
1	196	201	Canyon	0	0	0	0	100	30.78	39.27	0.092	0.093	1.16	1.30	2.04	20.72	63.83	66.34	0	0
2	373	390	Canyon	0	27	0	11	61	8.42	33.17	0.092	0.098	1.01	1.20	-22.89	2.63	45.77	48.67	0	0
3	170	245	Canyon	0	26	0	52	22	16.83	38.31	0.091	0.096	1.05	1.28	-16.29	30.64	64.47	69.66	0	0
4	300	308	Canyon	0	0	0	52	48	23.06	37.63	0.079	0.080	1.12	1.27	-22.44	-16.57	50.20	52.32	0	0
5	285	307	Canyon	0	10	3	14	73	18.25	41.86	0.075	0.083	1.05	1.34	-22.21	-0.07	51.96	57.76	0	0
6	155	167	Canyon	0	0	100	0	0	6.88	10.87	0.093	0.093	1.01	1.02	-7.98	-2.02	36.03	39.41	1	1
7	240	275	Canyon	12	14	7	7	60	3.09	45.54	0.143	0.153	1	1.43	-30.99	3.34	36.20	47.67	0	3
8	262	295	Canyon	4	5	0	51	41	10.44	37.63	0.080	0.086	1.02	1.27	-16.35	34.46	56.27	66.21	0	0
9	225	290	Canyon	0	23	11	25	41	2.77	40.87	0.082	0.093	1	1.33	-35.09	40.30	54.37	73.34	0	0
10	110	110	Shelf	0	96	0	0	4	0.05	2.09	0.116	0.128	1	1	-0.37	1.79	0.68	1.98	0	0
11	117	117	Shelf	0	100	0	0	0	2.22	3.72	0.131	0.132	1	1	-7.09	-5.38	8.51	9.54	3	4
12	98	101	Shelf	0	73	9	3	15	0.25	2.13	0.113	0.128	1	1	-0.49	1.35	3.65	5.24	0	7
13	100	100	Shelf	0	100	0	0	0	0.06	0.59	0.067	0.070	1	1	-0.48	-0.02	1.03	1.46	1	10
14	100	100	Shelf	100	0	0	0	0	0.26	2.58	0.063	0.065	1	1	-1.09	0.45	1.79	2.30	0	5
15	104	104	Shelf	0	100	0	0	0	0.03	0.43	0.075	0.077	1	1	-0.24	0.22	0.95	1.10	1	2
16	107	107	Shelf	0	100	0	0	0	0.02	0.28	0.089	0.089	1	1	-0.34	-0.19	0.70	0.80	0	0
17	106	107	Shelf	97	0	0	0	3	0.14	3.03	0.096	0.099	1	1	-0.17	2.97	1.18	3.18	0	0
18	112	116	Shelf	0	0	100	0	0	0.05	2.17	0.155	0.160	1	1	-2.11	6.79	3.03	15.72	0	2
19	102	102	Shelf	0	100	0	0	0	0.17	1.06	0.073	0.076	1	1	-0.36	0.56	1.12	1.44	0	9
20	111	111	Shelf	0	94	6	0	0	0.02	0.80	0.108	0.118	1	1	-0.34	6.51	1.18	13.26	1	2
21	120	149	Edge	0	0	100	0	0	3.39	23.43	0.163	0.170	1	1.09	-2.64	19.29	30.81	36.11	0	3
22	108	108	Shelf	0	99	0	1	0	0.10	3.00	0.115	0.120	1	1	-0.80	2.61	1.29	2.92	0	0
23	144	149	Edge	0	0	100	0	0	7.71	8.63	0.096	0.096	1.01	1.01	-17.45	-12.96	23.85	26.65	1	2
24	160	167	Canyon	0	1	68	32	0	0.96	3.72	0.132	0.145	1	1	-2.28	26.73	23.56	43.83	0	0
25	115	123	Shelf	0	84	16	0	0	0.17	4.62	0.142	0.154	1	1	0	10.76	1.27	21.14	0	1
26	95	110	Shelf	0	76	0	7	17	0.18	6	0.109	0.130	1	1.01	-3.95	2.76	4	7.99	0	2
27	128	147	Edge	0	23	77	0	0	0.88	14.08	0.117	0.120	1	1.03	-8.74	21.62	14.99	29.64	3	6
28	98	98	Shelf	0	100	0	0	0	0.03	0.68	0.068	0.073	1	1	0.06	0.40	0.79	1.35	2	14
29	99	101	Shelf	0	100	0	0	0	0.04	0.85	0.072	0.075	1	1	-0.62	0.05	0.96	1.28	1	12
30	153	160	Edge	100	0	0	0	0	9.96	15.96	0.106	0.107	1.02	1.04	-11.69	-6.72	20.58	22.37	5	5
31	137	137	Shelf	100	0	0	0	0	0.12	0.57	0.030	0.030	1	1	4.39	5.53	11.65	13.70	0	0

ROV	Depth (m)			Substrate type (%)					Slope (deg)		Current vel. (m/s)		Surface ratio		TPI (500m)		TRI (500m)		Fishing (n/5yr)	
dive	Min	Max	Location	Mud/Sand	Sand/Gravel	Gravel/Pebbles	Sub. rock	Rock	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
32	114	118	Shelf	0	80	0	1	19	0.17	6.22	0.076	0.082	1	1.01	2.46	9.36	4.51	10.58	0	1
33	129	150	Edge	9	56	15	5	16	0.62	31,47	0.038	0.046	1	1.25	-17.37	5.87	12.83	22.15	0	0
34	92	96	Shelf	94	4	0	0	2	0.09	8.83	0.014	0.016	1	1.01	-1.54	3.82	0.85	4.37	0	29
35	127	127	Shelf	0	87	0	1	12	0.14	8.07	0.068	0.071	1	1.01	-3.32	4.00	2.64	4.87	0	2
36	106	116	Shelf	1	34	44	0	21	0.09	7.95	0.087	0.090	1	1.03	-0.57	5.81	6.17	8.96	0	7
37	125	126	Shelf	0	82	16	0	1	0.10	1.83	0.051	0.053	1	1	0.18	1.08	1.29	2.03	0	0
38	124	127	Shelf	0	71	29	0	0	0.14	3.55	0.069	0.072	1	1	-0.64	2.54	3.75	5.03	0	0
39	127	156	Edge	5	78	4	8	5	0.25	29.11	0.032	0.042	1	1.16	-13.12	6.30	8.10	17.72	0	0
40	126	189	Edge	1	0	99	0	0	1.27	15.95	0.133	0.142	1	1.04	-14.30	8.66	16.67	20.54	1	17
41	95	97	Shelf	0	13	40	0	47	0.69	3.39	0.122	0.133	1	1	0.62	1.97	4.34	5.53	0	0
42	106	109	Shelf	3	73	21	0	3	0.17	2.88	0.156	0.163	1	1	-3.45	-0.32	3.58	4.93	0	1
43	87	88	Shelf	0	44	40	5	12	0.61	1.16	0.079	0.084	1	1	0.25	0.68	3.72	3.99	0	1
44	114	114	Shelf	0	0	100	0	0	0.66	1.63	0.160	0.162	1	1	0.60	1.93	5.27	9.12	0	0
45	95	99	Shelf	0	0	84	1	15	0.20	3.55	0.100	0.129	1	1	-0.67	1.58	3.60	4.49	0	0
46	96	102	Shelf	0	97	0	1	2	0.25	6.58	0.073	0.082	1	1.01	-1.64	1.53	2.30	3.65	0	1
47	105	107	Shelf	0	100	0	0	0	0.12	0.88	0.087	0.095	1	1	-1.46	0.24	1.34	1.83	1	5
48	103	106	Shelf	0	100	0	0	0	0.24	0.67	0.077	0.081	1	1	-0.83	0.60	1.25	1.60	2	8
49	126	131	Shelf	0	0	100	0	0	0.18	1.90	0.100	0.111	1	1	-0.31	1.89	2.25	3.54	4	13
50	99	116	Shelf	0	92	2	0	6	0.17	12.52	0.140	0.167	1	1.03	-4.74	9.04	3.03	10.10	0	19
51	96	97	Shelf	0	98	0	1	1	0.70	3.54	0.091	0.096	1	1	0.85	6.03	3.97	7.36	0	3
52	118	208	Edge	17	2	82	0	0	1.49	23.91	0.102	0.112	1	1.09	-9.99	20.81	34.89	51.23	0	0
53	97	97	Shelf	100	0	0	0	0	0.09	0.47	0.056	0.057	1	1	-0.10	0.19	1.43	1.52	0	0
54	123	125	Shelf	3	81	0	5	11	0.36	3.33	0.062	0.067	1	1	-3.57	-0.03	2.49	5.21	0	1
55	108	111	Shelf	0	100	0	0	0	1.04	2.19	0.081	0.084	1	1	-2.19	-1.06	5.69	5.98	1	2
56	98	100	Shelf	0	100	0	0	0	0.62	1.40	0.075	0.079	1	1	0.28	1.18	3.55	3.93	24	39
57	90	97	Shelf	100	0	0	0	0	0.20	1.13	0.049	0.064	1	1	-0.16	0.48	2.18	2.79	14	23
58	90	92	Shelf	100	0	0	0	0	0.05	0.69	0.015	0.020	1	1	-0.14	0.24	1.11	1.34	16	34
59	121	121	Shelf	100	0	0	0	0	0.28	0.80	0.009	0.011	1	1	-0.25	0.11	2.20	2.37	24	35
60	121	121	Shelf	0	100	0	0	0	0.00	0.62	0.042	0.048	1	1	-0.20	0.28	0.37	0.66	4	11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trachurus
clustering
haliclona
dbrda
lanice
distribution
canyon
megafauna
trisopterus
reyss
ecology
gravels
madrepora
echinus
conservation
permanova
helicolenus
desbruyères

Summary

The MSFD enforces all Member States to adopt an adaptive management strategy in their territorial waters grounded on an ecosystem-based approach, the creation and management of Marine Protected Areas (MPAs) as essential tools to reach a previously designed Good Environmental Status (GES), to which they must evolve as the result of policy measures that would only be enforced after the launching of a monitoring program based on previously selected indicators/parameters. This PhD thesis aims to contribute towards an effective implementation of the MSFD in the offshore area of Cap de Creus.

Based on a set of 60 ROV video transects performed on its continental shelf and submarine canyon, (1) the different megabenthic invertebrate assemblages were characterized using quantitative data, to later (2) relate the observed patterns with a set of environmental parameters, which included geological, oceanographical and human factors. Making use of the predictive power of the algorithm Random Forest, (3) a set of maps displaying the diversity and benthic assemblages were generated to help towards the implementation of ecosystem-based management measures. The activity of bottom trawlers was also related to the diversity, structure and key species of the megafauna dwelling on continental shelf to (4) identify biological indicators sensitive to such practices that could be used in a monitoring program. Aiming to facilitate the implementation of an adaptive management, (5) a detailed monitoring protocol together with quantitative baseline data is provided in order to evaluate the potential changes that may occur to the benthic ecosystem in the forthcoming years using imaging technology, once management plans are implemented. Since fish species are still the most valuable resource of the fishing industry, (6) the demersal fish assemblages that were observed in the video images are also described. Finally, and following the demands of the MSFD, (7) the amount and origin of marine litter observed in the ROV images is also provided.