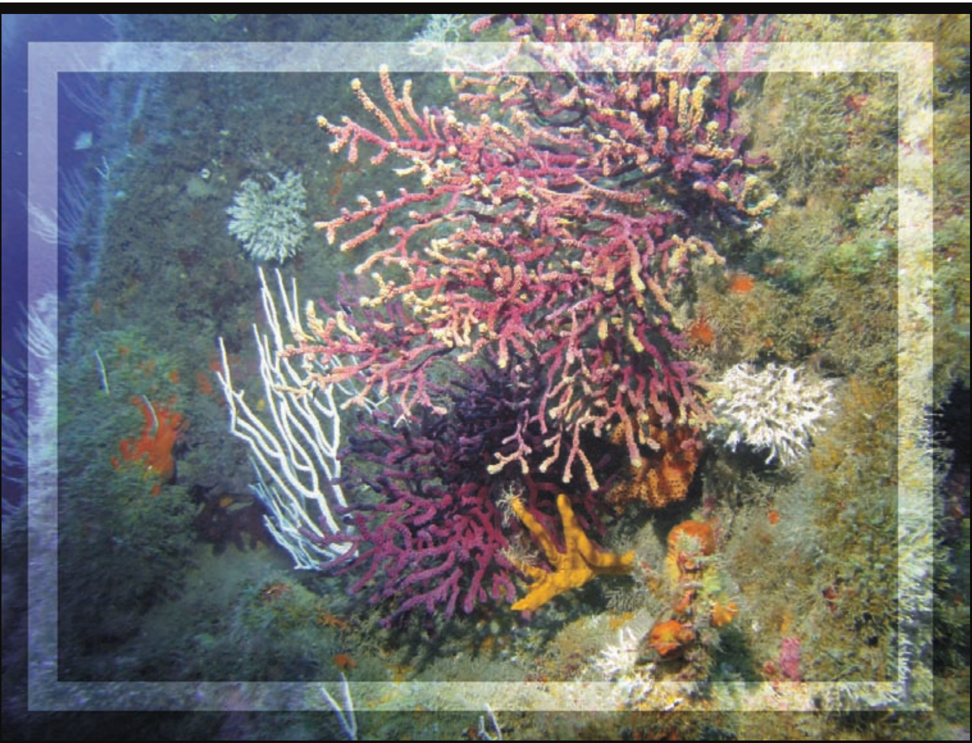

PhD Thesis

The importance of benthic suspension feeders in the biogeochemical cycles: active and passive suspension feeders in a coralligenous community

June 2015

Martina Coppari

Universitat Autònoma
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PhD THESIS

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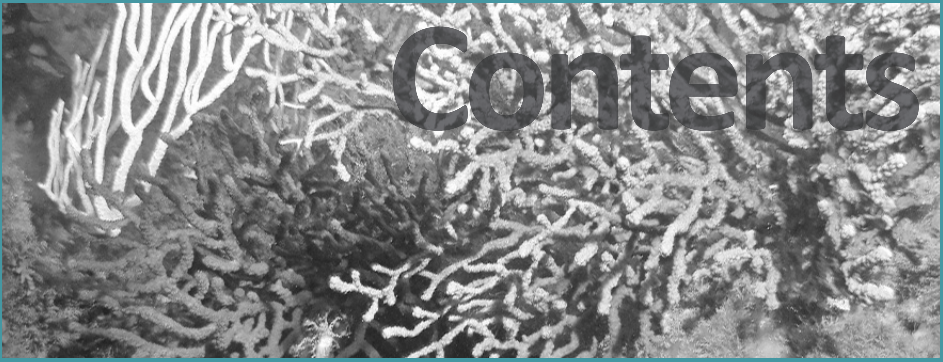
Para los que me han
acompañado en este viaje



Contents

Resumen

Hoy en día, el papel primordial jugado por los suspensívoros bentónicos en los ciclos biogeoquímicos y especialmente en la transferencia de materia y energía desde la columna de agua al bentos está más claro. Tales roles son diferentes, dependiendo de las estrategias de alimentación, las tasas de filtración y la abundancia de suspensívoros. Según sus estrategias de alimentación, los suspensívoros bentónicos se clasifican en dos grupos: suspensívoros activos y pasivos. La primera categoría incluye todos aquellos organismos que activamente bombean agua a través de un sistema de filtración que separa las partículas nutritivas del agua (el sistema de filtración es diferente entre especies); la última categoría, incluye a todos los organismos que dependen completamente de las corrientes para la provisión de alimento.

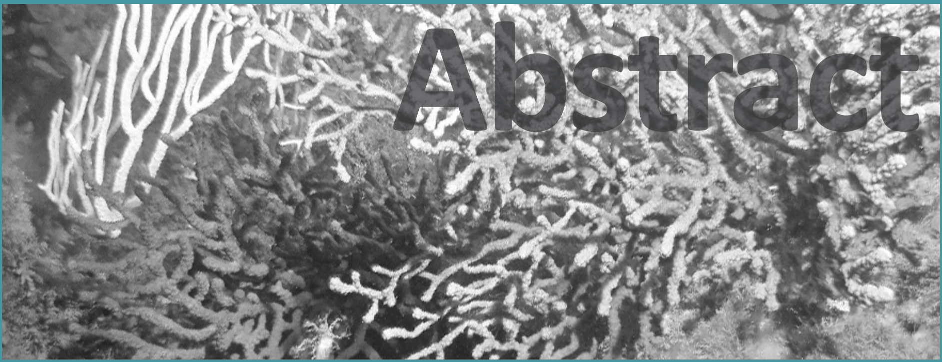
Existen muchos estudios centrados en la caracterización de los hábitos alimenticios, en la eficiencia de filtración y en la importancia del acoplamiento bento-pelágico en suspensívoros tanto activos como pasivos. Algunos de estos trabajos previos llevaron a cabo experimentos *in situ* que fueron particularmente útiles para entender hábitos alimenticios en condiciones naturales así como su variación estacional. Contrariamente, debido a la posibilidad de controlar las variables que influyen en el proceso experimental, los experimentos llevados a cabo en condiciones de laboratorio o mesocosmos son más precisos pero al mismo tiempo, con una menor interferencia con los sistemas naturales. Hay una falta general de información sobre la distribución a amplia escala de la fauna bentónica y los estudios previos acerca del impacto que los suspensívoros bentónicos tienen en el acoplamiento pelágico, normalmente se centran en pequeñas áreas.

El Vehículo de Operación Remota (ROV) permite explorar extensas y profundas áreas sin impacto en las comunidades bentónicas, por ello es la herramienta perfecta para llevar a cabo muestreos extensos y estudiar tanto el tamaño como las distribuciones espaciales y batimétricas de especies bentónicas.

La combinación de datos procedentes de ROV con datos de experimentos de alimentación permite estimar el impacto de los suspensívoros bentónicos sobre los procesos de acoplamiento bento-pelágico en áreas extensas.

En esta tesis, se han estudiado el tamaño y la distribución tanto batimétrica como espacial de tres suspensívoros bentónicos activos y tres pasivos, así como el papel que estas especies tienen en los procesos del acoplamiento bento-pelágico y como sumidero de Carbono (C) en la región del Cap de Creus (NO del Mar Mediterráneo). Como especies de suspensívoros activos, se seleccionaron para este estudio una ascidia (*Halocynthia papillosa*) y dos species de esponjas (*Aplysina aerophoba* y *Axinella polypoides*), mientras que tres especies de gorgonias (*Paramuricea clavata*, *Eunicella singularis* y *Corallium rubrum*) fueron seleccionadas como suspensívoros pasivos. Los suspensívoros bentónicos de este experimento son especies longevas de las cuales, en particular, las gorgonias pueden alcanzar los 100 años de edad, lo que permite que puedan acumular grandes cantidades de C y almacenarlo como biomasa.

Todas estas especies son comunes en las comunidades del coralígeno y precoralígeno Mediterráneo y su abundancia, especialmente las gorgonias, puede ser extremadamente alta. Las gorgonias son organismos arborescentes que crean, especialmente a altas densidades, un complejo ambiente tridimensional (conocido como bosque animal) en el que otros organismos pueden encontrar refugio, alimento y un lugar seguro en el que reproducirse. En consecuencia, la conservación de este bosque animal ayuda a mantener altos niveles de biodiversidad, un hecho que debe ser considerado en las decisiones sobre la gestión de estos hábitats.



Abstract

Nowadays, the paramount role played by benthic suspension feeders in the biogeochemical cycles and especially in the transfer of energy and matter from the water column to the benthos is more clear. Such roles are different, depending on the feeding strategies, the filtration rates and the abundances of these filter feeders. Based on the feeding strategies, benthic suspension feeders are classified in two groups: active and passive suspension feeders. The former category includes all the organisms that actively pump water through a filter system which separate the food particles from the water (the filtration system differs among species); the latter category includes all the organisms that completely depend on the water currents for the provision of food.

Many studies already focused on the characterization of the feeding habits, on the filtration efficiency and in the importance in the benthic-pelagic coupling of both active and passive suspension feeders. Some of these previous works performed in situ experiments, which are particularly useful to understand feeding habits in natural conditions and their seasonal variation. Contrarily, laboratories or mesocosm experiments allow for more precision, due to the possibility of controlling every variable influencing the experiment, but at the same time with less possibility of inference on natural systems. There is a general lack of information about the broad scale distribution of benthic fauna and the previous studies about the impact of suspension feeders on the benthic-pelagic coupling process normally focus on small patches.

Remotely Operate Vehicle (ROV) allows exploring extended and deep areas with no impact for the benthic communities, thus it is the perfect tool to perform extensive sampling in order to study the size, spatial and bathymetrical distribution of benthic species.

The combination of ROV data with data of feeding experiments allows estimating the impact on the benthic-pelagic coupling process played by suspension feeders in extended area.

In this thesis, the size and both spatial and bathymetrical distribution of three active and three passive suspension feeders were studied together with the role played by these species in the

benthic-pelagic coupling processes and as carbon (C) sinks in the Cap de Creus region (NW Mediterranean sea). As active suspension feeders, one ascidian (*Halocynthia papillosa*) and two sponge species (*Aplysina aerophoba* and *Axinella polypoides*) were selected for this study, whereas, as passive suspension feeders, three gorgonians species (*Paramuricea clavata*, *Eunicella singularis* and *Corallium rubrum*) were chosen. The benthic suspension feeders considered in this thesis are long-lived species, and, in particular gorgonians, could also reach 100 years of life span, fact that permit the accumulation of high amount of C, stored as biomass.

All these species are commonly founded in pre-coralligenous and coralligenous Mediterranean communities and especially gorgonians could reach extremely high abundances. Gorgonians are arborescent organisms that create, especially when they reach really high densities, a complex tridimensional environment (known as animal forests) in which other organisms, might find shelter, food and a safe site for reproduction. Consequently, preserving the animal forests help to maintain high levels of associated biodiversity, fact that need to be considered in management decisions.

Introduction

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On land ecosystems, the concept of forest is generally defined as a complex ecological system in which trees are the dominant life form. Trees create a three-dimensional environment that supports high levels of biodiversity, supplying structure, food and shelter to many other sessile and vagile organisms which establish more or less specialized relationships with both live or dead parts of the trees (FAO, 2006).

In marine environments, many ecosystems show the same level of complexity, biodiversity and structuring role respect to the terrestrial counterparts but contrarily to the land ones, they are animal, not vegetable, dominated systems. These animal-dominated ecosystems are called animal forests (Rossi 2013).

The three dimensional structure of the animal forests is normally given by sessile and long-living organisms, such as anthozoans (e.g. gorgonians or corals) (Smith 1978; Scinto et al. 2009) or arborescent sponges (Ponti et al. 2014). The three dimensional heterogeneous structure created by these organisms could host many other sessile species like bryozoan, ascidian, bivalves or hydrozoans, being also important as nursery or refuge area for pelagic or necto-bentonic fishes, molluscs, crustaceans, etc. (Marliave et al. 2009; Baillon et al. 2011; Bo et al. 2015). The high diversity recorded in these animal-dominated environments is due to the particular and local conditions created by these long-living organisms. They have been defined as “foundation species” (Dayton 1972) or “ecosystem engineer” (Jones 1994) due to their capability of modifying the surrounding habitat, reducing the current flow velocity with consequent decrease of re-suspension (Gacia and Duarte 2001), stabilization of soft substrata (Eckman et al. 1981), increase of local accumulations of fine particles and enhance residence time of food particles inside the animal forest (Gili and Coma 1998).

Animal forests are widespread all over the world, ranging from low (Mondal et al. 2014) to high latitudes (Arntz et al. 1997), from warm (Kleypas et al. 1999) to cold seas (Mortensen and Buhl-Mortensen 2004), and from shallow (McClanahan and Obura 1997) to deep bottoms (Bo et al. 2015). The last two decades observations led to the discovery of many unknown animal forests, indicating that their presence might be more conspicuous than previously thought (Rossi 2013).

One of the most common and peculiar animal forest is the tropical coral reef. Coral reefs are among the most productive and biologically diverse ecosystems of Earth (Connell, 1978), and they are mostly confined by narrow and stable range of environmental conditions, such as temperature, salinity, light penetration and nutrients concentration (Kleypas et al. 1999). The three-dimensional hard structure of the reef is given by scleractinians, corals that harbour photosynthetic microalgae (Fabricius and De'ath 2008).

However, corals do not just dominate shallow warm waters: the cold-water coral reefs (CWC) are comparable to their tropical counterpart, for their structural and functional role. They lack symbiotic zooxantellae, and they are distributed around the world mostly in the depth range of 200–1500 m (Mortensen et al. 2006; Roberts et al. 2006, 2009). Their occurrence is prevalently determined by specific hydrodynamic conditions, like the presence of intermediate and deep-water masses that supply food, together with a temperature ranging from 4° C to 12° C, rather than by depth per se (Roberts et al. 2006; Buhl-Mortesen et al. 2010).

Also at extremely high latitude, it is possible to find examples of animal forests dominated by benthic suspension feeders. Brey and Gerdes (1997) and Orejas et al. (2000) pointed out that Antarctic animal forests shows a slightly lower biodiversity than their tropical or temperate analogous, but their average biomasses are higher. However, more recently, many new species have been discovered (Clarke and Johnston 2003; Clarke et al. 2007), leading to the conclusion that Antarctic biodiversity of continental shelves is comparable with temperate and some non-tropical reefs (Clarke 2008).

Animal forests in warm-temperate seas: Mediterranean Sea as a case study

In the Mediterranean Sea, the knowledge about distribution and extension of different animal forests has increased in the last years. Many CWC reefs have been characterized both in the western and eastern basin (e.g. Orejas et al. 2009; Taviani et al. 2011). Black coral forests have been recorded in the Mediterranean twilight zone (Bo et al. 2009, 2011, 2014), as well as forest of the zoanthid *Savalia savaglia* growing on other gorgonians or black corals (Cerrano et al. 2010). The species forming these animal forests show high longevity (Roberts et al. 2006; Cerrano et al. 2010; Bo et al. 2015), hence they could play their ecological role for much longer temporal scales than their shallow analogous (e.g. coastal gorgonians), and for this characteristic they are also used as paleo-indicators (Roak et al 2009).

The most widespread and studied example of animal forest, in the Mediterranean Sea, is associated with the coralligenous bio-concretion. Coralligenous is defined as a hard substratum of biogenic origin, produced by the accumulation of calcareous encrusting algae growing in dim light conditions (Ballesteros 2006). Calcareous algae, sponges, cnidarians, bryozoan and tunicates are the most important species forming this assemblage (Ballesteros 2006). However, one of the main factors influencing the species composition of this assemblage is the food availability: in more eutrophic areas, gorgonians can dominate the animal-component of the community, whereas in very oligotrophic areas, sponges, bryozoan and small hexacorals are the dominant suspension feeders (Ballesteros 2006).

Coralligenous assemblages normally start to dominate (and replace the three-dimensional structure of photophilic-sciafilic algae) below 20 m depth, but can also develop in shallower areas with light conditions dim enough to permit the growth of calcareous algae. Coralligenous habitat has been identified as Mediterranean priority habitats by the EU Habitats Directive (92/43/CE) and harbour one of the richest assemblages found in Mediterranean, with approximately 10% of marine Mediterranean species (Pérès and Picard 1964; Ballesteros 2006).

What are the main factors threating Mediterranean animal forests?

Many direct and indirect anthropogenic impacts may threaten the integrity of the animal forests, reducing both structural and functional biodiversity, possibly causing a shift towards less complex communities with short-life cycles and opportunistic strategies (Trush and Dayton 2002).

It is well known that some fishing techniques (e.g. bottom trawling) have serious consequences on the sea bottoms. This is a non-selective fishing technique, which destroys the three-dimensional structure of the bottom (Trush and Dayton 2002) and homogenizes the habitats (Puig et al. 2012). Loss of habitat structure and/or patchiness results in a possible loss of ecological function of the impacted habitats, especially in the continental shelf and slope (Trush and Dayton 2002; Clark et al 2010). Sediments are also resuspended by trawling gears, and might be exported to other (generally deeper) locations (Martín et al. 2008; Pusceddu et al 2014); this resuspension might also cause the release of contaminants stored in sediments or toxic algal cysts (Brown et al. 2013) or have important effect on sediment organic decay and nutrient fluxes (Pilkskaln et al. 1998). In addition to bottom trawling, several other fishing apparatus also potentially modify the integrity of benthic assemblages, such as: gillnets, trammel nets, long lines and traps (Bo et al. 2014). The loss of these fishing nets is a common phenomenon on hard bottom (Chiappone et al. 2005), where the organisms that are more damaged by the lost nets are the arborescent ones (e.g. corals and gorgonians). Indeed, lost lines and nets may be among the main cause of mortality in the gorgonian *Paramuricea clavata* (Risso, 1826) (Bavestrello et al. 1997), causing mechanical injuries and lesions to the gorgonians coenechyme, and favouring the establishment of epibionts and the decrease of the gonadal output (Bavestrello et al. 1997; Tsounis et al. 2012).

Also the direct harvesting of species for the jewellery industry, has strongly affected several animal forests since ancient time (Tsounis et al. 2010). In the Mediterranean Sea, the red coral, *Corallium rubrum* (Linnaeus, 1758) represents one of the most emblematic examples of overharvested species, whose stocks have strongly declined in many areas, particularly in shallow waters (Weinberg 1991; Tsounis et al 2007).

Shallow animal forests can also be threated by recreational activities such as SCUBA diving. Previous studies highlighted that recreational diving may damage benthic communities, in particular arborescent species (e.g. gorgonians). Anchoring, grabbing, holding or knelling on benthic species, are the principal direct causes of detachment (Coma et al. 2004; Linares et al. 2012) or of tissue injuries that are normally followed by the attachment of numerous epibionts causing the weakening of the gorgonian skeleton, with increased possibilities of breakage (Bavestrello et al. 1997). Sites frequented by elevated number of divers may also show different distribution pattern of benthic species, with a preference for more protected and cryptic locations, less affected by divers (Sala et al. 1996; Luna-Perez et al. 2010). Divers may also cause indirect disturbance such as sediment resuspension that might clog the filtration system of benthic suspension feeders (Luna-Perez et al. 2010). Recent studies also highlighted the possible influence of diving activities in the reproductive success of surface brooders species (e.g. *Paramuricea clavata*) by removing the oocytes from the gorgonian branches as a consequence of bubbling (Tsounis et al. 2012).

Last in the order but not in importance, there are the consequences of the climate change. The impacts of climate change exceed the capacity of many organisms to adapt due to their highly rapid onset: animals and plants can adapt to environmental and biological changes, and acclimatise to specific situations, but never in the history of our planet, the environmental conditions have been changing so fast, and these phenomenon is also affecting several animal forests (Hughes et al. 2003). Three are the main effects of climate change on the marine animal forest: warming, acidification and rising sea levels, with all these impacts acting together (Anlauf et al. 2011).

In the Mediterranean Sea, one of the main consequences related to climate change is the increasing occurrence of mass mortalities of benthic suspension feeders. These events have been related with increased water temperature. In the Ligurian sea in 1999, sea water temperature anomalies, related to a period of less winds, high stability of the water column, and thermocline deepening (Romano et al. 2000), caused the mortality of 40–100% of the local gorgonian populations and it also affected several sponge species (Cerrano et al. 2000). The occurrence of such climatic anomalies in late summer implies prolonged exposure to summer conditions of organisms dwelling above the thermocline (high temperature, thus high respiratory demand, and low food availability) (Coma et al. 2009). The increased water temperature was also related to the proliferation of opportunistic pathogens such as fungi or protozoan ciliates that might easily cause infections, taking advantage of the host stressful condition (Cerrano et al. 2000). Although other more geographically restricted mass mortality events, occurred between 1999 and 2003 (e.g. in 2002 another mass mortality occurred in the Tyrrenian Sea; Sbrescia et al. 2008), in summer 2003 the largest mortality event registered in Mediterranean Sea, affected 25 benthic species belonging to five phyla: Cnidaria, Porifera, Bryozoa, and Mollusca (Garrabou et al. 2009). This events, as well as, other two sponge mass mortalities occurred in 2008 and 2009, were also related with increased water temperature.

Considering that global average temperature increased by 0.2° C (Hoegh-Guldberg and Bruno, 2010), these events might occur again becoming more frequent (Dequé 2007). Due to the high longevity, slow population dynamics with low growth, recruitment, and mortality rates (Garrabou and Harmelin 2002; Coma et al. 2004) and an increased frequency of such mortality events, the affected species might risk local extinction with consequent shift of the specific composition and structure of the animal forests, especially in shallow areas (Francour et al. 1994; Bianchi and Morri 2000).

What are we going to lose?

Marine ecosystems, as well as terrestrial ones, generate goods and benefits useful for the human welfare (the so called ecosystem services, ES). In this sense, animal forests are not an exception. As already pointed out, animal forests provide shelter and nursery areas for many species, several of them of commercial interest (Mortensen et al. 1995; Fosså et al. 2002); if the survival and the growth of these species' offspring are compromised due to the lack of suitable areas, then the natural turn over is at risk with consequences for the fishing stocks.

The aesthetic value of shallow animal forests supports another service: tourism activities. As explained above, in the last years diving tourism has increased consistently (Driedrich 2007). If human impacts cause the simplification of the animal forests with consequent shift from long-lived animal communities to fast living animal or algal communities, then the beauty related to the complexity of these environment might be lost, determining a decrease of the tourism interest as well as the its economic value.

The most important, but mostly neglected, ES generated by the animal forest are represented by the role in the benthic-pelagic coupling and as carbon (C) sinks.

The species forming the animal forests are responsible for the generation and the increase of nutrient exchange, for the capture and the retention of carbon (C), nitrogen (N) and other elements from the water column into their structure (Kinsey and Hopley 1991; Gili and Coma 1998; Rossi et al 2012). Due to the long life cycles of the species forming these animal-dominated ecosystems, they might accumulate elevated quantity of C as biomass. This C might last for decades, centuries or millennia, accumulated in the body structures of these species (Mallela and Perry 2007, Bo et al. 2015). Although some attempts of measuring these two ecological functions (impact on benthic-pelagic coupling and role as C sinks) have already been done at small scale (Coma et al. 1994, Rossi et al. 2004) or for vegetal ecosystems (i.e. the so called blue carbon; Duarte et al. 2010), there are almost no studies at broad scale for animal-dominated ecosystems.

In the last decades, the development of Remotely Operated Vehicles (ROV) and their decreased operational cost, have provided the opportunity to perform large scale detailed sampling of benthic species, from shallow to deep waters and with no impact for benthic communities (Teixidò et al. 2002; Mortensen and Buhl-Mortensen 2004; Rossi et al 2008). The analysis of ROV video transects provides both qualitative and quantitative data of species distribution and size class of the species populations (e.g. Mortensen and Buhl-Mortensen 2004; Rossi et al 2008; Bo et al. 2011; Gori et al. 2011a) as well as the presence of anthropogenic impacts and related damages to the benthic fauna (e.g. fishing lines or litter) (Bo et al. 2014; Mordecai et al. 2011; Pham et al. 2013). These broad scale data of size, spatial and bathymetrical distribution of benthic species, can be coupled with in situ feeding and respiration experiments to calculate the large scale impact of benthic suspension feeders on the benthic-pelagic coupling processes and consequently their role as C sinks.

OBJECTIVE AND STRUCTURE OF THE THESIS

The aim of this thesis is to understand and quantify the importance of representative benthic-suspension feeder species of the Mediterranean coastal areas in the benthic-pelagic coupling processes, and as potential C sink. The main focus is the understanding of the importance of such processes considering different feeding strategies and diets. Active suspension feeders pump water through a filtering structure that separates food particles from the water (Riisgård and Larsen 2000). Contrarily, passive suspension feeders completely rely on the current for food supply (Best 1988). In the present work, different benthic suspension feeders species that inhabit photofilic, pre-coralligenous and coralligenous communities were selected, taking into account their feeding strategies. As active suspension feeders, the ascidian *Halocynthia papillosa* and the sponges *Aplysina aerophoba* and *Axinella polypoides* were selected, whereas, as passive suspension feeders, the gorgonians *Paramuricea clavata*, *Eunicella singularis* and *Corallium rubrum* were chosen as study species.

Halocynthia papillosa (Linnaeus, 1767) (Order: Stolidobranchia, Family: Pyuridae) is a common species in Mediterranean pre-coralligenous, coralligenous and sciaphilic benthic bottoms (Turon 1990), normally living in non-perturbed rocky bottoms (Naranjo et al. 1996). *H. papillosa* mostly feeds on particulate organic matter (POM), both live and detrital, with this last category contributing the most to the diet of the species (Ribes et al. 1998).

Aplysina aerophoba (Nardo, 1833) (Orden: Verongida, Family: Aplysinidae) is a massive species, organized in chimney-like structures with high microbial abundance (HMA) (i.e. microbial densities 2 to 4 orders higher than that of the surrounding water; Friedrich et al., 2001), which is partially constituted by photosyntetic cyanobacteria (Vacelet, 1970). It is a mixotrophic species that inhabits shallow pre-coralligenous and photofilic rocky bottoms (Ballesteros 2006).

Axinella polypoides Schmidt, 1862 (Order: Halichondrida, Family: Axinellidae) is an erect, tree-like sponge, characterized by low microbial abundance (LMA) (i.e. same microbial density in their tissue as in the surrounding water; Hentschel et al., 2003) and completely heterotrophic feeding habits.

Paramuricea clavata (Risso, 1826) (Orden: Alcyonacea, Family: Plexauridae) is one of the most emblematic species of coralligenous bottoms. It normally inhabits vertical rocky bottoms dominated by strong currents (True 1970; Weinberg 1976; Gori et al 2011a). Lacking of symbionts, this species is completely heterotrophic and its diet is constituted by zooplankton and POM (both live an detrital) (Coma et al. 1994; Ribes et al. 1999).

Eunicella singularis (Esper, 1794) (Orden: Alcyonacea, Family: Gorgoniidae) is the only symbiotic gorgonian in the Mediterranean Sea. It presents two different morphotypes depending of the bathymetrical distribution: the shallow one presents candlestick-like shape with a brownish-white colour due to the presence of algae; whereas, the deep one (generally located below 35 m depth) has a variable colony shape and a bright white colour due to the lack of symbionts, thus it is completely heterotrophic (Théodor 1969; Gori et al. 2011b). A previous study on the feeding of shallow *E. singularis* highlighted that the autotrophic contribution to the diet, supplies more energy than the heterotrophic feeding (zooplankton) (Ferrier-Pagès 2015 et al. 2015; Coma et al. 2015). No previous data were available of the POM contribution in the diet of this species living in shallow waters, and no literature about the feeding habits of the deep *E. singularis* was available.

Corallium rubrum (Linnaeus, 1758) normally lives in dense patches in crevices, overhangs or cave entrances (Riedl 1984), between 7 and 200 m depth, with the bathymetrical range varying considerably between different areas (Carpine & Grasshoff 1975; Riedl 1984; Rossi et al. 2008). It is an heterotrophic species mostly feeding on detrital POM, followed by live POM and zooplankton (Tsounis et al. 2006; Picciano and Ferrier-Pagès 2007).

The main target of this thesis is the large-scale study of the role played on benthic-pelagic coupling processes, and as potential C sinks (C retention) by the above-mentioned benthic suspension feeders, characterized by diverse feeding strategies. The combination of in-situ and ex-situ experimental data with large-extent data about species distribution and population size structure obtained with ROV, results in the first large-scale estimation of the benthic-pelagic coupling process and C sequestration performed by benthic suspension feeders in a coastal area of the Mediterranean Sea.

The thesis is divided in four chapters:

Chapter 1 – Size, spatial and bathymetrical distribution of the ascidian *Halocynthia papillosa* in Mediterranean coastal bottoms: benthic-pelagic coupling implications

This chapter is focused on the study of the size, spatial and bathymetrical distribution of the ascidian *Halocynthia papillosa*, and on the quantification of its annual role in benthic-pelagic coupling processes and as C sinks, in the Cap de Creus area (NW Mediterranean Sea). Results of ROV video analysis were coupled with previous data about feeding and respiration of the species (Ribes et al. 1998; Coma et al. 2002).

Chapter 2 – The role of sponges in the benthic-pelagic coupling process in temperate coastal areas

This chapter is focused on the study of size, spatial and bathymetrical distribution of the sponges *Aplysina aerophoba* and *Axinella polypoides* and on the quantification of their influence in the benthic-pelagic coupling estimating the C flow induced by these two species in spring. In situ feeding experiment were performed by means of flow incubation chambers to measure the C ingested, and the results of these experiments were coupled with results of ROV video analysis performed in Cap de Creus area.

Chapter 3 – The importance of gorgonians in the benthic-pelagic coupling processes in Mediterranean coastal areas, or why do we have to preserve the animal forest

This chapter is focused on the quantification of the role played in the benthic-pelagic coupling and on the C sequestration in spring, by the gorgonians *Paramuricea clavata*, *Eunicella singularis*, and *Corallium rubrum*. All the previous literature about feeding and respiration rates of these species was reviewed and coupled with their abundance and population size structure recorded by ROV survey in the Cap de Creus area.

Chapter 4 - Species distribution models as a tool to estimate the benthic-pelagic coupling process in coastal areas

This chapter is focused on the performance of species distribution models (SDM) for three of the studied species: *Paramuricea clavata*, *Eunicella singularis* and *Axinella polypoides.* SDM assess the relationships between species presence and abundance, and several environmental and topographic features of marine bottoms to provide a full coverage predictive map of distribution of the abundances of the studied species all over the study area.

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chapter 1

Martina Coppari (2015) DOCTORAL THESIS pp. 29-54

Size, spatial and bathymetrical distribution of the ascidian *Halocynthia papillosa* in Mediterranean coastal bottoms: benthic-pelagic coupling implications

Benthic suspension feeders are abundant in Mediterranean coastal environments, though most of them are threatened due to overexploitation, climate change impacts, and unregulated diving or fishing practices. Little is known about most of the coastal communities in terms of large scale distribution and realistic benthic-pelagic coupling implications, which are keys to understand and manage those threatened ecosystems. The active suspension feeder *Halocynthia papillosa* (one of the most common ascidian species of the Mediterranean Sea) was selected as a model organism to help to understand the ecological role in benthic-pelagic coupling processes and its importance as a carbon sink (an essential ecosystem service). The spatial and bathymetrical distribution of this organism has been studied using Remotely Operated Vehicle (ROV) video transects. The species was distributed throughout the study area, with a maximum density of 4 specimens m^{-2}. The highest abundances and the biggest sizes were observed on the range of 20–50 m depth. The role as carbon and nitrogen sink of this suspension feeder has been quantified coupling distribution data with existing in situ studies of feeding and respiration. Along the 1.24 ha of the study area, *H. papillosa* yearly ingested 519.4 g C and 31.4 g N and retained 20.2 g C. As long as the physiological data are known, this new methodology could be very useful in assessing bentho-pelagic links and the capacity of being C and N sinks of a wide range of species. This new approach may be essential for future management of benthic communities.

ABSTRACT

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1 INTRODUCTION

Benthic suspension feeders are the main constituents of hard-bottom benthic communities in dark or low-light zones (Witman and Dayton 2000) in cold (Gili et al. 2001), temperate (Gili and Coma 1998, Orejas et al. 2009) and tropical environments (Lesser et al. 2006, Wild et al. 2011). These sessile organisms play a key role as ecosystems engineers (Jones et al. 1994), enhancing the structural heterogeneity of the community and thus promoting a diversification of the associated fauna (Krieger and Wing 2002, Henry and Roberts 2007, Rossi 2013). The three-dimensional complexity originated by their populations, may influence the water flow at a local scale and increase the residence time of suspended particles (Leicher and Witman 1997, Gili and Coma 1998). These complex and well-structured communities (mainly composed by sponges, gorgonians, corals, and bryozoans) are called 'animal forests', and probably accumulate one of the highest biomasses within world oceans (Rossi et al. 2012, Rossi 2013).

Depending on the feeding strategy, these animals can be classified as passive or active suspension feeders (Shimeta and Jumars 1991). Passive suspension feeders (e.g. gorgonians and corals) solely rely on the movement of water masses respect to their capturing structures, and on the particle concentration in the water for food capture (Best 1988). Conversely, active suspension feeders (e.g. sponges, bivalves, and ascidians) pump water through a filtering structures which separate food particles from the water; the particle capture mechanisms can be different depending on the organism, ranging from cirri trapping to mucus net filter feeding (see Riisgård and Larsen 2000 for further details). By means of their trophic activity, both passive and active benthic suspension feeders generate an important flux of energy and matter from the pelagic to the benthic ecosystems (Gili and Coma 1998). Their populations may act as carbon and/or nitrogen sinks by removing organic matter from the highly dynamic and yet poorly structured pelagic system (where both carbon and nitrogen are quickly transmitted and recycled through the trophic net), and incorporating this organic matter into the long-lived and highly structured benthic system (Smith et al. 1981, Graf 1992, Gili and Coma 1998, Coma et al. 2001, Rossi et al. 2012).

Ascidians show an heterogeneous diet mainly composed of particulate organic matter (POM) (Petersen and Riisgård 1992, Ribes et al. 1998, Petersen 2007). Active feeding behaviour allows many ascidian species to reach high filtration rates, especially if occurring in dense populations (Riisgård et al. 1995). In many hard bottom communities, ascidians act as key organisms in specific seasons contributing to control the density of phytoplankton (Petersen and Riisgard 1992), as well as reducing eutrophication or contaminant concentrations (Officer et al. 1982, Loo and Rosemberg 1989, Hily 1991, Naranjo et al. 1996). Ascidians may also be significant carbon sinks in areas where they occur in high densities (Petersen and Riisgard 1992).

In the Mediterranean Sea, *Halocynthia papillosa* (Linnaeus, 1767) (Order: Stolidobranchia, Family: Pyuridae) is one of the most common ascidian species inhabiting coralligenous and sciaphilic benthic communities (Turon 1985, 1990), especially in crevices and overhangs in non-perturbed rocky bottoms (Naranjo et al. 1996). The information about the distribution and abundance pattern of this species is currently restricted to the very shallow depths, lacking information about its populations below 30 m depth. The recent development of Remotely Operated Vehicles (ROV) and their decreased operational cost and increased accessibility makes it possible to quantitatively sampling rocky bottoms at depths that cannot be sampled with traditional SCUBA dive (Mortensen and Buhl-Mortensen 2004, Rossi et al. 2008, Gori et al 2011a). One of the main advantages of this methodology is that ROV sampling coupled with quantitative video analysis allows for the study of large extensions of sea-bottom with no impact on the benthic communities (Bianchi et al. 2004, Mortensen and Buhl-Mortensen 2004, Bianchi et al. 2012). On the other hand, ROV based studies have lower accuracy compared to SCUBA dive sampling especially with species that tends to occupy cryptic locations (Gili and Ballesteros 1991).

Recent studies using ROV have shown that some of the most common coastal benthic suspension feeder species display greater abundances below 30–40 m depth (Rossi et al. 2008, Bo et al. 2011, Gori et al. 2011a, Ambroso et al. 2013), highlighting the importance of studies beyond the SCUBA limit depth. The large-extent approach proposed in this work allowed to obtain quantitative information of the distribution pattern of benthic sessile species along the entire bathymetrical range. This knowledge is essential for the management of large areas (including Marine Protected Areas, MPA) and to obtain a clear picture of the overall system.

The availability of *in situ* experimental data on the clearance and respiration rates of *H. papillosa* (Ribes et al. 1999, Coma et al. 2002), allowed to explore the possible influence of trophic constrains on the species distribution, as well as to infer the role played by this active suspension feeder in coastal marine biogeochemical cycles over a large geographical and bathymetrical extension. The novelty of the methodology proposed here lays in combining these data with quantitative data on the distribution pattern and population structure. To reach the study goal three different steps have been performed: (1) Characterize the spatial distribution pattern of *H. papillosa* over a large geographical and bathymetrical extent, following previous ROV and quantitative video analysis methods (Gori et al. 2011a, Ambroso et al. 2013); (2) Describe the size structure of the studied species; (3) Estimate the trophic impact (in terms of carbon (C) and nitrogen (N) uptake) of this species over a large representative area of Mediterranean coastal bottoms. This is the first application of such large scale approach to one of the species of the coralligenous community as previous studies focussed on coral reefs (Kinsey and Hopley 1991), seagrass meadows (Duarte et al. 2010), or mangrove forests (Eong 1993). The results of this study will increase our understanding about the distribution pattern of one important species of the coralligenous community, and will provide some insight into the benthic-pelagic coupling in coastal areas. Both information sets have to be taken into account for the establishment of management and conservation measures, as well as to make accurate calculation of ecosystem services played by the animal forest as carbon sinks.

2 MATERIALS AND METHODS

2.1. Distribution and size structure of *Halocynthia papillosa*

2.1.1 Study area

The study area extends along the Catalan Coast (northwestern Mediterranean Sea), from the boundary with France, going southward across the Cap de Creus (42°19’ 12 N; 03°19’ 34 E) and the coast belonging to the natural park of Montgrí (42° 02’ 38 N; 03° 07’ 43 E) and the marine protected area of Medes Islands (40°

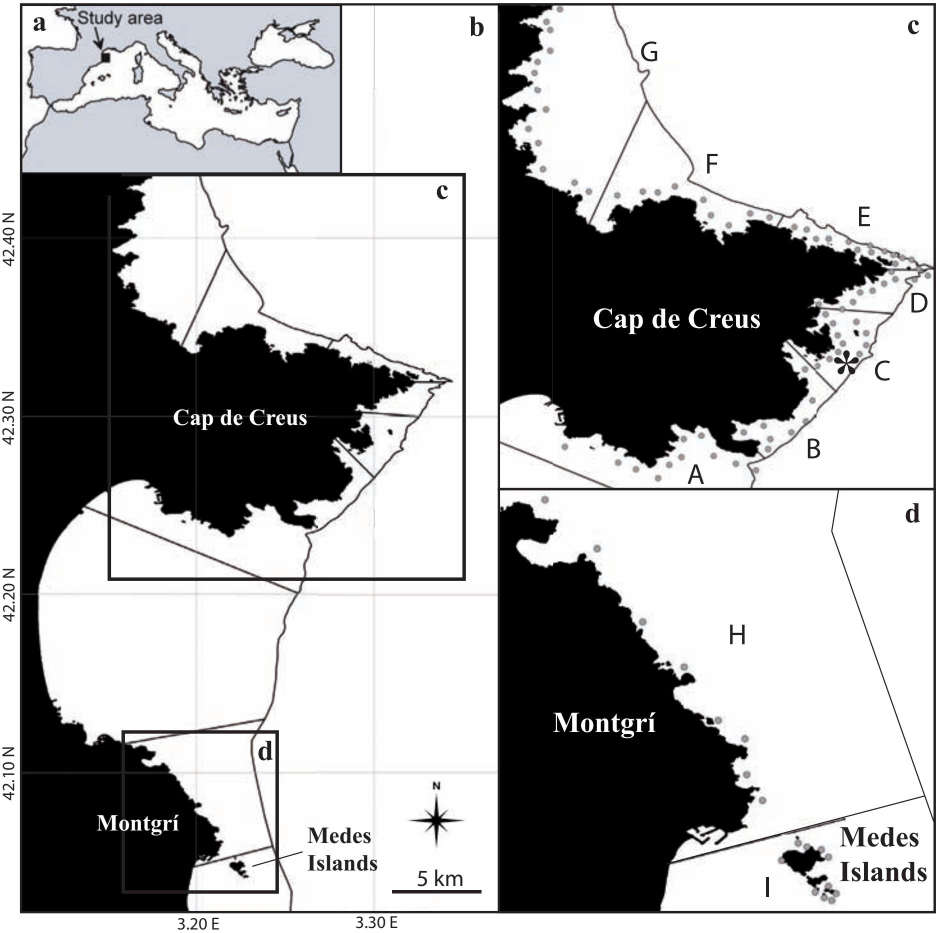

Fig. 1. Map of the study area: Location of the study area (**a**), study area in details (**b**), Cap de Creus area showing the seven sub-areas and the transect positions; the black star indicates the position of the sampling site Punta s'Oliguera (**c**), Montgrí and Medes Islands area showing the others two sub-areas and transect positions (**d**).

02' 55 N; 03° 13' 30 E). According to the main hydrodynamic patterns in the zone, and the specific features of the studied coast (Fig. 1), the study area was sub-divided into 9 sub-areas (A to I). Sub-areas from A to G are located in the Cap de Creus area (see also Gori et al. 2011a), sub-area H corresponds to the Montgrí, and sub-area I corresponds to the Medes Islands. The general circulation pattern is characterized by the dominance of the Liguro-Provençal-Catalan current (or Northern current) which flows south-westward creating an east-to west circulation (DeGeest et al. 2008). The study area receives sediment inputs from the northern Gulf of Lions (Durrieu de Madron et al. 2000), especially by the Rhone that supplies for about 90% of the total freshwater in the Gulf of Lions (Palanques et al. 2006). Besides, Medes Island are influenced by the Ter river, which show seasonal differences in runoff with heavy discharges between November and February and low discharges in spring-summer (Rossi et al. 2003). The most important winds influencing the Cap de Creus area are the northerly Tramuntana and the northwesterly Mistral that occur for 41% and 28% of the time respectively. Also in Montgrí and Medes Islands, Tramuntana and Mistral are the most important winds, although the northeasterly Gregal and the easterly Levante are the ones that bring the biggest storm waves, being as high as 5 meters (Pascual and Flos 1984). South-easterly and easterly marine winds are rare (<6% of the time) and brief (less than 3 days), in contrast to the northerly ones that can last up than one month (Ulses et al. 2008). Consequently, sub-area A is the most sheltered area of the surveyed coast; sub-areas B, C, D are affected only by easterly winds and they are not directly influenced by the main near-bottom currents (Ulses et al. 2008, DeGeest et al. 2008). Sub-areas E, F are directly exposed to the main winds and wave actions in the study area (Ulses et al. 2008), as well as to the main near-bottom currents that accelerate around the cape (DeGeest et al. 2008). Sub-area G is characterized by sediment deposition processes due to the reduced influence of the main near-bottom currents. The bionomic characteristics vary between different sub-areas of the Cap de Creus: the northern part is characterized by higher slopes and mostly rocky-bottom communities, while the southern part is much flatter and mostly contained soft bottom habitats (Sardà et al. 2012). Likewise, the Medes Islands topography is markedly asymmetrical, with prominent slopes on the northern side, and gentle slopes on the southern side (Gili and Ros 1985). Coralligenous and precoralligenous communities predominate on the east side of the Medes Island, whereas sand and shards bottoms predominate on the west side (Gili and Ros 1985). The Montgrí area is characterized by the presence of rocky bottoms close to the coast, whereas coralligenous and sandy bottoms gain presence alternating each other at increasing distance from the coast (Hereu et al. 2010).

2.1.2 Sampling procedure

Fieldwork was conducted in October-November 2004 and in November 2009 for the Cap de Creus and Montgrí-Medes areas, respectively. In Cap de Creus, transects were video recorded with the ROV Phanthom XTL equipped with a SONY FCB S3000P 3CCD camera (resolution of 700 horizontal lines), a depth sensor, a compass, and two laser beams that provide a scale to define a fixed width of the transects (0.5 m) for subsequent video analysis. In Montgrí-Medes, transects were video recorded with the ROV Triggerfish equipped with a DOE 18:1 colour zoom camera unit (resolution of 470 horizontal lines), a depth sensor, a compass, and two laser beams. Videos were recorded on tapes in DV format. In each sampled location, seabed video recording started at the deepest position and proceeded toward the shallows, until the ROV surfaced close to shore. The ROV speed was kept constant, approximately 0.4 knots for Cap de Creus and 0.5 knots for Montgrí-Medes. Transects started at a depth between 12 and 71 m in Cap de Creus, and between 10 and 52 m in Montgrí-Medes, depending on the geographical characteristics of each location. Transect length varied between 92.6 m and 907.1 m in Cap de Creus and between 13.9 m and 249.2 m in Montgrí-Medes.

On the whole, 76 video transects were recorded in the 2004 field campaign (Fig. 1c) and 18 in the 2009 (Fig. 1d) covering a total distance of 28.3 km in Cape de Creus and 2.1 km in Montgrí-Medes.

2.1.3 Video analysis

Quantitative video analysis was performed according the methodology described in Gori et al. 2011a and Ambroso et al. 2013. Videos were transferred from tapes to hard disk and then analysed using Final Cut Pro 6.06 software (Apple). As speed was constant, all the pauses in the movement of the ROV were removed to correctly estimate the total length of each transect. Sequences with poor image quality, due to bad visibility or too much distance from the bottom, were discarded from the analysis. Each transect has been divided in sampling units of 2.5 m² (0.5 m width and 5 m long); all the sampling units, except the ones that correspond completely or partially to no analysable sequences, have been examined. The sampling unit area was chosen from Weinberg 1978, who estimated that a sample size of 2 m^2 was a representative sampling area for studying invertebrates in the Mediterranean rocky substrata. A total of 4559 useful sampling units were obtained from the 76 transects of the Cap de Creus that correspond to 0.011 km^2 and 381 were obtained from the 19 transects of Montgrí-Medes equal to 0.0095 km^2. For each sampling unit the number of *Halocynthia papillosa* was determined, together with its depth and the percentage of the abundance of each seabed substrate type. Substrates were classified in five different categories: soft bottoms (mud, sand, detritic and *Posidonia oceanica* cover), maërl (species of coralline algae growing loosely in beds of fragmented nodules), pebbles, rock and coralline rock. The distance occupied by each substrate type has been recorded and then converted into % of coverage. The cumulative percentage was converted into number of sampling units considering 100% of a determinate substrate type as one sampling unit. To study the size structure of its populations, the maximum height of each observed *H. papillosa* was measured using the Carnoy software package (Schols and Smets 2001) on still images extracted from the videos. Only the organisms lain perpendicular to the video and on the same plane of the laser beams have been measured (Gori et al. 2011b), considering the distance between the laser beams as calibration for the extracted images. This methodological constraint entail that not all the specimens encountered could be measured, hence only a subsample of the *Halocynthia papillosa* observed has been included in the study of the size structure.

2.1.4 Data treatment

The presence of *H. papillosa* was quantified both by occupancy (frequency of occurrence in the set of sampling units) and by abundance (number of specimens per sampling unit). The spatial distribution was studied by mapping the densities observed in each sampling unit with respect to its position on a geographically referenced map using software Quantum Gis 1.7.2 (Quantum GIS Development Team 2009). The position of the sampling units was estimated from the recorded geographical coordinates of the initial and final point of each transect. The bathymetrical distribution of the species was studied in each sub-area, taking into account the average depth of each sampling unit and estimating the median density at depth intervals of 5 m. The R-language function Lm of the R software platform (R Development Core Team 2012) was used to perform multiple linear regressions between *H. papillosa* abundance, and depth and presence of hard substrate (rock and coralline rock). Afterwards, the R-language function Summary was used to test the adequacy of the obtained equation by means of an analysis of variance (ANOVA), calculate the multiple regression coefficient of determination (R^2), and assess the significance of each variable in the explanation of the species abundance.

The size structure of *H. papillosa* populations was analysed with descriptive statistics using distribution parameters such as skewness and kurtosis. Skewness is a measure of the symmetry of a distribution using its mean; if skewness is significant, the distribution is asymmetric. The prevalence of small size class in a population is indicated by positive skewness whereas negative skewness indicates the dominance of large size classes. Kurtosis indicates the peakedness of a distribution near its central mode. A significant value of kurtosis indicates that the variable has longer tails than a normal distribution and therefore the prevalence of a particular size class in a population. Skewness and kurtosis were calculated by the R-language functions Agostino.test (Komsta and Novomestky 2012) and Anscombe.test (Anscombe and Glynn 1983), which are available in the moments library of the R software platform.

2.2 Trophic impact of *Halocynthia papillosa*

2.2.1 Size *versus* dry weight

The relationship between *H. papillosa* size and dry weight (essential to calculate the biomass) was determined by sampling by SCUBA dive 13 ascidians ranging from small to medium and large size class (Punta s'Oliguera, Cap de Creus, 42° 17' 1.62 N, 3° 17' 57.18 E, Fig. 1c). Ascidians were photographed *in situ* with a ruler on their side, to infer the biomass from the image analysis performed with Carnoy software package (Schols and Smets 2001). Samples were collected at depths ranging from 10 to 30 m, in April 2012. Once in the laboratory, ascidians were freeze-dried, weighed, and then combusted for 5 h at 500°C and weighed again to determine their Ash Free Dry Weight (AFDW). The relationship between size and AFDW highlighted a lineal dependence between height and weight (Fig. 2). This relation was used to convert the size of the *H. papillosa* observed along the video transects, to their equivalent AFDW.

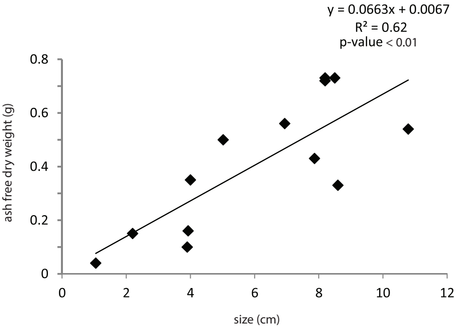

Fig. 2. Relationship between *Halocynthia papillosa* size (cm) and ash free dry weight (AFDW) (g).

2.2.2 Carbon and nitrogen estimation

Total amount and changes with depth in the seasonal ingested C and N, as well as respired C, were then estimated for the entire study area based on the distribution of *H. papillosa* by means of the data from previous *in situ* experiments (Ribes et al. 1998, Coma et al. 2002). By combining the above mentioned relationship between size and dry weight with the data about size and density of the species in the study area, the ingested C and N, and the respired C were estimated in 5 m depth intervals. Ingestion and respiration rates are related to ascidian size following an allometric relation: $F = aW^b$ where a is the filtration or respiration rate of an organism of 1 g, W is the

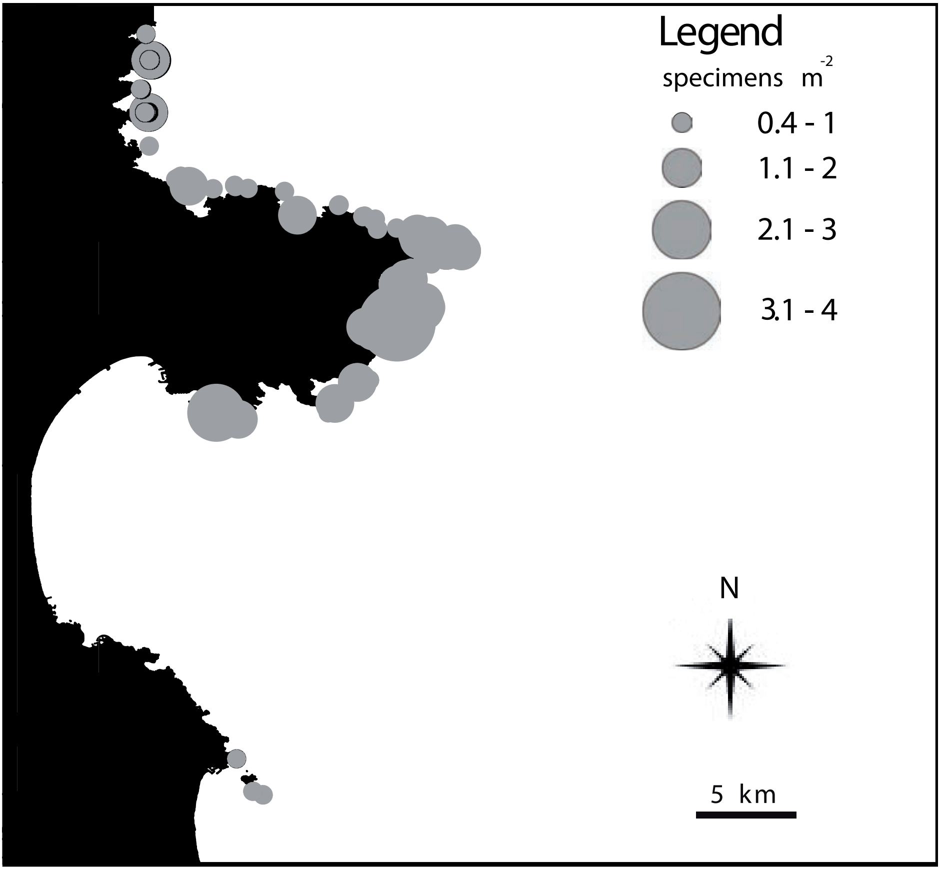

Fig. 3. Spatial distribution of *Halocynthia papillosa* in the study area. Bubbles indicates the density of *H. papillosa* (specimens m^{-2}).

weight of the individual and *b* is the rate of change of metabolic rates with size. Since the value of *b* is not known for *H. papillosa*, we used the value 0.67 and 0.70, for filtration and respiration respectively, based on previous studies on ascidians (Fisher 1976, Robbins 1983, Klumpp 1984, Jiang et al. 2008). Respiration also depends on seawater temperature (Riisgard and Larsen 2000), therefore respiration rates from Coma et al. 2002 were chosen based on the following seasonal temperatures: shallow ascidians (from 0 to 35 m depth) 16°C for spring, 20°C for summer, 16°C for autumn and 12°C for winter time; deep ascidians (deeper than 35 m) 14°C for spring, 15°C for summer, 16°C for autumn and 12°C for winter (Pascual and Flos 1984, Gori et al. 2012, Fiorillo et al. 2013). The seasonal oxygen consumption was converted into seasonal carbon dioxide production using the respiratory quotient (RQ

Sub-area	Sampling units			Max densities (Ascidians m^{-2})	Mean densities ± SD (Ascidians m^{-2})	Mean height ± SD (cm)
	Numbers	With ascidians	%			
A	803	10	1.3	2.8	0.8 ± 0.7	4.7 ± 1.6
B	456	28	6.1	2.0	0.7 ± 0.5	4.7 ± 1.1
C	630	87	13.8	4.0	0.7 ± 0.5	5.4 ±1.3
D	652	54	8.3	2.0	0.6 ± 0.4	5.5 ± 1.4
E	787	39	5.0	1.6	0.6 ± 0.3	6.2 ± 1.6
F	450	17	3.8	1.2	0.5 ± 0.2	6.2 ± 1.1
G	777	58	7.5	2.0	0.6 ± 0.4	6.9 ± 1.9
H	176	1	0.6	0.4	/	/
I	205	4	2.0	0.4	0.4	8.2 ±1.1

Table 1. Presence and spatial distribution of *Halocynthia papillosa* in the study area. Occupancy (frequency of occurrence in the set of sampling units), maximum and mean densities, and mean size are given for each sub-area. For sub-area H the mean density and the mean size are not reported because only one specimen was encountered. For sub-area I the SD of the mean density is not reported because the four sampling units with ascidians had the same density.

= $CO_{2\ evolved}$ / $O_{2\ consumed}$). Considering that most aquatic organisms, in particular ascidian species, release ammonia as excretory product (Markus and Lambert 1983), the RQ = 0.949 estimated for ammonotelic organisms (Lampert 1984) was chosen for our conversion.

2.3 Environmental conditions

Water temperature, salinity, and turbidity were measured monthly at 1 m depth intervals from 5 to 60 m with Seabird 25 and Seabird 19 conductivity temperature and depth sensors (CTDs) (see Gori et al. 2012 for further details). These data were used to characterize annual variation in water temperature, density and turbidity along the studied depth range using the Surfer 8 software (Surfer 8 Manual, 2006). Moreover, organic C/N composition of the POM was quantified seasonally. Water samples were collected at 20 and 60 m depth by means of a 5 l Niskin bottle. Three 800 ml replicates of sea water were filtered through GF/F pre-combusted (450°C for 5 h) glass fibre filters. Filters were immediately frozen in liquid nitrogen and stored at -80°C until POC (Particulate Organic Carbon) and PON (Particulate Organic Nitrogen) analysis were performed. Filters were acidified with HCl, dried at 60°C for 24 h, and therefore analysed using a C:N autoanalyser (Perkin-Elmer 2040).

3 RESULTS

3.1 Distribution and size structure of *Halocynthia papillosa*

Halocynthia papillosa was recorded in 6.45% of the total number of 4559 sampling units in Cap de Creus and in 1.31% of the total number of 381 sampling units in Montgrí-Medes area. A total of 483 specimens were counted and 169 of them measured in Cap de Creus, with the highest maximum density of 4 individuals m^{-2}. In Montgrí-Medes area, five specimens were counted and three measured. *H. papillosa* was observed all along the coast of the Cap de Creus (Fig. 3), with a higher density on the east

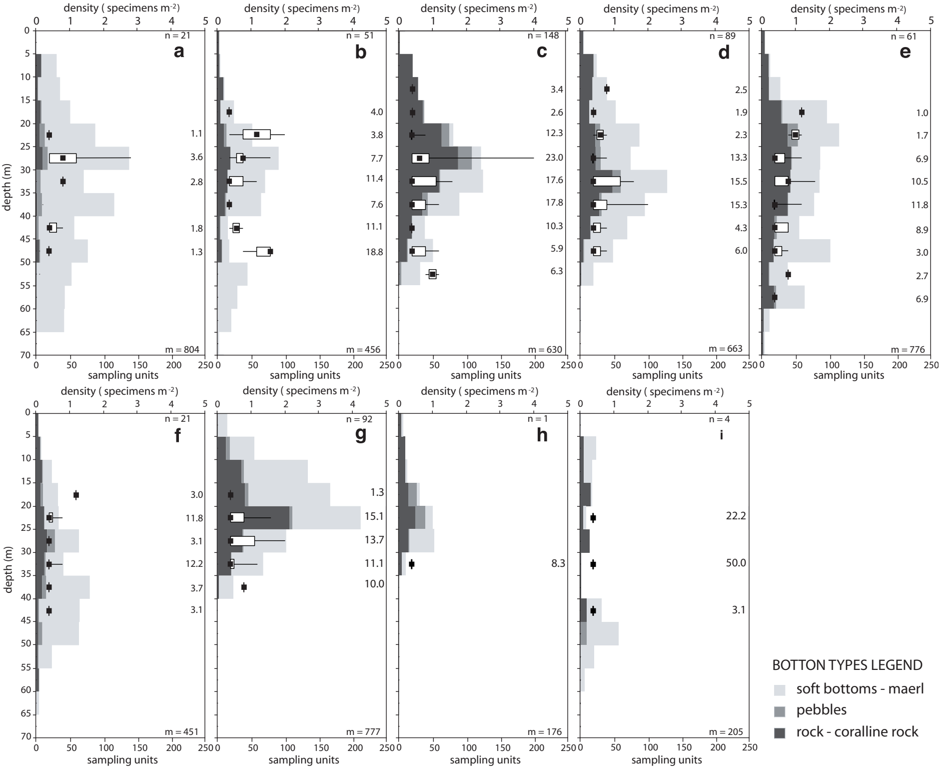

Fig. 4. Bathymetrical distribution of the density of *Halocynthia papillosa* in each sub-areas (**a-i**): the *black square* indicates the median value of the density parameter; the *box* indicates the first and the third quartiles of the density parameter; and the *line* indicates the range between minimum and maximum and median value. *Gray-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* at the right side indicate the percentage of sampling units with presence of the species. Total number of specimens (*n*) and sampling units (*m*) are indicated for each sub-area.

side of the cape (sub-areas C and D), and in some locations along the south side of the cape (sub-area A). The studied species was very rare in the Montgrí-Medes area (Fig. 3). Bathymetrical distribution of *H. papillosa* ranged from 17 to 58 m depth, with the highest frequencies and abundances observed between 20 m and 50 m depth (Fig. 4). Below this depth, sandy bottoms were dominant in almost all the areas. Subarea E was the unique subarea where the species was present at all depths. Multiple linear regressions adequately represented the relationship between *H. papillosa* abundance, depth and presence of hard substrate (y = -0.00378 + 0.00019 depth + 0.00033 rock + 0.00229 coralline rock; ANOVA, p-value <0.001), even if the regression coefficient of determination was low (R^2 = 0.163). Species abundance was not significantly related to depth (t test, p-value = 0.339), whereas it was significantly related to rock (t test, p-value <0.001) and coralline rock (t test, p-value <0.001) coverage.

Along all the study area, the smallest, the medium and the largest ascidians encountered had a dimension of 2.35 cm, 5.31 cm and 11.26 cm respectively. Medium sized specimens were dominant in all the sub-areas (but the sub-area I where only 3 specimens were observed), and small specimens of *H. papillosa* were only observed in the south and east coast of the Cap de Creus. Average heights of *H. papillosa* for each subarea are shown in Table 1. Skewness and kurtosis analysis were not significant in any of the subareas (Table 2), highlighting that the size structure of the populations of this species showed a normal distribution in all the subareas with the dominance of the medium size class (Fig. 5). Data for the subarea H were not shown because none of the encountered specimens could be measured. Skewness and kurtosis for subarea I could not be calculated because of the low number of specimens encountered and measured (Fig. 5, Table 2).

Sub-area	Skewness	p-value	Kurtosis	p-value
A	0.58	0.43	2.61	0.88
B	1.00	0.14	3.63	0.22
C	0.74	0.10	3.86	0.11
D	0.37	0.52	2.40	0.59
E	1.32	0.15	3.79	0.08
F	0.20	0.82	1.55	0.16
G	1.01	0.23	3.30	0.28
I	/	/	/	/

Table 2. Size structure distribution parameters of studied populations of *Halocynthia papillosa*: skewness and kurtosis. Skewness and kurtosis are considered significant if the *p-value* is equal or less than 0.05. Skewness and kurtosis for subarea I could not be calculated due to the few number of specimens measured.

Depth (m)	Spring		Summer		Autumn		Winter	
	I	R	I	R	I	R	I	R
0-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5-10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10-15	2.6	0.9	0.4	1.6	0.3	0.9	0.2	0.3
15-20	5.2	1.9	0.9	3.3	0.5	1.9	0.5	0.6
20-25	50.6	18.3	8.5	31.6	5.3	18.1	4.5	5.7
25-30	104.0	37.5	17.6	64.9	10.9	37.1	9.4	11.7
30-35	92.0	33.3	15.5	57.6	9.7	33.0	8.3	10.4
35-40	63.0	14.6	10.6	19.7	6.6	22.5	5.7	7.1
40-45	25.3	5.8	4.3	7.9	2.7	9.0	2.3	2.9
45-50	25.9	6.0	4.4	8.1	2.7	9.3	2.3	2.9
50-55	7.8	1.8	1.3	2.4	0.8	2.8	0.7	0.9
55-60	4.5	1.0	0.8	1.4	0.5	1.6	0.4	0.5
60-65	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
65-70	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOT (g C)	380.8	121.3	64.3	198.5	40.0	136.3	34.3	43.1

Table 3. Seasonal ingested (I) and respired (R) carbon (g) along the considered depth range in the study area, and their total (TOT).

Depth (m)	Spring	Summer	Autumn	Winter
0-5	0.0	0.0	0.0	0.0
5-10	0.0	0.0	0.0	0.0
10-15	0.2	0.0	0.0	0.0
15-20	0.3	0.1	0.0	0.0
20-25	3.0	0.7	0.2	0.3
25-30	6.3	1.3	0.4	0.6
30-35	5.5	1.2	0.3	0.5
35-40	3.8	0.8	0.2	0.4
40-45	1.5	0.3	0.1	0.2
45-50	1.6	0.3	0.1	0.2
50-55	0.5	0.1	0.0	0.0
55-60	0.3	0.1	0.0	0.0
60-65	0.0	0.0	0.0	0.0
65-70	0.0	0.0	0.0	0.0
TOT (g N)	22.9	4.9	1.4	2.3

Table 4. Seasonal ingested nitrogen (g) along the considered depth range in the study area, and its total (TOT).

Depth (m)	Season	C/N	
		mean	SD
20	Spring	11.40	4.12
	Summer	8.97	0.61
	Autumn	5.79	0.38
	Winter	8.18	2.26
60	Spring	10.19	1.08
	Summer	9.71	1.59
	Autumn	7.01	2.92
	Winter	8.57	3.43

Table 5. Seasonal organic C/N composition (mean ± SD) of the particulate organic matter at 20 m and 60 m depth.

3.2 Trophic impact of *Halocynthia papillosa*

The 172 *Halocynthia papillosa* observed and measured in the analysed sampling units (total area: 1.24 ha), ingested each year 519.4 g of C: 380.8 g C in spring, 64.3 g C in summer, 40.0 g C in autumn, and 34.3 g C in winter. A total of 499.2 g of C were estimated to be respired each year: 121.3 g C in spring, 198.5 g C in summer, 136.3 g C in autumn, and 43.1 g C in winter (Table 3). Regarding the N ingestion, *H. papillosa* ingested 22.9 g of N in spring, 4.9 g of N in summer, 1.4 g of N in autumn and 2.3 g of N in winter, accumulating a total of 31.4 g of N each year (Table 4). From these data, the balance between ingested and respired C results in a net surplus of 259.6 g of C during spring, whereas a deficit of 134.2 g results in summer, 96.2 g in autumn and 8.9 g in winter. Overall, the balance between ingested and respired C for *H. papillosa* in the study area results in a net surplus of 20.2 g of C per year.

The impact of this species per m^2 was also estimated: *H. papillosa* was able to ingest 1.28 ± 0.6 g C m^{-2} in spring, 0.22 ± 0.10 g C m^{-2} in summer, 0.13 ± 0.06 g C m^{-2} in autumn and 0.12 ± 0.05 g C m^{-2} in winter. Conversely this species respired 0.41 ± 0.21 g C m^{-2} in spring, 0.67 ± 0.37 g C m^{-2} in summer, 0.46 ± 0.22 g C m^{-2} in autumn and 0.15 ± 0.07 g C m^{-2} in winter. Regarding N ingestion, 0.08 ± 0.04 g N m^{-2} were ingested in spring, 0.017 ± 0.008 g N m^{-2} in summer, 0.005 ± 0.002 g N m^{-2} in autumn and 0.01 ± 0.003 g N m^{-2} in winter. The highest ingestion and respiration of C, as well as ingestion of N, occur between 20 and 35 m depth in spring (Figs. 6, 7 and 8), due to the elevated number of specimens observed in this depth range (Fig. 4).

3.3 Environmental conditions

Stratification of the water column began to develop in April with a thermo- and pycnocline developing at 30 m depth. The water column was fully stratified in July and August with a thermo- and pycnocline at 35 m depth,

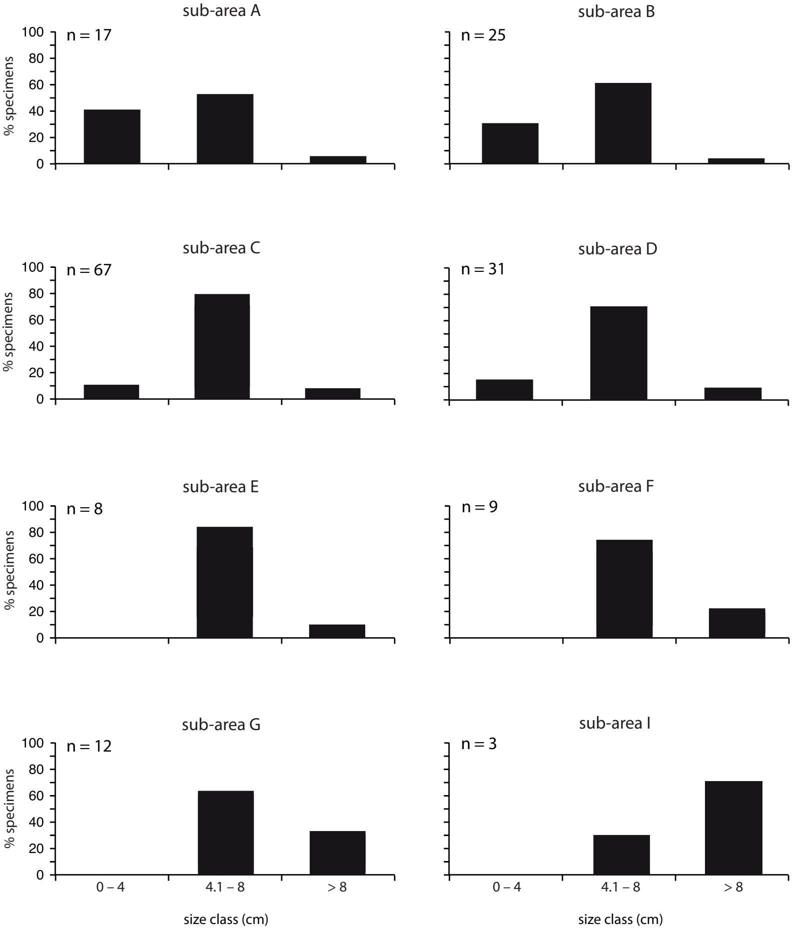

Fig. 5. Size-frequency distribution of *Halocynthia papillosa* in all the subareas. (*n* = number of specimens).

whereas stratification was stretched out in September (Fig. 9a, b). Turbidity was highly variable with the highest value in November and in April-June in shallow waters, and in September and May in deeper waters (Fig. 9c). Organic C/N composition of the POM changed during season but without significant difference between depths (Table 5).

4 DISCUSSION

This study assessed the distribution and population size structure of the active suspension feeder *Halocyntia papillosa* over a large Mediterranean coastal area and inferred the trophic impact and the potential role of this species as C and N sinks.

4.1 Large extent distribution of *Halocynthia papillosa*

H. papillosa has a more homogeneous distribution along the Cap de Creus coastline (Fig. 3) than previously studied passive suspension feeder species (*Corallium rubrum*, *Eunicella singularis*, *Paramuricea clavata*, *Leptogorgia sarmentosa*, *Alcyonium acaule*; Rossi et al. 2008, Gori et al. 2011a, Ambroso et al. 2013). These other species have their higher abundance and density concentrated on the north side of the cape, in the area directly exposed to the main currents (DeGeest et al. 2008, Ulses et al. 2008). The active feeding of *H. papillosa* may explain its more homogeneous distribution along the coast, independently from the main hydrodynamic conditions of the area (Ribes et al. 1999, Armsworthy et al. 2001, Petersen 2007). The maximum density of *H. papillosa* observed in this study (4 individuals m^{-2}) is in line with previous data from Banyuls-sur Mer (10–15 km north from Cap de Creus), were densities of ~3.5 specimens m^{-2} were found on walls and crevices, and 1 specimens m^{-2} on boulders (Weinberg 1978). This data confirms that even if the ROV-based approach has its limitations in accuracy, the numbers gathered with this methodology are realistic. In fact, similar densities of ascidian species have been recorded in other shallow water tropical, cold temperate or polar environments (McClintock et al. 1991, Riisgård et al. 1995, Shenkar and Loya 2008). High-density patches of *H. papillosa* were not observed in the present or in previous studies. This lack of patchiness could be related to the 12–24 h free swimming period of its larvae (Lubbering et al. 1993), which may allow large extent dispersion of the species (Fletcher et al. 2013).

The presence of the highest densities of *H. papillosa* between 20 and 50 m depth is in line with previous data about gorgonians, soft corals and hydrozoans (Rossi et al. 2008, Gori et al. 2011a, Ambroso et al. 2013, Cúrdia et al. 2013, Di Camillo et al. 2013). This highlights that maximum biomass of many coastal suspension feeder species may be concentrated in deep sublittoral bottoms, a largely disregarded area by previous studies, as well as by conservation and management decisions (Bongaerts et al. 2010, Bridge and Guinotte 2013). As the highest abundance of rock and especially coralligenous substrate suitable for the studied species is concentrated between 20 and 50 m in the study area (Sardà et al. 2012), substrate availability could explain the higher densities observed in this depth range (Fig. 4). The very low presence of *H. papillosa* in the Montgrí and Medes area contrasts with the occurrence and abundance of this species in Cap de Creus, and could be related to several factors. The elevated number of SCUBA divers visiting the Medes Islands each year (up to 10.000, Sala et al. 1996, Linares et al. 2012) might have impacted the studied species in the area, indeed SCUBA divers have a significant impact on the abundance of *H. papillosa* (Luna-Perez et al. 2010). However, no *H. papillosa* was observed along the Montgrí coast (Fig. 3), were diver pressure is one order of magnitudes lower than in the Medes Islands (Coma et al. 2004, Linares et al. 2012). The survey in Montgrí-Medes area was performed after the heavy storm that took place in 26th December 2008, which caused up to 80% of mortality rates of several coastal benthic organisms (Garcia-Rubies et al. 2009, Navarro et al. 2011, Teixidó et al. 2013). Although the storm might have affected the shallow organisms, deep *H. papillosa* would not have been affected by this punctual perturbation. The disappearance from the same area of the ascidian *Microcosmus sabatieri* long before this heavy storm (Martinez Ricart 2011), also reinforce the idea that other reasons may explain the sparse distribution of *H. papillosa* in Mont-

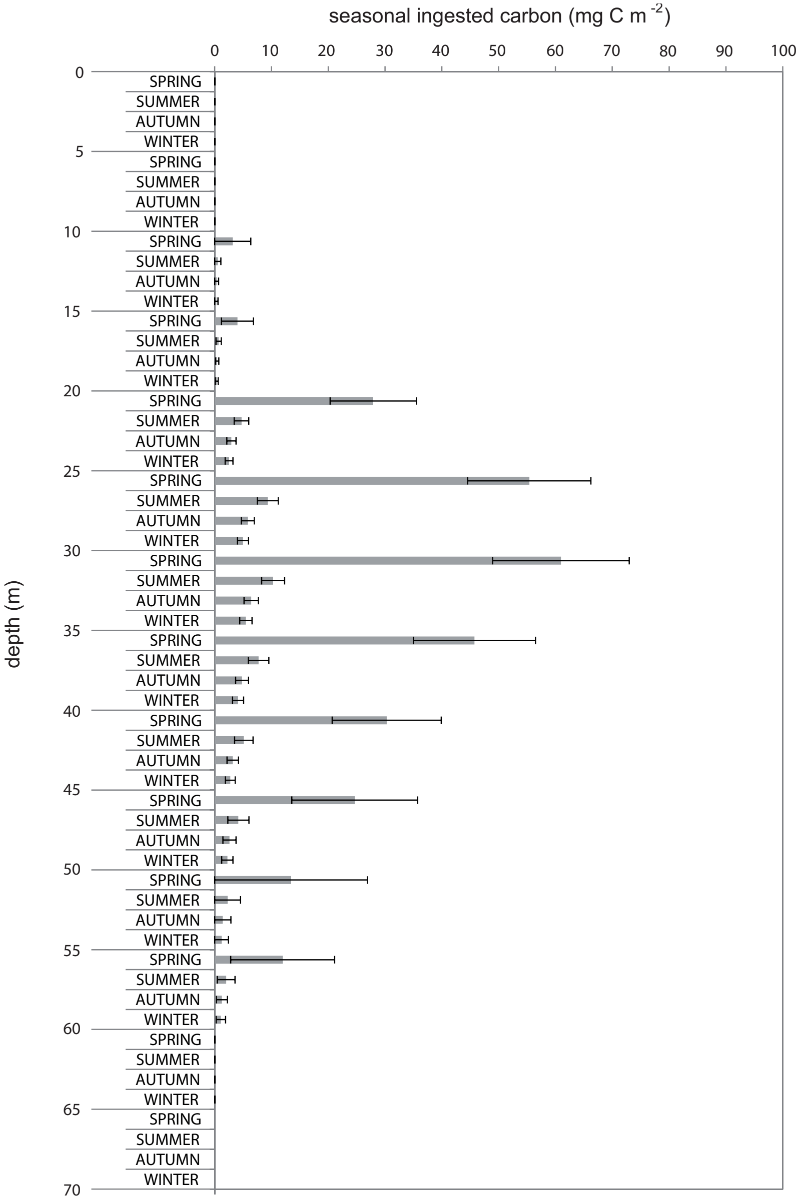

Fig. 6. Seasonal ingested carbon (mg C m^{-2}, mean ± SE) estimated every 5 m depth interval.

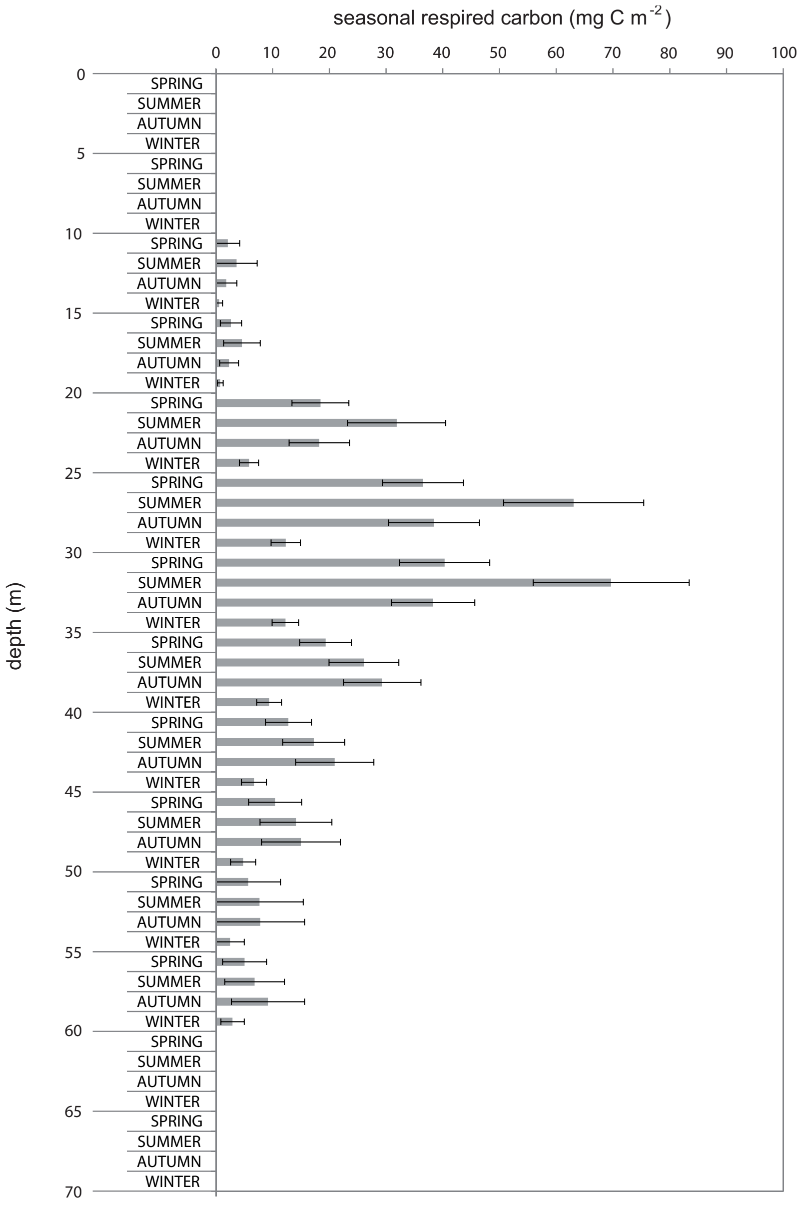

Fig. 7. Seasonal respired carbon (mg C m^{-2}, mean ± SE) estimated every 5 m depth interval.

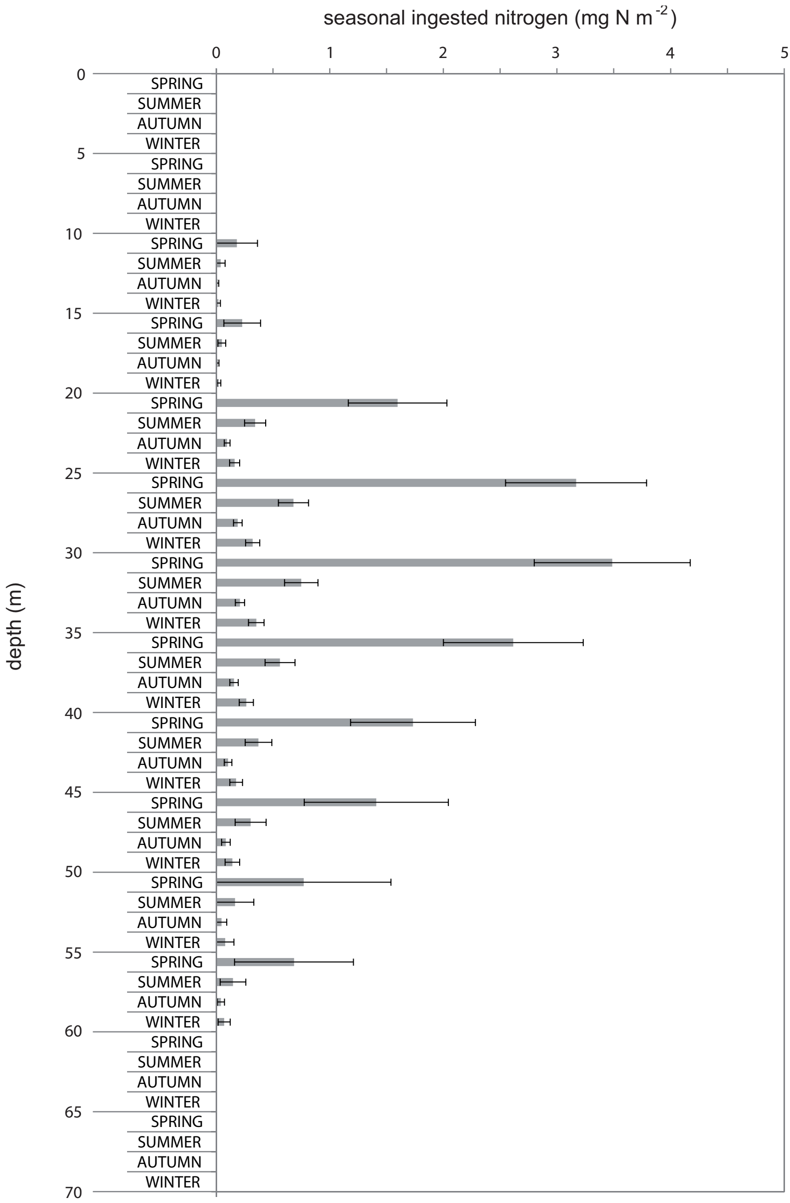

Fig. 8. Seasonal ingested nitrogen (mg N m^{-2}, mean ± SE) estimated every 5 m depth interval.

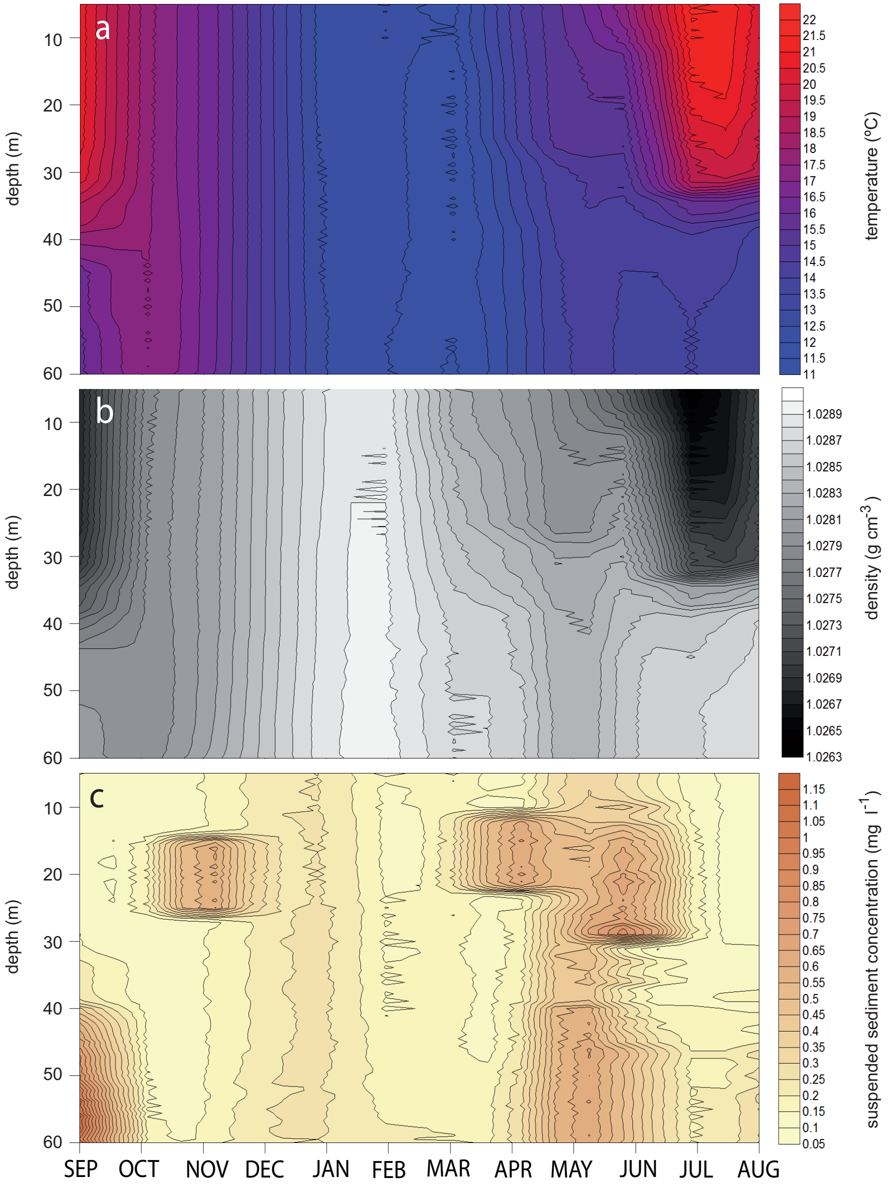

Fig. 9. Main environmental characteristics at the study area along an annual cycle: Water temperature in °C (**a**), density g/cm^3 (**b**), and water turbidity expressed in SSC (suspended sediment concentration) mg/l (**c**).

grí and Medes. It has been demonstrated that the abundances of many species has severely declined in Montgrí-Medes area, during the last two decades (Sala et al. 1996, Coma et al. 2004, Tsounis et al. 2006a, Teixidó et al. 2013). The institution of the MPA in Medes Islands in 1983, with consequent fishing prohibition, resulted in a sudden increase in fish density, which consequently increase the feeding pressure on benthic organisms (García-Rubies and Zabala 1990, Sala et al. 1998), and might have reduced the recruitment of suspension feeder species (Rius and Zabala 2008, Santangelo et al. 2012). Since the highest abundances of *H. papillosa* are concentrated on rocky and coralligenous bottoms at 20–50 m depth (Fig. 4, a-g), the lower presence of *H. papillosa* in Montgrí-Medes may also be related to the scarcity of these substrate in the 20–50 m depth range in this area (Fig. 4, h-i) (Gili and Ros 1985, Martinez Ricart 2011, Sardà et al. 2012).

4.2 Large scale trophic impact of an active suspension feeder

Our results shown that *H. papillosa* is not relevant in terms of global C coastal budget. This fact might be related with the low density and completely lack of patchiness of the studied species. *H. papillosa* yearly ingested 519.4g C and retained 20.2 g C along the 1.24 ha of the explored coastal area. The C retained represents the C sink (difference between ingested and respired C); this C could return to the system partially in the form of reproductive output (Becerro and Turon 1992). We found out that *H. papillosa* had a trophic impact as high as $5.194 \cdot 10^{-4}$ t C ha^{-1} y^{-1} and $3.14 \cdot 10^{-5}$ t N ha^{-1} y^{-1}, and that the retention is equal to $2.02 \cdot 10^{-5}$ t C ha^{-1} y^{-1}. Therefore, the trophic impact of *H. papillosa* is five orders of magnitude lower compared with previous studies performed on seagrass meadows (6.7 t C ha^{-1} y^{-1} of C stored in one year; Duarte et al. 2010) and mangrove forests (1.5 t C ha^{-1} y^{-1} of C stored in one year, Eong 1993).

Due to the higher densities and bigger specimens of *H. papillosa* concentrated between 20 and 50 m depth, this depth range shows the highest quantity of C retained and N ingested, compared to shallower or deeper bottoms where very low impact is recorded. The low presence of the species at depths shallower than 15–20 m may be due to high hydrodynamics and temperature constraints (shallow waters temperature rise up to 20–22°C in the study area, Rossi and Gili 2005, Gori et al. 2012, Fig. 9), as well as to the presence of faster growing seaweeds competing for space (Young and Chia 1984, Garrabou et al. 2002). Although the quality of food is similar in shallow and deep waters (Table 5), temperature rising in spring, summer and early autumn in shallow waters (Fig. 9) determines a negative balance between ingested and respired C at depths shallower than 15–20 m. In fact, respiration of *H. papillosa* increases in the warmer seasons (Coma et al. 2002), while clearance rates fall to suboptimal levels (Petersen 2007). This could also explain the low abundance of *H. papillosa* in shallow waters. Lesser (2006) provided evidence of a similar bottom-up control in sponge in coral reefs, where the availability of picoplankton is one of the key factors to understand their bathymetrical distribution.

Spring is the only period in which there is a clear positive balance between ingested and respired C, possibly due to the high quality and quantity of the near-bottom seston available for the species (Grèmare et al. 1997, Estrada et al. 1996, Ribes et al. 1999, Rossi et al. 2003). Indeed the studied species had to stand for the others periods of the year with the energy stored in this favourable season, allowing secondary production, growth and reproduction taking place in September until early November (Becerro and Turon 1992). In high seasonal environments, benthic suspension feeders can accumulate large quantities of lipids in favourable seasons to face the less favourable part of the year, where food input may be drastically lower (Cavalletto et al. 1999, Rossi et al. 2006, Elias-Piera et al. 2013).

5 CONCLUSION

The present study confirms that in Mediterranean Sea coastal areas, many benthic suspension feeder species concentrate their presence and abundance below 30 m depth (Rossi et al. 2008, Bo et al. 2011, Gori et al. 2011a, Ambroso et al. 2013). This should be carefully taken into account when conservation and management measures of coastal areas are established. Although our results shows that the studied species is not important in terms of C sink, it is the perfect case study to test the applicability of the novel approach proposed in this study for the quantification of the functional role of benthic species. The application of this methodology to the main species that compose the animal forests will provide a quantitative evidence of the ecosystem services performed by these assemblages in the overall budget of C as they may retain organic matter during decades or, in some cases, even centuries.

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chapter 2

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The role of sponges in the benthic-pelagic coupling process in warm temperate coastal areas

Sponges are important components of marine benthic communities with a worldwide distribution ranging from polar to tropical regions. Through their active suspension feeding, they play a key role in benthic-pelagic coupling processes providing a trophic link between the benthos and the overlying water column. Little is known about their broad-scale distribution and feeding ecology, instead, there is a general tendency to quantify their trophic impact through small patch estimations. In this work, two of the most abundant sponges in Mediterranean coastal bottoms (*Aplysina aerophoba* and *Axinella polypoides*) were studied combining Remotely Operated Vehicle (ROV) survey with in situ feeding experiments. Spatial, bathymetrical distribution and population size structure of these species were analysed, together with their trophic ecology, in spring and autumn. *A. aerophoba* is distributed between 5 and 20 m depth, with maximum densities of 1.6 sponges m^{-2}. This species ingested 0.12–0.39 mg of carbon (C) g AFDW-1 (Ash Free Dry Weight) day^{-1} in spring and 0.09–0.13 mg C g AFDW^{-1} day^{-1} in autumn. Conversely, *A. polypoides* occurred between 10 and 70 m depth, with maximum densities of 7.6 sponges m^{-2}. This species ingested 0.07–0.17 mg C g AFDW^{-1} day^{-1} in spring, and 0.18–0.60 mg C g AFDW^{-1} day^{-1} in autumn. The highest uptake of C concentrated between 5–15 m depth for A. aerophoba and between 65–70 m depth for A. polypoides. In the 1.14 ha of studied coastal bottom, *A. aerophoba* ingested 0.79 g C during spring and 0.08 g C during autumn, whereas *A. polypoides* 13.60 g C and 29.36 g C during spring and autumn, respectively. The present approach allowed a spatially explicit quantification of benthic-pelagic coupling processes produced by two of the most common sponges in a Mediterranean coastal area. This methodology, applied to benthic communities, mirrors similar approaches used in terrestrial forestry studies for C flux estimation.

ABSTRACT

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1 INTRODUCTION

Sponges (Porifera) have been important components of benthic fauna since the Early Cambrian (Zhang and Pratt, 1994). There are more than 8000 described species (World Porifera Database; www.marinespecies.org/porifera) distributed worldwide in marine and freshwater systems (Hooper and Van Soest, 2002). These metazoans became dominant during climate change shifts, forming transitional reefs that substituted calcium carbonate bioconstructions (Copper, 1994).

Sponges play different functional and structural roles (Bell, 2008), either bio-eroding or consolidating substrata, providing protection from predation and enhancing survival of associated species, thus increasing biodiversity (Marliave et al., 2009). They represent important carbon (C) sinks, accumulating biomass in three-dimensional or encrusting long-lived structures (Maldonado et al., 2012), and play an important role in the biogeochemical cycles of C, nitrogen (N) or silicon (Si) (Nixon et al., 1976; Richter et al., 2001; Maldonado et al., 2005; de Goeij et al., 2008). Although sponges can use food sources ranging from dissolved organic matter (DOM) (de Goeij et al., 2008) to small crustaceans (< 1 mm) (Vacelet and Boury-Esnault, 1995), they primarily feed on picoplankton (< 2 µm) with efficiencies up to 99 % (Pile et al., 1996; Ribes et al., 2005).

The C transfer from pelagic to benthic system has been estimated in shallow (Ribes et al., 1999a; Ribes et al., 2005) and deep environments (Pile and Young, 2006; Yahel et al., 2007). However, much work has to be done to quantify the influence of sponges in the benthic-pelagic coupling and biogeochemical cycles on a broad spatial scale. Indeed, most studies have quantified the C captured per m^{-2} only on small patches (Rossi et al., 2004). If these estimations are coupled with species distribution, density and population structure data over large areas, the impact of the sponge feeding can be quantified at the ecosystem-level. This approach is commonly used in landscape ecology (Bekkby et al., 2002) to estimate the role of forests, crops or grasslands as C sinks. This approach might help to bridge the gap of knowledge in between landscape and seascape ecology (Pittman et al., 2011).

Two of the most common Mediterranean coastal sponges, *Aplysina aerophoba* (Nardo, 1833) (Orden: Verongida, Family: Aplysinidae) and *Axinella polypoides* Schmidt, 1862 (Order: Halichondrida, Family: Axinellidae) (Fig. 1) were studied to understand their role in benthic-pelagic coupling processes. The two species show a different morphology, feeding and physiological strategies: the first is characteristic of Mediterranean photofilic algae and pre-coralligenous communities, whereas the second is among the main constituents of the coralligenous community (Ballesteros, 2006; Gili et al., 2014). *A. aerophoba* is a massive species, organized in chimney-like structures with high microbial abundance (HMA), which is partially constituted by photosyntetic cyanobacteria (Vacelet, 1970). *A. polypoides* is an erect, tree-like sponge with low microbial abundance (LMA). In their tissues, HMA sponges have microbial densities 2 to 4 orders higher than that of the surrounding water (Vacelet and Donaday, 1977; Friedrich et al., 2001). Conversely, LMA have the same microbial density in their tissue as in the surrounding water (Hentschel et al., 2003). Different densities of bacteria in the sponge tissue contribute to determine the distribution pattern of the species as well as its clearance rates and feeding strategy (Vacelet and Donaday, 1977; Friedrich et al., 2001), depending on the phototrophic contribution of endosymbiotic bacteria or algae (Wilkinson, 1983).

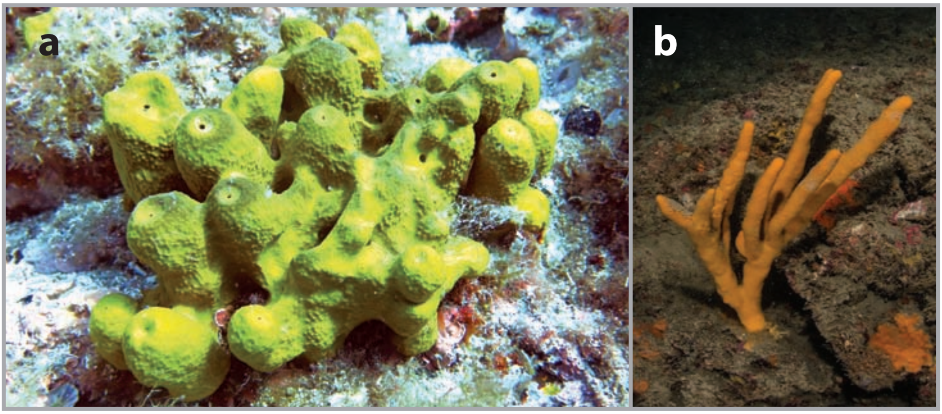

Fig. 1. Two studied species: *Aplysina aerophoba* (**a**), and *Axinella polypoides* (**b**). Photos by Núria Viladrich and Federico Betti.

Based on Coppari et al. (2014), quantitative analysis of video transects performed by Remotely Operated Vehicle (ROV), were coupled to data of in situ feeding experiments to estimate the trophic impact and the C flux mediated by these Mediterranean coastal sponges over a large extent. ROV surveys allow to assess the abundance and distribution pattern of megabenthic species over large areas and depths that cannot be sampled by SCUBA diving (Mortensen and Buhl-Mortensen, 2004; Gori et al., 2011a), and where several benthic suspension feeders often display their highest abundances (Rossi et al., 2008; Bo et al., 2011; Gori et al., 2011a; Ambroso et al., 2013; Coppari et al., 2014). In situ experiments allow determining the feeding habits of benthic suspension feeders under natural conditions (Ribes et al., 1999b; Tsounis et al., 2006) taking into account seasonal changes in food availability and retention rates (Ribes et al., 1998).

This study was organized as follows: 1) characterize the spatial distribution pattern of *A. aerophoba* and *A. polypoides* over a broad geographical and bathymetrical extent; (2) describe their population size structure; (3) perform in situ experiments to quantify the feeding habits and C uptake of the two species in spring and autumn and to test the effect of the seasonality; (4) estimate the total amount and the changes with depth in the C ingested by the studied species over the entire study area.

This study will increase our understanding of the distribution pattern of two important sponge species of the Mediterranean coastal bottoms, and will provide quantitative data about the importance of these species in the benthic-pelagic coupling process. Both information data sets are crucial in order to establish correct management and protection strategies.

2 MATERIALS AND METHODS

2.1 Study area

Fieldwork was performed in Cap de Creus (42° 19′ 12″ N; 003° 19′ 34″ E), the northern extreme of the Catalan Coast (northwestern Mediterranean Sea), bordering with France. According

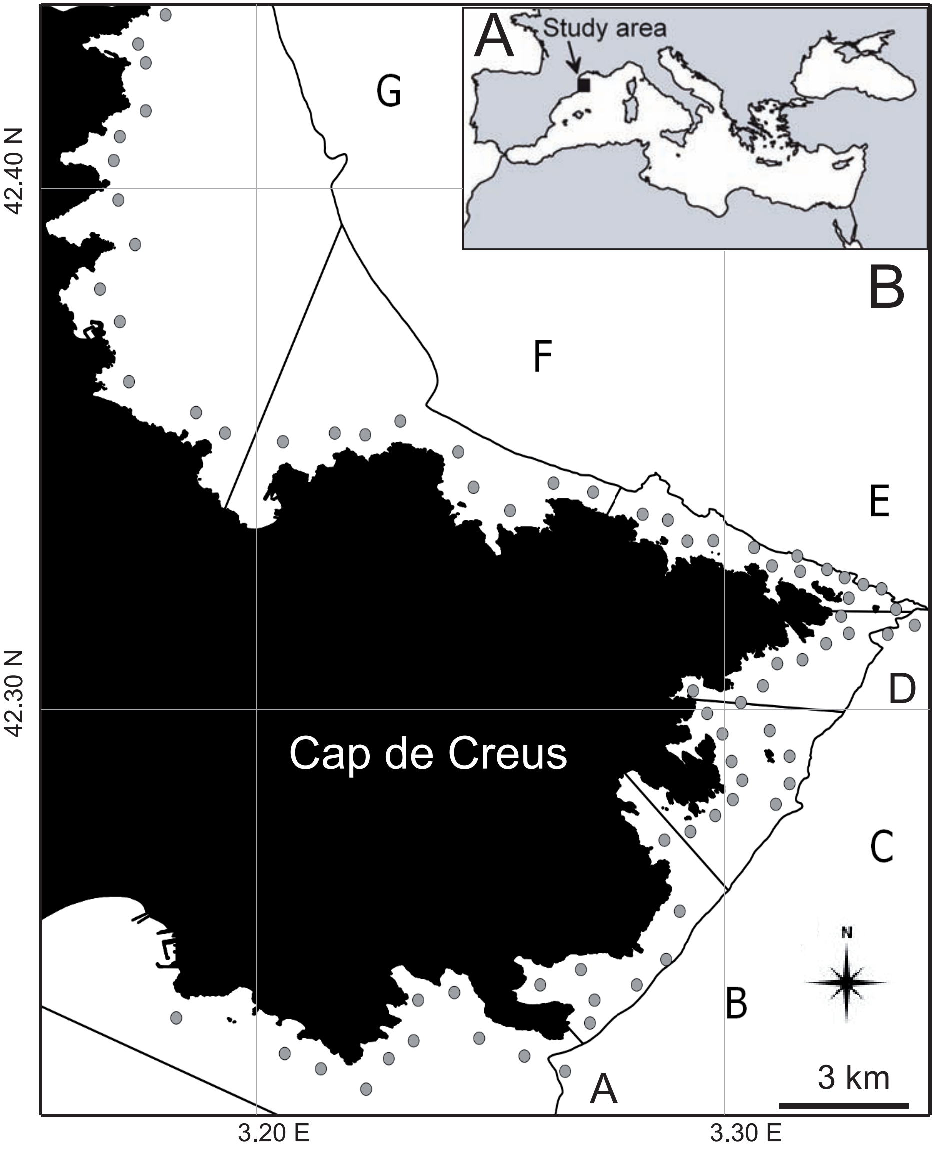

Fig. 2. Map of the study area: Location of the study area (**a**), Cap de Creus area showing the seven sub-areas and the transect positions; the black star indicates the position of the sampling site Punta s'Oliguera (**b**).

to the main hydrodynamic patterns in the zone, and the specific features of the studied coast (Fig. 2), the study area was sub-divided into 7 sub-areas, from A to G (see also Gori et al., 2011a). The general circulation pattern is characterized by the dominance of the Liguro-Provençal-Catalan current (or Northern current), which flows south-westward creating an east-to west circulation (DeGeest et al., 2008). The study area receives sediment inputs from the northern Gulf of Lions (Durrieu de Madron et al., 2000), especially by the Rhone river that supplies for ~90% of the total freshwater in the gulf (Palanques et al., 2006). The most important winds influencing the study area are the northerly Tramuntana and the northwesterly Mistral that occur for 41% and 28% of the time respectively. Strong south-easterly and easterly marine winds are rare (<6% of the time) and brief (less than 3 days), in contrast to the northerly ones that can last up than one month (Ulses et al., 2008). Consequently, sub-area A is the most sheltered area of the surveyed coast; sub-areas B, C, D are affected mainly by easterly winds and are not directly influenced by the main near-bottom currents (Ulses et al., 2008, DeGeest et al., 2008). Sub-areas E and F are directly exposed to the main winds and wave actions in the study area (Ulses et al., 2008), as well as to the main near-bottom currents which accelerate around the cape (DeGeest et al., 2008). Due to the reduced influence of the main near-bottom currents, sub-area G is characterized by sediment deposition processes.

2.2 Sponges distribution and size structure

2.2.1 Sampling procedure

Fieldwork was conducted in October-November 2004. Video transects were performed with the ROV Phanthom XTL equipped with a SONY FCB S3000P 3CCD camera (resolution of 700 horizontal lines), a depth sensor, a compass, and two laser beams that provide a scale to define a fixed width of the transects (0.5 m) for subsequent video analysis. The ROV speed was kept constant, approximately 0.4 knots. In each sampled location, seabed video recording started at the deepest position and proceeded toward the shallows, until the ROV surfaced close to shore. Depending on the geographical characteristics of each location, transects started at a depth between 12 and 71 m, and their length varied in between 92.6 m and 907.1 m. On the whole, 76 video transects were recorded (Fig. 2b) covering a total distance of 28.3 km.

2.2.2 Video analysis

Quantitative video analysis was performed according the methodology described in Gori et al. (2011a). Videos were analysed using Final Cut Pro 6.06 software (Apple). As speed was constant, all the pauses in the movement of the ROV were removed to estimate the total length of each transect. Sequences with poor image quality, due to bad visibility or too much distance from the bottom, were discarded from the analysis. Each transect was divided in sampling units of 2.5 m^2 (0.5 m width and 5 m long). The sampling unit area was chosen from Weinberg (1978), who estimated a sample size of 2 m^2 as representative for studying invertebrates in the Mediterranean rocky substrata. A total of 4559 useful sampling units were obtained from the 76 transects. For each sampling unit, the number of *Aplysina aerophoba* and *Axinella polypoides* was determined, together with their depth and the percentage of abundance of each seabed substrate type classified in five categories: soft bottoms (mud, sand, detritic and *Posidonia oceanica* cover), maerl (species of coralline algae growing loosely in beds of fragmented nodules), pebbles, rock and coralline rock. The distance occupied by each substrate type was converted into percentage of coverage. The cumulative percentage was converted into number of sampling units considering 100% of a determinate substrate type as one sampling unit.

To study the size structure of their populations, the maximum height of each observed *A. aerophoba* and *A. polypoides* was measured on still images extracted from the videos using the Macnification 1.8 software (Schols and Lorson, 2008). Only the organisms situated perpendicular to the video and on the same plane of the laser beams were measured, considering the distance between the laser beams as calibration for the still images (Gori et al., 2011b). This methodological constraint entails that only a subsample of the observed sponges could be measured for the study of the size structure (see Results).

2.2.3 Data treatment

The presence of *A. aerophoba* and *A. polypoides* was quantified by occupancy (frequency of occurrence in the set of sampling units) and by abundance (number of specimens per sampling unit). The spatial distribution was studied mapping the densities observed in each sampling unit on a geographically referenced map using Quantum Gis 1.7.2 software (Quantum GIS Development Team 2009). The position of the sampling units was estimated from the recorded geographical coordinates of the initial and final point of each transect. The bathymetrical distribution of the species was studied in each sub-area, taking into account the average depth of each sampling unit and estimating the median density at 5 m depth intervals.

Skewness and kurtosis were used to analyse the size structure of the sponge populations. Skewness is a measure of the symmetry of a distribution using its mean; if skewness is significant, the distribution is asymmetric. The prevalence of small size class in a population is indicated by positive skewness, whereas negative skewness indicates the dominance of large size classes. Kurtosis indicates the peakedness of a distribution near its central mode. A significant value of kurtosis indicates that the variable has longer tails than a normal distribution and therefore the prevalence of a particular size class in a population. Skewness and kurtosis were calculated by the R-language functions agostino.test (Komsta and Novomestky, 2012) and anscombe.test (Anscombe and Glynn, 1983), which are available in the moments library of the R software platform (R Development Core Team 2012).

2.3 Trophic impact

2.3.1 *In situ* feeding experiments

In situ feeding experiments were conducted at 5 m depth (Punta s'Oliguera, 42° 17' 1.62" N; 003° 17' 57.18" E, Fig. 2b) in May and November 2013 for *A. aerophoba*, and in November 2013 and May 2014 for *A. polypoides*. This timing was chosen to obtain feeding rate data for both species in spring and autumn, which are the most contrasting seasons in the Mediterranean Sea in terms of seston quantity and quality (Rossi et al., 2003; Rossi and Gili, 2005). Experiments were performed following Ribes et al. (2000) and Tsounis et al. (2006) using incubation chambers of approximately 4.5 l volume each (Science O'Matic, www.science-o-matic.com) (Fig. 3 and Supplementary data, see Appendix 1 in Supplementary data). The system is designed to switch from open circulation (with water continuously entering the chamber from outside) to close recirculation of the water inside each chamber. For each species and season, three

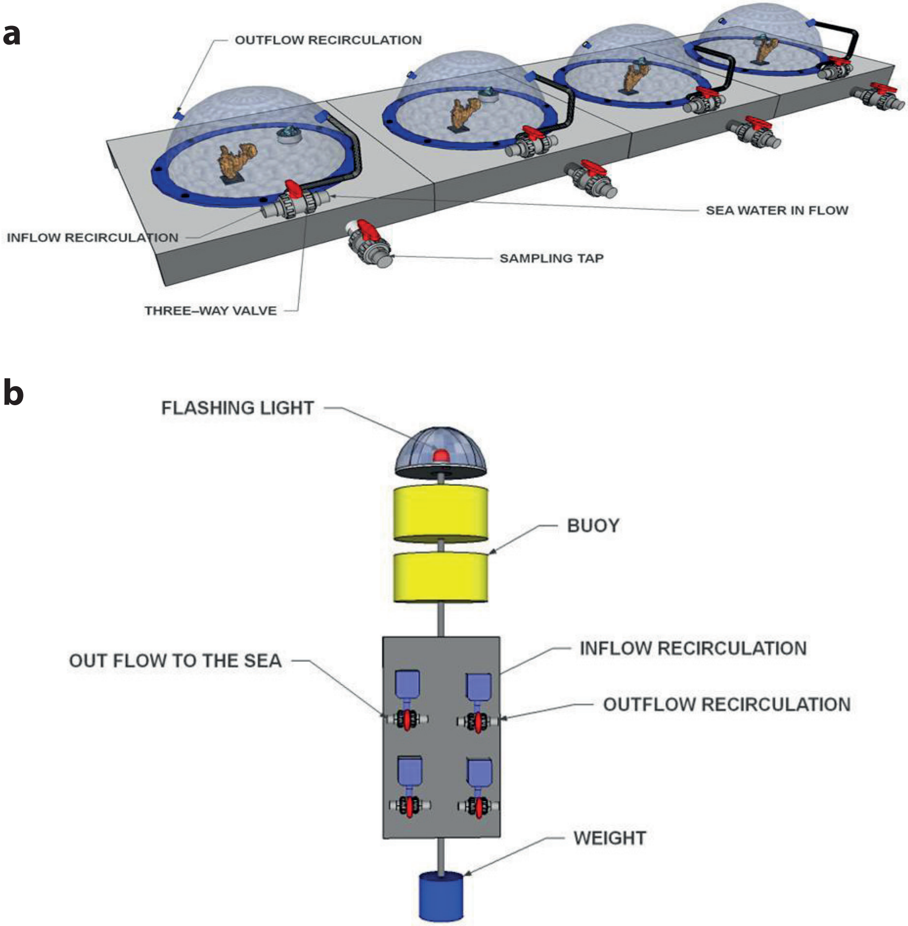

Fig. 3. In situ incubation chambers: the three way valve allow switching from open to close recirculation, the sampling tap allow the plastic bag opening and consequently the final water sampling (**a**); floating system equipped with 4 pumps and 4 taps (one for each chamber); the "outflow to the sea" indicates where the initial and final water were sampled (**b**).

experiments with sponges (n=3) and three controls without sponges (n=3) were performed. Sponges were collected by SCUBA diving, positioned into the chambers, and left for one hour with the system in open circulation mode for acclimation before the experimental started. After that, 3 initial samples of 1.8 ml of water were collected from each chamber and fixed for further analysis (see below), then the system was switched to close recirculation mode. Two hours later, 3 final samples of 1.8 ml of water were collected from

a)

Sub-area	Sampling units			Max densities (sponges m^{-2})	Mean densities ± SD (sponges m^{-2})	Mean height ± SD (cm)
	Numbers	With sponges	%			
A	803	-	-	-	-	-
B	456	10	2.19	1.6	0.96 ± 0.43	10.07 ± 5.61
C	630	-	-	-	-	-
D	652	6	0.92	1.6	0.73 ± 0.53	8.42 ± 6.34
E	787	3	0.38	1.2	0.67 ± 0.46	3.31 ± 1.21
F	450	-	-	-	-	-
G	777	8	1.03	0.8	0.6 ± 0.21	5.68 ± 2.45

b)

Sub-area	Sampling units			Max densities (sponges m^{-2})	Mean densities ± SD (sponges m^{-2})	Mean height ± SD (cm)
	Numbers	With sponges	%			
A	803	11	1.37	5.6	2 ± 1.46	11.31 ± 6.42
B	456	18	3.95	1.6	0.84 ± 0.43	8.6 ± 4.09
C	630	151	23.97	4	0.96 ± 0.76	12.08 ± 6.99
D	652	43	6.66	2.8	0.87 ± 0.62	12.02 ± 7.09
E	787	76	9.66	5.2	0.96 ± 0.81	13.2 ± 7.57
F	450	47	10.44	7.6	1.17 ± 1.37	9.75 ± 7.71
G	777	4	0.51	2	0.8 ± 0.8	2.61 ± 1.08

Table 1. Presence and spatial distribution of *Aplysina aerophoba* (**a**) and *Axinella polypoides* (**b**) in the study area. Occupancy (frequency of occurrence in the set of sampling units), maximum and mean densities, and mean size are given for each sub-area.

each chamber. Water samples were immediately fixed with 1 % paraformaldehyde + 0.05 % gluteraldehyde, frozen in liquid nitrogen and stored at -80°C until analysis by means of a Becton Dickson FACSCalibur flow cytometer to quantify the abundance of heterotrophic and autotrophic bacteria, and autotrophic pico- and nanoplankton following Gasol and Morán (1999). Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll), and green fluorescence (from DNA stained with SYBR Green) were collected through band-pass interference filters at 650, 585 and 530 nm, respectively. The five measured parameters, forward and right-angle light scatter (FALS and RALS), and orange, red and green fluorescence were recorded on 3-decade logarithmic scales, sorted in list mode, and analysed using Flowing Software (Perttu, Turku Centre for Biotechnology, Finland,). Differences between cell counts from initial and final water samples were tested with generalized linear models (GLM) assuming a negative binomial error family distribution with log-link (Zuur et al., 2009). Models were fitted using the function glm.nb available in MASS package of the R software platform (Venables and Ripley, 2002). Grazing rates were calculated following

Ribes et al. (1998), and the number of cells removed converted to equivalent content of C using the following conversion factors: 10 fg C cell^{-1} for heterotrophic bacteria (Gundersen et al., 2002); 46 and 470 fg C cell^{-1}, respectively for *Prochlorococcus* sp. and *Synechococcus* sp. (Campbell and Vaulot, 1993; Bertilsson et al., 2003); finally, C conversions for pico- and nanoeukaryotes were based on their mean biovolume, 5.13 µm^3 and 20 µm^3, respectively (Montagnes et al., 1994; Caron et al., 1995). Difference in ingested C between *A. aerophoba* and *A. polypoides* in spring and autumn were tested using ANOVA. Data were log-transformed to meet the assumptions of normality and homogeneity of variance.

2.3.2 Sponge size *versus* dry weight

To determine the relationship between *A. aerophoba* and *A. polypoides* size and dry weight, 11 *A. aerophoba* (4–16 m depth) and 26 *A. polypoides* (10–30 m depth) were sampled in May 2014 (Punta s'Oliguera, Fig. 2b). Sponges were photographed with a ruler on their side to infer their height from the image analysis performed with the Macnification software (Schols and Lorson, 2008). Once in the laboratory, sponges were freeze-dried, weighed, and then combusted for 5 h at 500°C and weighed again to determine their ash free dry weight (AFDW). Height and AFDW were linearly related in *A. aerophoba*, whereas an exponential relation was obtained for *A. polypoides* (Fig. 4). These relations were used to convert size of the specimens observed along the video transects to their equivalent AFDW as in Coppari et al. (2014).

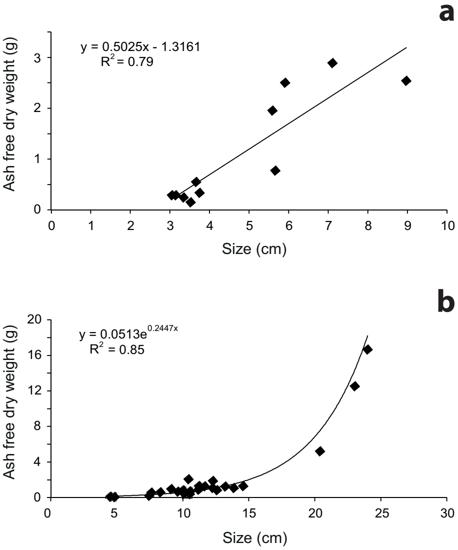

Fig. 4. Relationship between *Aplysina aerophoba* size (cm) and AFDW (g) (**a**), relationship between Axinella polypoides size (cm) and AFDW (g) (**b**)

2.3.3 Carbon fluxes estimation

Total amount and changes with depth of the seasonal ingested C were estimated for the entire study area based on the distribution of *A. aerophoba* and *A. polypoides* and the results from the in situ feeding experiments. By combining the above-mentioned relationship between sponge size and AFDW, with the data about their size and density in the study area, the ingested C was estimated in 5 m depth intervals. Ingestion rates (F) are related to sponge size following an allometric relation:

$$F = aW^b$$

where a is the filtration rate of an organism of 1 g, W is the weight of the individual and b is the rate of change of metabolic rates with size. Since the value of b is not known for the two study species, we used the value 0.914 based on previous study from Thomassen and Riisgard (1995).

3 RESULTS

3.1 Sponges distribution and size structure

A total of 56 *A. aerophoba* were observed and 20 measured, whereas a total of 1050 *A. polypoides* were observed and 703 measured. The highest density recorded was 1.6 *A. aerophoba* m^{-2} and 7.6 *A. polypoides* m^{-2} (Table 1). *A. aerophoba* mainly occurred in the south and east side of the cape, whereas *A. polypoides* was distributed throughout all the study area, with maximum abundances in subareas C and D (Fig. 5). Bathymetrical distribution of *A. aerophoba* ranged from 5 to 20 m depth, whereas *A. polypoides* was encountered between 10 and 70 m depth. Below this depth, sandy bottoms, unsuitable for both species, were dominant in almost all study areas (Figs 6 and 7).

Size of *A. aerophoba* specimens varies between 2.38 and 20.86 cm height, whereas *A. polypoides* varies between 1.07 and 46.20 cm height. In subarea B the medium sized sponges dominated, whereas in subarea D, small and big specimens were found in the same percentage; in subarea E only small sponges were encountered and, in subarea G, small and medium sized specimens were found in the same percentage. Small sized specimens of *A. polypoides* dominated all but subarea E, were the medium sized sponges were dominant (Table 2). Skewness and kurtosis were not calculated for *A. aerophoba* in any of the subareas, and for *A. polypoides* in the subarea G due to the low number of specimens encountered and measured. Skewness and kurtosis for *A. polypoides* were both significant in subareas C, D and F highlighting the dominance of small

a)

Sub-area	n	%		
		Small	Medium	Large
A	-	-	-	-
B	11	18	45	36
C	-	-	-	-
D	2	50	-	50
E	3	100	-	-
F	-	-	-	-
G	4	50	50	-

b)

Sub-area	n	%		
		Small	Medium	Large
A	38	51	41	8
B	34	68	32	-
C	240	45	41	14
D	80	48	42	10
E	186	38	42	20
F	118	60	29	11
G	8	100	-	-

Table 2. Size frequency distribution of *Aplysina aerophob*a in each subarea (**a**) with specimens classified as follows: small (0–5 cm), medium (5–10 cm), large (> 10 cm); size frequency distribution of *Axinella polypoides* in each subarea (**b**) with specimens classified as follows: small (0–10 cm), medium (10–20 cm), large (> 20 cm).

Sub-area	Skewness	p-value	Kurtosis	p-value
A	1.027	0.086	3.830	0.152
B	0.260	0.641	2.068	0.135
C	1.297	< 0.001	5.368	< 0.001
D	1.594	0.002	6.419	0.001
E	0.723	0.012	2.911	0.981
F	1.327	0.001	4.859	0.004
G	-	-	-	-

Table 3. Size structure distribution parameters of studied populations of *Axinella polypoides*; skewness and kurtosis are considered significant if the p-value is equal or less than 0.05.

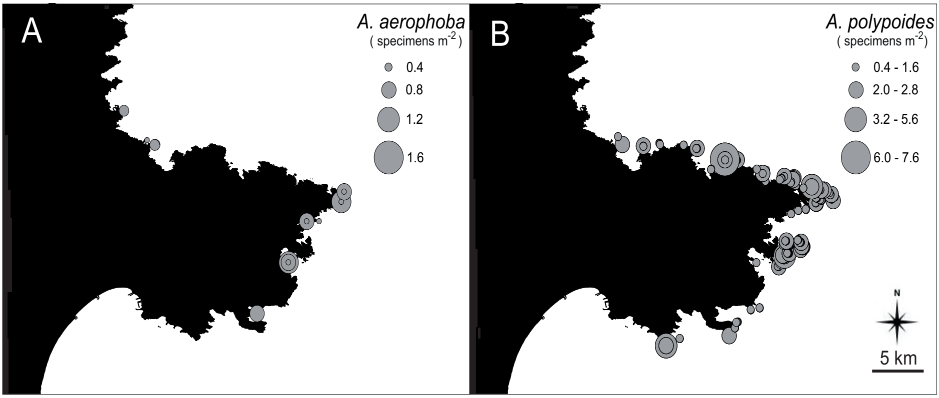

Fig. 5. Spatial distribution of *Aplysina aerophoba* (**a**) and *Axinella polypoides* (**b**) in the study area. *Bubbles* indicate the density of the two sponge species (specimens m^{-2}).

size specimens. Conversely, both skewness and kurtosis were not significant in subareas A and B highlighting a normal distribution of the sizes and dominance of medium size specimens. In the subarea E only skewness was significant, probably due to the absence of large specimens (Table 3).

3.2 *In situ* feeding experiment

In spring, the presence of *A. aerophoba* caused a significant reduction in all prey items analysed ($p < 0.05$) except for heterotrophic bacteria ($p = 0.96$) (Table S1a), whereas the presence of *A. polypoides* caused a significant reduction in all prey items analysed ($p < 0.05$) (Table S2a). *Prochlorococcus* sp was not detected in any of the spring samples. In autumn, the presence of both species caused a significant reduction only in the concentration of *Synecococcus* sp. ($p < 0.05$), and *A. polypoides* significantly removed also heterotrophic bacteria ($p < 0.05$) (Table S1b and S2b).

No significant difference in the amount of C acquired were observed between seasons ($p = 0.13$), but a significant difference in the C ingested was observed between species, as *A. polypoides* ingested significantly more C than *A. aerophoba* ($p < 0.05$). The interaction between species and seasons was significant ($p < 0.05$) (Fig. 8 and Table S3), highlighting an opposite trend in the C consumed: *A. aerophoba* ingested more C in spring, whereas *A. polypoides* in autumn. Overall, *Synecococcus* sp. (in spring) and heterotrophic bacteria (in autumn) constituted the major food source of *A. aerophoba* in terms of particle abundance, even though the main source of C originated from *Synecococcus* sp. in spring and from autotrophic nanoeukaryotes in autumn. Heterotrophic bacteria were the major food source in terms of particle abundance also for *A. polypoides* (both in spring and autumn), but the main source of C originated from nanoeukaryotic cells. *A. aerophoba* ingested 0.12–0.39 mg C g $AFDW^{-1}$ day^{-1} in spring and 0.09–0.13 mg C g $AFDW^{-1}$ day^{-1} in autumn, whereas *A. polypoides* ingested 0.07–0.17 mg C g $AFDW^{-1}$ day^{-1} in spring and 0.18–0.60 mg C g $AFDW^{-1}$ day^{-1} in autumn. The impact of the two sponge species per m^2 was also estimated: *A. aerophoba* was able to ingest 0.31 ± 0.09 mg C m^{-2} day $^{-1}$ (medium ± SE) in spring

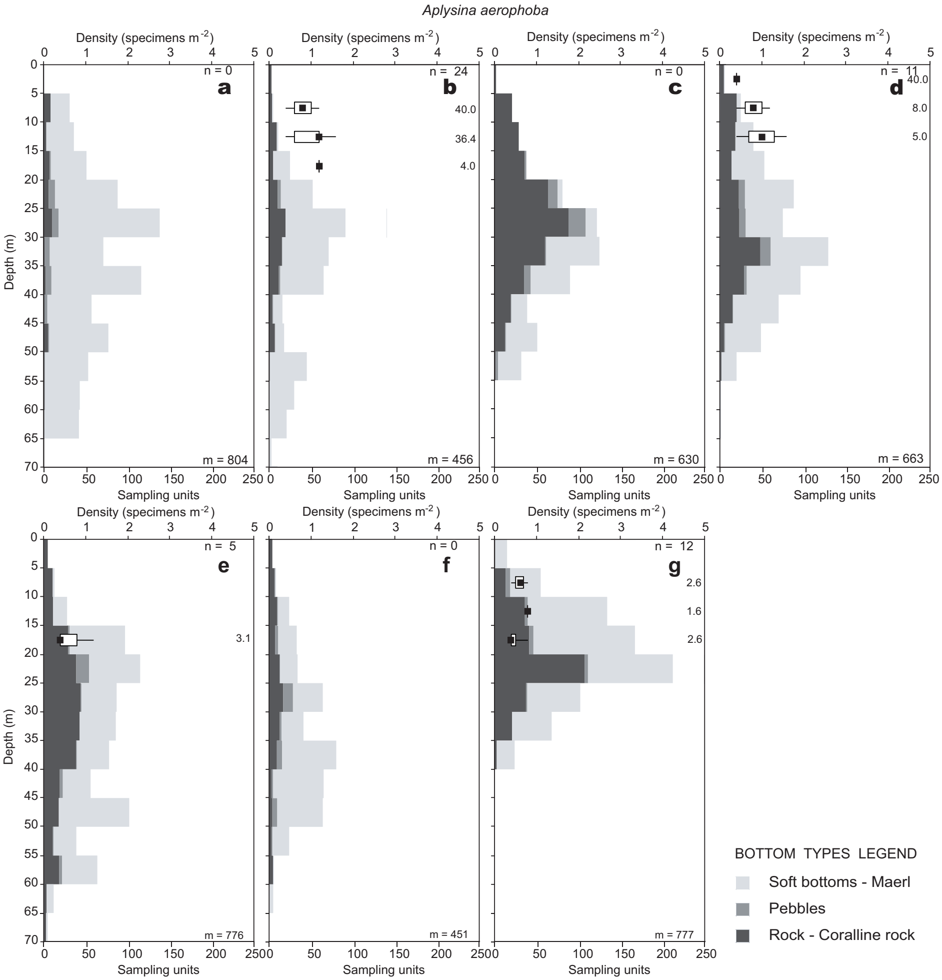

Fig. 6. Bathymetrical distribution of the density of *Aplysina aerophoba* in each sub-areas (**a-i)**: the *black square* indicates the median value of the density; the *box* indicates the first and the third quartiles; and the line indicates the range between minimum and maximum and median value. *Gray-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* at the right side indicate the percentage of sampling units with presence of the species. Total number of specimens (*n*) and sampling units (*m*) are indicated for each sub-area.

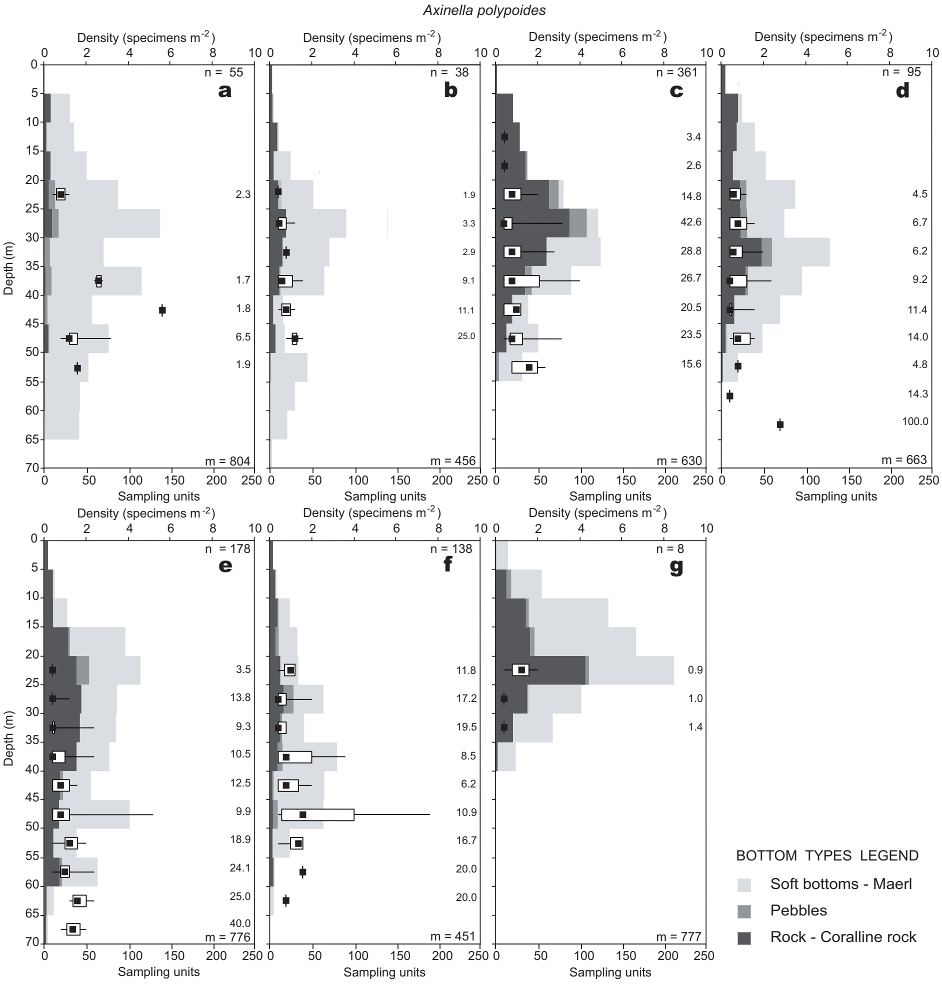

Fig. 7. Bathymetrical distribution of the density of *Axinella polypoides* in each sub-areas (**a-i**): the *black square* indicates the median value of the density; the *box* indicates the first and the third quartiles; and the line indicates the range between minimum and maximum and median value. *Gray-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* at the right side indicate the percentage of sampling units with presence of the species. Total number of specimens (*n*) and sampling units (*m*) are indicated for each sub-area.

and 0.03 ± 0.01 mg C m^{-2} day $^{-1}$ in autumn. *A. polypoides* ingested 0.19 ± 0.02 mg C m^{-2} day $^{-1}$ in spring and 0.42 ± 0.04 mg C m^{-2} day $^{-1}$ in autumn.

3.3 Carbon fluxes estimation

Over the entire study area (1.14 ha), the 20 measured *A. aerophoba* ingested 0.79 g of C in spring and 0.08 g in autumn (Table 4a); whereas the 703 measured *A. polypoides* ingested 13.60 and 29.36 g of C in spring and autumn, respectively (Table 4b). Highest trophic impact concentrated at 5–15 m depth for *A. aerophoba* (Fig. 9), and 65–70 m depth for *A. polypoides* (Fig. 10).

4 DISCUSSION

The studied species shown contrasting spatial and bathymetrical distribution. *Aplysina aerophoba* was mainly concentrated on the east side of the cape, probably sheltered from strong hydrodynamics caused by wind waves (Ulses et al., 2008). As a matter of fact, HMA sponges that need light for their symbiotic autotrophic bacteria prefer sheltered, low hydrodynamic conditions to survive (Imhoff and Trüper, 1976) as well as shallow environment to maintain the photosynthetic activity (Becerro et al., 2003; Pfannkuchen et al., 2009; Perez Castro 2014). On the other hand, *Axinella polypoides* presents a more homogenous distribution along the studied coast, similarly to the ascidian *Halocynthia papillosa* (Coppari et al., 2014). This continuous distribution of sponges and ascidians contrasts the abundances of passive suspension feeders mainly distributed in the northern face of the cape (Rossi et al., 2008; Gori et al., 2011a; Ambroso et al., 2013), in zones directly exposed to the main near-bottom currents (DeGeest et al., 2008). In point of fact, active suspension feeders may be more independent from near-bottom currents for food supply (Ribes et al., 1999a; Armsworthy et al., 2001).

Maximum density of *A. aerophoba* observed in this study (1.6 individuals m^{-2}) was much lower than previously reported (11 individuals along 5 m transect, Becerro et al., 2003), possibly due to differences between the studied areas or to differences in the methodology applied. Conversely, maximum density of *A. polypoides* recorded in this study (7.6 individuals m^{-2}) was higher than previously reported (0.5 individuals m^{-2}, at 23 m depth in Banyuls sur Mer, Weinberg, 1978) possibly because the use of the ROV allowed to reach and quantitatively study the depth range where this species shows its highest densities (Fig. 7). The bathymetrical distribution of *A. polypoides* concentrated below 35 m depth, might be related to its arborescent-shape morphology, which can be easily break down in wave-exposed shallower depths (Bell and Barnes, 2000; Bell, 2007). Moreover, similarly to other LMA species, *A. polypoides* mainly depends on near bottom live and detrital POM for its nutrition (Weisz et al., 2008; Hadas et al., 2009), resulting particularly affected by the summer oligotrophic con-

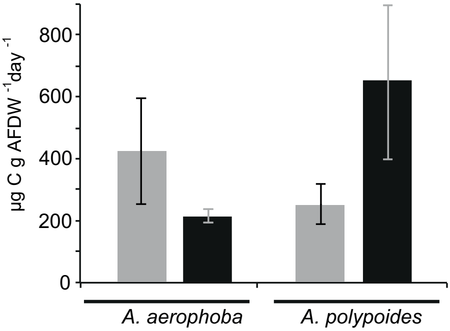

Fig. 8. Ingested C (μg C g AFDW^{-1} day-1) (mean ± SE) in spring (grey histogram) and autumn (black histogram) by the two sponge species.

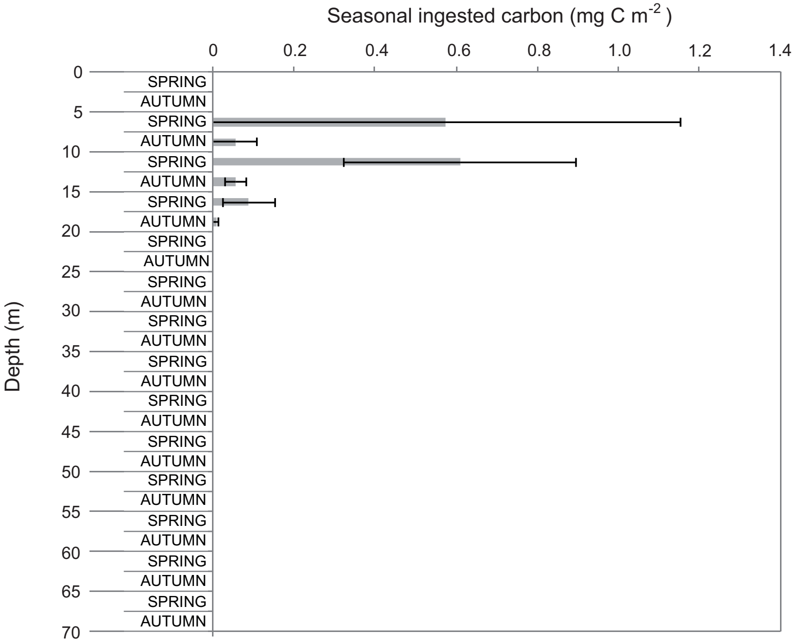

Fig. 9. Seasonal ingested carbon (mg C m^{-2}, mean ± SE) of *Aplysina aerophoba* estimated every 5 m depth interval.

dition of shallow Mediterranean waters (Rossi and Gili, 2005). The observed bathymetrical distribution of *A. polypoides* supports the recent observation that high biomass of coastal megabenthic suspension feeders is generally concentrated in deep sublittoral bottoms (30–90 m depth) (Gori et al., 2011a; Ambroso et al., 2013; Coppari et al., 2014), possibly due to the major stability of the main environmental features under the summer thermocline (Gori et al., 2012; Coppari et al., 2014).

In accordance to Ribes et al. (1999a), both studied species showed a clear seasonal change in their feeding habits, mainly related to the natural variation in the composition of the near bottom seston in the Mediterranean Sea (Ribes et al., 1999b; Rossi and Gili, 2005). According to Ferrier-Pagès et al. (1998) and Ribes et al. (1999b), no *Prochlorococcus* sp. was detected in spring. Conversely, *Prochlorococcus* sp. was observed in autumn with abundances one order of magnitude less than in Ribes et al. (1999a). This discrepancy was also observed for picoeukaryotes, whose low concentration in the ambient water might impede to detect any possible decrease due to sponge feeding. In the Mediterranean Sea autumn storms and river runoff result in high concentration of low quality POM mainly composed by inorganic sediment (Grémare et al., 2003; Rossi et al., 2003). Most of the C ingested by *A. aerophoba* derived from *Synecococcus* sp., as previously observed by Pile et al. (1996, 1997), and nanoeukaryotes, whilst C mainly proceeded from nanoeukaryotes in *A. polypoides*,

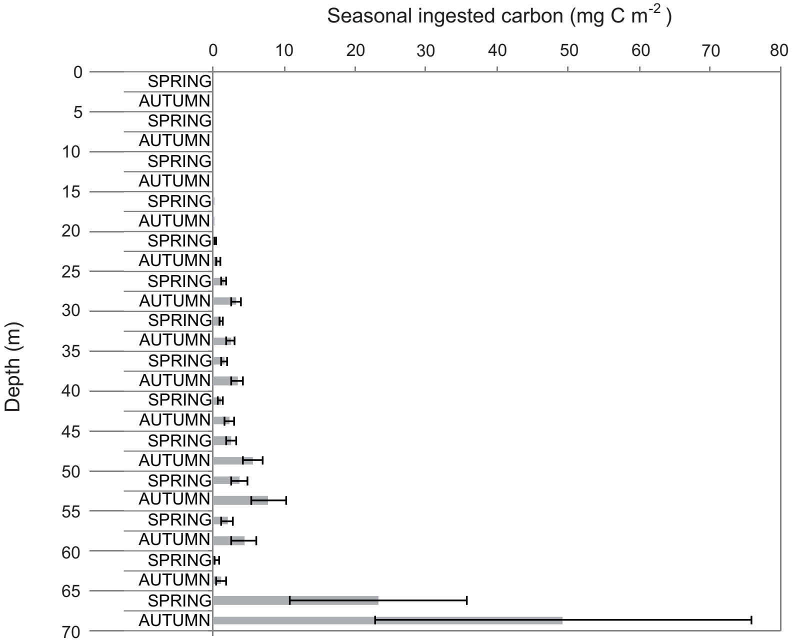

Fig. 10. Seasonal ingested carbon (mg C m^{-2} mean ± SE) of *Axinella polypoides* estimated every 5 m depth interval.

as described by Topçu et al. (2010) for *Spongia officinalis*. Detrital component of POM and DOM have also been considered important C input for sponges (de Goeij et al., 2008; Hadas et al., 2009). In our study, no evidence of detrital POM feeding by both sponges was detected (data not shown); in accordance to Jimenez (2011) flow incubation chambers did not allow to detect differences neither in detrital POM nor in DOM between initial and final water samples. Even if other methodologies (e.g. InEx; Yahel et al., 2005) can be more appropriate to completely identify all the food sources of sponge species, they can only be applied to species with osculum diameter big enough to allow for sampling of exhalant water (see Yahel et al., 2005 for further details), and this was not the case of *A. polypoides*.

The lack of difference in the C ingested between seasons is consistent with previous study performed on *D. avara* (Ribes et al., 1999a), whereas the higher C intake of *A. polypoides* respect to *A. aerophoba* might be related to its deeper bathymetrical distribution concentrated at depths where food availability is constant throughout the year (Gori et al., 2012; Coppari et al., 2014). Basile et al. (2009) demonstrated that *A. polypoides* has an elevated growth rate possibly related to the lack of chemical defences against microbial fouling and feeding deterrence against predators, since chemically-undefended species might invest more energy in growth and reproduction while tolerating partial predation as a cost of being only marginally defended by means of physical defence such as spicules (Haber et al., 2011). Contrarily, *A. aerophoba* produces bromotyrosine alkaloids (BAs) with cytotoxic, algicide, antibacterial and anti-predators effects (Sacristian-Soriano et al., 2011). A consume of DOC by *A. aerophoba* could be hypothesized as already demonstrated in other HMA species (de Goeij et al., 2008), and this additional source of food, together with the possible nutritional contribution from symbiotic cyanobacteria, could explain the lower C ingested by this species.

Considering the density of the studied species and their population size structure *A. aerophoba* removed 0.31 ± 0.09 mg C m^{-2} day^{-1} in spring and 0.03 ± 0.01 mg C m^{-2} day^{-1} in autumn, whereas *A. polypoides* removed 0.19 ± 0.02 mg C m^{-2} day^{-1} in spring and 0.42 ± 0.04 mg C m^{-2} day^{-1} in autumn. These are low values compared to other sponges (*Mycale lingua*: 29 mg C m^{-2} day^{-1}, Pile et al. (1996); *Sericolophus hawaiicus*: 55 mg C m^{-2} day^{-1}, Pile and Young (2006)), octocoral species (*Leptogorgia sarmentosa*: 2.3–16.8 mg C m^{-2} day^{-1}, Rossi et al. (2004); *Corallium rubrum*: 0.4–9.6 mg C m^{-2} day^{-1}, Tsounis et al. (2006)), or ascidians (*Halocynthia papillosa*: 13.9 mg C m^{-2} day^{-1} and 1.5 mg C m^{-2} day^{-1}, in spring and autumn respectively, Coppari et al. (2014)). This discrepancy could be related to the low densities of both species in the study area, or to the neglecting of some of the potential food sources. Overall, along all the Cap de Creus coast *A. aerophoba* removed 0.79 g C during spring and 0.08 g during autumn, whilst *A. polypoides* removed 13.60 g C during spring and 29.36 g C during autumn, as a consequence of the higher abundance and the larger specimens of *A. polypoides* compared to *A. aerophoba*.

During the last decade, a effort has been made to understand the role of terrestrial ecosystems in capturing part of the anthropogenic produced C (Bellassen and Luyssaert, 2014) by coupling forest ecology to broad-scale cartography and landscape studies based on remote sensing (Janssens et al., 2004). As proposed in this study, in seascape ecology similar results may be achieved by coupling large-scale quantification of species distribution pattern, to in situ assessment of their trophic impact (Coppari et al., 2014). This approach allows the quantification of the role played by the main components of the marine animal forests in the benthic-pelagic coupling process (Rossi, 2013). The knowledge of the distribution and the role played by benthic organisms is a fundamental information in which protection strategies might be based. The results of this work contribute to create an exhaustive ecological data set of the study area and could be used to supervise the effects of conservation and management plans.

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chapter 3

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The importance of gorgonians in the benthic-pelagic coupling processes in Mediterranean coastal areas, or why do we have to preserve the animal forest

Marine ecosystems, as well as terrestrial ones, provide various ecosystems services (ES). The role played by benthic suspension feeders in the benthic-pelagic coupling processes as well as the capability of these organisms to retain carbon (C), represent two of the main ES provided by sessile animal-dominated ecosystems (animal forests). Gorgonians were selected as case study to quantify both the impact in the benthic-pelagic coupling process and the amount of C stored. These arborescent organisms are common inhabitants of Mediterranean coralligenous communities. Three species may be considered really conspicuous: *Paramuricea clavata*, *Eunicella singularis* and *Corallium rubrum*. The first step was collecting all the previous literature about size and spatial distribution for the three species in the Cap de Creus region, as well as all the information available about feeding and respiration. These data were combined to estimate the retained C (difference between ingested and respired) by the three species in spring (the most productive season in warm-temperate seas). The present results shown that, due to their high abundances, the three gorgonians species deeply affect the benthic-pelagic coupling processes in spring and might also retain a high quantity of C (two orders of magnitude less than terrestrial ecosystems, such as rain forests). Consequently, we need to reconsider the importance of the animal forests in the C sequestration as well as guarantee the appropriate management measures to protect these species especially in that areas and depths in which their abundance are concentrated.

ABSTRACT

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1 INTRODUCTION

Several marine benthic communities dominated by animals, play a significant role in the benthic-pelagic coupling processes (Lesser 2006; Pile and Young 2006) and marine communities dominated by vegetal species contribute to the sequestration of atmospheric CO_2, thus acting as C sinks (Eong 1993; Duarte et al. 2010). The capability of retaining C is considered an important Ecosystem Services (ES, the goods and benefits obtained from the ecosystems; Farber et al. 2002) and in the last years it has been included in environmental policies and management.

The carbon retained by aquatic organisms (commonly called "Blue Carbon") is one of the main ES claimed to be essential in contrasting the green-house effect (Laffoley and Grimsditch 2009). The term Blue Carbon is normally referred to the carbon sequestered by vegetal coastal ecosystems, specifically mangrove forests, seagrass beds and salt marshes (Mcleod et al. 2011); but important C accumulation might also occur in marine animal dominated ecosystems in form of structural molecules (organic or inorganic) that might last for decades, centuries, or even millions of years (e.g. in the case of calcium carbonate structures) (Goreau 1963; Mallela and Perry 2007). Indeed, many works already highlighted the ecological importance of the animal dominated communities (e.g. animal forests, Rossi et al. 2012) (e.g. Cerrano et al. 2010; Bo et al. 2015), constituted by corals, gorgonians, sponges, bryozoan or ascidians (among other organisms). Animal forests are probably constituting one of the biggest biomass reservoir in the world oceans (Rossi 2013), responsible for the generation and the increase of nutrient exchange, and for the capture and the retention of C, nitrogen (N) and other elements from the water column, thus influencing the benthic-pelagic coupling processes (Sorokin 1973; Kinsey and Hopley 1991; Gili and Coma 1998; Rossi 2013). But what is the real impact of animal forests on the benthic-pelagic coupling processes? How much is the C sequestered by these complex ecosystems? To answer these questions, data about species distribution and abundance need to be crossed with those about C intake balance (C ingested – C consumed). Reliable data about marine benthic species distribution and abundance over large geographical extents might be obtained by means of quantitative video analysis of transects performed with Remotely Operated Vehicles (ROV), which allow to study large areas and reach high depths, with no impact on benthic communities (e.g. Teixidó et al. 2002; Mortensen and Buhl-Mortensen 2004). ROV samplings are thus the ideal tool to describe both qualitatively and quantitatively the health status of benthic communities (Rossi et al. 2008; Bo et al. 2015), and allow characterizing the distribution patterns, the population size structure (e.g. Watanabe et al. 2009; Gori et al. 2011a; Coppari et al. 2014) as well as the presence of fishing impacts (Bo et al. 2014). Contrarily, data about C balance are obtained by in situ or laboratories feeding and respiration experiments. Both the two methodologies show advantages and disadvantages: in situ experiments allow determining the feeding or respiration under natural conditions, highlighting the importance of seasonal changes in the environmental features (e.g. light, food availability and composition, temperature) (Ribes et al. 1998; Tsounis et al. 2006a). Contrarily, laboratories or mesocosm experiments allow for more precise estimates, due to the possibility of controlling every variable influencing the experiment, but, at the same time, reduce the possibilities to incorporate natural conditions (Worm et al. 2000).

One of the most conspicuous organisms in the animal forests are gorgonians, which are widespread all over the world (Gili and Coma 1998; Gili et al. 2001; Ballesteros 2006; Wild et al. 2009), being one of the most abundant components of benthic communities in tropical, temperate and polar areas (Loya 1972, Arntz et al. 1999; Harmelin & Garrabou 2005). Gorgonians are passive suspension feeders that rely completely on the ambient flow and on the particle abundances in the water masses for feeding (Best 1988). Their diet is mainly composed of zooplankton (Coma et al 1994; Rossi et al. 2004; Coma et al. 2015), microplankton (Ribes et al. 1999; Ribes et al. 2003; Picciano and Ferrier-Pagés 2007) and particulate organic matter (POM) (Lasker 1981; Tsounis et al. 2006a). Gorgonians also release mucus and metabolic wastes, easily recycled by bacterioplankton, thus influencing the benthic-pelagic coupling process (Marcus and Boero 1998). These anthozoans are long-lived, slow growing organisms with a life span sometimes beyond 100 years (Riedl 1984, Weinberg 1991; Linares et al. 2007), being particularly sensible to environmental and human-induced disturbances (Cerrano et al. 2000; Garrabou et al. 2009).

In the Mediterranean Sea, gorgonians can constitute up to 40 % of the rocky sublittoral biomass (Ballesteros 2006); in particular three species are among the most abundant in coastal areas in pre-coralligenous and coralligenous communities: *Paramuricea clavata* (Risso, 1826), *Eunicella singularis* (Esper, 1794) and *Corallium rubrum* (Linnaeus, 1758) (Weinberg 1976; Ballesteros 2006). *Paramuricea clavata* is a non-symbiotic species that normally inhabits vertical rocky bottoms areas in which strong currents dominated (True 1970; Weinberg 1975; Gori et al 2011b). This species may reach large size (Coma et al. 1994; Harmelin and Marinopoulos 1994; Linares et al. 2008), especially in deep areas (Bo et al. 2012), having a non-neglectable role in benthic-pelagic coupling processes. Zooplankton is the main component of the diet of *P.clavata* (Coma et al. 1994), together with microplankton (both live and detrital) (Ribes et al. 1999). *Eunicella singularis* is the only symbiotic species in the Mediterranean Sea. This species presents two different morphotypes depending on the bathymetrical distribution range: the shallow one presents candlestick-like shape with a dirty white color due to the presence of algae, while the deep one (generally located below 35 m depth) has a variable colony shape and a bright white color due to the lack of symbionts (Théodor 1969; Gori et al. 2011a). Finally, *Corallium rubrum* normally lives in dense patches in crevices, overhangs or cave entrances (Riedl 1984), between 7 and 200 m depth, with the bathymetrical range varying considerably between different areas (Carpine and Grasshoff 1975; Riedl 1984; Rossi et al. 2008). Its diet is mainly based on detritus and resuspended organic matter (Tsounis et al. 2006a). Whilst the main perturbation suffered by *P. clavata* and *E. singularis* may rise from climate change (i.e. mass mortalities, Garrabou et al. 2009) and a inadequate tourism and fisheries management (Coma et al. 2004; Tsounis et al. 2012), *C. rubrum* has been also commercially harvested since ancient time and it is now overexploited in the Mediterranean basin, especially in shallow bottoms (Tsounis et al. 2010).

Following the methodology of Coppari et al. (2014), previous data about size, spatial and bathymetrical distribution of the three studied species, were combined with previous data about their feeding and respiration rates in order to estimate the role played by these species in the benthic-pelagic coupling in a Mediterranean coastal area, and, as possible C sinks during spring. It has been shown that spring is the moment in which, in warm temperate seas, benthic suspension feeders have the highest impact on the water column, accumulating matter and energy that may be subsequently used during the rest of the year (Coma et al. 1998b; Rossi et al. 2006; Coppari et al. 2014). To achieve this goal, the present work has been organized as follow: 1) collect all the previous literature data about distribution, feeding and respiration for the three studied species; 2) calculate the total biomass of the three species in the study area, based on distribution and abundance data as well as on the respective population size structure; 3) estimate the role that the three gorgonian species play in benthic-pelagic coupling processes and as potential C sink (balance between C ingested and respired).

2 MATERIALS AND METHODS

2.1 Study area

The study area is located in Cap de Creus (42° 19′ 12″ N; 003° 19′ 34″ E), in the northwestern Mediterranean Sea (Fig. 1). According to the main hydrodynamic patterns in the zone, and the specific features of the studied coast, the study area was sub-divided into 7 sub-areas, from A to G. The general circulation pattern is characterized by the dominance of the Liguro-Provençal-Catalan current (or Northern current), which flows south-westward creating an east-to west circulation (DeGeest et al. 2008). The study area receives sediment inputs from the northern Gulf of Lions (Durrieu de Madron et al. 2000), especially by the Rhone river that supplies for ~ 90% of the total freshwater in the gulf (Palanques et al. 2006). The most important winds influencing the study area are the northerly Tramuntana and the north-westerly Mistral that occur for 41% and 28% of the time respectively. Strong south-easterly and easterly marine winds are rare (< 6% of the time) and brief (less than 3 days), in contrast to the northerly ones that can last up than one month (Ulses et al. 2008). Consequently, sub-area A is the most sheltered area of the surveyed coast; sub-areas B, C, D are affected mainly by easterly winds and are not directly influenced by the main near-bottom currents (Ulses et al. 2008, DeGeest et al. 2008). Sub-areas E and F are directly exposed to the main winds and wave actions in the study area (Ulses et al. 2008), as well as to the main near-bottom currents which accelerate around the cape (DeGeest et al. 2008). Due to the reduced influence of the main near-bottom currents, sub-area G is characterized by sediment deposition processes. 76 transects (Fig. 1) were recorded in the study area by means of ROV survey (see also Gori et al. 2011b) covering a total surface of 1.14 ha extending from 0 to 70 m depth.

2.2 Previous literature

Previous literature about spatial and bathymetrical distribution, population size structure, feeding and respiration rates was compiled for each studied species.

2.2.1 *Paramuricea clavata*

Data on spatial and bathymetrical distribution of *P. clavata* in the study area were available in Gori et al. (2011b). All the *P. clavata* observed in the study area were classified as shallow (0–35 m depth) or deep (> 35 m depth). For shallow populations, the size structure obtained by Linares et al. (2008) were applied to the total number of shallow gorgonians observed in Gori et al. (2011b) (for each of the 76 video transects recorded in the study area, the nearest size structure from Linares et al. 2008 was used). Conversely, for deep populations the size of the deep colonies were measured and their population size structure assessed as in Gori et al. (2011a).

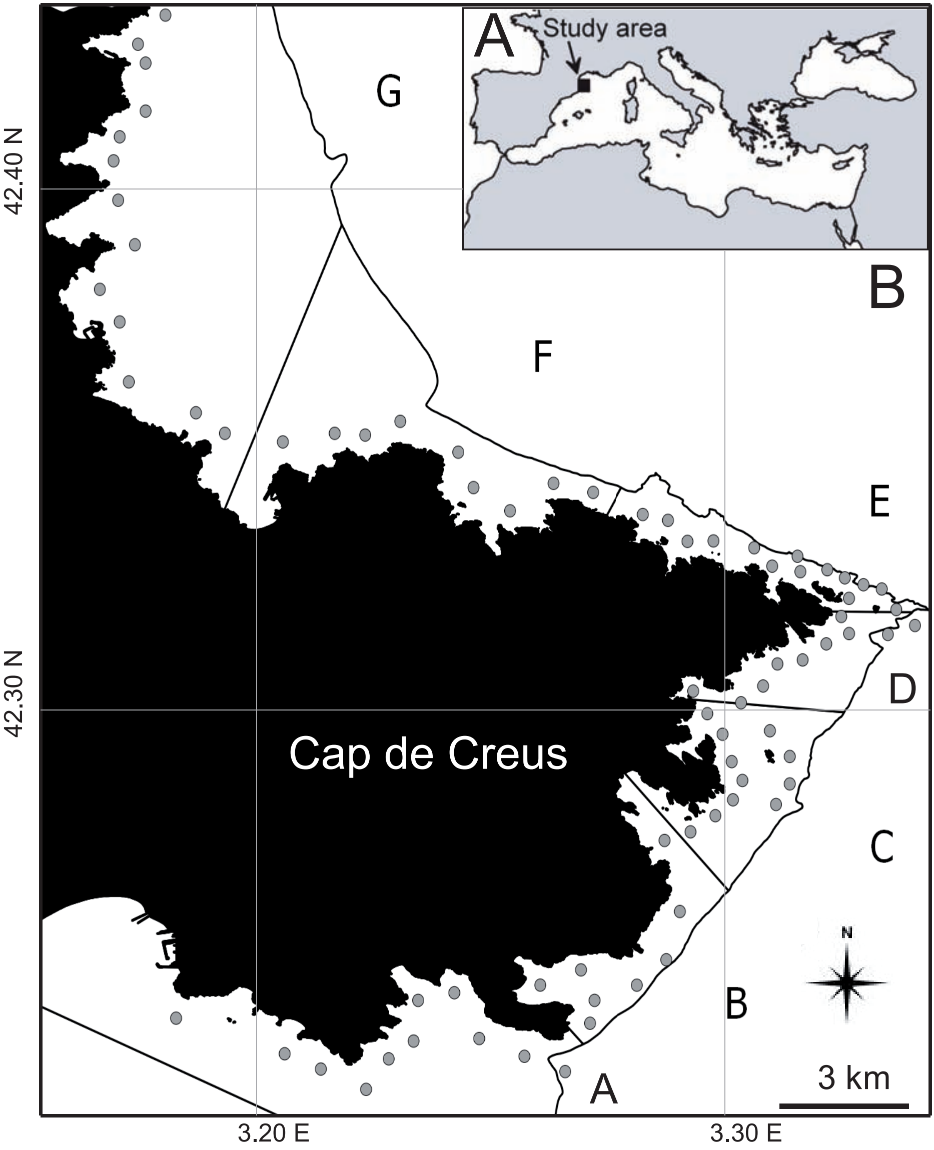

Fig. 1. Map of the study area: Location of the study area (**a**), Cap de Creus area showing the seven sub-areas and the transect positions (**b**).

For each typology of suitable hard substrata for *P. clavata*, its mean density (colonies m^{-2}) was calculated and this density used to estimate the total number of colonies of *P. clavata* in all the study area, based on Sardà et al. (2012), who quantified the extension (ha) of the suitable hard bottom. From the total estimated abundance, the potential amount of C retained by the species was calculated.

Ingestion rate of detrital POC, pico-, nano-, and microplankton of *P. clavata* were studied in Ribes et al. (1999), whereas the zooplankton contribution to the diet of this species was assessed by Coma et al. (1994). Respiration rates were obtained from Previati et al. (2010). Since temperature in Mediterranean Sea varies with depth and season (Pascual and Flos, 1984), and temperature significantly affects respiration (Riisgård and Larsen 2000), we selected respiration values assessed at 16° C for the shallow populations and at 14° C for deep populations (Table 1). For all the three gorgonian species, oxygen consumption was converted into C respired using the conversion factor 0.281 (McCloskey et al. 1978).

2.2.2 *Eunicella singularis*

Data on spatial and bathymetrical distribution of *Eunicella singularis* in the study area were available in Gori et al. (2011b). Again, all the *E. singularis* observed in the study area were classified as shallow (0–35 m depth) or deep (> 35 m depth). The population size structures found by Linares et al. (2008) were used to estimate the size of all the shallow colonies, whereas the population size structure found by Gori et al. (2011a) were used to estimate the size of the deep colonies observed on the subareas E and F. Finally, thanks to the availability of the video transect analysed by Gori et al. (2011a), we accomplish the study of the population size structure for the deep colonies located in the other subareas, following the same approach as in Gori et al. (2011a).

For each typology of suitable hard substrata for *E. singularis*, its mean density (colonies m^{-2}) was calculated and, as in *P. clavata*, this density was used to estimate the total number of colonies of *E. singularis* in all the study area, based on Sardà et al. (2012) who quantified the extension (ha) of the suitable hard bottom. From the total estimated abundance, the potential amount of C retained by the species was calculated.

Since shallow colonies of *E. singularis* host zooxanthellae, both the autotrophic (Ferrier-Pagès et al. 2015) and the heterotrophic (Coma et al. 2015) supply of C were considered to quantify the total amount of C inputs. Unfortunately no previous data was available about feeding on detrital POC, pico-, nano-, and microplankton for this species, thus, for our estimation, data of C ingestion in the gorgonian *Leptogorgia sarmentosa* (Ribes et al. 2003)

Paramuricea clavata	**Spatial & bathymetrical distribution**	**Size distribution**	**C ingestion**	**C respiration**
Shallow populations	Gori et al. 2011b	Linares et al. 2008	Coma et al. 1994 Ribes et al. 1999	Previati et al. 2010
Deep populations	Gori et al. 2011b	This study	Coma et al. 1994 Ribes et al. 1999	Previati et al. 2010

Table 1. Previous literature used in this study for *Paramuricea clavata*.

were used. Similarly, since no information were available about the feeding of deep population of *E. singularis*, the same value of heterotrophic feeding as for the shallow population (Ribes et al. 2003; Coma et al. 2015) were used, even though we are conscious of the introduction of some bias in this estimate. Respiration rates at 16° C were obtained from Previati et al. (2010), and applied to the shallow colonies. Conversely, the respiration rates of the non-symbiotic species *E. cavolinii* at 14° C (Previati et al. 2010) were applied to the deep colonies (Table 2).

2.2.3 *Corallium rubrum*

Data on spatial distribution of *C. rubrum* located at 20–50 m depth in the study area were available in Tsounis et al. (2006b), whereas data about deep populations located at 50–80 m depth were available in Rossi et al. (2008). Unfortunately, for this species it was not possible to achieve the same level of resolution and details as for the previous two gorgonian species. Indeed, using the densities of the above-mentioned works and the total suitable substrata from Sardà et al. (2012), we calculated the total number of colonies of *C. rubrum* in all the study area and the potential C retained. For both shallow and deep population of *C. rubrum,* we applied the size distribution recorded by García-Rodríguez and Massó (1986).

Ingestion rate was obtained from previous study of Picciano and Ferrier-Pagès (2007) for pico-and nano plankton contribution, whereas the amount of ingested detrital POC, phyto- and zooplankton were obtained from Tsounis et al (2006a). Respiration rates were obtained from Previati et al. (2010), at 16° C for shallow populations, and at 14° C for deep populations (Table 3).

Eunicella singularis	**Spatial & bathymetrical distribution**	**Size distribution**	**C ingestion**	**C respiration**
Shallow populations	Gori et al. 2011b	Linares et al. 2008	Ferrier-Pagès et al. 2015 Coma et al. 2015 Ribes et al. 2003	Previati et al. 2010
Deep populations	Gori et al. 2011b	Gori et al. 2011a This study	Coma et al. 2015 Ribes et al. 2003	Previati et al. 2010

Table 2. Previous literature used in this study for *Eunicella singularis*.

Corallium rubrum	**Spatial & bathymetrical distribution**	**Size distribution**	**C ingestion**	**C respiration**
Shallow populations	Tsounis et al. 2006b	García-Rodríguez & Massó 1986	Picciano & Ferrier-Pagès 2007 Tsounis et al. 2006a	Previati et al. 2010
Deep populations	Rossi et al. 2008	García-Rodríguez & Massó 1986	Picciano & Ferrier-Pagès 2007 Tsounis et al. 2006a	Previati et al. 2010

Table 3. Previous literature used in this study for *Corallium rubrum*.

2.3 Biometric relationship and biomass calculation

Data collected from the literature were presented with different normalization; hence we first needed to apply transformations based, either on the already available biometric relationships for the three studied species, or on new ones, calculated in this study.

2.3.1 *Paramuricea clavata*

Ingestion rates of *P. clavata* were presented in µg C polip^{-1} d^{-1}, whereas respiration rates were presented in mg O_2 g^{-1} ash free dry weight (AFDW) h^{-1}. Consequently the relationships between colony height (cm) and total number of polyps, and total colony AFDW needed to be calculated.

Colony size was converted into number of polyps using the relationship obtained by Coma et al. (1998). By means of the relationship obtained by Coma (1994), colony height (cm) was converted into colony length (cm), which was subsequently converted into AFDW by means of a relationship between AFDW and cm of gorgonian branch obtained in this study. To calculate the AFDW, 15 pieces of shallow *P. clavata* colonies were haphazardly collected *in situ* in spring 2012 (Punta s'Oliguera, Cap de Creus, 42° 17′ 1.62″ N, 003° 17′ 57.18″ E) by SCUBA diving, at a depth of 15–20 m depth. Once in laboratory, the tip part of the collected branches was removed, and the remaining portion was measured, dried at 90° C for 24 h, weighed, and then combusted for 5 h at 450 °C and weighed again to determine their AFDW. The AFDW was then normalized by the size of the portion collected. The AFDW/cm measured for *P. clavata* was 18.35 mg /cm, and was used for both the estimation of the total biomass in the study area and the respiration calculation.

2.3.2 *Eunicella singularis*

Ingestion rates of *E. singularis* were presented in µg C polyp^{-1} h^{-1} and in µg C cm^{-2} d^{-1} for the zooplankton (Coma et al. 2015) and the autotrophic contribution (Ferrier-Pagès et al. 2015), respectively. Data about microplankton ingestion (Ribes et al. 2003) and respiration rates (Previati et al. 2010) were presented as µg C g AFDW^{-1} h^{-1} and mg O_2 g AFDW^{-1} h^{-1}, respectively. Consequently the relationships between colony height (cm) and total colony polyps, total colony area (cm^2), and total colony AFDW needed to be calculated.

For shallow populations, the first two relationships were obtained by means of image analysis: 28 colonies were photographed *in situ* with a ruler on their side, and the maximum height (i.e. the maximum distance between the base of the stem and the tips of the farthest branches) and the length of all the branches was measured with Macnification 1.8 software (Schols and Lorson, 2008). This relationship resulted as:

$$y= 0.2869x^{1.9652}, \text{ with } R^2= 0.76$$

Total colony length was then converted into number of polyps by means of the relationship obtained by Coma et al. (2015). By means of image analysis we also measured the diameter and the length of each branch in order to calculate the colony surface, resulting in the relationship between colony height (cm) and total colony area (cm^2):

$$y= 0.0609x^{2.4655}, \text{ with } R^2= 0.81$$

Photos of deep population of *E. singularis* were obtained from still images extracted from the videos, and measured following the same method, obtaining the relationship between colony height (cm) and total colony length (cm) for deep colonies:

$$y= 0.4669x^{1.8432} \text{ with } R^2= 0.66$$

The AFDW of shallow *E. singularis* was obtained as explained above for *P. clavata*, sampling 15 portions of randomly selected colonies of this species. For deep populations, 15 pieces of *E. singularis* were randomly collected in situ in spring 2011 (Es Forcats, Cap de Creus, 42° 18′ 44″N, 003° 19′ 05″ E) at a depth of 50–60 m. The AFDW/cm of shallow colonies of *E. singularis* was 5.69 mg AFDW/cm, whereas for deep colonies was 4.43 mg/cm. The relationship between height and AFDW of the shallow colony was as follows:

$$y= 1.6323x^{1.9652} \text{ with } R^2= 0.78$$

Contrarily, for deep colonies the same relationship was:

$$y= 2.0685x^{1.8432} \; R^2= 0.66$$

2.3.3 *Corallium rubrum*

Ingestion rates of *C. rubrum* were presented as ng C polyp^{-1} d^{-1} (Tsounis et al. 2006a), thus we first calculated, from unpublished data of Rossi S., the biometric relationship between colony height (cm) and colony length (cm) that was as follow:

$$y= 1.3185x-4.6692 \text{ with } R^2 = 0.67$$

Total colony length was then converted into number of polyps by means of the relationship obtained by Bramanti et al. (2005).

The AFDW of *C. rubrum* was calculated as for the other two species sampling 15 portions of randomly selected colonies. The AFDW/cm was 5.82 mg/cm and it was used for both the estimation of the total biomass in the study area and the respiration calculation.

3 RESULTS

3.1 *Paramuricea clavata*

A total of 269 *P. clavata* colonies were observed in shallow waters (Gori et al. 2011b), accounting for a total biomass of 1236 g AFDW (Table 4a). The total ingestion of C was 10.04 g C d^{-1}: feeding on zooplankton accounted for 5.90 g C d^{-1} (2.4 µg C polyp^{-1} d^{-1} x total number of polyps) whereas feeding on microplankton accounted for 4.14 g C d^{-1} (1.69 µg C polyp^{-1} d^{-1} x total number of polyps). The total C respired by the shallow *P. clavata* colonies was 5.00 g C d^{-1} (0.1686 mg C g AFDW^{-1} d^{-1} x total g AFDW), and the balance between ingested and respired C was 5.04 g C d^{-1}, equivalent to 453.45 g C in spring. A total of 366 *P. clavata* colonies were observed in deep waters, which corresponded to 1213 g AFDW (Table 4b). The total ingested C was 9.73 g C d^{-1}: feeding on zooplankton accounted for 5.76 g C d^{-1} (2.4 µg C polyp^{-1} d^{-1} x total number of polyps), whereas feeding on microplankton accounted for 4.02 g C d^{-1} (1.69 µg C polyp^{-1} d^{-1} x total number of polyps). The amount of C respired by the deep colonies was 4.91 g C d^{-1} (0.1686 mg C g AFDW^{-1} d^{-1} x total g AFDW), and the balance between ingested and respired C for all the deep colonies of *P. clavata* was 4.82 g C d^{-1}, which corresponded to 433.73 g C in spring. Together, all the observed shallow and deep colonies of *P. clavata* retained a total of 0.89 kg C in spring.

The estimated abundance of *P. clavata* in the shallow bottoms of the study area was 163795 colonies (0.449 colonies m^{-2} x 36.5 ha of suitable substrate < 35 m depth, Sardà et al. 2012), corresponding to a total biomass of 752.86 kg AFDW. Conversely, the estimated abundance of this species in the deep sublittoral bottoms was 909279 colonies (1.132 colonies m^{-2} x 80.3 ha of suitable substrate > 35 m depth, Sardà et al. 2012) corresponding to a total biomass of 3015.45 kg AFDW. All these estimated colonies ingested a total of 2.72 t C, and respired a total of 1.37 t C in spring, resulting in a total retention of 1.35 t C, 276.11 and 1077.55 kg C for shallow and deep colonies, respectively. Considering a total suitable substrate for this species in the study area of 116.8 ha (Sardà et al. 2012), *P. clavata* retained 0.012 t C ha^{-1} in spring (Fig. 3).

3.2 *Eunicella singularis*

In the 76 ROV transects, 2540 *E. singularis* were recorded at shallow depth (Gori et al. 2011b), representing a total biomass of 1607 g AFDW (Table 5a). The total amount of C ingested was 35.28 g C d^{-1}: heterotrophic diet accounted for 19.56 g C d^{-1} whereas the autotrophic ingestion of C accounted for 15.71 g C d^{-1} (48.4 µg C cm^{-2} d^{-1} x total colonies surface). Finally the C ingested by zooplankton feeding was 1.47 g (0.168 µg C $polyp^{-1}$ d^{-1} x total number of polyps). The total C ingested by feeding on microplankton accounted for 18.09 g C d^{-1} (11256 µg C g $AFDW^{-1}$ d^{-1} x total g AFDW). The total C respired was as high as 4.28 g C d^{-1} (2.63 mg C g $AFDW^{-1}$ d^{-1} x total g AFDW). The balance between ingested and respired C for all the shallow *E. singularis* of Cap de Creus was 31.05 g C d^{-1} equivalent to 2794.28 g C in spring. We recorded 1863 deep *E. singularis* with a total

SUBAREA	Number of colonies	Biomass (g)	Number of polyps	C ingested (g C d^{-1})	C respired (g C d^{-1})	C sink (g C d^{-1})
A	5	43.38	87688	0.36	0.18	0.18
B	/	/	/	/	/	/
C	15	169.47	339245	1.39	0.69	0.7
D	39	411.30	825086	3.37	1.66	1.71
E	152	309.99	602818	2.46	1.25	1.21
F	58	302.27	602883	2.46	1.23	1.23
G	/	/	/	/	/	/

Table 4a. Observed shallow colonies of *Paramuricea clavata*: number of colonies, total biomass, C ingested, respired and retained are listed for each subarea.

SUBAREA	Number of colonies	Biomass (g)	Number of polyps	C ingested (g C d^{-1})	C respired (g C d^{-1})	C sink (g C d^{-1})
A	9	25.53	49597	0.2	0.1	0.099
B	/	/	/	/	/	/
C	13	56.41	113566	0.46	0.23	0.23
D	27	66.08	128396	0.52	0.27	0.25
E	204	729.69	1431968	5.85	2.95	2.9
F	114	336.07	657937	2.69	1.36	1.33
G	/	/	/	/	/	/

Table 4b. Observed deep colonies of *Paramuricea clavata*: number of colonies, total biomass, C ingested, respired and retained are listed for each subarea.

biomass of 458 g AFDW (Table 5b). The total C ingested was 5.70 g C d^{-1}: 0.54 g C d^{-1} by zooplankton feeding (0.168 µg C $polyp^{-1}$ d^{-1} x total number of polyps) and 5.16 g C d^{-1} by microplankton feeding (11256 µg C g $AFDW^{-1}$ d^{-1} x total g AFDW). The total C respired by the observed *E. singularis* was 0.62 g C d^{-1} (1.35 mg C g $AFDW^{-1}$ d^{-1} x total g AFDW). The balance between ingested and respired C for all the deep *E. singularis* of the Cap de Creus was 5.08 g C d^{-1} equivalent to 457.15 g C in spring. The counted colonies in the study area, *E. singularis* of the Cap de Creus area retained 3.25 kg C in spring.

The estimated abundance of *E. singularis* in the shallow bottoms of the study area was 10108233 colonies (0.332 colonies m^{-2} x 617.8 ha of photophilic rock, 2.449 x 199.3 ha of precoralligenous bottom, 1.970 x 170.6 ha of coralligenous < 35 m depth, Sardà et al. 2012), corresponding to a total biomass of 6395.25 kg AFDW. Conversely, the estimated abundance of this species in the deep sublittoral bottoms was 5125029 colonies (6.382 colonies m^{-2} x 80.3 ha of suitable substrate > 35 m depth, Sardà et al. 2012) corresponding to a total biomass of 1259.94 kg AFDW. All these estimated colonies ingested a total of 14.06 t C, and respired a total of 1.68 t C in spring, resulting in a total retention of 12.38 t C, 11120.16 and 1257.61kg C for shallow and deep colonies, respectively. Considering the total suitable substrate for this species has an extension of 1068 ha, *E. singularis* retained 0.012 t C ha^{-1} in spring (Fig. 3).

3.3 *Corallium rubrum*

The estimated abundance of *C. rubrum* in the shallow bottoms of the study area was 2725740 colonies (3.42 colonies m^{-2} x 79.7 ha of suitable substrate < 35 m depth, Sardà et al. 2012), corresponding to a total biomass of 131.69 kg AFDW. Conversely, the estimated abundance of this species in the deep sublittoral bottoms was 18340790 colonies (7.31 colonies m^{-2} x 25.1 ha of suitable substrate > 35 m depth, Sardà et al. 2012) equivalent to 886.09 kg AFDW. All these estimated colonies ingested a total of 1.99 t C, and respired a total of 0.19 t C in spring, resulting in a total retention of 1.80 t C, 229.28 and 1569.63 kg C for shallow and deep colonies, respectively. Considering a total suitable substrate for this species in the study area of 330.6 ha, *C. rubrum* retained 0.005 t C ha^{-1} in spring (Fig. 3).

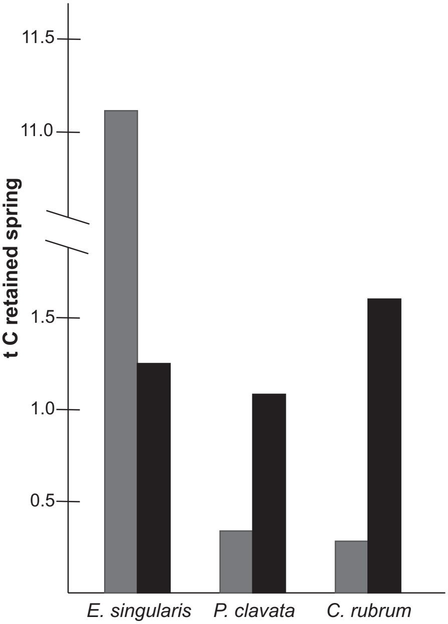

Fig. 2. Potential amount of C retained (t C) in spring by the estimated number of colonies of the three gorgonian species (*Eunicella singularis*, *Paramuricea clavata*, *Corallium rubrum*) in the study area. The grey bar indicates the C retained in shallow bottoms, the black bar indicates the C retained in deep bottoms.

4 DISCUSSION

This study assessed the importance of three of the main gorgonian species in Mediterranean coastal areas in the benthic-pelagic coupling processes, and inferred the potential role of these species as C sinks.

Eunicella singularis was the most abundant gorgonian species in the study area, but despite its highest number of observed colonies (4403 vs 636 of *Paramuricea clavata*), this species did not represent the highest biomass (2065 g vs 2449 g of *P. clavata*). Indeed *P. clavata* had a quantity of organic material (AFDW) cm^{-1} three times higher than *E. singularis*. The observed colonies of the two species, in spring, are responsible for a C flux of 3.76 kg C 1.78 kg C, respectively for *E. singularis* and *P. clavata*. Spring is the most favourable season for Mediterranean benthic species due to the high quality and quantity of the near-bottom seston (Grèmare et al .1997) which allow an increase in activity and in secondary production (Coma et al. 2000) as well as the accumulation of high quantities of lipids essential to face less favourable seasons (Cavaletto and Gardner 1999; Rossi et al. 2006). The autotrophic contribution obtained by *E. singularis* from symbiotic algae (Ferrier-Pagès et al. 2015), together with its higher abundances, allow this species to retain more C than *P. clavata* (3.25 kg C retained by *E. singularis* vs 0.89 kg C retained by *P. clavata*). In fact, the recent work of Ferrier-Pagés et al. (2015) highlights the importance of the autotrophic C budget for this mixotrophic species, which, in shallow areas, has a low C input from the zooplankton and other large preys (Coma et al 2015). The mixotrophic habits of *E. singularis* might explain its wider distribution, only limited by the presence of suitable hard substrata, whereas *P. clavata*, which rely on zooplankton as the main food source, needs vertical hard bottoms exposed to strong currents that ensure the provision of food (Weinberg 1976; Linares et al. 2008; Gori et al 2011b). It has to be highlighted that not all the amount of C retained, is used for growth and thus fixed as biomass. As a point of fact, a portion of the C ingested is used for reproduction, and it will be released in the water column as gametes or larvae (Coma et al. 1998). Consequently, as already observed in other suspension feeders (Ribes et al 1998; Ribes et al 1999; Coppari et al. 2014), it is also probable that these high quantities of C retained in spring will be used during the rest of the year, consistently decreasing the amount of C sequestered.

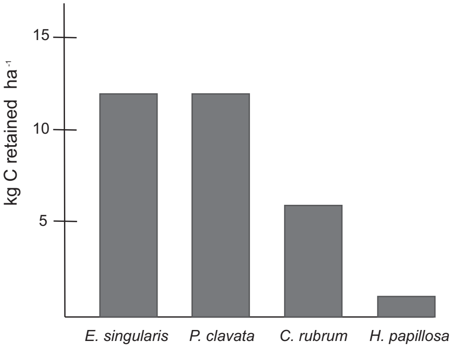

Fig. 3. Potential amount of C retained (kg C ha^{-1}) in spring by the three gorgonian species *Eunicella singularis*, *Paramuricea clavata*, *Corallium rubrum* and the ascidian *Halocynthia papillosa*.

By taking into account both the abundance and the population size structure of the observed colonies of the studied gorgonians, these study showed that the hot-spots for C retention can vary spatially according to the main environmental factors. Indeed, shallow colonies of *P. clavata* were more important as C sink in the subarea D, and deep colonies in subarea E (Table 4 a, b). Conversely, shallow *E.*

singularis colonies shown the highest amount of C retained in subarea G, whereas the deep ones concentrated more C in subareas E and F (Table 5 a, b). The general pattern of increased quantity of C retained from the south to the north of the study area, is in line with the distribution of these two gorgonian species (Linares et al. 2008, Gori et al. 2011b) that shown the highest densities in the northern coast of the Cap de Creus, due to the exposure to the main currents, wind and wave actions in the area (DeGeest et al. 2008, Ulses et al. 2008).

Regarding the broad-scale estimation of gorgonian abundances in the study area (based on their mean density, and the total abundance of suitable substrate), *C. rubrum* shown the highest number of colonies in all the study area (Table 6) compared to *E. singularis* and *P.clavata*. However, in terms of C fluxes, *E. singularis* ingested the highest quantity of C, followed by *P. clavata* and *C. rubrum* (Table 6). *E. singularis* is the species that retain the highest quantity of C, followed by *C. rubrum* and *P. clavata*. The lower importance of *P. clavata* as C sink compared to the other two species, could be expected since previous works already pointed out the higher respiration rate of this species (Coma et al. 1998). Due to the dominance of small and medium size colonies in *C. rubrum* populations (García-Rodríguez and Massó, 1986; Tsounis et al. 2006b; Rossi et al. 2008), the total biomass of this species in the study area was much lower than *E. singularis* and *P. clavata*. In this sense, the impact of overharvesting on the biomass of *C. rubrum* in coastal areas (Garrabou and Harmelin 2002; Tsounis et al. 2007, 2010; Santangelo et al. 2007) can finally have significant repercussions also on its capability to play as C sink.

Fig. 2 shows the different impact in the C sequestration caused by the three gorgonians species in shallow and deep bottoms. Shallow colonies of *E. singularis* accounted for the higher retention due to their abundance in the study area. Deep colonies of the three gorgonian species retained almost the same quantity of C, and for *P. clavata* and *C. rubrum* the role played as C sinks is much bigger in deep than shallow bottoms. This result is in line with previous works that highlighted the importance of benthic suspension feeders in the twilight zone (below 50 m depth) (Bo et al. 2009, 2011; Gori et al. 2011b), and as already pointed out by Bo et al. (2009), an extension of the lower depth limit of protection in Mediterranean sea, should be considered.

Can we consider Mediterranean suspension feeders as contributor to the Blue Carbon retention?

The amount of C retained by the gorgonians in the study area can be compared with the quantity retained in the same area by the ascidian *Halocynthia papillosa* (see chapter one). The estimated abundance in the Cap de Creus area of this active suspension feeder, is responsible for the C sequestration of 0.77 t C in spring, whereas the C retained by the three gorgonians is 15.53 t C in spring.

Previous literature estimate the role as C sink of both land and marine ecosystems: Grace et al. (1995) pointed out that undisturbed Amazonian forests retain 1.02 t C ha^{-1} year, Duarte et al. (2010) calculate the amount of C accumulated by seagrass meadows being as high as 6.7 t C ha^{-1} year, Eong (1993) found out that mangrove forests retain 1.5 t C ha^{-1} year. The three gorgonians retain 1.4 x 10^{-2} t C ha^{-1} in spring (Fig. 3), thus two orders of magnitude lower than the above-mentioned ecosystems. Although this is a rough estimate, we believed that these results highlight the importance of benthic suspension feeders as C sinks. Even if the C per hectare is lower compared to other land or marine ecosystems, the importance of gorgonians as C sinks might be higher than previously thought, if we consider the extension of unexplored oceans.

Overall, our results highlighted the importance of Mediterranean coastal gorgonians in the benthic-pelagic coupling and confirmed that crossing data from experimental feeding experiments with quantitative data about marine species abundance is a useful methodology to make realistic estimations of benthic-pelagic coupling processes over large areas.

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chapter 4

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Species distribution models as a tool to estimate the benthic-pelagic coupling processes in coastal areas

Distribution and abundance of the gorgonians *Eunicella singularis* and *Paramuricea clavata* and of the sponge *Axinella polypoides* were obtained by means of quantitative video analysis and coupled with environmental descriptors, such as high-resolution swath bathymetry, sediment hardness and hydrodynamic variables (near bed orbital velocity). Statistical computations were used to find relationships between species distribution and environmental factors. Random forest (RF) statistical model was used to build predictive maps of distribution and abundance for the three species. The use of species distribution models (SDM) can represent a cost-effective strategy to predict where the studied species are most concentrated, specifically in not surveyed regions. Moreover, some of the environmental variables that SDM took into account to explain the species distribution, were not previously considered as important factors for the studied species, likely producing even more accurate predictive maps.

ABSTRACT

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1 INTRODUCTION

Benthic suspension feeders are widely distributed in coastal marine benthic communities, being dominant in low-light conditions where algae and seagrass cannot grow (e.g. Witman and Dayton 2000; Gili and Coma 1998; Wild et al. 2011). By filtering particulate organic matter (POM) and capturing plankton suspended in the water (Wildish and Kristmanson 1997), benthic suspension feeders determine an important energy and transfer of matter between the pelagic and benthic systems (Gili and Coma 1998; Van Oevelen et al. 2009). Moreover, they take part in the biogeochemical cycles of important elements such as carbon (C) and nitrogen (N) (Maldonado et al. 2005; de Goeij et al. 2008). Benthic suspension feeders may act as C and/or N sinks by removing organic matter from the highly dynamic pelagic system, and incorporating this organic matter into the long-lived and highly structured benthic system (Smith 1981; Graf 1992; Gili and Coma 1998; Coma et al. 2001; Rossi et al. 2012). Most sessile benthic suspension feeders may also play a key role as ecosystems engineers (sensu Jones et al. 1994), enhancing the structural heterogeneity of the community, and providing suitable habitat for hundreds of associated species (Henry and Roberts 2007; Rossi 2013). Their complex three-dimensional frameworks may induce current variations and sediment resuspension, generally enhancing food availability by increasing the residence time of suspended particles (Leicher and Witman 1997; Gili and Coma 1998).

Food supply is one of the main factors driving the distribution of sessile benthic suspension feeders (Wildish and Kristmanson, 1997), but also the feeding strategy may affect their spatial distribution patterns. Passive suspension feeders (such as corals and gorgonians) trap food particles transported by water currents by means of capturing structures (Best 1988; Leonard 1989). Consequently, these species commonly concentrate in areas, bottoms types or exposures where locally intense currents enhance food availability (e.g. Thiem et al. 2006; Davies et al. 2009; Orejas et al. 2009; Gori et al. 2011). Conversely, active suspension feeders (such as sponges, bivalves and ascidians) actively pump water flows through their filtering structure (Riisgård and Larsen 2000, and reference therein), thus are less dependent upon the current flow for food capture (Wildish and Miyares 1990). Interactions among bottom types, slope gradients, elevation in comparison with the surrounding areas and exposure to the main hydrodynamic patterns may be then highly significant in driving the spatial distribution patterns of different benthic suspension feeder species.

Species distribution models (SDMs) have been used to assess the relationships between species presence/abundance and several environmental and topographic features of marine bottoms (Guinan et al. 2009; Brown et al 2011; Robert et al. 2014). By means of this correlation, SDMs provide a full coverage predictive map also for the areas that could not be surveyed (Robinson et al. 2011; Robert et al. 2014). To understand and explain which physical factor drives the distribution of benthic species is a central question in ecology (Ives and Klopfer 1997; Currie 2007), and has a significant importance in the establishment of cost-effective management and conservation plans (Margules and Austin 1994; Fortin and Dale 2005). This is particularly important in marine ecosystems, which are under increasing human pressure (Halpern et al. 2008) as a consequence of fishing activities (e.g. Fosså et al. 2002; Bo et al. 2014), eutrophication (Conley et al. 2009), harvesting (Tsounis et al. 2010) or climate change (e.g. Hoegh-Hulberg et al. 2007). In the Mediterranean Sea, active and passive sessile suspension feeders are among the most important animal species in the coralligenous communities (Ballesteros 2006), which corresponds to a hard substratum of biogenic origin that is mainly produced by the accumulation of calcareous encrusting algae that growth in dim light conditions (Ballesteros 2006). Coralligenous communities probably harbour more species than any other Mediterranean community (Ballesteros 2006) and is one of the most threatened benthic ecosystems (Garrabou and Harmelin 2002; Coma et al. 2004; Gatti et al 2015). Due to the wide bathymetric distribution of the coralligenous biocenosis (20–120 m depth, Ballesteros 2006) it is particularly difficult to obtain data about species distribution over large spatial extents. However, the increased application of multibeam sonar for scientific purpose combined with ground-truthing remotely operated vehicles (ROVs) survey, allowed producing full coverage bathymetric maps and extensive groundtruthing of marine bottoms from coastal environment to high depths (e.g. Urgeles et al. 2002; Mortensen and Buhl-Mortensen 2004; Lo Iacono et al. 2008). In this way, recent works along the Cap de Creus continental shelf (NW Mediterranean) assessed the presence and abundance of the gorgonians *Eunicella singularis* and *Paramuricea clavata* (Gori et al. 2011) and the erected sponge *Axinella polypoides* (Coppari et al. submitted), which are characteristics of the Mediterranean coralligenous community over a large extent of coastal and shelf bottoms, from 0 to 70 m depth. By combining this information about species abundance (obtained by ROV surveys), with high-resolution bathymetry and data on sediment hardness (measured by multibeam) (Lo Iacono et al. 2012), and data about the main hydrodynamic conditions, this study aims to deepen into the environmental features driving the distribution patterns of the studied species in the Mediterranean coastal bottoms. Moreover, the prediction of species abundance over large geographical and bathymetrical extension may be crucial to estimate the role played by these benthic suspension feeders in the benthic-pelagic coupling occurring in coastal areas of the Mediterranean Sea. Indeed, estimations based on predicted species distribution coupled with in situ feeding and respiration rates, may allow to quantify the amount of C and N transferred by these species from the pelagic to the benthic system, where it can be retained during several decades (Coppari et al. 2014).

2 MATERIALS AND METHODS

2.1 Data collection

Data about *Eunicella singularis*, *Paramuricea clavata* and *Axinella polypoides* abundance in the Cap de Creus area were obtained by means of quantitative video analysis of 76 video transects recorded in the study area (Fig. 1) (see Gori et al. 2011; Coppari et al. submitted for further details). Each transect was divided into 2326 sampling units of 2 m^2 (Gori et al. 2011), or into 4559 sampling units of 2.5 m^2 (Coppari et al. submitted), and species abundance in each sampling unit was assessed together with their geographic coordinates and depth.

2.2 Data spatial autocorrelation assessment

The results about spatial distribution of the abundances of the studied species were first explored in order to detect the presence of spatial autocorrelation. Spatial autocorrelation is frequently encountered in ecological data, and if not properly considered, it can influence the statistical inference of species distribution models (Dormann, 2007). Spatial autocorrelation was analysed by calculating Moran's I (Moran 1950) using the functions 'moran.test'

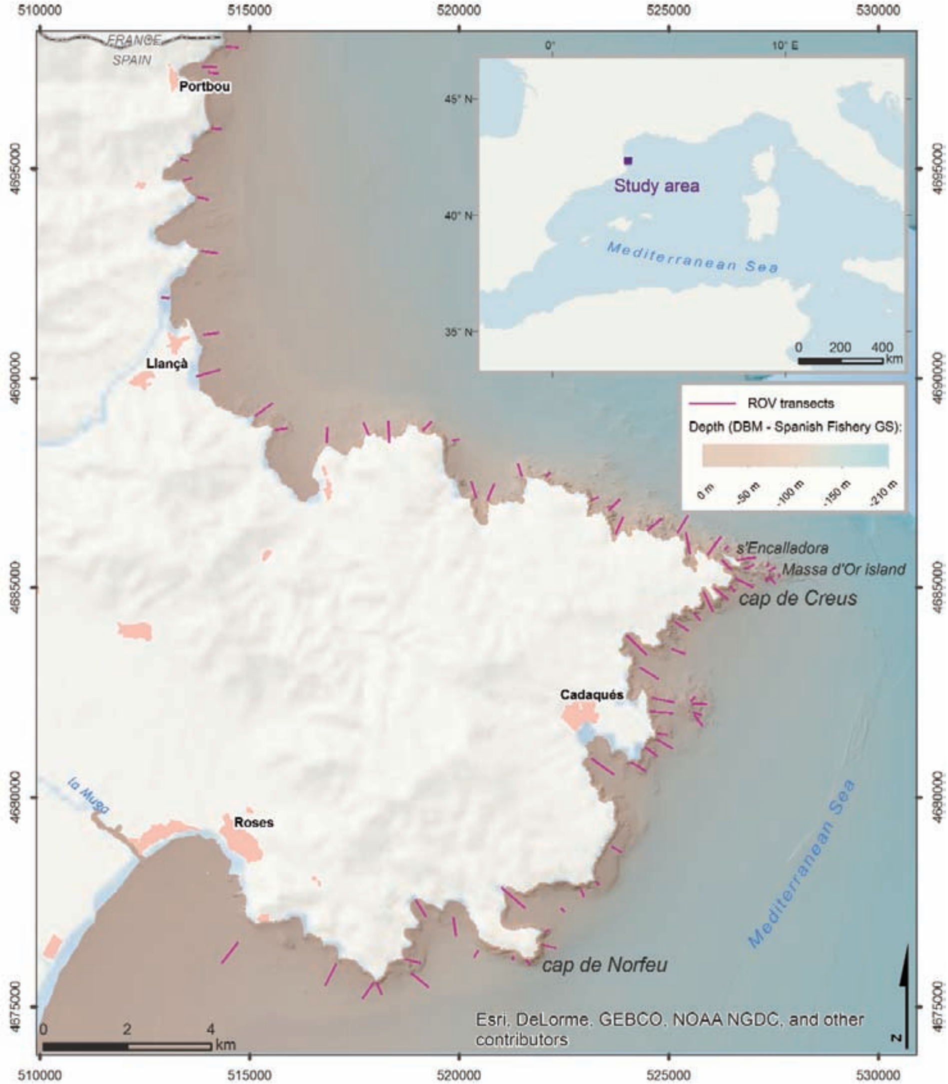

Fig. 1. Map of the studied area showing the transect positions. Own elaboration with cartographic data of GADM, DBM–DEM, GEBCO, NGDC–NOAA, DELORME. Spatial reference system: UTM 31N- WGS84.

in the R-package 'spdep' (R version 3.1.3, R Development Core Team). Moran's I ranges from -1 (perfect dispersion) to +1 (perfect correlation), with values around zero indicative of a random spatial pattern.

2.3 Environmental and hydrodynamic descriptors

To model the distribution of the species abundances, we used both morphological and and hydrodynamic descriptors. Environmental descriptors such as slope, rugosity (TRI, measure of the terrain irregularity), bathymetric position index (BPI, measure of the relative height of a pixel compared with the surrounding pixels), curvature (highlight the presence of convex and concave slopes) and surface (ratio between the area of a sloping pixel and a flat pixel) were derived from bathymetric map of the study area (Wilson et al. 2007) using ArcGIS 10.3 (ESRI 2014). Multibeam data for the available bathymetric map were acquired by the Spanish Fishery General Secretary-ESPACE Project (http://www.ieo.es/ESPACE/descripcion_ESPACE.htm). Multibeam data cover a depth range of 5–80 m and bathymetric models have been obtained at a spatial resolution of 10 m.

The bionomic map of the study area from Sardà et al. 2012 describing the different biocenosis and their relative extensions in the natural park of Cap de Creus and surrounding area was also used in our model.

The hydrodynamic variable considered in this study was the near bed orbital velocity. The maximum (within a wave period) near bed orbital velocity, u, is the wave induced velocity of the water in the proximity of the bed. It is given (Dalrymple and Dean, 1991) by:

$$u = (gHT)/(2L \cosh(2ph/L))$$

where g is the gravity acceleration ($g = 9.8$ m/s^2), H is the wave height, T is the wave period, h is the local depth, and L is the wave length. Further, wavelength was solved in an iterative way as a function of the wave period and water depth from the dispersion equation:

$$2pL = gT^2 \tanh(2ph/L)$$

Data of wave period, height and incident direction, used to calculate the near bed velocity were provided by the public port authority "Puertos del Estado". The wave data corresponds to the WANA analysis model (developed by "Puertos del Estado" and the Spanish meteorological office "Agencia Estatal de Meteorología"), which gives the data at some points, so-called "WANA nodes", every three hours. Near bed orbital velocity was computed for all the year before fieldworks, i.e., from October 2003 to September 2004, at 2.5 m, 7.5 m, 12.5 m, up to 67.5 m. The values at 2.5 m are representative for depths from 0 to 5m, those at 7.5 m for depths from 5 m to 10 m and so on. The yearly average value of this parameter for every depth range was used for the model.

2.4 Model construction

Random Forest (RF), a statistical model adopting a machine learning approach, was selected for our predictions, due to its strength in analysing correlate variables (Archer and Kimes, 2008) and coping with several non-detection of the studied species (zero data). RF is a technique whereby many multiple decision trees are built based on random subsamples of the data and the environmental predictors, leading to the construction of a 'forest' (Breiman 2001). Trees are built by binary splits such that the data are recursively separated into smaller and smaller groups based on the best predictor variable available. As each tree will be built based on a different set of samples and environmental predictors, each tree will be different and once grown can be used to make predictions based on the rules developed at each node (Cutler et al. 2007). Each tree provides an answer and the average (in the regressive case) is the expected value. The interpretation of the tree increases in complexity as the number of terminal nodes increases (Cutler et al. 2007). For each of the biological characteristics measured, a total of 1500 trees were built with 10 variables being randomly selected at each node. RF was performed in R using the functions 'randomForest'. RF was used for both distribution and abundance models.

2.5 Model evaluation and calibration

Survey data were divided into training and test data by randomly selected 70 % of the data for model performance and 30% of the data for model evaluation (Araújo and Guisan, 2006). Training data were used to run the model, whereas test data where used to check for the prediction accuracy: we assessed the performance of distribution models based on the accuracy of predictions for both the training and the test data, and report the area under the receiver-operating characteristic curve (AUC) as discrimination performance criteria. AUC ranges from 0 to 1, with values below 0.6 indicating a performance no better than random, values between 0.7–0.9 considered as useful, and values > 0.9 as excellent (Oppel et al. 2012). All the model evaluation was performed in R using the package 'PresenceAbsence'. In addition to discrimination, which measures a model's ability to discriminate between presence and absence locations, calibration measures how well the frequency of observations in test data agrees with predicted probabilities of occurrence. Pearson correlation coefficient was used for prediction accuracy in abundance models.

a) ***Eunicella singularis***

	PCC	Sensitivity	Specificity	Kappa	AUC
Train data	0.99	0.98	1	0.99	0.99
Test data	0.85	0.43	0.96	0.47	0.89

b) ***Paramuricea clavata***

	PCC	Sensitivity	Specificity	Kappa	AUC
Train data	1	1	1	1	1
Test data	0.97	0.30	0.99	0.40	0.93

c) ***Axinella polypoides***

	PCC	Sensitivity	Specificity	Kappa	AUC
Train data	0.99	0.91	0.99	0.95	0.99
Test data	0.93	0.45	0.96	0.45	0.91

Table 1. Model evaluation and calibration of the RF for *Eunicella singularis* (a), *Paramuricea clavata* (b), *Axinella polypoides* (c): PCC = overall percentage correctly classified; Sensitivity = percentage of presences correctly classified; Specificity = percentage of absences correctly classified; Kappa = a measure of agreement between predicted presences and absences with actual presences and absences corrected for agreement that might be due to chance alone; AUC = area under the receiver-operated characteristic curve.

3 RESULTS

RF performed well for all the three study species in discriminating presences and absences (all the AUC for test data were > 0.75, Table 1). Little spatial autocorrelation was found for the three species: for *Eunicella singularis* Moran's I = 0.38, $p < 0.05$; for *Paramuricea clavata* Moran's I = 0.27, $p < 0.05$; for *Axinella polypoides* Moran's I = 0.17, $p < 0.05$. The results of the distribution model for the three species are listed in Table 1. Predictive maps for distribution models shown the same pattern as the ones from abundance models, thus we decided to represent only the results of this second model (see below). Results of the abundance models showed that RF explained the 40 % of the variation in recorded abundance data for *E. singularis*, the 16 % for *P. clavata* and no more than 4 % for *A. polypoides*. Results of RF highlighted that for *E. singularis* the longitude was the most important variable, followed by the TRI (100 m) (Fig. 1); for *P. clavata* BPI (100 m) was the most important factor followed by TRI (100 m) (Fig. 2), whereas for *A. polypoides* the most affecting factors were substrate and depth (Fig. 3).

For any of the three species, near bed orbital velocity did not exceed 15 % of the increase of the mean squared error if removed from the model (Figs. 1, 2 and 3).

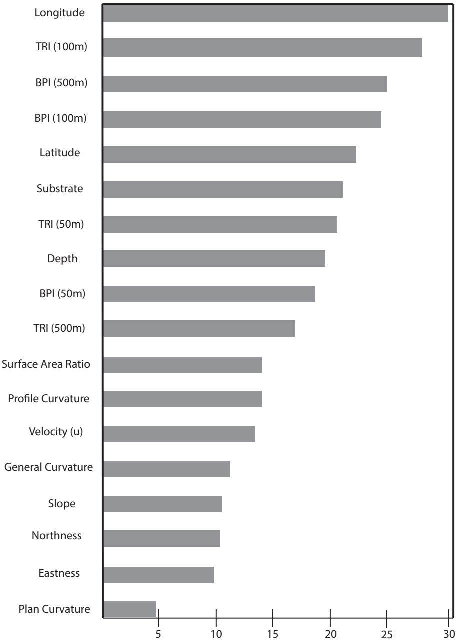

Fig. 2. Variable importance (reported as percentage increase in mean squared errors) for the random forest models for abundance in *Eunicella singularis*.

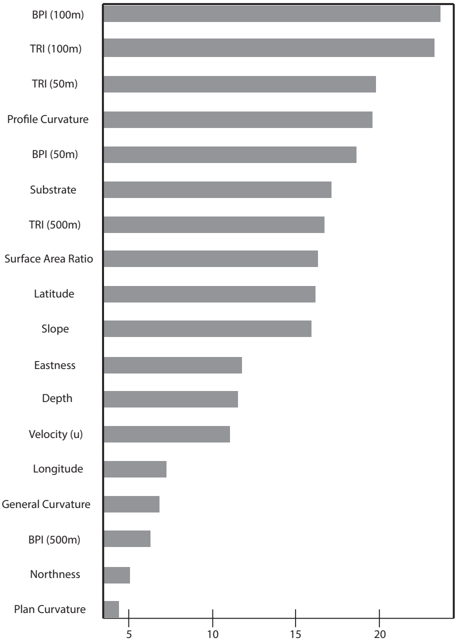

Fig. 3. Variable importance (reported as percentage increase in mean squared errors) for the random forest models for abundance in *Paramuricea clavata*.

Figs. 4, 5, 6 shown the predictive distribution maps of the three study species. All the three species presented their potential abundances concentrated on the northern side of the Cap de Creus. In particular RF predict the highest abundances for the two gorgonian species around the Massa d'Oros island (Fig. 4, 5).

4 DISCUSSION

Recent works pinpoints that environmental data, combined with accurate ROV transects or similar visual tools may be essential to build SDMs in order to determinate where the suspension feeding species are more abundant (Robert et al. 2014). In this study, SDMs have been performed to assess the main factors driving the distribution patterns and the estimated abundances of the three target species in a broad-scale area. Based on the resulting estimated abundance, the impact of the three species in the benthic-pelagic coupling can be calculated, as well as their role as C sinks.

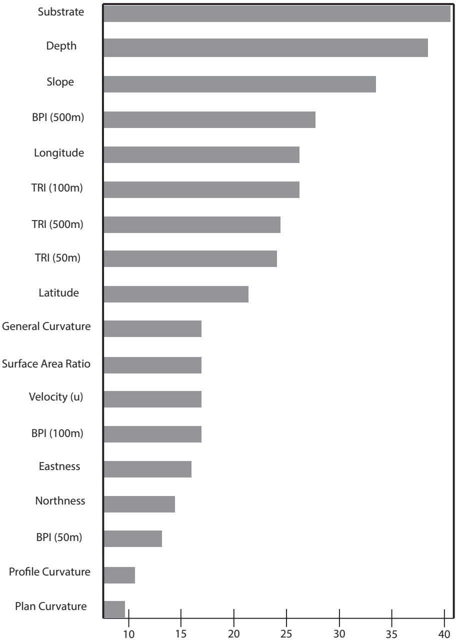

Fig. 4. Variable importance (reported as percentage increase in mean squared errors) for the random forest models for abundance in *Axynella polypoides*.

4.1 Critical evaluation of models performances

The fact that environmental variable were spatial autocorrelated was not unexpected, due to the methodology used for sampling (video transects) (Kenchington et al. 2014). As expected, the model shown better predictions in the areas where more transects were recorded, whereas show more weakness moving away from the coast. Indeed, far from the coast, the model generated different artifacts displayed as a band of solid color. These artifacts might be probably related with the wrong association of abundances with unsuitable substrate or for the strong influence of longitude (Figs. 5, 6, 7). This wrong association of species abundances with unsuitable substrate might be caused by the lower resolution of the bionomic map used in this study (Sardà et al. 2012) compared with the other environmental variables. This problem might be solved introducing in the model, the seafloor backscatter data, which is considered as a substrate surrogate, providing then information from the seafloor at a high resolution (variation in substrate and roughness among others) (de-Moustier 1985; Lo Iacono et al. 2008).

The strong importance of longitude in the predicted abundance of *E. singularis* was unexpected, but could be related with the morphology of the study area: the presence of the cape impedes an equal distribution of the species westward and eastward, constraint that is detected by the model as the effect of

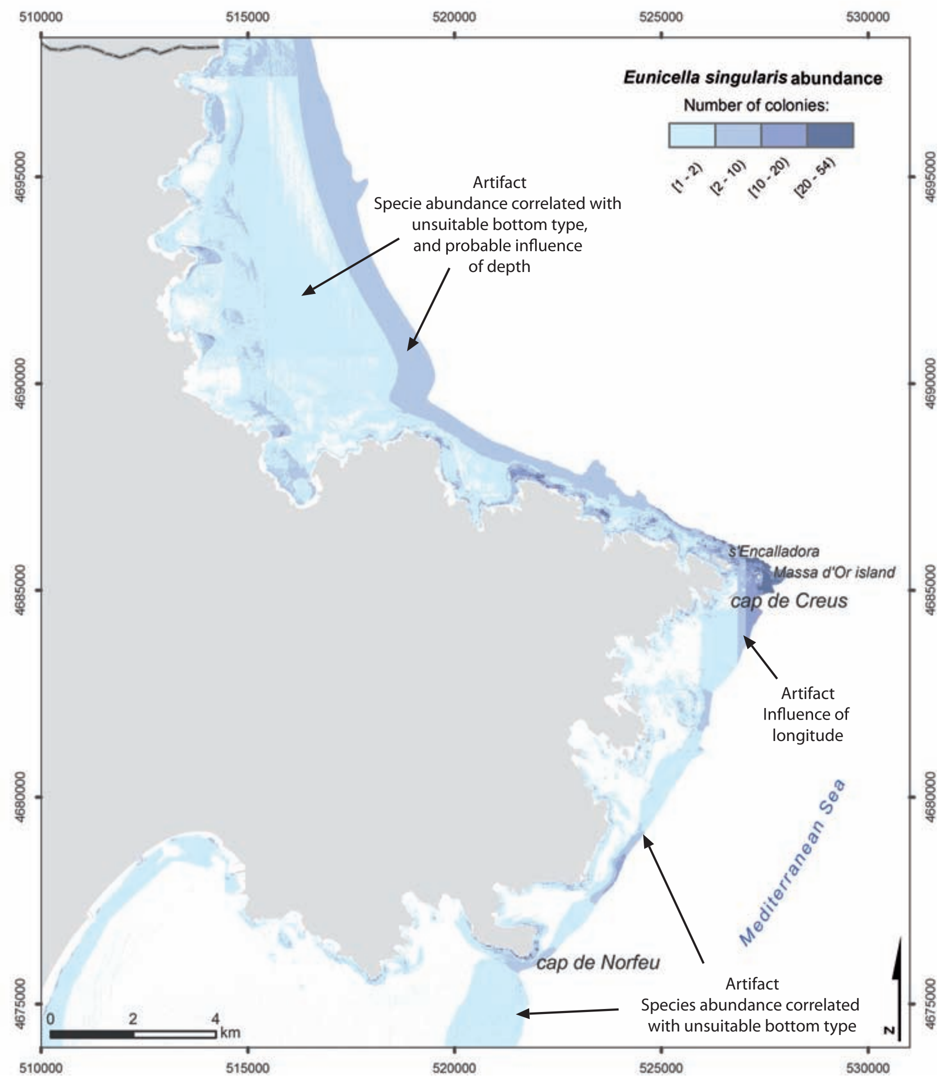

Fig. 5. Model output for predicting *Eunicella singularis* abundance. Cartographic data from Cartographic and Geological Institute of Catalonia (ICGC). Spatial reference system: UTM 31N- WGS84.

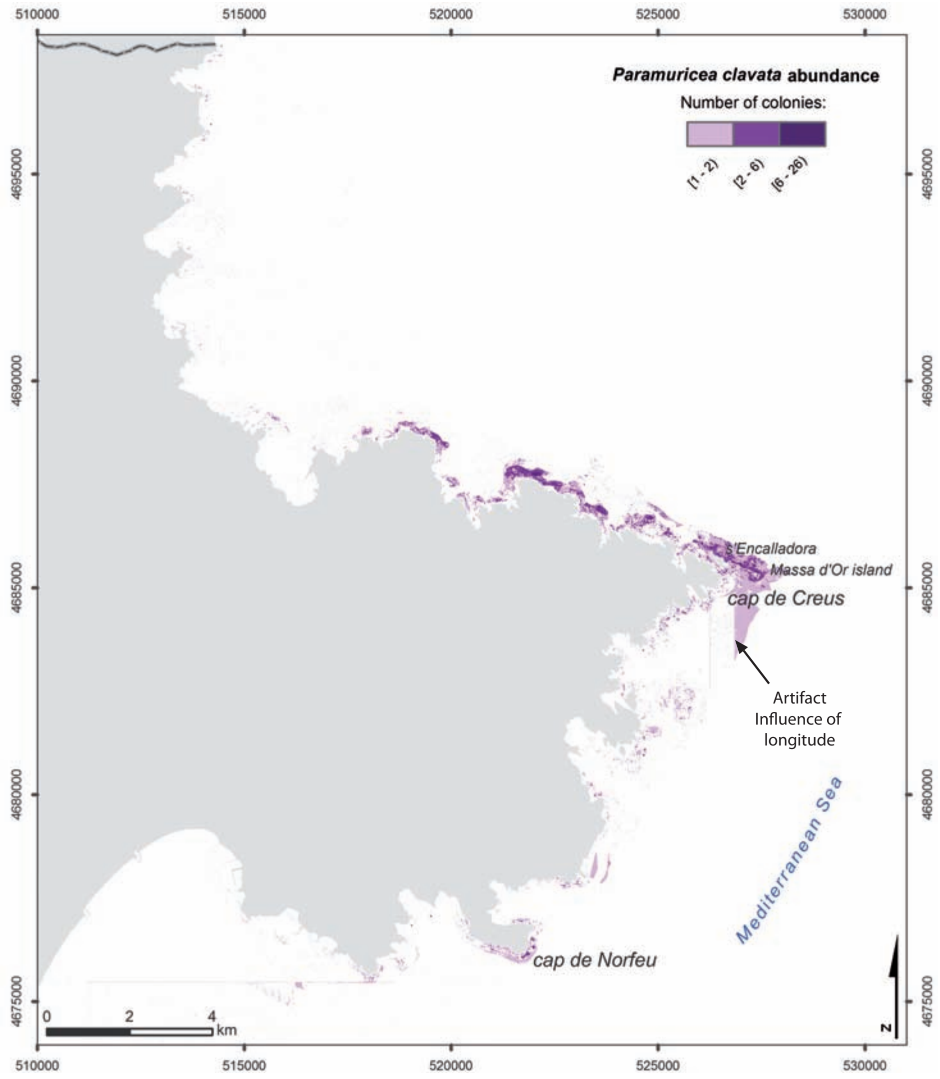

Fig. 6. Model output for predicting *Paramuricea clavata* abundance. Cartographic data from Cartographic and Geological Institute of Catalonia (ICGC). Spatial reference system: UTM 31N- WGS84.

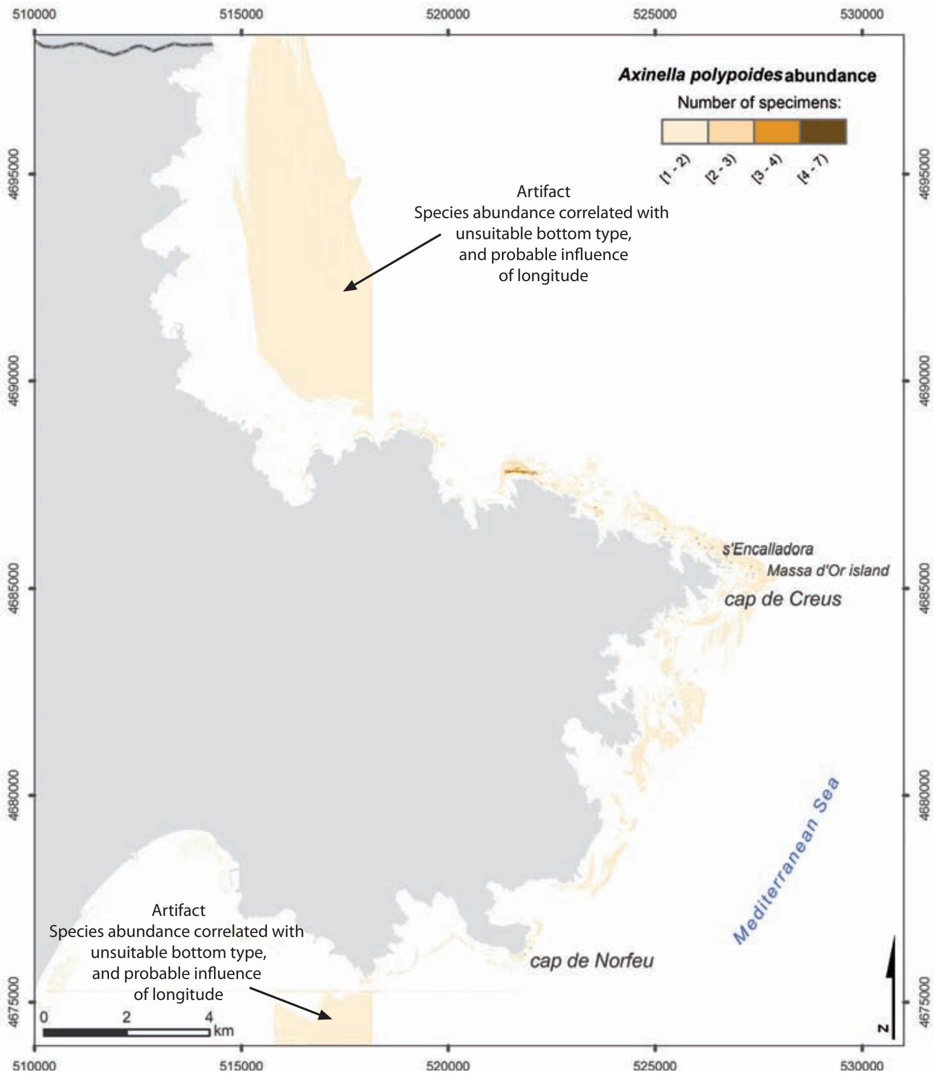

Fig. 7. Model output for predicting *Axinella polypoides* abundance. Cartographic data from Cartographic and Geological Institute of Catalonia (ICGC). Spatial reference system: UTM 31N- WGS84.

the longitude. A possible solution for this problem, which will also reduce the above-mentioned artifacts, might be to georeference the colonies not by geographical coordinates but with the distance from the coastline (Gori et al. 2011). This solution would reduce the effect of longitude introducing in the model that the species might be distributed only eastward from the coast.

Better results in prediction of the gorgonian abundances were related to higher number of sampling units showing species presence (value = 1), compared with distribution data of *A. polypoides* which mostly shown absences (value = 0). Presence of zero sampling in a model (i.e. random zero that emerge where conditions are suitable for the specie but absence is observed) could not be handled by all the predictive models as pointed out by Sileshi et al. (2009). This author suggested the use of 'Zero Inflated distribution' (i.e. distribution describing the presence of many zeros in the response variable; commonly applied in analyzing sampling data of rare species) (Sileshi et al. 2009; Zurr et al. 2009). Although different model were performed using various distributions (data not shown), RF was the one that better predicted both presence/absence and abundances. RF can handle the presence of strong interaction among the variables or cases in which the number of predictor variables exceeded the number of the observations (De'ath and Fabricius 2000; Cutler et al. 2007). Another explanation of the low response of the abundance prediction of *A. polypoides* could be the different resolution of the environmental data compared with species distribution data: our data of abundance is detected every 2.5 m^2 whereas the bathymetry and all the other related environmental variables had a resolution of 10 m^2. This situation might cause, the non-coincidence between the correct environmental parameters and the species presence. Although it would be ideal to perform the model with all the data at the same resolution level, sometimes this step represents a limitation, either because data do not exist in such detail, or due to their inaccessibility (Rodríguez et al. 2007).

4.2 Factors driving species distribution

RF predicted distribution and abundance considering environmental variables (TRI, BPI, Curvature as an example) that were not previously taken into account in the study of species distribution of the Cap de Creus shelf (Gori et al. 2011; Coppari et al. submitted). Thus, it allowed deepening into the factors that constrain the presence and abundance of the studied species at a local scale. Topographic variability (TRI and BPI) have a strong influence on the abundance of the two gorgonian species, result that is in line with previous finding in which a positive association between these environmental variable and the occurrence of cold-water corals was highlighted (Henry et al. 2010). Moreover, in the Cap de Creus submarine canyon located a few km to the north respect to the study area, cold-water corals seems to have similar preferences respect to topography and slope (Orejas et al. 2009; Gori et al. 2011). The importance of TRI in the abundance of gorgonians species might be explained by the preference of larvae to settle on rough substrate (Petersen et al. 2005; Burt et al. 2009), whereas their presence along positive reliefs (BPI), might be induced by the role of morphological highs in reducing the current flows and increasing the turbulence, thus facilitating the suspension and capture of food (Gili and Coma 1998; Orejas et al. 2009). On the other hand, the prevalent influence of substrate and depth on the abundance of *A. polypoides* was not surprisingly since this sponge species inhabits only rocky bottoms below 20 m depth (see chapter two).

The majority of the SDMs were performed including only seafloor related environmental variables; nonetheless, we also considered hydrodynamic variables (e.g. waves regimes) as they are substantially important for the development and distribution of benthic suspension feeders (Carballo et al. 2008). Indeed, wave disturbance associated with winter storms may be responsible for enhancing sediment transport (Bavestrello et al .1991) or resuspension affecting benthic secondary production (Emerson 1989). Our results reveal an unexpected reduced impact of near bed orbital velocity on the abundance of any of the three species. This is especially true, for the shallow *E. singularis*, being the influence of near bed orbital velocity higher at shallow depths. According to the model outputs, the local wave regime is not driving the presence-absence and abundance of suspension feeding organisms. Consequently, the asymmetric patterns of distribution observed in the study area, might be more influenced by the quality and quantity of the near bottom seston (Rossi and Gili 2009) than by physical the hydrodynamic disturbance. Resuspension caused by waves, might better explain the observed distribution patterns.

4.3 Species abundances in the study area and their role in benthic-pelagic coupling

Once the main artifacts in the model functioning will be solved, and model functioning will be improved, the potential abundance of the studied species all over the study area predicted by the model, may be used to refine the previous calculated impact in the benthic-pelagic coupling process as well as the role as C sink played by the three species all over a large area of Mediterranean coastal bottoms (see chapter 3).

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Synthesis of the results and general discussion

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The present study is the first making a broad-scale approach of the benthic-pelagic coupling processes in different organisms representative of the Mediterranean pre-coralligenous and coralligenous assemblages. This study gives a new perspective of the roles played by benthic suspension feeders in the benthic-pelagic coupling processes and as C sinks. The novelty lies in the methodology used, that cross in-situ and ex-situ experiments results, with the quantification of the presence, density and population size structure (obtained by means of quantitative analysis of ROV video transects) of the studied species along a large Mediterranean coastal area.

Size, spatial and bathymetrical distribution of the ascidian *Halocynthia papillosa* in Mediterranean coastal bottoms: benthic-pelagic coupling implications

- *Halocynthia papillosa* had a homogeneous distribution along the Cap de Creus coastline with maximum density of 4 specimens m^{-2}. Conversely, passive suspension feeders showed an asymmetric distribution, being more abundant in the north face of the study area. This could be explained by the active feeding of *H. papillosa*, which allow for the necessary food supply independently from the main hydrodynamic conditions of the area.

- The highest abundances of *H. papillosa* were recorded between 20 and 50 m depth in hard substrate (rock and coralline rock). The availability of suitable substrata in this bathymetrical range might explain the higher densities recorded in this depth range. This also highlights the importance of considering the deep (below 40 m depth) sublittoral coastal areas to correctly assess the distribution patterns of coastal species.

- Medium sized specimens (4.1–8 cm) dominated the *H. papillosa* populations in all the study area.

- *H. papillosa* was very rare in the Montgrí-Medes area compared to Cap de Creus. The low abundance might be related to: 1) the elevated number of divers visiting this area every year; 2) the consequences of a heavy storm that took place one year before the sampling; 3) the increased number of fishes in the area (due to the protection) with consequent increase of feeding pressure on the benthic fauna, especially on new recruits. The presence and abundance asymmetry between Cap de Creus and Montgrí-Medes (both protected areas but with different management strategies) of this species may be a good key to further investigate if the different approaches to management of Marine Protected Areas may be affecting the animal forest.

- The *H. papillosa* observed and measured in the study area ingested each year 31.4 g N, 519.4 g C, and respired 499.2 g C.

- The balance between ingested and respired C resulted in a net surplus of the quantity of C ingested only in spring, whereas a deficit was observed in the other seasons. This demonstrates that spring is the season in which this species gather and store the energy that will be used all year long, and invested in reproduction and growth.

- In the study area, *H. papillosa* retained a total of 20.2 g C per year.

- This study allow to test the applicability of a new approach, based on combining broad-scale data of distribution with experimental results, to quantify the functional role of benthic species. Importantly, the methodology used could be essential to understand how this and other active suspension feeders impact on the water column at a wide spatial scale, and how part of the C could be potentially retained in their structures.

The role of sponges in the benthic-pelagic coupling process in warm temperate coastal areas

- The two studied species (*Aplysina aerophoba* and *Axinella polypoides*) shown different spatial distribution pattern. *A. aerophoba* was recorded between 5 and 20 m depth, whereas *A. polypoides* was found between 10 and 70 m depth. This asymmetric distribution of the two species reflects their morphological and physiological characteristics. The mixotrophy of *A. aerophoba* determines its needing of well-lighted bottoms, to maintain the photosynthetic activity. This sponges is massive, well adapted to the higher hydrodynamism of shallow areas. *A. polypoides* shown a distribution limited to deeper waters, as a result of its arborescent-shape morphology that might be easily break down in wave-exposed shallower depths, and due to its heterotrophic condition. Indeed, near bottom live and detrital POM remains constant throughout the year in deeper waters, whilst in shallow waters it is particularly affected by the summer oligotrophic conditions.

- The highest densities recorded were 1.6 and 7.6 specimens m^{-2} for *A. aerophoba* and *A. polypoides,* respectively. *A. aerophoba* mainly occurred in the south and east side of the cape. This area is more exposed to light and generally sheltered from the frequent north winds, being the distribution of this species related to more calm waters. *A. polypoides* was distributed throughout all the study area. As an active suspension feeder, like *Halocyntia papillosa*, it seems reasonable to think that this organism is more independent from the main currents, being distributed throughout the entire coast and not only in the area more exposed to the active hydrodynamism (north face).

- *A. aerophoba* ingested 0.12–0.39 mg C g AFDW^{-1} day^{-1} in spring and 0.09–0.13 mg C g AFDW^{-1} day^{-1} in autumn, whereas *A. polypoides* ingested 0.07–0.17 mg C g AFDW^{-1} day^{-1} in spring and 0.18–0.60 mg C g AFDW^{-1} day^{-1} in autumn. It seems that there is a slightly different impact on the water column of the two species, being the spring more profitable for *A.aerophoba* and the autumn the season where *A.polypoides* has a higher capture rates.

- In the study area, the observed and measured *A. aerophoba* shown an ingestion of 0.79 g of C in spring and 0.08 g in autumn, whereas the observed and measured *A. polypoides*, accounted for an ingestion of 13.60 and 29.36 g of C in spring and autumn, respectively. The higher density of *A. polypoides* and its wider distribution give to this species a more prominent role in benthic-pelagic coupling processes in the studied area. However, the role of photosynthesis and the C translocation have to be investigated to understand how much C could be retained in the mixotrophic species.

- *A. aerophoba* removed 0.31 ± 0.09 mg C m^{-2} day^{-1} in spring and 0.03 ± 0.01 mg C m^{-2} day^{-1} in autumn, whereas *A. polypoides* removed 0.19 ± 0.02 mg C m^{-2} day^{-1} in spring and 0.42 ± 0.04 mg C m^{-2} day^{-1} in autumn. The low quantities of C ingested by both species compared to other tropical sponges or Mediterranean octocorals and ascidians might be related with the neglecting of some food sources or with the general lower densities of the two species in the study area.

The importance of gorgonians in the benthic-pelagic coupling processes in Mediterranean coastal areas, or why do we have to preserve the animal forest

- *Eunicella singularis* shown the highest biomass in the study area as well as the highest impact in benthic-pelagic coupling processes and as potential C sink.

- *Corallium rubrum* shown the highest number of colonies but accounted for the lowest biomass. This is related with its population size structure shifted towards small colonies, as a probable consequence of the overharvesting. Natural non-harvested populations are not present in the study area, indicating a dramatic bias toward immature populations that may not play their real role as potential C sinks.

- *Paramuricea clavata* was the species that retain less C, due to its high respiration rates.

- Shallow colonies of *E. singularis* accounted for the highest quantity of C retained (one order of magnitude higher than the shallow colonies of the other two species) due to the elevated number of colonies recorded in the study area. The autotrophic input seems also to be determinant in this species, which may have an advantage in shallow waters respect to the other two species. Interestingly, the deep colonies of the three species retained almost the same quantity of C.

- Compared with other coastal and marine ecosystem (e.g. mangrove or seagrass meadow), the three studied gorgonians species account for two orders of magnitude less C sequestered than the above–mentioned ecosystems. This is the first time that such large-scale approach has been done.

Species distribution models as a tool to estimate the benthic-pelagic coupling processes in coastal areas

- Spatial distribution models performed with Random Forest (RF), highlighted that the variation in the abundance of *Eunicella singularis*, was determined by longitude and topographic variability (TRI and BPI), whereas *Paramuricea clavata*, was mostly influenced by BPI and TRI. Indeed gorgonians, might prefer hard rough substrata to settle whereas the presence of prominent substrate compared to the surrounding ones (BPI), might facilitate the supply of food slowing the current flow and increasing the turbulence, thus facilitating the capture of food. *Axinella polypoides* is mostly influenced by substrate and depth, in fact this sponge species lives prevalently on rocky bottoms below 20 m depth.

- Near bed orbital velocity did not significantly influence any of the three species. This is interesting, because the initial hypothesis was that hydrodynamism might be one of the keys to understand species distribution. Possibly, the near bed orbital velocity is not the correct parameter to be measured for this purpose; maybe resuspension which influence the availability of food (quantity and quality) is more important to understand benthic suspension feeders distribution.

- In this chapter the strengths and weakness of our SDMs results are discussed, highlighting the advantages of using this tool to quantify large-scale benthic pelagic coupling processes.

Future perspectives

This thesis highlighted the importance of some of the most important species of Mediterranean benthic suspension feeders in the benthic-pelagic coupling processes, in coastal bottoms.

This study evidenced that accurate results from in situ and ex situ feeding experiments are needed to be coupled with spatial data of species distribution. At the same time, this thesis highlighted that many experiments still need to be performed to make the C flux and C sinks estimations more realistic. As an example, for the studied sponges, we could only estimate their influence in the C flux comparing two different seasons. It would be really interesting to perform in situ measurements of respiration, and to study the reproductive cycles of these two sponges to understand which is the amount of C returned to the system in the form of organic matter.. Also the possibility of performing feeding experiments during all the different seasons will elucidate the complete seasonality on the feeding rates during an annual cycle.

Regarding the estimation of the impact of gorgonians species in the benthic-pelagic coupling, results need to be refined: for every species, it would be really interesting to consider the amount of C returned to the water column through reproduction as well as to quantify the C that is fixed by growth. We need to understand if the quantity of microplankton ingested by *Leptogorgia sarmentosa* is a good representation for shallow and deep *Eunicella singularis*. And for deep population of *E. singularis* we need to explore if the contribution of zooplankton to the diet is comparable with the shallow ones. In this work, the deep populations demonstrated to be very different in its impact on the water column respect the shallow ones. The vertical homogenization of the results is not a good tool to understand the real role of the animal forest in the benthic-pelagic coupling processes.

The predictive abundances obtained for *E. singularis*, *Paramuricea clavata* and *Axinella polypoides* in the fourth chapter will be used again to calculate the potential C retained in all the study area, and this new estimation will be compared with the one obtained in the third chapter to check how much difference exist between two different methodology.

This thesis has given the opportunity to answer many question related to the importance of benthic suspension feeders species in the benthic-pelagic coupling and as C sinks. But at the same time this study only focused on some of the species of the coralligenous community. To really understand the trophic impact of the animal forests associated with this community, many other species should be studied such as bryozoan, hexacorals, soft corals among others. Coralligenous communities might show changes in their structure over time, thus, a temporal comparison, by repeating the same transect after several years, will provide crucial information of long-term changes in the community structure. But also a spatial comparison between coralligenous communities located in different areas of the Mediterranean Sea, will allow for a wider vision about the role played by this community in the benthic-pelagic coupling in the Mediterranean Sea, and will help to understand if there is any change in the trophic impact depending on the more eutrophic or oligotrophic condition of the study area.

Finally, it will be of high interest to try to apply this methodology to other Mediterranean animal forests located in deeper areas, such as black coral forests or cold-water corals reefs, and understand if their impact in the benthic-pelagic coupling processes and as C sinks is higher or comparable with shallower communities.

Nowadays, the paramount role played by benthic suspension feeders in the biogeochemical cycles and especially in the transfer of energy and matter from the water column to the benthos is more clear. Such roles are different, depending on the feeding strategies, the filtration rates and the abundances of these filter feeders. Based on the feeding strategies, benthic suspension feeders are classified in two groups: active and passive suspension feeders. The former category includes all the organisms that actively pump water through a filter system which separate the food particles from the water (the filtration system differs among species); the latter category includes all the organisms that completely depend on the water currents for the provision of food.

Many studies already focused on the characterization of the feeding habits, on the filtration efficiency and in the importance in the benthic-pelagic coupling of both active and passive suspension feeders. Some of these previous works performed in situ experiments, which are particularly useful to understand feeding habits in natural conditions and their seasonal variation. Contrarily, laboratories or mesocosm experiments allow for more precision, due to the possibility of controlling every variable influencing the experiment, but at the same time with less possibility of inference on natural systems. There is a general lack of information about the broad scale distribution of benthic fauna and the previous studies about the impact of suspension feeders on the benthic-pelagic coupling process normally focus on small patches.

Remotely Operate Vehicle (ROV) allows exploring extended and deep areas with no impact for the benthic communities, thus it is the perfect tool to perform extensive sampling in order to study the size, spatial and bathymetrical distribution of benthic species.

The combination of ROV data with data of feeding experiments allows estimating the impact on the benthic-pelagic coupling process played by suspension feeders in extended area.

In this thesis, the size and both spatial and bathymetrical distribution of three active and three passive suspension feeders were studied together with the role played by these species in the benthic-pelagic coupling processes and as carbon (C) sinks in the Cap de Creus region (NW Mediterranean sea). As active suspension feeders, one ascidian (*Halocynthia papillosa*) and two sponge species (*Aplysina aerophoba* and *Axinella polypoides*) were selected for this study, whereas, as passive suspension feeders, three gorgonians species (*Paramuricea clavata*, *Eunicella singularis* and *Corallium rubrum*) were chosen. The benthic suspension feeders considered in this thesis are long-lived species, and, in particular gorgonians, could also reach 100 years of life span, fact that permit the accumulation of high amount of C, stored as biomass.

All these species are commonly founded in pre-coralligenous and coralligenous Mediterranean communities and especially gorgonians could reach extremely high abundances. Gorgonians are arborescent organisms that create, especially when they reach really high densities, a complex tridimensional environment (known as animal forests) in which other organisms, might find shelter, food and a safe site for reproduction. Consequently, preserving the animal forests help to maintain high levels of associated biodiversity, fact that need to be considered in management decisions.