The ecology of deep sublittoral populations of Mediterranean gorgonians

L'ECOLOGIA DE LES POBLACIONS SUBLITORALS PROFUNDES DE LES GORGÒNIES MEDITERRÀNIES

ANDREA GORI

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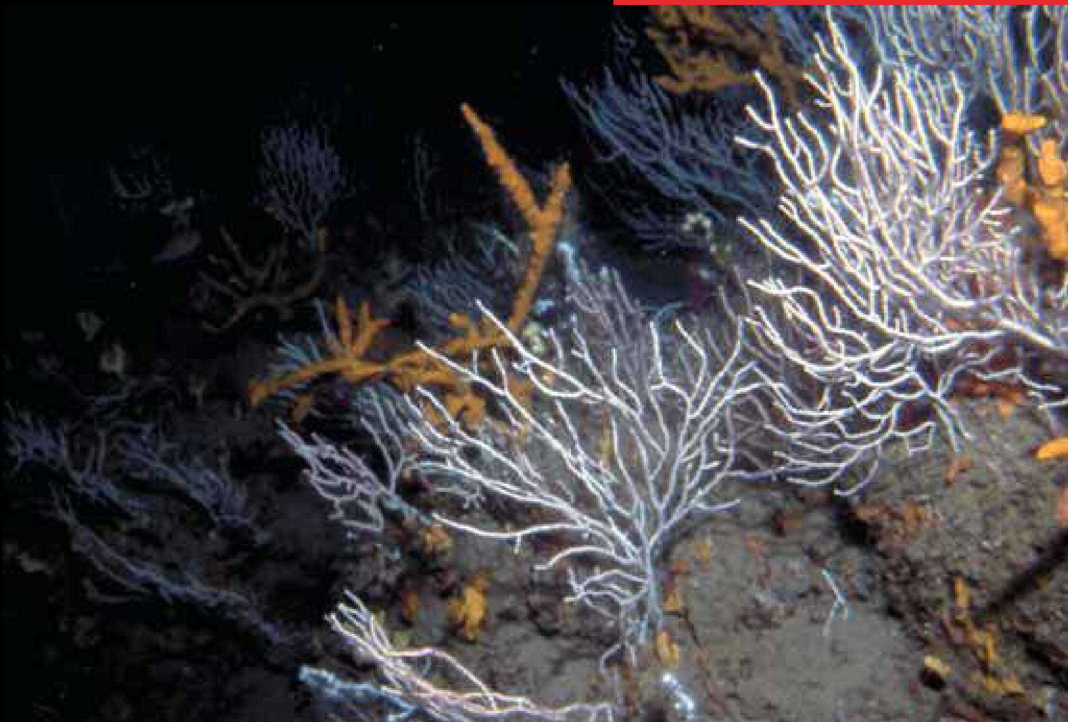

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Doctoral Thesis

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The ecology of deep sublittoral populations of Mediterranean gorgonians

L'ECOLOGIA DE LES POBLACIONS SUBLITORALS PROFUNDES DE LES GORGÒNIES MEDITERRÀNIES

Memòria presentada per Andrea Gori per optar al títol de Doctor per la Universitat de Barcelona

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Fe no és esperar,
fe no és somniar.
Fe és penosa lluita per l'avui i pel demà.
Fe és un cop de falç,
fe és donar la mà.
La fe no és viure d'un record passat.

No esperem el blat
sense haver sembrat,
no esperem que l'arbre doni fruits sense podar-lo;
l'hem de treballar,
l'hem d'anar a regar,
encara que l'ossada ens faci mal.

No somnien passats
que el vent s'ha emportat.
Una flor d'avui es marceix just a l'endemà.
Cal que neixin flors a cada instant.

Fe no és esperar
fe no és somniar.
Fe és penosa lluita per l'avui i pel demà.
Fe és un cop de falç,
fe és donar la mà.
La fe no és viure d'un record passat.

Enterrem la nit,
enterrem la por.
Apartem els núvols que ens amaguen la claror.
Hem de veure-hi clar,
el camí és llarg
i ja no tenim temps d'equivocar-nos.

Cal anar endavant
sense perdre el pas.
Cal regar la terra amb la suor del dur treball.
Cal que neixin flors a cada instant.

(*Cal que neixin flors a cada instant* - Lluís Llach)

A la Laia,
a la meva família,
als meus amics
i als meus mestres

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Informe dels Directors de la Tesi Doctoral

En Josep-Maria Gili Sardà de l'Institut de Ciències del Mar (CSIC) i en Sergio Rossi Heras de l'Institut de Ciència i Tecnologia Ambientals (UAB), directors de la tesi de doctorat elaborada per Andrea Gori, amb el títol "The ecology of deep sublittoral populations of Mediterranean gorgonians - L'ecologia de les poblacions sublitorals profundes de les gorgònies mediterrànies",

INFORMEN

Que els treballs de recerca duts a terme per Andrea Gori com a part de la seva formació predoctoral i inclosos a la seva tesi doctoral han donat lloc a 2 publicacions i 2 manuscrits enviats a revisió. A continuació es detalla la llista d'articles així com els índex d'impacte (segons el Journal Citation Reports 2009) de les corresponents revistes.

Gori A, Rossi S, Berganzo E, Pretus JL, Dale MRT, Gili JM (2011) Spatial distribution patterns of the gorgonians *Eunicella singularis, Paramuricea clavata* and *Leptogorgia sarmentosa* (Cap de Creus, Northwestern Mediterranean Sea). Marine Biology 158:143-158.
L'índex d'impacte de la revista Marine Biology és a l'actualitat 2,011. Aquesta revista està inclosa a la categoria "Marine and Freshwater Biology", que té una mediana d'índex d'impacte de 1,380 i inclou un total de 88 revistes. Tenint en compte l'índex d'impacte de Marine Biology, aquesta ocupa el lloc 22 de la seva categoria.

Gori A, Bramanti L, Lopez-Gonzalez P, Thoma J, Gili JM, Grinyó J, Uceira V, France S, Rossi S (submitted) Morphological variation across a depth gradient: rediscovery and characterization of the two morphotypes of the Mediterranean gorgonian *Eunicella singularis*. Marine Biology
L'índex d'impacte de la revista Marine Biology és a l'actualitat 2,011. Aquesta revista està inclosa a la categoria "Marine and Freshwater Biology", que té una mediana d'índex d'impacte de 1,380 i inclou un total de 88 revistes. Tenint en compte l'índex d'impacte de Marine Biology, aquesta ocupa el lloc 22 de la seva categoria.

Gori A, Rossi S, Linares C, Berganzo E, Orejas C, Dale MRT, Gili JM (2011) Size and spatial structure in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). Marine Biology DOI 10.1007/s00227-011-1686-7
L'índex d'impacte de la revista Marine Biology és a l'actualitat 2,011. Aquesta revista està inclosa a la categoria "Marine and Freshwater Biology", que té una mediana d'índex d'impacte de 1,380 i inclou un total de 88 revistes. Tenint en compte l'índex d'impacte de Marine Biology, aquesta ocupa el lloc 22 de la seva categoria.

Gori A, Viladrich N, Gili JM, Kotta M, Cucio C, Magni L, Rossi S (submitted) Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). Coral Reef
L’índex d’impacte de la revista Coral Reef és a l’actualitat 3.780. Aquesta revista està inclosa a la categoria “Marine and Freshwater Biology” que té una mediana d’índex d’impacte de 1,380 i inclou un total de 88 revistes. Tenint en compte l’índex d’impacte de Coral Reef, aquesta ocupa el lloc 3 de la seva categoria.

A més CERTIFIQUEN

Que Andrea Gori ha participat en el desenvolupament del treball de recerca associat a cadascun dels articles, així com en la seva elaboració. En concret, la seva participació en cadauna de les tasques ha estat la següent: plantejament inicial dels objectius de cadascun dels treballs, disseny experimental, realització dels mostrejos de camp, processat i anàlisi de les mostres obtingudes, càlcul de resultats i anàlisi estadístic de les dades, redacció dels articles i seguiment del seu procés de revisió.
Una part d’aquesta tasca va comportar una estada a la University of Northern British Columbia (UNBC) per l’aprenentatge de metodologies i interpretació de resultats de temàtiques afins a la tesi.

Finalment, certifiquen que cap dels coautors dels articles abans esmentats i que formen part de la tesi doctoral d’Andrea Gori ha utilitzat o bé té pevist utilitzar, implícita o explícitament, aquests treballs per a l’elaboració d’una altra tesi doctoral.

Atentament,

Josep-Maria Gili Sardà Sergio Rossi Heras

Introduction

Sublittoral marine ecosystems extend from the coastal line to the continental shelf break. They are characterized by many soft-bottom communities, and by rich hard-bottom communities. One of the factors that contribute more significantly to the richness of the hard bottom communities is the presence of sessile species which create three-dimensional structures. The heterogeneity of these generated structure, provides refuge and habitats to a rich associated fauna. Apart from algae meadows, sessile invertebrates such as corals and gorgonians are among the most representative structural species in marine benthos.

CORALS AND GORGONIANS

Corals and gorgonians belong to different orders within the class Anthozoa, phylum Cnidaria (except fire corals, which belong to the order Filifera, class Hydrozoa). Hard corals species (Scleractinia) exhibit very different forms, from solitary polyps to very large colonies made up by hundreds to thousands of polyps, whereas blue corals (Helioporacea), soft corals and gorgonians (Alcyonacea) as well as black corals species (Antipatharia) are always colonial organisms.

All corals and gorgonians build a skeleton that, depending on its composition in calcium carbonate and/or protein, can be more or less flexible. Owning a hard skeleton allows most corals and gorgonians to keep an erect position, which endows them with an important role as ecosystem engineers (Jones et al. 1994) being essential contributors in the construction of complex three-dimensional structures. Wide assemblages of coral species (coral reefs) are known mainly from warm waters (Fig. 1), but also from temperate and cold water areas (Roberts et al.

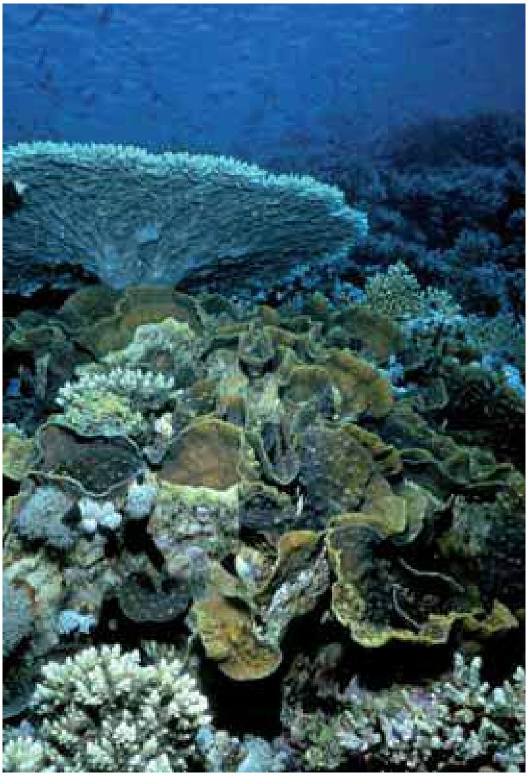

Fig. 1 - Tropical coral reef in the Red Sea.

2009; Wild 2011), and gorgonians are common and conspicuous members of most coral reefs (Fig. 2) (Lasker and Coffroth 1983; Brazaeu and Lasker 1989; Mortensen et al. 1995; Ben-David-Zaslow and Benayahu 1999). In coral reefs, coral species grow forming complex three-dimensional frameworks, which provide different habitats for hundreds of associated organisms, thus environmental factors such as water current, food availability and sediment resuspension vary greatly between and within the habitats generated in the coral reef (Connell 1978; Kim and Lasker 1997; Wild 2011). The heterogeneity of the structure generated by corals and gorgonians, commonly enhances the abundance and functional diversity of associated fauna (Mortensen et al. 1995; Witherell and Coon 2000; Roberts et al. 2002; Henry and Roberts 2007), making the coral reef one of the most diverse ecosystems on the planet (Connell 1978). Corals and gorgonians fulfill their energetic demand through the symbiosis with zooxanthellae and/or by filtering particulate organic matter (POM) and capturing plankton (Wildish and Kristmanson 1997). This way, they often initiate element cycles via the release of organic matter, particularly mucus (Wild et al. 2004; Crossland 1987), whereas capturing POM and plankton they determine an important energy and matter transfer between the pelagic and benthic systems (Gili and Coma 1998).

Fig. 2 - Tropical coral reef in the Red Sea.

MEDITERRANEAN GORGONIANS

Out of the approximately 20 gorgonian species which inhabit the Mediterranean Sea (Carpine and Grasshoff 1975), *Eunicella singularis* (Esper, 1791), *Eunicella cavolinii* (Koch, 1887), *Paramuricea clavata* (Risso, 1826), *Leptogorgia sarmentosa* (Esper, 1789), and *Corallium rubrum* (Linnaeus, 1758) are the most frequent species in the sublittoral zone, from the surface down to the edge of the continental shelf (Harmelin 1995; Gili et al. 1989). *E. singularis, E. cavolinii* and *P. clavata* are among the most conspicuous and abundant structural species on the rocky sublittoral bottoms characterized by the Mediterranean coralligenous and precoralligenous communities (Gili and Ros 1985; Harmelin 1995; Ballesteros 2006). In the coralligenous and precoralligenous communities, these three gorgonians can develop very dense populations (Harmelin and Garrabou 2005) and play an important role as ecosystem engineers (Jones et al. 1994) by providing biomass and structural complexity (Fig. 3 and 4) (True 1970). Gorgonians represent the most erect stratum of this community (Gili and Ros 1985), whose diversity and richness in animal species has been compared to those of the tropical coral reefs (Ros et al. 1985; Ballesteros 2006). The high biodiversity of the coralligenous community among the Mediterranean benthic communities, is mainly due to the great heterogeneity and microhabitat diversity originated by its complex three-dimensional structure (Laborel et al. 1961; Gili and Ros 1985; Harmelin 1995). *C. rubrum* is a gorgonian species often found in the coralligenous community, although it is also a

Fig. 3 - Mediterranean gorgonian population (*Paramuricea clavata*) in Cap de Creus.

characteristic species of the semi-dark caves community (Gili and Ros 1985). Conversely, *L. sarmentosa* is a gorgonian commonly found on soft-gravel bottoms where it can develop very dense populations (Weinberg 1979; Mistri 1995).

Mediterranean gorgonian species share common traits in their reproductive ecology such as gonochoric colonies and annual sexual reproduction with longer oogenesis as compared to spermatogenesis (Coma et al. 1995; Ribes et al. 2007; Rossi and Gili 2009). *E. singularis* and *C. rubrum* are brooder species in which the embryogenesis takes place inside the female polyps and the mature planulae larvae are released from the polyp mouth (Weinberg and Weinberg 1979; Vighi 1970). Differently, *P. clavata* is a surface brooder thus embryogenesis takes place on the surface of female colonies (Coma et al. 1995). Up to this date, there is no clear information about *E. cavolinii's* reproductive mode, even thought it was reported to be a brooder species (Rossi 1959), and *L. sarmentosa* is supposed to be a broadcast spawner species since no larva has ever been observed inside the female polyps or on the surface of female colonies (Rossi and Gili 2009). *E. singularis* is the only mediterranean gorgonian showing symbiosis with zooxanthellae (Rossi 1959; Carpine and Grasshoff 1975; Weinberg 1976), whereas all the other species are completely heterotrophic and feed on a wide spectrum of food sources that ranges from nanoeukaryotes to copepods, being the microplankton and detrital particulate organic matter the bulk of their diet (Coma et al. 1994; Ribes et al. 1999, 2003; Rossi et al. 2004; Tsounis et al. 2006a; Picciano and Ferrier-Pagès 2007). The estimated capture rates suggest that gorgonian grazing on planktonic communities and suspended detrital matter gives rise to a substantial depletion of these components in the water column (Ribes et al. 1999, 2003) and, consequently, they play a paramount role in sublittoral food webs, since they cause an important flow of energy and matter from the plankton to the benthos system (Gili and Coma 1998). Mediterranean gorgonians are long-lived species displaying slow growth rates and low natural mortality rates (Weinberg and Weinberg 1979; Mistri and Ceccherelli 1993; Harmelin 1995; Coma et al. 1998, 2004; Linares et al. 2007; Bramanti et al. 2009); these slow population dynamics make gorgonians especially vulnerable to increasing anthropogenic disturbances (Dayton et al. 1995; Garrabou and Harmelin 2002; Santangelo et al. 2007; Linares and Doak 2010). Among disturbances of direct anthropogenic origin, anchors, fishing nets, divers and pollution have been recorded as threats for gorgonian species (Harmelin and Marinopoulos 1994; Bavestrello et al. 1997; Coma et al. 2004); whereas indirect perturbations such as recent mass mortality events (Linares et al. 2005; Coma et al. 2006; Garrabou et al. 2009), appear to be related to climatic anomalies indirectly linked to human activities.

Fig. 4 - Mediterranean gorgonian population (*Eunicella singularis*) in Cap de Creus.

BACKGROUND HYPOTHESES

The hypotheses that are at the base of the present thesis, originate from the results of recent investigations on the spatial variability in the ecology of Mediterranean gorgonians, as well as from the identification of an important gap in their knowledge.

Spatial variability

The main environmental factors influencing suspension feeders' ecology in the Mediterranean coastal areas are very variable at a small spatial scale (Rossi et al. 2003), which in turn may have repercussions on the feeding, reproduction, growth, mortality and energy storage capabilities of gorgonians species. Spatial comparisons are becoming increasingly important to understand the potential variability and adaptability of species to local conditions, and all in all, to achieve a comprehensive understanding of their ecology. A previous study (included in this thesis as an annex, pag. 133) focusing on the spatial variability in the reproductive traits of *E. singularis* and *P. clavata*, reported strong differences regarding the energy storage and an offset in the reproductive cycles of both gorgonians between populations located 600 km apart (Gori et al. 2007, Additional Resource 1). Large spatial comparisons showed strong variations in the population size structure of *P. clavata*, *E. singularis* and *C. rubrum* among different localities in the Western Mediterranean (Santangelo et al. 2003; Tsounis et al. 2006b; Linares et al. 2008; Bramanti et al. 2009), similarly, the vertical distribution of *P. clavata* was reported to significantly change along a latitudinal gradient in response to water temperature and light (Linares et al. 2008).

Lack of knowledge on intermediate depths

In the study of marine hard-bottom communities, intermediate depths, particularly coastal rocky bottoms in the 40–150 m depth range, have received relatively little attention because they lie below scuba depth (Menza et al. 2008; Rooney et al. 2010), while most submersible-based research was traditionally conducted at depths below 150 m (Sink et al. 2006; Virgilio et al. 2006; Hinderstein et al. 2010).

Research on tropical coral and gorgonian ecology has been mainly focused on coral reefs within the depth range of traditional scuba diving (Menza et al. 2008; Rooney et al. 2010), and much of our understanding of coral reef ecology in general is based on these relatively shallow depths (Menza et al. 2008). Far less is known on coral reefs located in the mesophotic zone (Menza et al. 2008), defined as the deeper part of the photic zone in which light-dependent coral communities develop (Ginsburg 2007). This zone ranges from between 30-40 m depth to the bottom of the photic zone, which varies by location and extends to over 150 m in some regions (Hinderstein et al. 2010). Mesophotic coral ecosystems harbor coral species found in their shallower counterparts, but they may also present a number of depth-restricted species (Hinderstein et al. 2010). Overall, this depth range accounts for two-thirds of the total depth range of zooxanthellate coral environments (Pyle 1996, 2000), but to this day it has remained largely unexplored (Bongaerts et al. 2010). Enabled by technological advances, mesophotic coral ecosystems studies are revealing extensive, productive and rich communities, which differ significantly from their shallow-water counterparts (Kahng et al. 2010). More importantly, the processes occurring in the mesophotic zone might have a global relevance that has yet to be understood (Buesseler et al. 2007; Hinderstein et al. 2010).

In a similar way, although Mediterranean sublittoral gorgonians are known to be present from shallow waters down to 100 m depth (Rossi 1959; Laborel et al. 1961; Carpine and Grasshoff 1975; Weinberg 1976), the great majority of studies carried out to this date were focused on shallow water populations. Environmental features change considerably with depth in the Mediterranean Sea (Garrabou et al. 2002; Rossi and Gili 2009), thus shallow and deep sublittoral gorgonian populations will be exposed to different environmental conditions. Shallow habitats are subject to strong hydrodynamic forces (Hiscock 1983; Weinbauer and Velimirov 1996) and are mainly exposed to bidirectional currents (Riedl 1971), whereas deeper near-shore habitats are more affected by unidirectional water movements (Riedl 1971; Hiscock 1983) and are largely sheltered from the direct physical damage that may be caused by strong storm-induced waves (Bongaerts et al. 2010). Light intensity decreases exponentially with depth (Drew 1983; Mass et al. 2007), and high irradiance in the summer is conducive to strong stratification of the water column, which can result in a severe depletion of suspended materials in shallower waters during the summer (Coma et al. 2000; Rossi and Gili 2009). Overall, variability in environmental conditions is dampened at greater depth in the nearshore bottoms of the Mediterranean Sea (Garrabou et al. 2002) because temperature, currents, and other water column features are more constant below the summer thermocline (Cebrián et al. 1996; Ballesteros 2006; Rossi et al. 2008). Such differences in the main environmental features could induce differences among gorgonian populations at different depths. For instance, a shift in the timing of *C. rubrum* reproductive cycle, and a significant difference in the reproductive output were reported between populations located at 18 and 40 m depth (Tsounis et al. 2006c). Trophic ecology was observed to change significantly with depth in *C. rubrum* (Tsounis et al. 2006a; Rossi and Tsounis 2007), and both *P. clavata* and *C. rubrum* present population size structures dominated by bigger colonies in deep than in shallow populations (Harmelin and

Marinopoulos 1994; Tsounis et al. 2006b; Rossi et al. 2008).

The observation of such a great spatial variability in the ecology of Mediterranean gorgonian species, both in horizontal and vertical comparisons, makes the investigation of the deep sublittoral gorgonian populations located between 40 and 150 m depth an important advance in the study of Mediterranean gorgonians, in order to achieve a comprehensive understanding of their ecology.

OBJECTIVES OF THE THESIS

The present thesis addresses a first description of the ecology of deep sublittoral populations of Mediterranean gorgonians by means of four chapters containing new data and results, followed by a fifth chapter containing an overall discussion.

Chapter 1 - Spatial distribution patterns of the gorgonians *Eunicella singularis, Paramuricea clavata* and *Leptogorgia sarmentosa* (Cap de Creus, northwestern Mediterranean Sea)

This chapter focuses on the exploration of the Cap de Creus's (northwestern Mediterranean Sea) sublittoral zone, in order to investigate the occurrence and spatial distribution patterns of deep sublittoral populations of Mediterranean gorgonians. This study was prompted by the lack of quantitative data on the presence of the *Eunicella singularis*, *Paramuricea clavata*, and *Leptogorgia sarmentosa* species over their entire bathimetrical range of distribution. The aims of this chapter are (1) to quantify the occurrence and abundance of the main gorgonian species in northwestern Mediterranean benthic communities over a large geographic (60 km of coastline) and bathymetrical (0–70 m deep) extent, (2) to assess the spatial distribution patterns of their abundance, and (3) to discuss the main environmental determinants of such distributions.

Chapter 2 - Morphological variation across a depth gradient: rediscovery and characterization of the two morphotypes of the Mediterranean gorgonian *Eunicella singularis*

Eunicella singularis occurs at high densities in sublittoral waters ranging from shallow to deep (15–70 m depth). However, the appearance of its colonies seem to change with increasing depth: colonies shallower than 30–35 m display the common candlestick-like colony morphology and a dirty grayish-white color from the presence of symbiotic algae, whereas colonies located deeper than 30–35 m show a more variable colony morphology and bright white color, being identified as the *E. singularis* aphyta described by Théodor (1969). Combining scuba sampling and remotely operated observations, this chapter focuses on a detailed examination of *E. singularis* colonies located from 20 to 60 m depth. The aims of this chapter are to quantify the variation with depth of (1) the gorgonian colony shape, (2) the sclerite size and shape, (3) the differences in genetic markers, and (4) the presence of symbiotic algae in the gorgonian tissue.

Chapter 3 - Size and spatial structure in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea)

Since abiotic factors change considerably with depth in the Mediterranean Sea, shallow and deep sublittoral *Eunicella singularis* populations are exposed to different environmental conditions. These changing conditions could result in differences in the suitability and stability of habitats, thus inducing differences between gorgonian populations located at different depths. The aims of this chapter are to explore the existence of differences in (1) the size structure, and in (2) the spatial structure of shallow versus deep sublittoral populations of the two morphotypes of *E. singularis*.

Chapter 4 - Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea)

Differences in the environmental conditions experienced by gorgonian populations located above and below the summer thermocline, may potentially affect their reproductive cycle, trophic ecology, and energy storage. This chapter focuses on the study of the reproductive cycle of two *Eunicella singularis* populations, located respectively at 20 and 60 m depth, and on the study of their trophic ecology by means of the examination of multiple indirect trophic markers. The aims of this chapter are to explore the existence of differences in (1) the gonadal output and reproductive cycle, (2) the energy storage capability, and (3) the food sources along a seasonal cycle of shallow versus deep sublittoral populations of the two morphotypes of *E. singularis*.

COMMENTS

Pictures in Figures 1 to 4 were made by Josep-Maria Gili, Sergio Rossi Heras and Andrea Gori.

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Chapter 1

Spatial distribution patterns of the gorgonians *Eunicella singularis*, *Paramuricea clavata* and *Leptogorgia sarmentosa* (Cap de Creus, northwestern Mediterranean Sea)

Additional Resource 2

Marine Biology (2011) 158:143-158

RESUM

La major part dels coneixements actuals sobre les gorgònies de la Mediterrània es limita a les investigacions de les poblacions que es troben en les aigües poc profundes del sublitoral, per tant existeixen poques dades sobre les poblacions situades per sota de la profunditat de busseig. Per superar aquesta falta d'informació, la presència i abundància de les gorgònies *Eunicella singularis* (Esper, 1791), *Paramuricea clavata* (Risso, 1826) i *Leptogorgia sarmentosa* (Esper, 1791), van ser investigades en les comunitats bentòniques de la Mediterrània nord-occidental en una àmplia extensió geogràfica (60 km de costa) i batimètrica (0-70 m de profunditat) utilitzant un vehicle operat per control remot (ROV). La major presència i abundància de *E. singularis* i *P. clavata* es va concentrar en les àrees que estan directament exposades a fortes corrents properes al fons. *E. singularis* ha resultat ser l'espècie més comuna i més abundant; ha mostrat una gran plasticitat i amplitud en els seus requeriments ambientals. En canvi, *P. clavata* ha mostrat una distribució molt irregular estrictament associada amb les parets rocoses verticals. A l'àrea d'estudi, només s'han observat colònies aïllades de *L. sarmentosa*. Les màximes abundàncies d'*E. singularis* i *P. clavata* s'han identificat per sota de 40 m de profunditat, cosa que demostra la importància d'estudiar la distribució de les espècies bentòniques en una àmplia extensió geogràfica i batimètrica.

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ABSTRACT

Most of the current knowledge on Mediterranean gorgonians is restricted to investigations of those populations found within shallow sublittoral waters, and only limited data are available for populations located below scuba depth. To overcome this lack of information, the occurrence and abundance of the gorgonians *Eunicella singularis* (Esper, 1791), *Paramuricea clavata* (Risso, 1826) and *Leptogorgia sarmentosa* (Esper, 1791) were investigated in northwestern Mediterranean benthic communities over a wide geographical (60 km of coastline) and bathymetrical (0-70-m deep) extent using a remotely operated vehicle (ROV). The greatest occurrence and abundances of *E. singularis* and *P. clavata* were concentrated in areas that are directly exposed to strong near-bottom currents. *E. singularis* was the most common and abundant species and displayed great plasticity and amplitude in its environmental preferences. Conversely, *P. clavata* showed a very patchy distribution that was associated with vertical rocky walls. Only isolated colonies of *L. sarmentosa* were observed in the study area. Hot spots of abundance of *E. singularis* and *P. clavata* were identified below a depth of 40 m, which demonstrates the importance of studying the distribution of benthic species over a wide geographical and bathymetrical extent.

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INTRODUCTION

Characterizing and explaining the spatial variation of the abundance of species is a central question in ecology (Ives and Klopfer 1997; Currie 2007), and an understanding of the distribution patterns of species in space and time is of significant importance in the establishment of management and conservation plans (Ives and Klopfer 1997; Fortin and Dale 2005). Although variation in environmental factors is often considered the most significant factor in determining abundance patterns within a species (Whittaker 1975; Brown et al. 1995), spatial patterns can also arise in the absence of environmental variations (Ives and Klopfer 1997; Currie 2007) due to biological interactions among organisms (Borcard et al. 1992; Frelich et al. 1993) and historical factors such as low-frequency disturbances.

In sessile marine invertebrates, the spatial distribution is determined by the combined effects of biological (Glynn 1976; Neudecker 1979; Sheppard 1979) and environmental factors (Glynn 1976; Adjeroud 1997) that can affect the birth, growth, and death rates of individuals in single-species populations (Hutchinson 1953). Recruitment determines the spatio-temporal distribution patterns of new individuals (Chiappone and Sullivan 1996; Edmunds 2000; Baird et al. 2003) and is influenced by the characteristics of the reproductive cycle of the species, the dispersal abilities of the larvae, the features of the substrate, hydrodynamic patterns, and stochasticity. Recruitment is patchy in time and space across a variety of scales (Dunstan and Johnson 1998; Edmunds 2000). Environmental factors, the food supply, biological interactions (intra and inter-specific), and perturbations determine the survival of new individuals and affect biological processes such as growth and reproduction. The interaction between the recruitment and survival of individuals determines the spatial patterns of the distribution of sessile organisms (Gaines and Roughgarden 1985; Dunstan and Johnson 1998). It has been pointed out that in sessile marine organisms, this interaction frequently results in patchy distribution patterns (Sebens 1991) and spatially structured populations (Karlson 2006). Such patterns will have a fundamental influence both on future processes within each species (feeding, growth, reproduction, intra-specific competition) and on their interactions with other species (predation, inter-specific competition) (Dale 1999) because the spatial structure determines ecological processes in the short term, and ecological processes modify the spatial structure in the long term (Illian et al. 2008).

Among the benthic sessile invertebrates of Mediterranean sublittoral communities, gorgonian corals play an important role as ecosystem engineers (Jones et al. 1994) by providing biomass, structural complexity, and biodiversity in benthic communities (True 1970; Gili and Ros 1985; Gili et al. 1989; Ballesteros 2006). Three species of gorgonians are most abundant in the Western Mediterranean Sea: *Eunicella singularis* (Esper, 1791), *Paramuricea clavata* (Risso, 1826), and *Leptogorgia sarmentosa* (Esper, 1791) (Carpine and Grasshoff 1975; Weinberg 1976). In contrast, *Eunicella cavolinii* (Koch, 1887) has been found to be very common only in the eastern part of the Western Mediterranean Sea, whereas it is absent or very rare along the coasts located west of Marseille (Weinberg 1976).

Our understanding of the ecology of these species has improved during the last two decades (e.g., Mistri and Ceccherelli 1993; Coma et al. 1995; Linares et al. 2007; Ribes et al. 2007), and previous studies have indicated likely environmental parameters that influence the ecological range of each species (Weinberg 1978a, 1979, 1980). Thus far, the majority of studies have been conducted on shallow water populations of gorgonians (depths of less than 40 m), whereas limited information is available for rocky coast assemblages that are present in deeper waters (Virgilio et al. 2006; Rossi et al. 2008). Although gorgonians are known to be present in Western Mediterranean deep sublittoral zone (Rossi 1959; Carpine and Grasshoff 1975; Weinberg 1976), coastal rocky bottoms below depths of 40 m are poorly understood because they lie below scuba depth, and most submersible-based research is conducted at depths below 150 m (Sink et al. 2006, Virgilio et al. 2006). Thus, there is a paucity of information on the bottoms in the 40-150 m deep range (Sink et al. 2006).

In recent years, the development of remotely operated vehicles (ROVs) has allowed quantitative sampling and detailed observations in areas that could not be sampled using traditional methods (scuba, trawl) due to the depth or the roughness of the terrain (Mortensen and Bull-Mortensen 2004; Rossi et al. 2008). In addition, video surveys have the advantage of sampling large areas without affecting benthic communities (Bianchi et al. 2004; Mortensen and Buhl-Mortensen 2004). Such a large spatial extent is a central requirement for the correct estimation of general patterns of species distributions, because observations conducted over smaller extents may not reflect the patterns and processes occurring over larger scales (Gotelli and Ellison 2004). The slow population dynamics of gorgonians make them susceptible to many direct and indirect anthropogenic effects, such as ship anchoring and fishing activities (Harmelin and Marinopoulos 1994; Bavestrello et al. 1997), diving (Linares et al. 2008a), and mass mortalities (Cerrano et al. 2000; Perez et al. 2000; Linares et al. 2005; Garrabou et al. 2009). Therefore, a detailed and wide-ranging knowledge of the abundance distributions of each species is fundamental to assess ongoing human effects and to propose scientifically valid conservation and management measures (Fraschetti et al. 2002; Benedetti-Cecchi et al. 2003). To date, knowledge is lacking regarding the abundance of gorgonians in the Mediterranean benthic community below depths of 40 m, the general patterns of the distribution of each species over its entire bathymetrical range, and the depth ranges in which the main densities of each species are concentrated. To approach these questions, the aims of the present study were: (1) to quantify the occurrence and abundance of the main gorgonian species in northwestern Mediterranean benthic communities over a large geographic (60 km of coastline) and bathymetrical (0-70 m deep) extent, (2) to assess the spatial distribution patterns of their abundance, and (3) to discuss the main environmental determinants of such distributions.

MATERIALS AND METHODS

Study area

The study area is located in the northwestern region of the Western Mediterranean Sea, south of the Gulf of Lions, and it comprises the Cape of Creus (42º19’12’’ N; 03º19’34’’ E) and the coast up to the boundary with France (Fig. 1). More than 60 km of coast were sampled by means of 76 video transects perpendicular to the coastline. The study area was sub-divided into seven sub-areas (A to G). The general circulation pattern in the area is dominated by the Northern current, which flows southwestward and thus creates an overall east-to-west circulation (Millot 1990; DeGeest et al. 2008). The wind regime in the study area is characterized by frequent and strong northern winds (Mistral and Tramuntana 28% and 41% of the time, respectively) and rare southeasterly and easterly marine winds (<6% of the time) (Ulses et al. 2008). Both Mistral and Tramuntana wind regimes transport coastal water and suspended sediment toward the southwest (DeGeest et al. 2008; Ulses et al. 2008). Consequently, the southern coast (sub-area A) is the most sheltered section of the surveyed coast. The eastern coast of the cape (sub-areas B, C, and D) is not influenced by direct near-bottom currents and is only affected by the infrequent winds from the east (Ulses et al. 2008; DeGeest et al. 2008). The north face of the cape (sub-areas E and F) is directly exposed to the main winds and wave action in the study area (Ulses et al. 2008), as well as to a strong near-bottom current that accelerates around the cape (DeGeest et al. 2008). Finally, the northern coast (sub-area G) is not influenced by direct near-bottom currents, and the deposition of sediments predominates in this section of the surveyed coast (DeGeest et al. 2008).

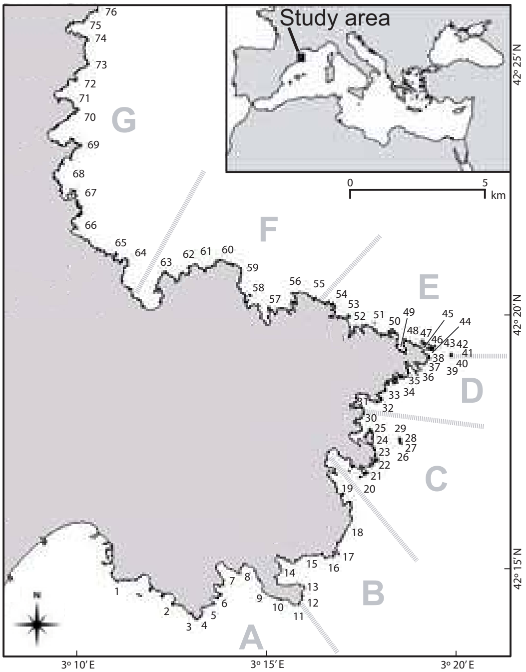

Fig. 1 - Map of the study area showing the study sites and the seven sub-areas (A-G).

Sampling procedure

Fieldwork was conducted over a total of 21 days in October and November 2004. Transects were video recorded with the ROV Phanthom XTL equipped with a SONY FCB S3000P 3CCD (700 horizontal lines) camera, a depth sensor, a compass, and two parallel laser beams that provide a scale to define a fixed width of the transects (0.5 m) for subsequent video analysis. Videos were recorded on video tapes in DV format. In each sampled location, seabed video recording was started at the deepest point of the transect and proceeded toward the shallows until the ROV surfaced close to shore (except in the case of a few transects that were terminated before reaching the sea surface). The ROV speed was constant (approximately 0.4 knots) during all transects. The initial depth of each transect varied between 12 and 71 m depending on the geographical characteristics of each location. The maximum depth assessed in this study was selected to cover almost all of the sublittoral zone in the study area, from the littoral to the start of the continental shelf. The transect length ranged from 92.6 m to 907.1 m, and a total distance of 28.3 km along the seabed was recorded.

Video analysis

Videos were transferred from tapes to a hard disk, and video analysis was performed with Final Cut software (Apple). Pauses in the movement of the ROV were eliminated from each video to correctly estimate the total length of each transect, assuming a constant speed. In the initial stage of the video analysis, sequences for which the video record was not useful were identified (i.e., regions of poor visibility, or periods during which the ROV was at a high altitude from the seabed). The remaining useful sequences comprised 86.1% of the total recorded material and corresponded to a total distance of 24.5 km and an area of approximately 12.2 km^2. Starting from the beginning of each transect, sampling units of 2 m^2 (0.5 m width and 4 m long) were defined every 10 meters along each transect. Sampling units that correlated with an unusable section of the video record were discarded from the analysis. The sampling unit area was chosen to be comparable with previous studies that estimated a minimum sample size of 1.75 m^2 for *Eunicella singularis* and 1.5 m^2 for *Paramuricea clavata* (Coma et al. 2006; Linares et al. 2008b) and a representative area of 2.0 m^2 for invertebrates in the rocky substrata of the Mediterranean (Weinberg 1978b).

A total of 2326 useful sampling units were obtained from the 76 transects. In each of these units, the gorgonian abundance was assessed by counting the number of colonies of *E. singularis*, *P. clavata* and *Leptogorgia sarmentosa*. Each sampling unit was also characterized according to environmental variables (percentage of the abundance of each seabed substrate type, seabed slope class, and the average depth) and spatial variables (geographical coordinates, transect and sub-area to which the sampling unit belongs, distance along the coast from the southern first transect, and distance from the coastline). The seabed substrate types were classified into the following categories: soft bottoms (mud, sand and detritic), maerl (species of coralline algae growing loosely in beds of fragmented nodules), pebbles, rock, coralline rock, and *Posidonia oceanica* cover. Each video frame was assigned to only one seabed substrate type, and in video frames with the simultaneous presence of more than one substrate type, only the most abundant substrate type was recorded. Similarly, according to previous studies investigating Mediterranean assemblages (Weinberg 1979, Virgilio et al. 2006), the seabed slope in each video frame was classified as flat (0º-30º), sloping (30º-80º), or vertical (80º-90º). The slope was estimated by looking at the distance between the two parallel laser beams on the video screen and the depth sensor data.

Data treatment

The presence of gorgonians was quantified both by occupancy (frequency of occurrence in the set of sampling units) and by abundance (number of colonies per sampling unit). The spatial distribution of each species was studied by mapping the densities observed in each sampling unit in relation to its position on a geographically referenced map using Quantum GIS 0.11 software (Quantum GIS Development Team 2009). The position of each sampling unit was estimated from the recorded geographical coordinates of the initial and final points of each transect. Using Passage 2 software (Rosenberg 2008), the Gi* statistic was used to identify the locations of highest abundance (hot spots) (Getis and Ord 1996). This statistic provides a measure of the density variation within a fixed distance (1000 m) from each sampling unit in relation to the density magnitude within the entire study area. The bathymetrical distribution of each 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 spatial and bathymetrical distribution of young and adult colonies of *E. singularis* were plotted separately due to the large number of young colonies found for this species. Young colonies were considered as the smallest colonies that could be distinguished by the video analysis (2-5 cm in height); they are non-reproductive colonies (Ribes et al. 2007) that have no ramifications and are <10% of the maximum height of the species (Weinberg 1976; Linares et al. 2008b) (Fig. 2).

The relationships among gorgonian abundance and environmental and spatial variables were analyzed by redundancy analysis (RDA) (ter Braak 1994) using the ordination software Canoco 4.5 (ter Braak and Smilauer 2002). RDA assumes a linear model for the relationship between the response of each species and the ordination axes. The eigenvalue of an ordination axis in RDA is the proportion of the total variance explained by that axis and indicates its relative importance. Gorgonian densities, as quantitative data, were included as response variables; substrate type, seabed slope, and depth were included as environmental variables; geographical coordinates, transects, sub-areas, distance along the coast, and distance from the coastline were included as spatial variables. Forward selection of environmental and spatial variables was used to ascertain the minimum set of variables that best explained the species density. The statistical significance of the environmental and spatial variables in the RDA was determined using a Monte Carlo permutation test with 999 permutations. All of the multivari-

Fig. 2 - Young colonies of *Eunicella singularis* (2-5 cm in height); they are non-reproductive colonies, and are <10% of the maximum height of the species.

ate analyses were performed using log-transformed data, and the species were centered and standardized. Because spatial structure is a source of spurious correlations due to the spatial autocorrelation between variables (Legendre and Legendre 1998; Currie 2007), we partialled out the spatial component of the species matrix variation using partial redundancy analysis (Borcard et al. 1992; Peres-Neto et al. 2006). According to this method, four fractions of variance can be identified: (a) variance explained by the environmental variables independently of any spatial structure, (b) the spatial structure of the species data that is shared by environmental variables, (c) the spatial pattern of the species data that is not shared by environmental variables, and (d) the unexplained variance (Borcard et al. 1992; Peres-Neto et al. 2006). The amount of each fraction was calculated using the R-language function Varpart, which is available in the Vegan library (Oksanen et al. 2005) of the R software platform (R Development Core Team 2007). A species matrix was created by considering only the sampling units in which gorgonian species were present. For this matrix, two RDAs were generated that were respectively constrained by the sets of environmental and spatial variables to ascertain the minimum set of environmental and spatial variables that best explained the species density data. Subsequently, a partial RDA was generated for the species matrix, constrained by the previously selected environmental variables while controlling for the effects of the previously selected spatial variables. The

Table 1 - Gorgonian presence and spatial distribution in the study area. Occupancy (frequency of occurrence in the set of sampling units) is given for each sub-area and species; abundance (number of colonies) and maximum density of each species is given per each sub-area.

Sub-area	**Sampling units**			**Species**	**Sampling units with species**		**Colonies**		**Max Density**
	Number	with gorgonians	(%)		Number	(%)	Number	(%)	(colonies m^{-2})
A	411	20	(4.9)	*E. singularis*	14	(3.4)	109	(84.5)	20.5
				P. clavata	2	(0.5)	15	(11.6)	4.5
				L. sarmentosa	5	(1.2)	5	(3.9)	0.5
B	233	23	(9.9)	*E. singularis*	22	(9.4)	172	(99.4)	30.5
				P. clavata	0	(0.0)	0	(0.0)	0.0
				L. sarmentosa	1	(0.4)	1	(0.6)	0.5
C	332	68	(20.5)	*E. singularis*	53	(16.0)	206	(81.7)	15.0
				P. clavata	8	(2.4)	28	(11.1)	4.0
				L. sarmentosa	13	(3.9)	18	(7.1)	1.5
D	342	73	(21.3)	*E. singularis*	58	(17.0)	593	(86.6)	28.5
				P. clavata	11	(3.2)	67	(9.8)	10.0
				L. sarmentosa	20	(5.8)	25	(3.6)	1.5
E	403	128	(31.8)	*E. singularis*	120	(29.8)	1598	(81.4)	34.0
				P. clavata	39	(9.7)	360	(18.3)	17.5
				L. sarmentosa	5	(1.2)	5	(0.3)	0.5
F	236	54	(22.9)	*E. singularis*	51	(21.6)	794	(81.4)	30.0
				P. clavata	19	(8.1)	174	(17.8)	18.5
				L. sarmentosa	4	(1.7)	7	(0.7)	1.5
G	369	119	(32.2)	*E. singularis*	117	(31.7)	920	(99.7)	37.0
				P. clavata	0	(0.0)	0	(0.0)	0.0
				L. sarmentosa	3	(0.8)	3	(0.3)	0.5

results of the partial RDA were interpreted to identify the environmental factors that better explain the variation in the presence and abundance of the species after controlling for the effects of the spatial structure.

RESULTS

Occupancy and abundance

Gorgonians were recorded in 20.9% of the total number of 2326 sampling units, and a total of 5100 colonies were observed along all of the transects. *Eunicella singularis* was the most frequent and abundant species, representing 86.1% of the total gorgonian colonies observed and occurring in 18.7% of the sampling units. Young colonies of *E. singularis* accounted for 18.3% of the colonies of this species and were observed in 8.2% of the sampling units. *Paramuricea clavata* was the second most frequent and abundant species, and it accounted for 12.6% of the observed colonies and was present in 3.4% of the total sampling units. *Leptogorgia sarmentosa* represented 1.3% of the total observed colonies and occurred in 2.2% of the total sampling units. *E. singularis* showed the highest maximum density (37.0 colonies m^{-2}), which was twice the maximum density determined for *P. clavata* (18.5 colonies m^{-2}). *L. sarmentosa* showed lower and more constant density values (0.5-1.5 colonies m^{-2}) (Table 2).

Spatial and bathymetrical distribution

The north coast of the Cape of Creus showed the highest presence of gorgonians species with the highest densities (Fig. 3, Table 1). *E. singularis* was the only species that was present throughout the entire study area. This species showed

Table 2 - Maximum densities reported for the studied gorgonian species in other locations in Western Mediterranean Sea.

Species	Maximum density (colonies m^{-2})	Geographyc location	Author
Eunicella singularis	56.4	Cape of Creus	Linares et al. 2008a
	4.76	Montgrí	“
	41.91	Medes Islands	“
	47.55	Tarragona	“
	1.67	Valencia	“
	18.6	Gandia	“
	41.25	Cape of Palos	“
	> 50	Banyuls-sur-Mer	Weinberg 1976
	> 84	Banyuls-sur-Mer	Weinberg 1978b, 1979
Paramuricea clavata	52	Cape of Creus	Linares et al. 2008a
	52.5	Medes Islands	“
	30.82	Tamariu	“
	53.13	Columbretes Islands	“
	37.33	Cape of Palos	“
	59	Port-Cros (Ilot de Gabinière)	Linares et al. 2005
	38	Port-Cros (Montrémian)	“
	32	Medes Islands	Coma et al. 1994
	55.8	Medes Islands	Coma et al. 1995
	> 55	Banyuls-sur-Mer	Weinberg 1978b, 1979
	46	Banyuls-sur-Mer	Weinberg 1976
	33	Gulf of La Spezia (Tino Island)	Cupido et al. 2008
	52	Gulf of La Spezia (Tinetto Island)	“
	50	Gulf of La Spezia (Tinetto Shoal)	“
Leptogorgia sarmentosa	> 12	Banyuls-sur-Mer	Weinberg 1976
	17	Banyuls-sur-Mer	Weinberg 1978b, 1979

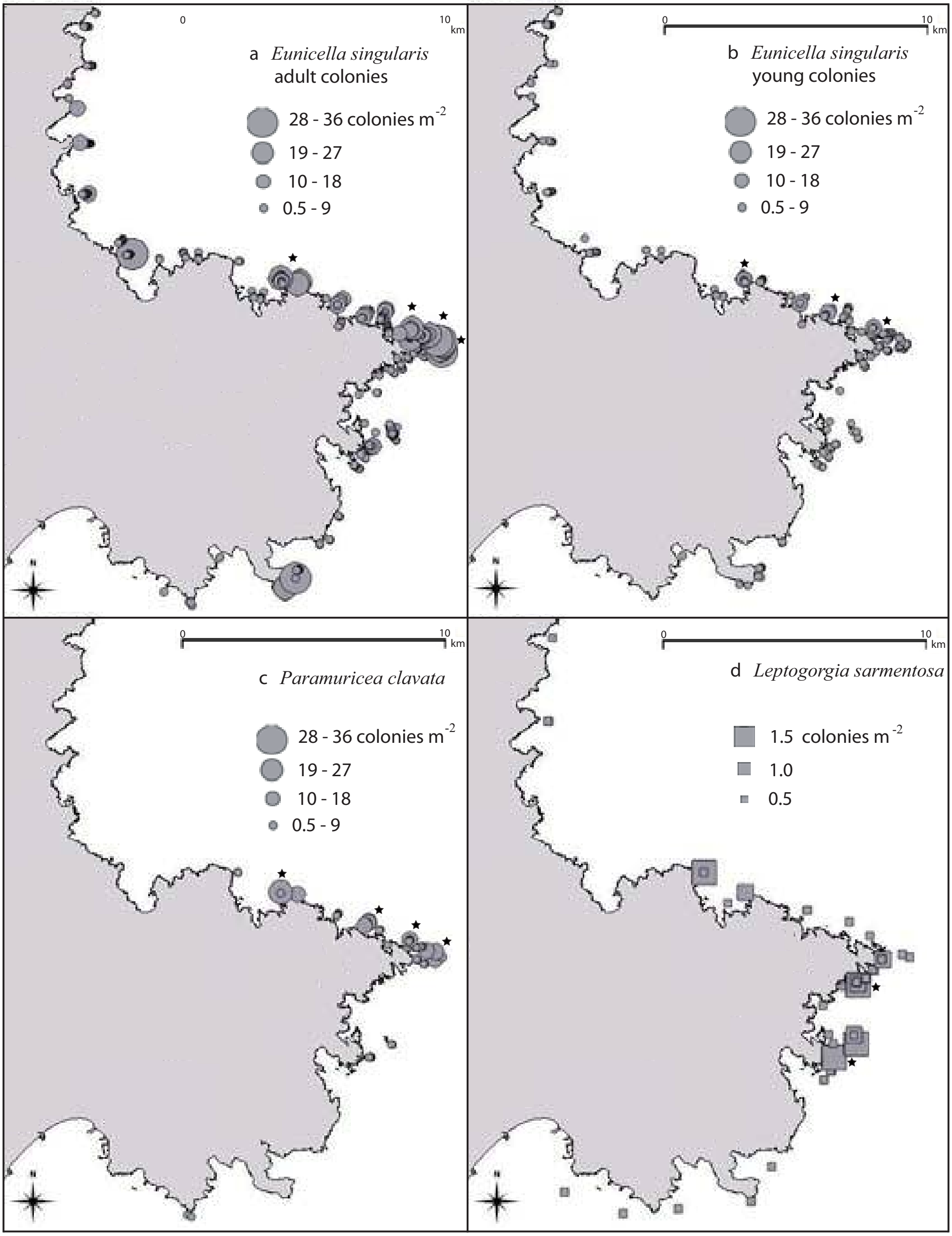

Fig. 3 - Spatial distribution of *Eunicella singularis* adult (a) and young (b) colonies, *Paramuricea clavata* (c), and *Leptogorgia sarmentosa* (d) in the study area. *Squares* are used instead of *bubbles* in Fig. 2d to highlight the lower magnitude of the density values. *Black stars* indicate hot spots of species abundance, which were identified as the sampling units that displayed the highest 10% of the Gi* values. The spatial distribution of each species can be displayed on Google Earth by means of Additional Resources 3 to 6.

the highest frequency of occurrence and the highest densities on the north side of the cape, and in a few locations on the south coast of the cape. Across all of the other sub-areas, even though *E. singularis* was present at lower densities, it remained the most abundant of the studied species (Fig. 3a,b, Table 1). Adult colonies of *E. singularis* appeared to be widely distributed (Fig. 3a, Additional Resource 3), whereas young colonies were more concentrated in areas with high densities of adult colonies (Fig. 3b, Additional Resource 4). The distribution of *P. clavata* was also concentrated on the north coast, where it displayed the highest densities, whereas this species was present in only a few locations along the south and east coasts (Fig. 3c, Additional Resource 5, Table 1). The distribution of this species appeared to be restricted, because it was present only in some contiguous locations. In contrast, *Leptogorgia sarmentosa* was the only species that was more common on the east side of the cape, where it showed the highest frequency of occurrence and abundance, although it was widely present along the entire coast as isolated colonies (Fig. 3d, Additional Resource 6, Table 1).

The bathymetrical distribution of *E. singularis* ranged from depths of 6 to 67 m (Figs. 4-5). *E. singularis* was frequent on the north face of the cape (sub-areas E and F) and showed high densities within all depth ranges, achieving a maximum depth of 67 m. In the northern part of the study area (sub-area G), this species was frequent at all depths, showing small median density values but very high maximum values. In the other sub-areas (A to D), *E. singularis* was frequent at depths between 15 and 25 m, but on the east coast of the cape (sub-areas C and D) the species displayed high median and maximum density values below a depth of 40 m (Fig. 4). The occurrence of young colonies of *E. singularis* was very variable among the sub-areas, but in sub-areas E, F, and G they were more frequent and presented a distribution in the same depth range in which the adult colonies presented high density values (Fig. 5). The bathymetrical distribution of *P. clavata* ranged from a depth of 15 to 62 m but was mainly restricted to depths from 20 to 50 m, where the species showed the highest density and frequency of occurrence in sub-areas D, E and F (Fig. 6). In sub-area F, the deepest depth of this species was observed, with medium densities and frequencies of occurrence. *L. sarmentosa* was documented at depths from 11 to 61 m, and it consistently showed low densities and frequencies of occurrence. In sub-areas C and D, this species was slightly more frequent, and the highest density values were observed; only a few isolated colonies were found in the other sub-areas (Fig. 7).

Hot spots (identified as the sampling units that displayed the highest 10% of the Gi* values) of *E. singularis* and *P. clavata* were concentrated on the north side of the cape (Fig. 3), which represented the area with the highest values of abundance within the entire study area. The bathymetrical distribution of the hot spots showed that the highest abundances of *E. singularis* were concentrated between depths of 32.5 and 47 m (first and third quartile) with a median at a depth of 39 m for adult colonies and between 31 and 55.7 m (first and third quartile) with a median at 40.2 m for young colonies. For *P. clavata*, the bathymetrical distribution of the hot spots ranged between depths of 26.1 and 40.1 m (first and third quartile) with a median at 33 m. In contrast, hot spots of *L. sarmentosa* were identified on the east side of the cape (Fig. 3) and in shallower waters between a depth of 16 and 31.5 m (first and third quartile) with a median at 25 m.

Relationship with environmental variables

The variation of the species matrix resulted partitioned as follows: (a) non-spatial environmental variation, 10.0%; (b) spatially structured environmental variation, 11.1%; (c) spatial species variation that is not shared by environmental variables, 19.2%; and (d) unexplained variation and stochastic fluctuations, 59.7%. Environmental variables explained 21.1% of the variance in the species matrix, but 50.7% of this amount could also be predicted by the spatial structure, which showed that the species and

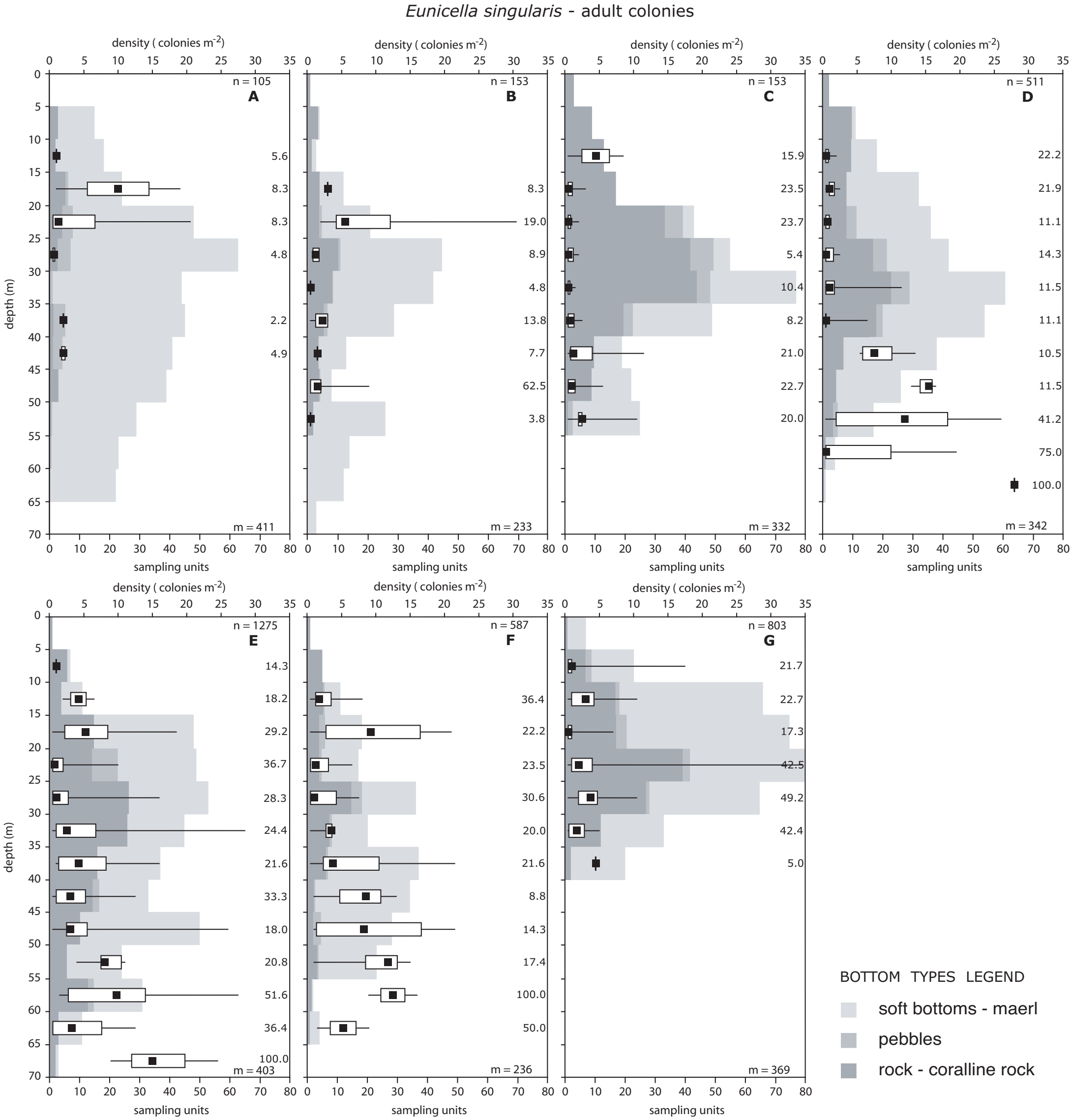

Fig. 4 - *Eunicella singularis* - Bathymetrical distribution of the density of adult colonies in each sub-area (A-G): the *black square* indicates the median value; the *box* indicates the first and third quartiles; and the *line* indicates the range between minimum and maximum values. *Grey-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* on the right indicate the percentage of sampling units with presence of the species. Total number of colonies (*n*) and sampling units (*m*) are indicated for each sub-area.

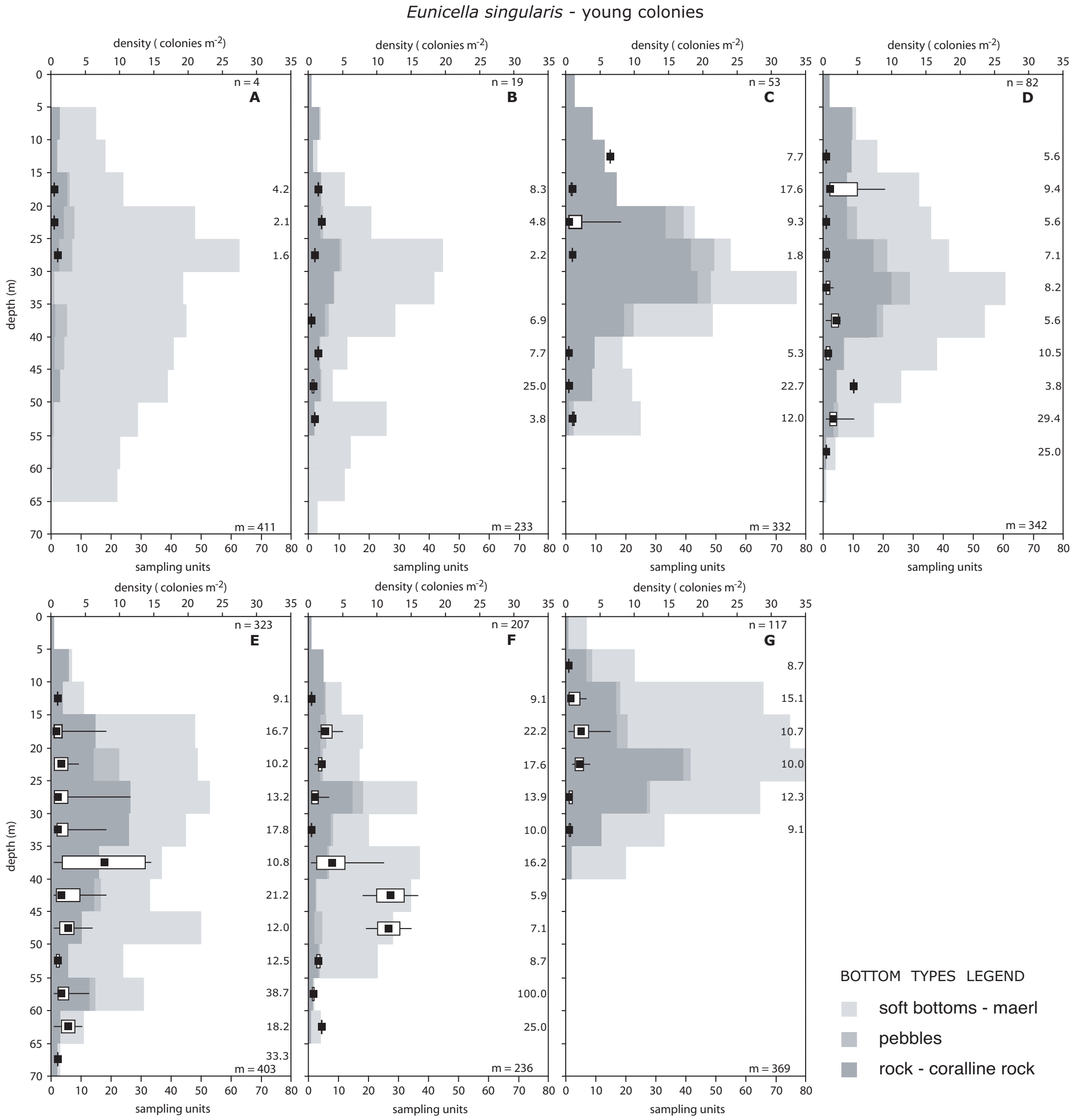

Fig. 5 - *Eunicella singularis* - Bathymetrical distribution of the density of young colonies in each sub-area (A-G): the *black square* indicates the median value; the *box* indicates the first and third quartiles; and the *line* indicates the range between minimum and maximum values. *Grey-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* on the right indicate the percentage of sampling units with presence of the species. Total number of colonies (*n*) and sampling units (*m*) are indicated for each sub-area.

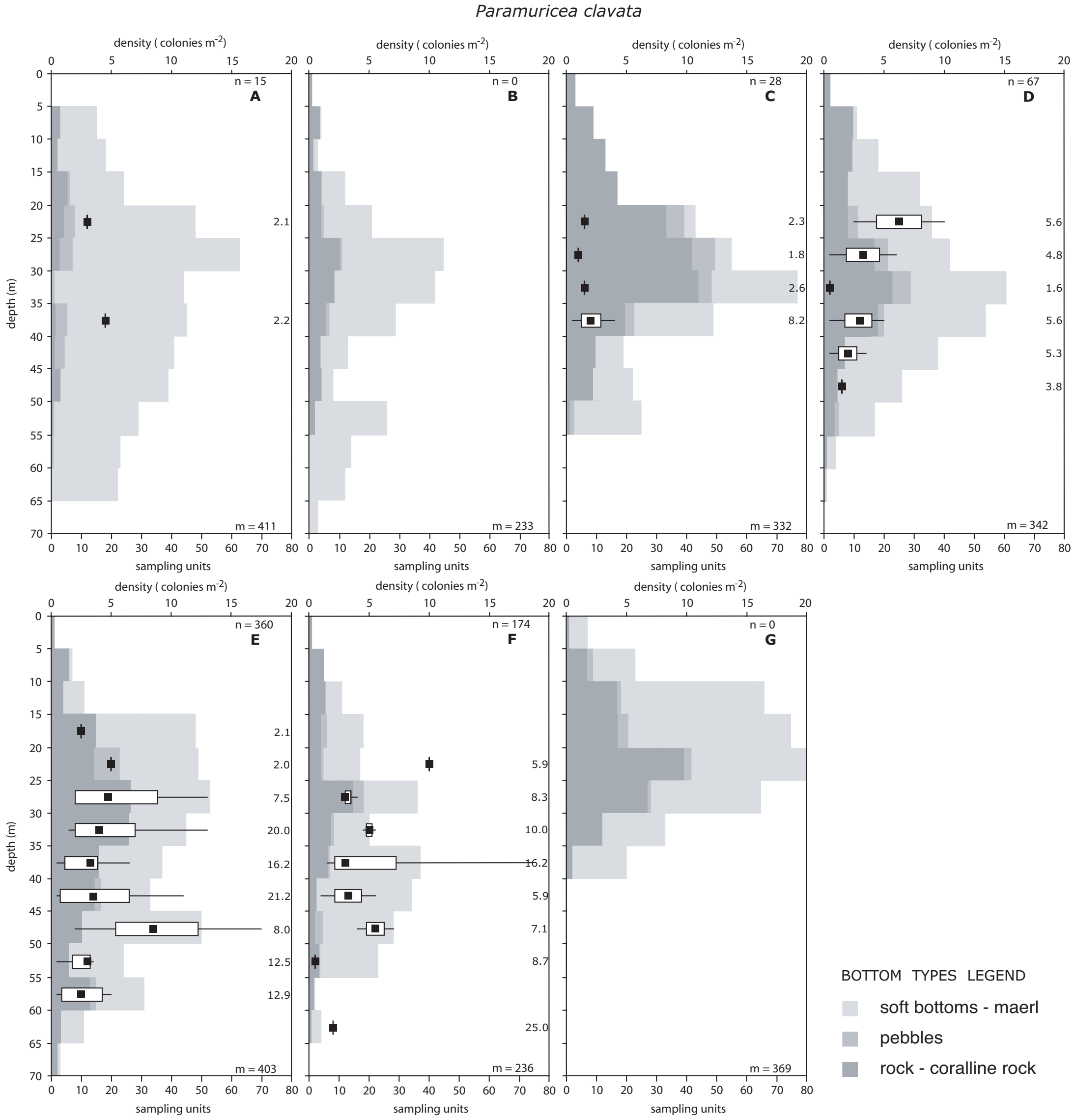

Fig. 6 - *Paramuricea clavata* - Bathymetrical distribution of the density of colonies in each sub-area (A-G): the *black square* indicates the median value; the *box* indicates the first and third quartiles; and the *line* indicates the range between minimum and maximum values. *Grey-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* on the right indicate the percentage of sampling units with presence of the species. Total number of colonies (*n*) and sampling units (*m*) are indicated for each sub-area.

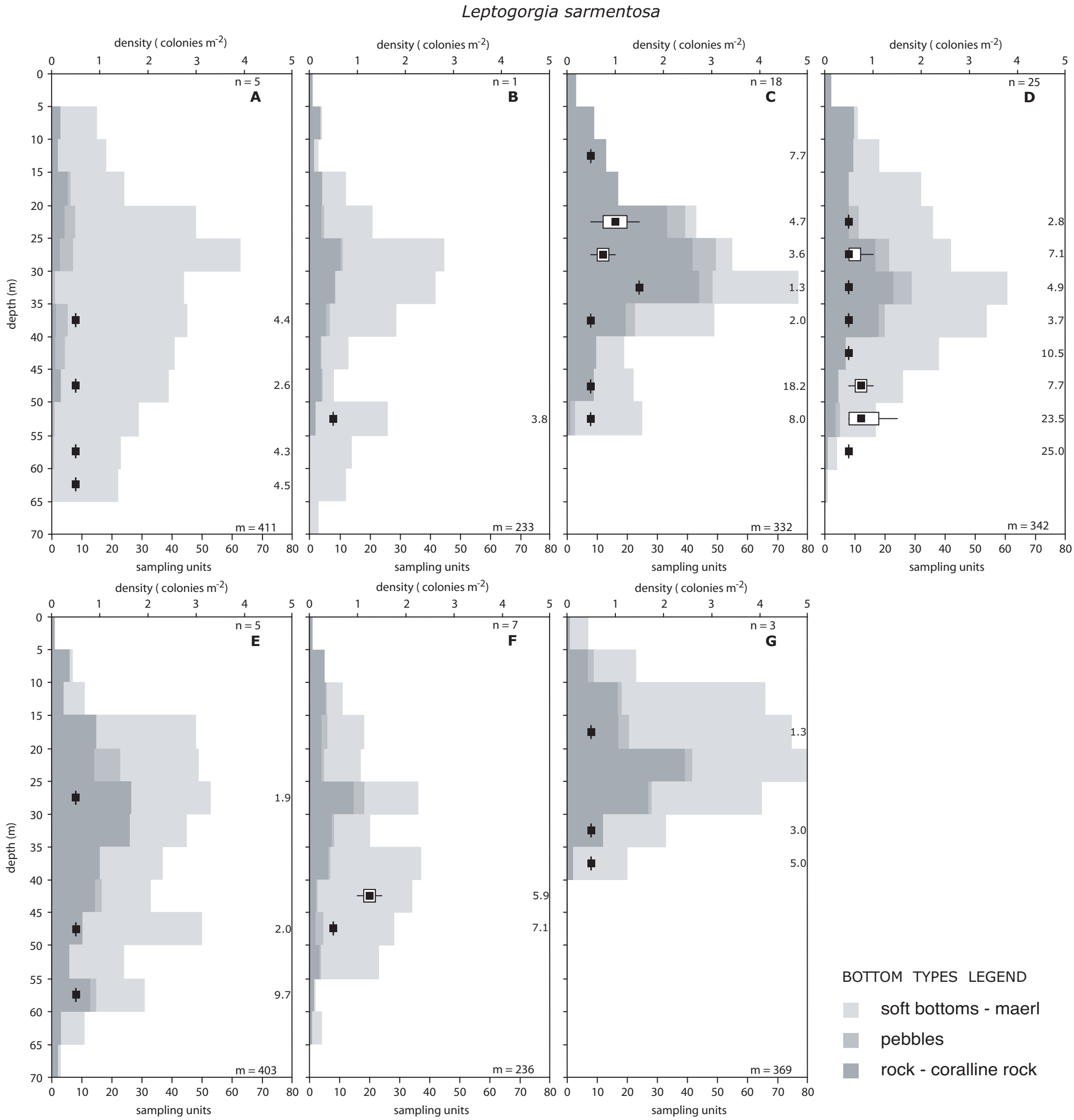

Fig. 7 - *Leptogorgia sarmentosa* - Bathymetrical distribution of the density of colonies in each sub-area (A-G): the *black square* indicates the median value; the *box* indicates the first and third quartiles; and the *line* indicates the range between minimum and maximum values. *Grey-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* on the right indicate the percentage of sampling units with presence of the species. Total number of colonies (*n*) and sampling units (*m*) are indicated for each sub-area.

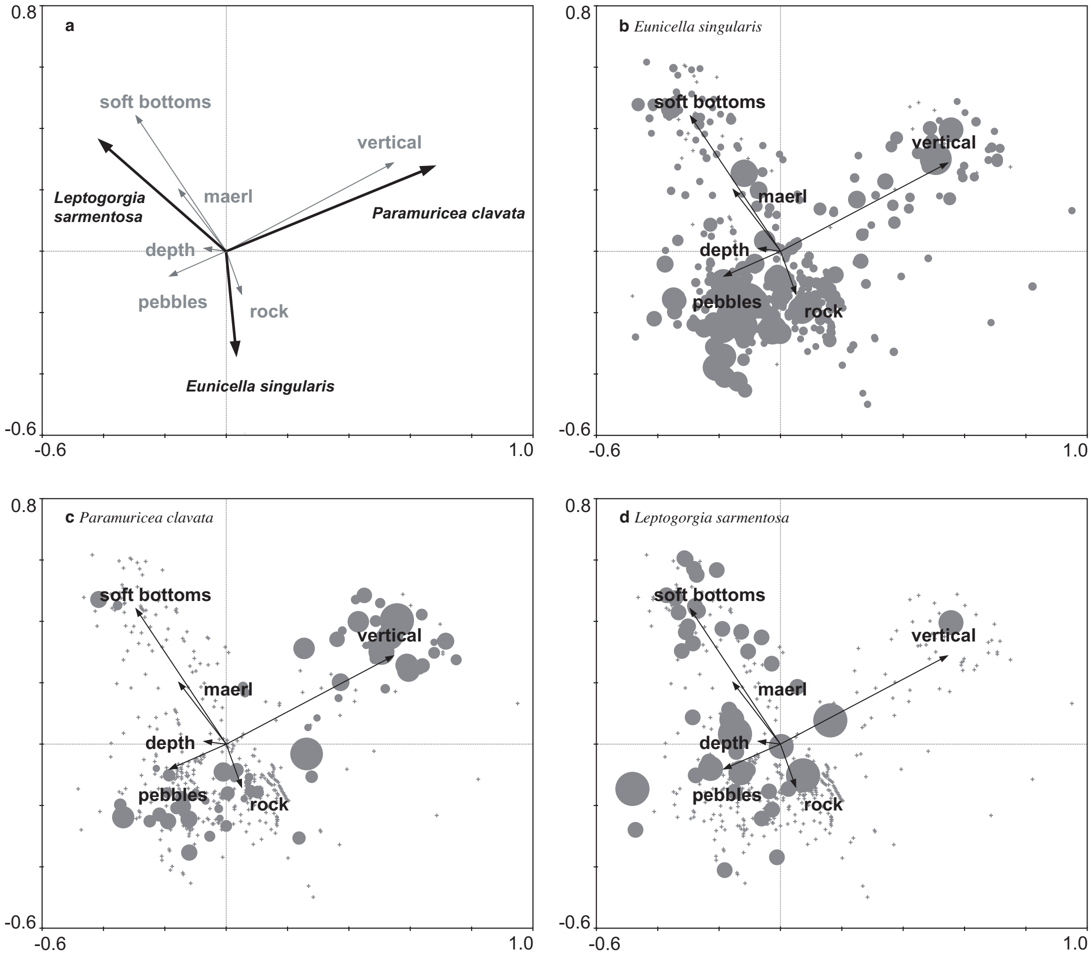

Fig. 8 - Partial redundancy analysis (RDA): (a) biplot showing the ordination of gorgonian species and the roles of the significant environmental variables; (b, c, d) attribute plots of the abundance of each species in the two-dimensional space determined by the partial RDA. *Crosses* represent empty sampling units, whereas in those units in which the species is present, the *circle diameter* is proportional to the species abundance from the minimum up to the maximum value (*Eunicella singularis*: 0.5 - 37 colonies m^{-2}; *Paramuricea clavata*: 0.5 - 18.5 colonies m^{-2}; *Leptogorgia sarmentosa*: 0.5 - 1.5 colonies m^{-2}).

environmental data had a fairly similar spatial structure. The variance explained by the spatial variables that could not be related to the measured environmental variables (fraction c) could be due to unmeasured environmental variables or to biotic processes. The unexplained variance (fraction d) was probably due to some overlooked factors (e.g., historical factors, food availability), to an incomplete description of the spatial structure or to a large amount of random variation (Borcard et al. 1992). The first two axes of the partial RDA controlled for the spatial structure, explained 12.8% of the species data variance; the first axis explained 6.8%. Six environmental variables were statistically significant according to the Monte Carlo permutation test ($p<0.05$) (Fig. 8). The seabed substrate types clearly divided *L. sarmentosa*, which appeared to be strongly associated with soft bottoms and maerl (Fig. 8a, d), from *E. singularis*, which was associated with a rocky substrate (Fig. 8a, b). The slope of the seabed was strongly significant in determining the presence and abundance of *P. clavata* (Fig. 8a, c).

DISCUSSION

The occurrence and abundance of gorgonians in the study area are consistent with previous data from other locations within the Western Mediterranean Sea (Table 2). Although differences in the applied methodology posed difficulties in making comparisons among studies, the density values documented for the Mediterranean species (Table 2) are comparable to those reported for tropical (Kinzie 1973; Lasker and Coffroth 1983; Yoshioka and Yoshioka 1989a; Chiappone et al. 2003) and temperate species (Grigg 1975, 1977). Conversely, research conducted in deep-sea (Mortensen et al. 1995; Mortensen and Buhl-Mortensen 2004) and polar areas (Orejas et al. 2002) demonstrated a much lower occurrence and abundance of gorgonians in those regions. In contrast with the high diversity of species in tropical gorgonian communities (Lasker and Coffroth 1983; Yoshioka and Yoshioka 1989b; Chiappone et al. 2003), the Mediterranean gorgonian assemblages are mostly monospecific, and the coexistence of multiple species has been observed only sporadically (*Eunicella singularis* and *Paramuricea clavata* on rocky walls, and deep horizontal rocky bottoms (Fig. 9a, b); *Leptogorgia sarmentosa* and *E. singularis* on shallow rocky bottoms (Fig. 9c)). Indeed, the three gorgonian species exhibit different spatial distribution patterns. *E. singularis* is widely distributed throughout the studied area as well as in other locations in the Western Mediterranean Sea (Weinberg 1979, 1980; Linares et al. 2008b), where it can be considered the most common and abundant gorgonian species (Table 2). Its distribution is mainly driven by the requirement for hard bottom substrates without a slope angle preference, and high densities of *E. singularis* are also observed on vertical walls independently of the

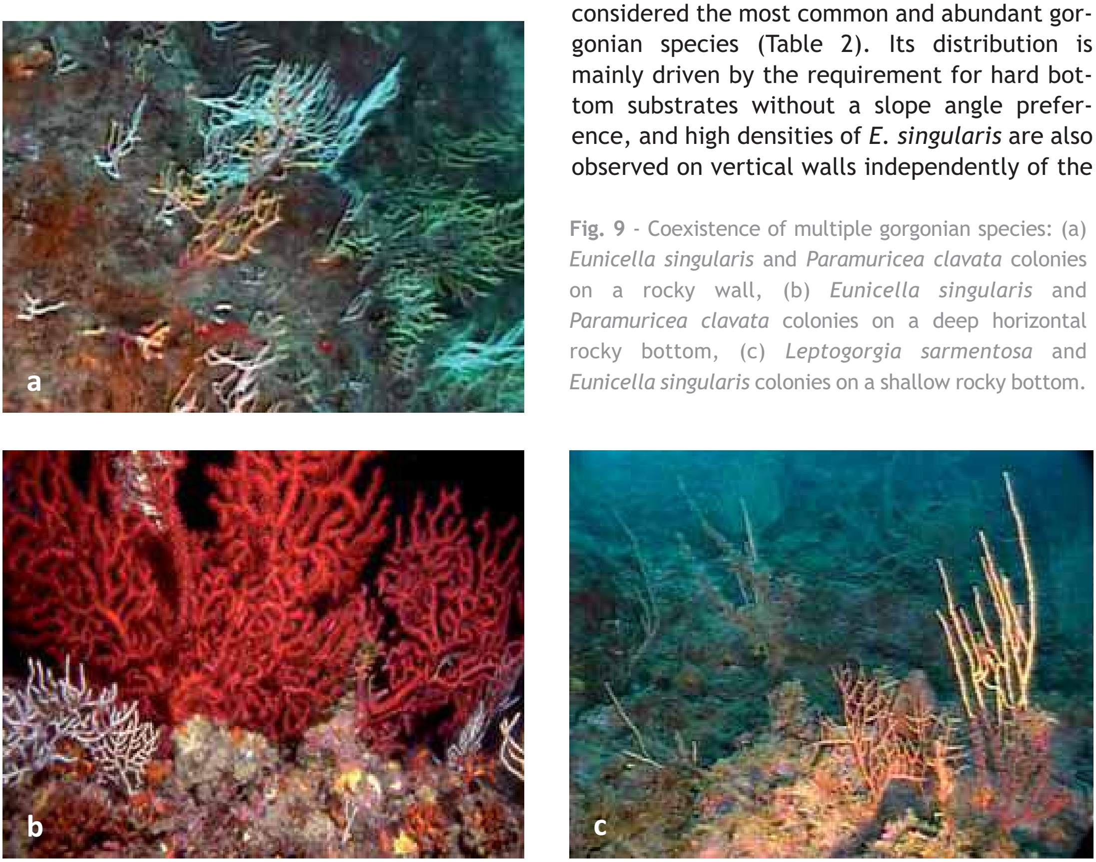

Fig. 9 - Coexistence of multiple gorgonian species: (a) *Eunicella singularis* and *Paramuricea clavata* colonies on a rocky wall, (b) *Eunicella singularis* and *Paramuricea clavata* colonies on a deep horizontal rocky bottom, (c) *Leptogorgia sarmentosa* and *Eunicella singularis* colonies on a shallow rocky bottom.

light exposure. Conversely, *P. clavata* presented a strongly patchy distribution that was mainly associated with vertical rocky walls (Fig. 8) (Carpine and Grasshoff 1975; Weinberg 1976; Linares et al. 2008b), and this strong association could be the main explanation for the observed confinement of populations of this species to a few sites (Fig. 3). According to Linares et al. (2008b), the extensive distribution of *E. singularis* might be related to its tolerance to a wide range of environmental conditions, in contrast to the contagious distribution of *P. clavata*, which seems to be highly dependent on the vertical slope of the rocky bottom and on low light conditions. The lower maximum densities reported in this study in comparison to other locations (Table 2) might reflect a constraint of the video technique employed, which allows an extended sampling coverage but loses accuracy in measuring density when compared to other methods. However, the observed range of variability in the density of both species in the Cape of Creus area is within the same order of magnitude as the variability previously reported for different locations in the Western Mediterranean Sea (Tables 1, 2) (Coma et al. 2006; Linares et al. 2005, 2008b; Cupido et al. 2008), which suggests that this is the natural range of the density of these species over a large extent. The scattered distribution of *L. sarmentosa*, with few colonies that were mainly dispersed on soft bottoms and maerl, is consistent with previous observations of this species (Carpine and Grasshoff 1975; Weinberg 1976; Gili et al. 1989; Mistri and Ceccherelli 1993; Rossi and Gili 2009). However, the low-density values determined for the Cape of Creus are different from those of other locations in which dense populations (up to 17 colonies m^{-2}) have been recorded (Weinberg 1979; Mistri 1995).

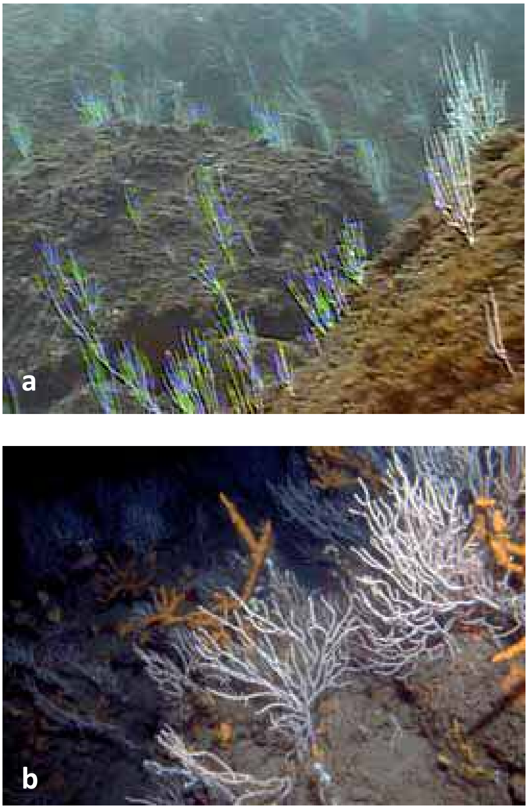

Fig. 10 - *Eunicella singularis* colonies in (a) shallow, and (b) deep waters.

The highest occurrences and abundances of *E. singularis* and *P. clavata* were concentrated on the northern coast of the Cape of Creus in the area directly exposed to the main near-bottom currents, wind and wave action (Millot 1990; DeGeest et al. 2008; Ulses et al. 2008; Rubio et al. 2009). In contrast, hot spots of *L. sarmentosa* were located along the more sheltered east side of the cape, where there is a turbulent circulation but no strong near-bottom currents (DeGeest et al. 2008; Ulses et al. 2008). Hydrodynamic patterns are a key factor in understanding the distribution of benthic suspension feeders (Gili and Ballesteros 1991; Wildish and Kristmanson 1997), and higher densities are often found in areas exposed to higher hydrodynamism and particulate organic matter concentrations (Cocito et al. 1997). The influence of topography on current patterns, and consequently on the food supply, is thought to determine the distribution of gorgonians and cold-water corals on both large and small scales (Cocito et al. 1997; Wildish and Kristmanson 1997; Mortensen and Buhl-Mortensen 2004). The diet of *P. clavata* consists mainly of microzooplankton (Coma et al. 1994; Ribes et al. 1999), and the preference for a moderate-high current regime and only moderate resuspension could explain the distribution of this species on vertical rocky walls. Shallow colonies of *E. singularis* host zooxantellae, and consequently, their distribution might be mainly related to light exposure; in contrast, data regarding the diet of deep sublittoral *E. singularis* colonies is lacking. The distribution of *L. sarmetosa* on soft bottoms might be related to a higher degree of resuspension (Rossi et al. 2003), which correlates with the increased importance of resuspended particles in the diet of this species (Ribes et al. 2003; Rossi et al. 2004).

The amount of variation accounted for by space alone shows that the substrate type, seabed slope, and depth were insufficient to completely explain the observed spatial structure of gorgonian abundance. This strictly spatial variation may be due to some overlooked environmental factors, or it may reflect some contagious biological process (Borcard et al. 1992). A short dispersal range of the larvae and settlement near the parental colony has been observed in *E. singularis* (Théodor 1967; Weinberg and Weinberg 1979) and *P. clavata* (Coma et al. 1995; Linares et al. 2007, 2008c), and the effect of variability in the dispersion capability of larvae on the resulting spatial structure has been reported for tropical gorgonians and corals (Carlon and Olson 1993; Jordán-Dahlgren 2002; Baird et al. 2003). Many young colonies of *E. singularis* were observed along the Cape of Creus, whereas populations of *P. clavata* and *L. sarmentosa* consisted almost exclusively of adult colonies. These results agree with the extremely rare recruitment reported for *P. clavata* (Linares et al. 2008b) and *L. sarmentosa* (Rossi and Gili 2009) and the very low survival of new *P. clavata* settlers (Linares et al. 2008c). The observed large number of young *E. singularis* colonies seems to indicate a high survival of its recruits (Linares et al. 2008b) and continuous recruitment over time (Ribes et al. 2007; Linares et al. 2008b). Conversely, the distribution patterns of *P. clavata* and *L. sarmentosa* seem to be more dependent on periodic occurrences of successful recruitments.

Previous knowledge on the bathymetrical distribution of *E. singularis* (depths of 7 to 54 m) and *P. clavata* (5 to 100 m depth) was based mainly on irregular observations (Laborel et al. 1961; Carpine and Grasshoff 1975; Weinberg 1976), but to date, there is still very little quantitative information on the bathymetrical distribution patterns of these species (Bo et al. 2009). Our results demonstrated a high abundance of *P. clavata* at depths from 25 to 50 m (Fig. 6) and high densities of *E. singularis* from 15 to 70 m (Figs. 4, 5). *E. singularis* colonies that were present at depths shallower than 30-35 m displayed the common candlestick-like colony morphology, with a few long and straight branches that run parallel to one another and exhibit a dirty grayish-white color (Weinberg 1976) (Fig. 10a). Conversely, colonies present at depths deeper than 30-35 m displayed a more variable colony morphology and bright white color (Théodor 1969; Weinberg 1976), and they should be considered as *E. singularis aphyta*, as previously described by Théodor (1969) (Fig. 10b). Further studies are needed to elucidate the classification of these colonies as a subspecies or variety (Théodor 1969; Carpine and Grasshoff 1975, Weinberg 1976). The observation of high-density populations of *P. clavata* and *E. singularis* at locations below 40 m depth, highlight as the research on Mediterranean gorgonians to date has focused only on a fraction of the species populations (the shallower ones). To achieve a complete understanding of the ecology of the main Mediterranean gorgonians species, it will now be important to explore the ecological characteristics of the deep sublittoral gorgonian populations, the possible connectivity with shallow populations, and the properties of these spatially structured population systems (Thomas and Kunin 1999). New studies are necessary to explore whether these deep sublittoral populations are exposed to more stable environmental conditions and whether these populations might play a role in the re-colonization of the shallower areas in which gorgonians are exposed more frequently to less stable conditions and to frequent perturbations. This aspect appears to be extremely important in light of the episodes of mass mortality that have affected the shallow populations of gorgonians in the Western Mediterranean (e.g., Cerrano et al. 2000; Perez et al. 2000; Linares et al. 2005; Cupido et al. 2008; Garrabou et al. 2009) and in consideration of the sporadic mortality events caused by perturbations, such as the extremely strong storm that severely affected many shallow populations of *E. singularis* and *P. clavata* along the Catalan coast in December 2008 (Linares, personal communication). The reported deep sublittoral populations of *E. singularis* and *P. clavata* demonstrate the importance of studying the distribution of benthic species over a great spatial and bathymetrical range and highlight how ROV studies can provide information that is complementary to that obtained by scuba divers.

COMMENTS

In the published version of this work, Figures 2, 9 and 10 are not included. Consequently, Figures 3, 4, 5, 6, 7 and 8 of the present work correspond respectively to Figures 2, 3, 4, 5, 6 and 7 in the published version. Pictures in Figures 2, 9 and 10 were made by Sergio Rossi Heras and Julian Gutt.

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Chapter 2

Morphological variation across a depth gradient: rediscovery and characterization of the two morphotypes of the Mediterranean gorgonian *Eunicella singularis*

Marine Biology (submitted)

RESUM

En els darrers 40 anys els estudis científics sobre les espècies de gorgònies del sublitoral ha estat centrat, principalment, en la franja batimètrica de fins a 40 metres. El recent desenvolupament de la tecnologia aplicada a l'exploració submarina ha permes l'extensió de l'observació científica més enllà dels 40 m de profunditat. A la Mediterrània, aquesta nova franja d'exploració ha permes registrar la presència de denses poblacions d'*Eunicella singularis* (Esper, 1791) entre el 10 i 70 m de profunditat. Aquesta és l'única espècie de gorgònia del Mediterrani que té algues simbiòtiques. Una subespècie d'*E. singularis* sense algues simbiòtiques (*E. aphyta singularis*), va ser descrita el 1969, però ja que aquesta forma aposymbiontica és rara en la franja de 0-40 m de profunditat, cap estudi es va centrar en ella fins avui. En el present estudi, la combinació de mostrejos fets bussejant i d'observacions fetes amb un vehicle de control remot, ha permès un examen detallat de les colònies d'*E. singularis* situades entre 20 i 60 m de profunditat. L'anàlisi de la forma de la colònia, la variabilitat de les espícules, els marcadors genètics i la presència d'algues simbiòtiques, suggereix l'existència de dos morfotipus diferents: un "morfotipus superficial" (colònies situades entre 20 i 30 m de profunditat) amb algues simbiòtiques que corresponen a la comunament coneguda *E. singularis*, i un "morfotipus profund" (colònies situades entre 40 i 60 m de profunditat) amb colònies més ramificades i sense algues simbiòtiques que corresponen a la varietat aphyta.

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ABSTRACT

In the last 40 years, scientific studies on sublittoral gorgonians species mainly focused on the bathymetric range up to 40 meters. The recent development of technology applied to underwater exploration allowed the extension of the scientific observation below 40 m depth. In the Mediterranean Sea this new range of exploration allowed to record the presence of dense populations of *Eunicella singularis* (Esper, 1791), the only Mediterranean species hosting symbiotic algae, between 10 and 70 m depth. A subspecies without symbiotic algae (*E. singularis aphyta*) was described in 1969; however, since this aposymbiontic form is rare in the 0-40 m depth range, no study focused on it until today. In the present study the combination of scuba sampling to remotely operated observations allowed for a detailed examination of *E. singularis* colonies located from 20 to 60 m depth. The analysis of colony's shape, sclerite variability, genetic markers and presence of symbiotic algae, suggested the existence of two different morphotypes: a "shallow morphotype" (20 and 30 m depth colonies) with symbiotic algae that correspond to the commonly known *E. singularis*, and a "deep morphotype" (40 to 60 m depth colonies) with more ramified colonies and without symbiotic algae that correspond to the aphyta variety.

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INTRODUCTION

Morphological variation is ubiquitous within coral species, creating significant challenges for the advance of taxonomic, evolutionary, and ecological studies (Vermeij et al. 2007). Variations in growth form differ from genus to genus; while individuals of the same species may display variation in growth forms correlated with environmental features such as light or the intensity of water movement (Lewis and Von Wallis 1991), others species are more conservative in their general shape, even under varying regimes of current speed and direction (Weinbauer and Velimirov 1998; Rodríguez-Lanetty et al. 2003). Actually, morphological plasticity has been shown in many gorgonian (Brazeau and Lasker 1988; West et al. 1993; Sánchez et al. 2007) and coral species (Muko et al. 2000; Todd et al. 2004; Einbinder et al. 2009), with morphology changes along the depth gradient reflecting the changes in hydrodinamic and light regimes (Sebens 1987).

Among the aproximately 20 gorgonian species which inhabit the Mediterranean Sea, four belong to the genus *Eunicella* (Bérenguier 1954; Rossi 1959; Carpine and Grasshoff 1975; Weinberg 1976). This is a genus of sea fans belonging to the family Gorgoniidae which currently includes about 35 species mainly distributed in shallow waters along the Eastern Atlantic and Mediterranean Sea, as well as a few deep-sea Atlantic and Pacific species (Grasshoff 1992). Both *Eunicella filiformis* (Studer, 1878) and *Eunicella verrucosa* (Pallas, 1766) have a main Atlantic distribution, being reported in the Mediterranean Sea with rare and patchy populations (*E. verrucosa*), or being only present in the Straits of Gibraltar area and in the Alboran Sea (*E. filiformis*) (Stiasny 1938; Rossi 1959; Carpine and Grasshoff 1975; Grasshoff 1992). Conversely, *Eunicella cavolinii* (Koch, 1887) and *Eunicella singularis* (Esper, 1791) are very common species in the Western Mediterranean Sea (Rossi 1959; Carpine and Grasshoff 1975), where they represent one of the main structural species of the rocky sublittoral bottoms characterized by the coralligenous and precoralligenous communities (Gili and Ros 1985; Harmelin 1995; Ballesteros 2006). *E. cavolinii* is only common in the eastern part of the Western Mediterranean basin and in the Adriatic Sea, whereas it is absent or very rare along the coasts located west of Marseille (Carpine and Grasshoff 1975; Weinberg 1976). Colonies are ramified on a plan with many short primary branches and their color ranges from faint yellow to orange (Rossi 1959; Weinberg 1976). In the coastal zone this species dwells mainly on vertical rocky walls, while on coralligenous formations it can be found as deep as 150 m (Rossi 1959; Carpine and Grasshoff 1975). *E. singularis* is the only Mediterranean gorgonian species hosting symbiotic algae; it is a very common species in the Western Mediterranean and in the Adriatic Sea, while it is rare in the Eastern Mediterranean (Rossi 1959; Carpine and Grasshoff 1975; Grasshoff 1992). Colonies have long primary branches arranged in parallel to each other, and very scant ramifications (Rossi 1959; Weinberg 1976). This species can be commonly found on rocky bottoms in shallow waters (Carpine and Grasshoff 1975; Weinberg 1976), as well as on coralligenous formations in deeper sublittoral bottoms (Rossi 1959; Gori et al. 2011). In 1969 a subspecies of *E. singularis* without symbiotic algae (*E. singularis aphyta*) was described (Théodor 1969). However, while some authors recognize the existence of this subspecies (Weinberg 1976), other authors do not agree with this differentiation into subspecies due to the coexistence of the two forms, preferring a differentiation into two varieties (Carpine and Grasshoff 1975). Since this aposymbiontic form of *E. singularis* is quite rare in the depth range where the investigation on Mediterranean gorgonians concentrated for the last 40 years (0-40 m depth), the argument about the existence and classification of this form lost interest. Knowledge on the distribution and ecology of shallow *E. singularis* populations increased considerably in the last decades (e.g. Weinberg and Weinberg 1979; Ribes et al. 2007; Linares et al. 2008; Gori et al. 2007, 2011), nevertheless there has been a significant lack of studies focusing on

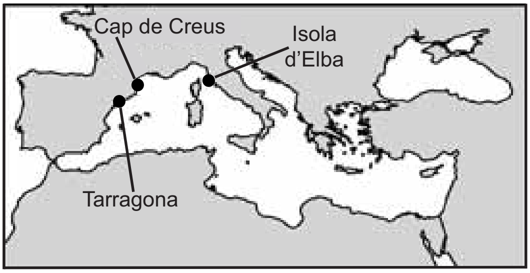

Fig. 1 - Map of the sampling locations.

nearshore rocky bottoms in the 40-150 m deep range (Virgilio et al. 2006; Sink et al. 2006), and there is currently no information available on the deep sublittoral colonies attributable to the *E. singularis* aposymbiontic form (Théodor 1969). The recent development of technology applied to underwater exploration facilitated the scientific observation of sea bottoms located below 40 m depth, allowing new studies focused on the benthic communities of the deep sublittoral rocky bottoms. In the study of tropical corals and gorgonian species the attention of scientists to the populations located in the mesophotic zone, defined as the deeper part of the photic zone in which light-dependent coral communities develop (approximatelly 40-150 m depth), has recently increased (Ginsburg 2007; Hinderstein et al. 2010). Mesophotic coral ecosystems harbor species found in their shallower counterparts, but they may also present a number of depth-restricted species (Hinderstein et al. 2010). These studies are revealing extensive, productive and rich communities, which significantly differ from their shallow-water counterparts (Kahng et al. 2010), and processes occurring in the mesophotic zone might have a global relevance in the ecology of tropical and subtropical coral regions (Buesseler et al. 2007; Hinderstein et al. 2010). In the Mediterranean Sea, as a consequence of such an extension in the scientific observation of deep sublittoral rocky bottoms, dense populations of gorgonian have been reported between 60 and 100 m depth (Bo et al. 2009, 2011; Cerrano et al. 2010), and *E. singularis* have been observed to develop very dense populations from shallow waters down to 70 m depth (Gori et al. 2011). Morphology of *E. singularis* colonies were observed to vary with depth, showing the common candlestick-like colony morphology and a dirty greyish-white colour (Weinberg 1976) in colonies located from the surface down to 30-35 m depth, while a more variable morphology and bright white colour were observed in colonies located from 30-35 m to 70 m depth (Gori et al. 2011). The later were considered to belong to the aposymbiontic form of *E. singularis* described by Théodor (1969), rising again the interest on the argument on the existence of the aposymbiontic form.

Aiming to shed light on this topic, we examined the variation with depth of *E. singularis* colonies dwelling in the bathymetric range from 20 to 60 meters, and compared them to the other Mediterranean species *E. cavolinii* and *E. verrucosa*. In order to quantify differences, we examined the variation of the following gorgonian descriptors: (1) colony shape, (2) sclerite size and shape, (3) differences in genetic markers, and (4) presence of symbiotic algae in the tissue.

MATERIALS AND METHODS

Colony shape

Pictures of *Eunicella singularis* colonies for the shape analysis were obtained in the north and east side of the Cap de Creus (42°18'49'' N; 003°19'23'' E), in the northwestern Mediterranean Sea (Fig. 1). Pictures of colonies from 20 m, 30 m, 40 m, 50 m and 60 m depth were obtained in November 2004 with the ROV Phanthom XTL equipped with a SONY FCB S3000P 3CCD (700 horizontal lines) camera, and two parallel laser beams providing a scale for the images. In each picture the colonies were perpendicular to the camera, and the laser beams were in the same plane as the colony. A total of 100 pictures of *E. singularis* colonies (20 for each depth) were selected for the analysis. Pictures of *Eunicella cavolinii* colonies (20-25 m depth) were obtained in March 2009 in the north coast of the Isola d'Elba (42°49'18'' N; 010º09'52'' E), in the northern Tyrrhenian Sea

(Fig. 1) by scuba diving with a CANON G10 digital camera enclosed in a IKELITE underwater housing and equipped with a IKELITE DS 51 underwater flash. A ruler placed near each colony provided a scale for the pictures. A total of 20 pictures of *E. cavolinii* colonies were selected for the analysis. Finally, pictures of *Eunicella verrucosa* colonies (15-25 m depth) were obtained in April 2010 in Tarragona (41º06'07'' N; 001º15'12'' E) (Fig. 1) also by scuba diving and with the same equipment used for the Isola d'Elba pictures. A total of 20 pictures of *E. verrucosa* colonies were selected for the analysis. All the selected pictures were analysed with Carnoy (Schols and Smets 2001) and ImageJ (Abramoff et al. 2004) softwares, calibrating each image using the laser beams or the ruler as reference.

For each picture, the gorgonian colony's maximum height and maximum width were measured; mean width was calculated as the mean of three measurements taken at equidistant positions and perpendicular to the height; the fan surface area was measured as described by Weinbauer and Velimirov (1995); the number of ramifications, and the number of branches of each order were counted according to Brazeau and Lasker (1988); the length of all the primary branches was measured. For each colony the following shape features were calculated: height to width ratio, height to mean width ratio, ramification density (number of ramifications/fan surface area), primary branches density (number of primary branches/fan surface area), order of the colony's base, the primary branches' maximum length, the primary branches' mean length, the bifurcation ratio ($R_b = \frac{1}{m}\sum_{i=1}^{m}\frac{n_i}{n_i+1}$ where n is the number of branches of order i, and m is the total number of orders) (Strehler 1957; Brazeau and Lasker 1988), the tributary to source ratio of primary and secondary order branches (defining as "source" any branch that joins another branch of equal order, and as "tributary" any branch that joins a higher order branch) (Brazeau and Lasker 1988). Distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001; McArdle and Anderson 2001) was employed to test the null hypothesis of no significant differences between depths and species. Each term of the analysis was tested using 9,999 random permutations of appropriate units (Anderson and ter Braak 2003), and significant terms relevant to hypothesis were investigated using *a posteriori* pair-wise comparisons with the PERMANOVA *t* statistic and 9,999 permutations. The analysis was performed using the PERMANOVA.exe software (Anderson 2005). Data were standardized in respect to their mean absolute deviation ($MAD = \frac{1}{n}\sum_{j=1}^{n}|x_{ij} - \bar{x}_i|$ where x_i is the value of the i variable observed in the n colonies) (García Pérez 2005), and an Euclidean distances matrix was build based on the standardized data. Furthermore, an ordination of all the analyzed colonies (*n* = 140) based on the Euclidean distances was obtained with a principal component analysis (PCA) performed with the R-language function Princomp which is available in the Vegan library (Oksanen et al. 2005) of the R software platform (R Development Core Team 2007). Finally a clustering of all the analyzed colonies (*n* = 140) based on the Euclidean distances was obtained with a cluster analysis performed with the R-language function As.phylo which is available in the Ape library (Paradis et al. 2004) of the R software platform (R Development Core Team 2007), using a Ward aggregation for the ordination performance. In order to assess the number of significant clusters produced, a simprof analysis was performed with the R-language function Simprof which is available in the Clustsig library (Whitaker and Christman 2010) of the R software platform (R Development Core Team 2007). The simprof analysis was obtained with 10,000 generated similarity profiles for creating the expected distribution of the data, and 9,999 similarity profiles generated for use in comparing the observed test statistic with its null distribution.

Sclerite variability

Portions of *E. singularis* colonies for the analysis of sclerite size and shape were obtained samplings in the east side of the Cap de Creus (42º18'44'' N; 003º19'05'' E) (Fig. 1). A portion of primary branch from 20 colonies from 20 m, 30 m, 40 m, 50 m and 60 m depth were collect-

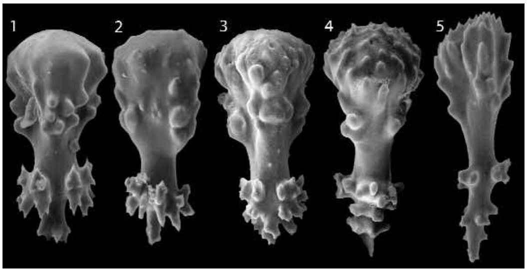

Fig. 2 - The five roughness degrees of the balloon club head.

ed by scuba diving in June 2010, and stored in ethanol. A portion of a primary branch from 20 colonies of *E. cavolinii* (20-25 m depth) were collected in March 2009 in the northern side of the Isola d'Elba (Fig. 1) by scuba diving, and stored in ethanol. Finally, a portion of primary branch from 20 colonies of *E. verrucosa* (15-25 m depth) were collected in January 2009 in Tarragona (Fig. 1) also by scuba diving, and were also stored in ethanol.

The collected branch portions were immersed in bleach until organic matter was disolved and sclerites were disaggregated, cleaned with distilled water, deposited on a glass cover attached to an aluminium stub with colloidal silver, and then sputter-coated with Au-Pd. Sclerite observations were performed with a Scanning Electron Miscroscope (SEM) HITACHI S-3500 N at 5.0 KV. Measures from 20 balloon clubs and 10 spindles for each colony were taken at X1000 and X400 respectively. From each balloon club the following measures were recorded: length, width, spiny end and collar widths, as described by Weinberg (1976); each balloon club head was classified into one of five categories according to its roughness' degree (Fig. 2). From each spindle the following measurements were recorded: length, and maximum width, as described by Weinberg (1976). For each colony the following sclerite features were calculated: mean length of the balloon club, mean width of the balloon club, mean width of the balloon club spiny end, mean width of the balloon club collar, mean roughness of the balloon club heads, mean length of the spindles, and mean width of the spindles. As for the colony shape data, a distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson 2001; McArdle and Anderson 2001) was employed to test the null hypothesis of no significant differences between depths and species. Data were standardized respect to their MAD, and an Euclidean distances matrix was built based on the standardized data. An ordination of all the analyzed colonies (n = 70) based on the Euclidean distances was obtained with a PCA, and a clustering of the colonies was obtained with a cluster analysis performed with the R software platform (R Development Core Team 2007) in the same way as for the colony shape analysis.

Genetic markers

A double molecular approach was carried out in this study: the analysis of the mitochondrial *msh1*, and the exploration of the variable nuclear ITS regions. Mitochondrial mismatch repair gene homolog (*msh1*) was chosen to be sequenced as it exhibits a relatively high rate of substitution in the Octocorallia (France and Hoover 2001; McFadden et al. 2010; van der Ham et al. 2009). Though variation in *msh1* may not distinguish among all congeners (Lepard 2003; McFadden et al. 2010), it is the most variable molecular marker available for the study of multiple octocorallian species (McFadden et al. 2010; Thoma et al. 2009). Nuclear ITS sequences (even using the secondary structure of these segments) were chosen to be sequenced because this DNA segment have been recently used for molecular phylogenetic and taxonomic study in gorgonians (Aguilar and Sánchez 2007; Grajales et al. 2007; Dueñas and Sánchez 2009), as well as because it is the best represented ITS segment in available databases (GenBank) and permits a framework of comparison. Taking into account the possible grade of variability, ITS sequences have also been considered useful to discover simple phenotypic morphologic variability (Sánchez et al. 2007). However, different authors clearly explained that the intragenomic variation in ITS sequences was widely ignored in phylogenetic studies, while it should be correctly evaluated (Harris and Crandall 2000; Calderón

Table 1 - Pairwise test results for the colony shape and the sclerite size comparison among depths and species.

Depth / Species	Colony shape			Spicule size		
	t	p		t	p	
20 m - 30 m	1.161	0.2485		1.063	0.3365	
20 m - 40 m	2.448	0.0011	*	2.804	0.0001	*
20 m - 50 m	2.356	0.0023	*	3.698	0.0001	*
20 m - 60 m	3.240	0.0001	*	3.497	0.0001	*
20 m - EC	5.539	0.0001	*	3.559	0.0001	*
20 m - EV	5.688	0.0001	*	7.019	0.0002	*
30 m - 40 m	1.582	0.0594		2.244	0.0059	*
30 m - 50 m	1.661	0.0479		3.739	0.0002	*
30 m - 60 m	2.287	0.0019	*	3.430	0.0001	*
30 m - EC	4.812	0.0001	*	3.461	0.0002	*
30 m - EV	4.865	0.0001	*	6.940	0.0001	*
40 m - 50 m	0.643	0.7605		2.508	0.0021	*
40 m - 60 m	0.880	0.5071		2.040	0.0071	*
40 m - EC	3.161	0.0001	*	2.144	0.0064	*
40 m - EV	3.106	0.0002	*	5.731	0.0001	*
50 m - 60	1.169	0.2418		1.183	0.2193	
50 m - EC	2.870	0.0002	*	1.604	0.0382	
50 m - EV	2.863	0.0005	*	5.786	0.0001	*
60 m - EC	3.068	0.0001	*	0.962	0.4278	
60 m - EV	2.726	0.0001	*	4.559	0.0001	*
EC - EV	1.893	0.0148		3.981	0.0001	*

et al. 2006). Samples for the genetic markers analysis were taken from the same sampling used for the spicule analysis; DNA sequences from 2 colonies for each depth or species were analysed.

For the first molecular approach (*msh1*), total genomic DNA was extracted from ethanol (EtOH)-preserved material using a CTAB (2% hexadecyltrimethylammonium bromide) protocol (with proteinase K in final concentration of 167 µg/mL) and a single chloroform-only extraction (modified from France et al. 1996). Using the polymerase chain reaction (PCR), we amplified approximately 700 bp of the 5'-region of *msh1* for 14 specimens using forward primers ND4L2475F or ND42625F (Brugler and France 2008), ND42599F (France and Hoover 2002), or MSH3010F (Thoma et al. 2009) and reverse primers MSH3101R (McFadden et al. 2010) and MUT3458R (Sánchez et al. 2003). PCR reactions used 0.5 U of either TaKaRa Ex Taq™ polymerase or DyNAzyme II™ DNA polymerase with the following reaction components: 1X TaKaRa Ex Taq buffer [Mg^{2+} free, with added $MgCl_2$ of 1.5 mM] or 1X Optimized DyNAzyme buffer [includes 1.5 mM $MgCl_2$], 0.4 mM dNTPs, 0.24-0.32 µM of each primer, 0-2.5 µg acetylated bovine serum albumin [Promega], 7-90 ng of genomic DNA, and brought to a final volume of 25 µl with dH_20. PCRs were run using the following cycle profile: initial denaturation at 94°C for 2 to 3 min followed by 30 to 40 cycles of denaturation at 94°C for 20 to 30 s, annealing at 45 to 50°C for 30 to 45 s, extension at 72°C for 50 s to 1 min, and a final extension at 72°C for 6 min. PCR products were purified by enzymatic digestion (1.52 U of ExoI and 0.15 U FastAP™ thermosensitive alkaline phosphatase [Fermentas] per 1 µl PCR product; modification of Werle et al. 1994) or from low melting point (LMP) agarose by digestion with agarase (5 U per 100 µl melted 1% LMP agarose; Sigma-Aldrich). Purified PCR reactions were cycle-sequenced at the University of Louisiana at Lafayette using ABI BigDye® Terminator v1.1 Cycle Sequencing Kit (1/4 reactions) and purified with Sephadex G-50 columns (GE Healthcare). Purified products were electrophoresed on an ABI PRISM® 3130*xl* Genetic Analyzer and sequence traces were edited using Sequencher™ v4.7 (Gene Codes). DNA sequences from 2 colonies for each depth or species were submitted to GenBank. A multiple alignment comprising *msh1* nucleotide sequences of all the analyzed colonies was created using MUSCLE (Edgar 2004). This alignment was submitted to DnaSP v5.00.07 (Librado and Rozas 2009) to generate a sequence file designating unique genetic types (i.e. haplotypes); sites with missing data were excluded.

For the second molecular approach (ITS regions) total genomic DNA was extracted from ethanol (EtOH)-preserved material using the E.Z.N.A.

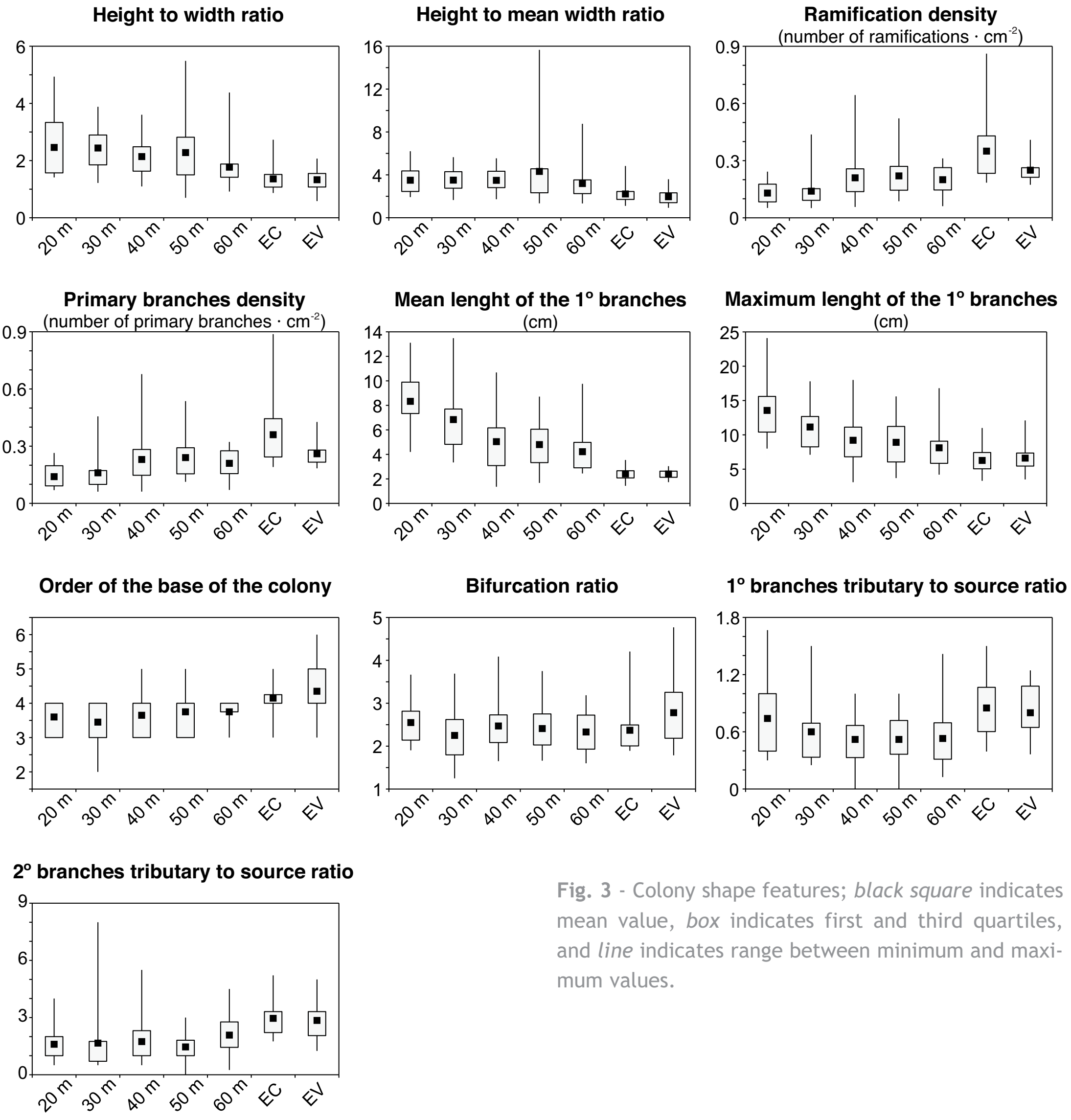

Fig. 3 - Colony shape features; *black square* indicates mean value, *box* indicates first and third quartiles, and *line* indicates range between minimum and maximum values.

DNA kit (OmegaBiotech) following the manufacturer's instructions. The full ITS region, including the 5.8S ribosomal (r)RNA gene (approximately 650 bp), was amplified using the primers ITS5: 5'-GGA AGT AAA AGT CGT AAC AAG G-3' and ITS4: 5'-TCC TCC GCT TAT TGA TAT GC-3' (White et al. 1990). Amplifications were arried out in a 50-µl volume reaction, with 0.75 µl (2.6 units) of Expand High Fidelity Enzyme Mix ® DNApolymerase (Roche), 1 µl (10mM) of dNTPs and 1,5 µl (10µM) of each primer. PCR was run using the following cycle profile: initial denaturing at 94°C for 2 min, 35 amplification cycles (94°C for 30 s, 54°C for 30 s, 72°C for 1 min 45 s), and a final extension at 72°C for 5 min. Amplifications were carried out in a MyGene^{TM} Series Peltier Thermal Cycler (Model MG 25+). PCR prodicts were purified using the Wizard® Genomic DNA Purification Kit. Chromatograms obtained from the automated sequencer were read and contigs assembled using the sequence editing software sequencher™ 4.0.

Presence of symbiotic algae

Samples for the analysis of symbiotic algae presence in the *E. singularis* colonies proceed from the same sampling made for the sclerite and genetic analysis. Ten *E. singularis* colonies for

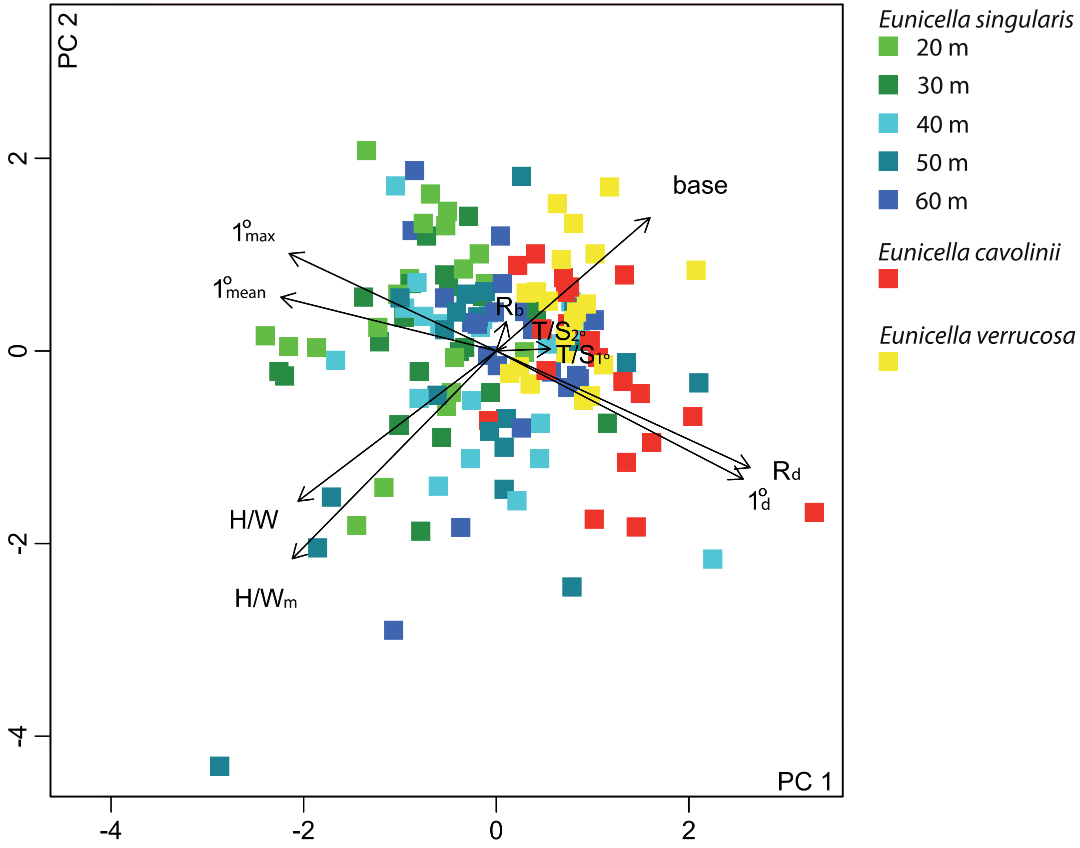

Fig. 4 - Principal component analysis (PCA) biplot showing the ordination of the studied colonies regarding their colony shape, and the roles of the analyzed features; H/W = height to width ratio, H/Wm = height to mean width ratio, Rd = ramification density, 1°d = primary branches density, base = order of the colony's base, 1°max = the primary branches' maximum length, 1°mean = the primary branches' mean length, Rb = the bifurcation ratio, $T/S_{1°}$ the tributary to source ratio of primary branches, $T/S_{2°}$ the tributary to source ratio of secondary branches.

each depth were preserved in 10% formol, rinsed in distilled water, decalcified in a 10% formic acid solution and then dehydrated in a set of ethanol solutions with increasing concentrations (70%, 96% and 100% ethanol). Samples were subsequently submerged in a mixture of 50% ethanol (100%) and 50% resin (Technovit 7100) during 2 hours, and then embedded in a bifasic resin (Technovit 7100) and stored in the dark at 4ºC during 48 hours. After the dehydration, samples were included in resin (Technovit 7100) and left hardening during 3 days at room temperature. Longitudinal 3 µm thick sections of the branches were cut and stained with haematoxylin and eosin. Sections were observed at microscope to determine the presence of the symbiotic algae in the tissue of each one of the analyzed colonies (*n* = 50).

Environmental features

The variation with depth of the main environmental features was recorded monthly from September 2009 to August 2010 at Cap de Creus (42º18'44'' N; 003º19'05'' E) (Fig. 1). Temperature, salinity, density, and photosynthetically active radiation (PAR, 400 to 700 nm) were measured at 1 m depth intervals from 5 to 60 m depth with a Seabird 19 and a Seabird 25 CTDs equipped respectively with a Biospherical Instruments Inc QSP-2300 and a Li-Cor underwater spherical quantum sensor LI-193.

RESULTS

Colony shape

Colony shape was significantly different among depths and species (PERMANOVA, Pseudo-F =

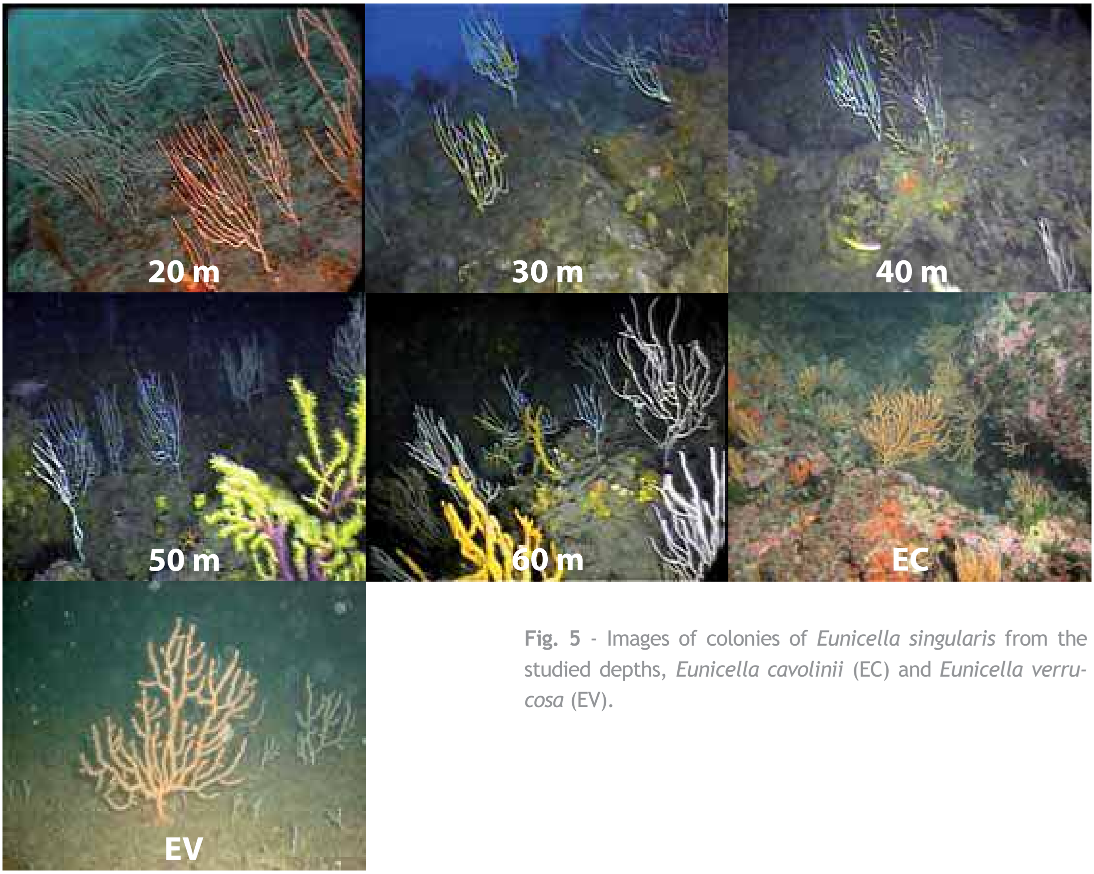

Fig. 5 - Images of colonies of *Eunicella singularis* from the studied depths, *Eunicella cavolinii* (EC) and *Eunicella verrucosa* (EV).

8.75, p<0.01). The *Eunicella cavolinii* and *Eunicella verrucosa* colonies shared a similar colony shape, which was significantly different from all the *Eunicella singularis* colonies, independently of depth (Fig. 3 and Table 1). The *E. singularis* colonies showed a gradient in the shape going from shallow to deep (Fig. 3 and Table 1). There were no significant differences between colonies from 20 and 30 m depth, whereas colonies from 20 m depth were significantly different from any other colonies. On the contrary, colonies from 30 m depth were similar to colonies from 40 and 50 m depth, but statistically different from the 60 m depth colonies. Finally, the 60 m depth colonies were similar to colonies from 40 and 50 m depth (Fig. 3 and Table 1). The first two principal components of the PCA explained 77.9% of the data variance; the first axis explained 55.9%. The PCA showed that these differences were mainly due to four groups of features, while the bifurcation ratio and both the tributary to source ratios did not contribute to the explanation of the variance (Fig. 4). There was a clear covariance between the mean and maximal length of the primary branches, the height to width and mean width ratios, and the ramification and primary branch densities (Fig. 4). Shallow *E. singularis* colonies were mainly characterized by large primary branches, high values of height to width ratios (colonies taller than wide), a low ramification and primary branch density, and a low order of the colony base (Fig. 3, 4 and 5). On the contrary, the *E. cavolinii* and *E. verrucosa* colonies showed a large number of short primary branches, values of height to width ratios close to 1 (colonies almost as tall as wide), and a higher order of the colony base (Fig. 3, 4 and 5). *E. singularis* colonies from 40, 50 and 60 m depth showed intermediate characteristics between the shallow shape and the *E.verrucosa* and *E. cavoli-*

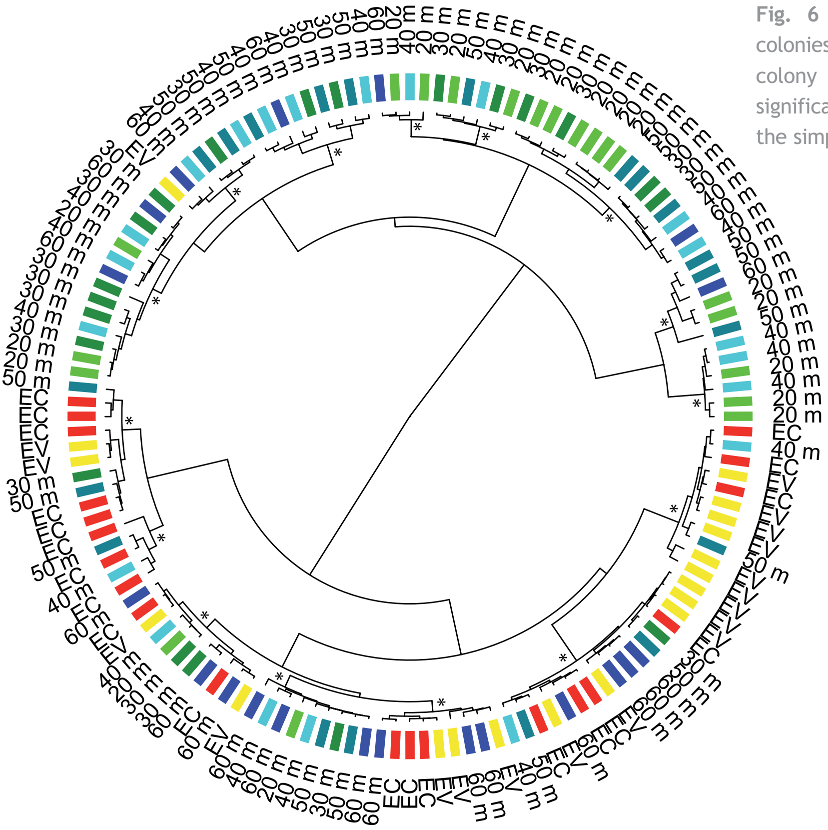

Fig. 6 - Cluster of the studied colonies aggregated regarding their colony shape; *asterisk* indicates significant clusters determined by the simprof test ($p < 0.05$).

ni shape, with the 50 m depth colonies showing the greatest variability in shape (Fig. 3, 4 and 5). The cluster analysis based on the colony shapes aggregated all the studied colonies into two main groups: one group containing all the *E. cavolinii* (20 out of 20) and *E. verrucosa* colonies (19 out of 20), and another group with almost all the shallow *E. singularis* colonies from 20 m (18 out of 20) and 30 m depth (15 out of 20) (Fig. 6). The colonies from 40 m depth were mainly grouped together with the shallow *E. singularis* colonies (14 out of 20) as well as the colonies from 50 m depth (12 out of 20), while the colonies from 60 m depth were mainly grouped with colonies of *E.cavolinii* and *E. verrucosa* (13 out of 20) (Fig. 6).

Sclerite variability

Sclerite size was significantly different among depths and species (PERMANOVA, Pseudo-F = 14.65, $p<0.01$). The shallow *E. singularis* colonies from 20 and 30 m depth shared similar sclerites, while the colonies from 40 m depth as well as the *E. verrucosa* colonies showed significantly different sclerites (Fig. 7 and Table 1). Finally, the *E. singularis* colonies from 50 and 60 m depth had similar sclerites to the *E. cavolinii* colonies (Fig. 7 and Table 1). The first two principal components of the PCA explained 76.1% of the data variance; the first axis explained 60.6%. The PCA showed that these differences were mainly due to three groups of features, with a clear covariance between the balloon club and spindle lengths, and a slight covariance among the balloon club, collar, spiny end and spindle widths (Fig. 8). Shallow *E. singularis* colonies from 20 and 30 m depth were mainly characterized by large and wide sclerites, with balloon clubs showing large collars and spiny ends, and very smooth heads (Fig. 7, 8, 9 and 10). Colonies from 40 m depth mainly had scle-

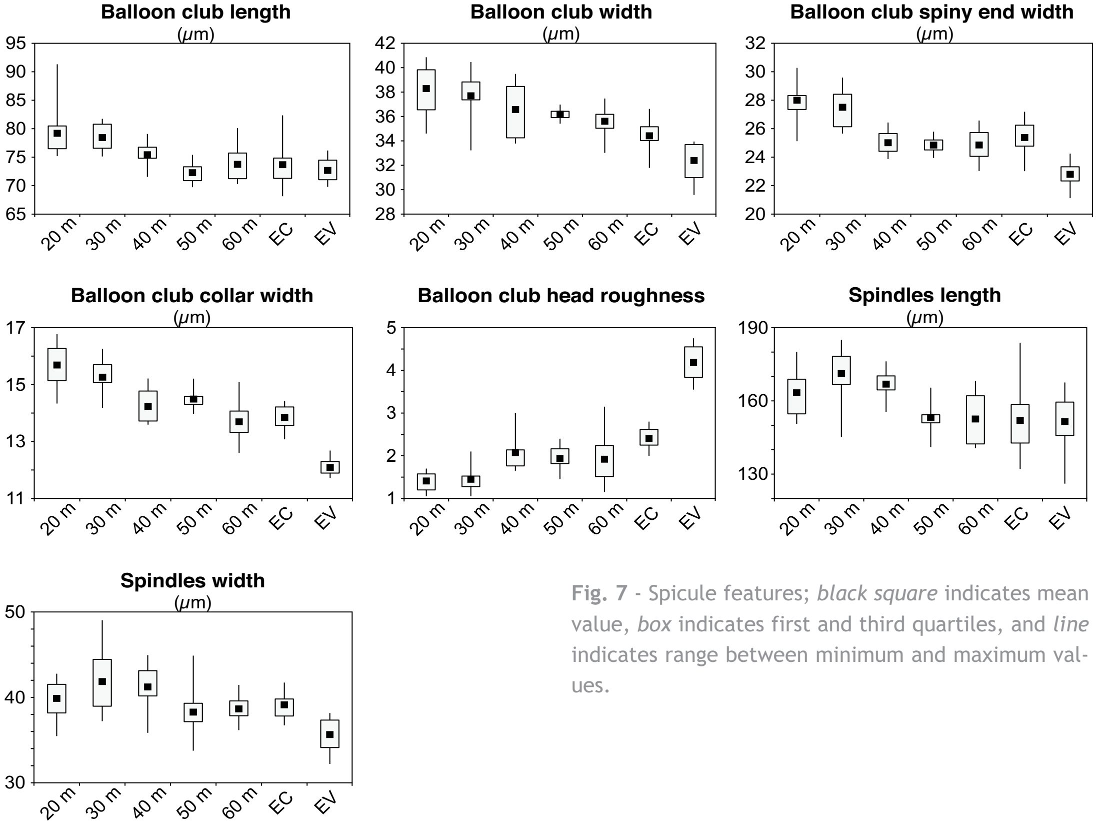

Fig. 7 - Spicule features; *black square* indicates mean value, *box* indicates first and third quartiles, and *line* indicates range between minimum and maximum values.

rites as large as the shallow colonies but much thinner as well as rougher balloon club heads (Fig. 7, 8 and 11), while the *E. singularis* colonies from 50 and 60 m depth and the *E. cavolinii* colonies had smaller sclerites with rough balloon club heads (Fig. 7, 8, 12, 13 and 14). Finally, the *E. verrucosa* colonies had very thin sclerites, with very rough balloon club heads (Fig. 7, 8 and 15). The cluster analysis aggregated together all the shallow *E. singularis* colonies from 20 (10 out of 10) and 30 m depth (8 out of 10), and almost half of the 40 m depth colonies (4 out of 10) (Fig. 16). In the same way, the analysis grouped together all the *E. verrucosa* colonies (10 out of 10), and related this group to a another big group composed of the remaining *E. singularis* colonies from 40 m depth (6 out of 10), and almost all the 50 m (9 out of 10), 60 m depth (10 out of 10), and the *E. cavolinii* colonies (9 out of 10) (Fig. 16).

Genetic markers

All the 14 *msh1* sequences obtained in this study representing the different depths or species were identical: only a single *msh1* haplotype was observed and shared among all the analyzed colonies. On the contrary, all the ITS2 sequences obtained in this study representing the different depths or species fall within the wide range of variability showed by previous

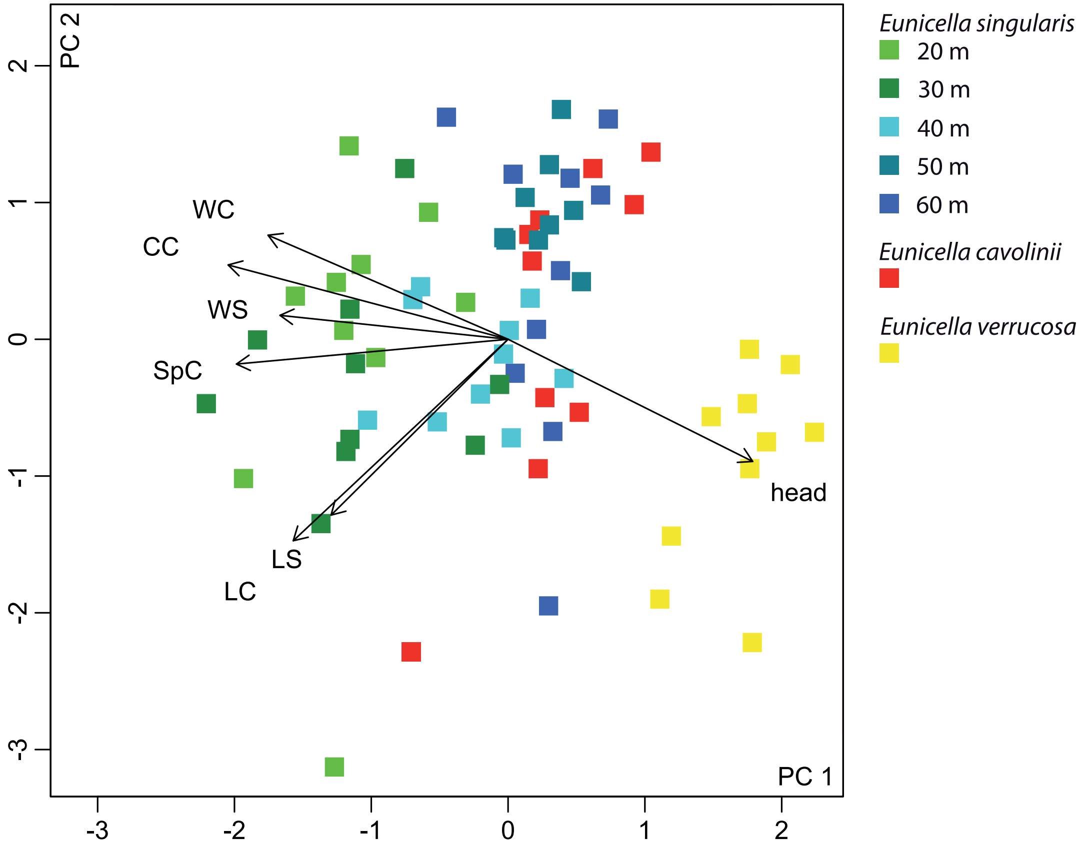

Fig. 8 - Principal component analysis (PCA) biplot showing the ordination of the studied colonies regarding their spicule, and the roles of the analyzed features; LC = mean lenght of the balloon club, WC = mean width of the balloon club, SpC = mean width of the balloon club spiny end, CC = mean width of the balloon club collar, head = mean roughness of the balloon club head, LS = mean lenght of the spindles, WS = mean width of the spindles.

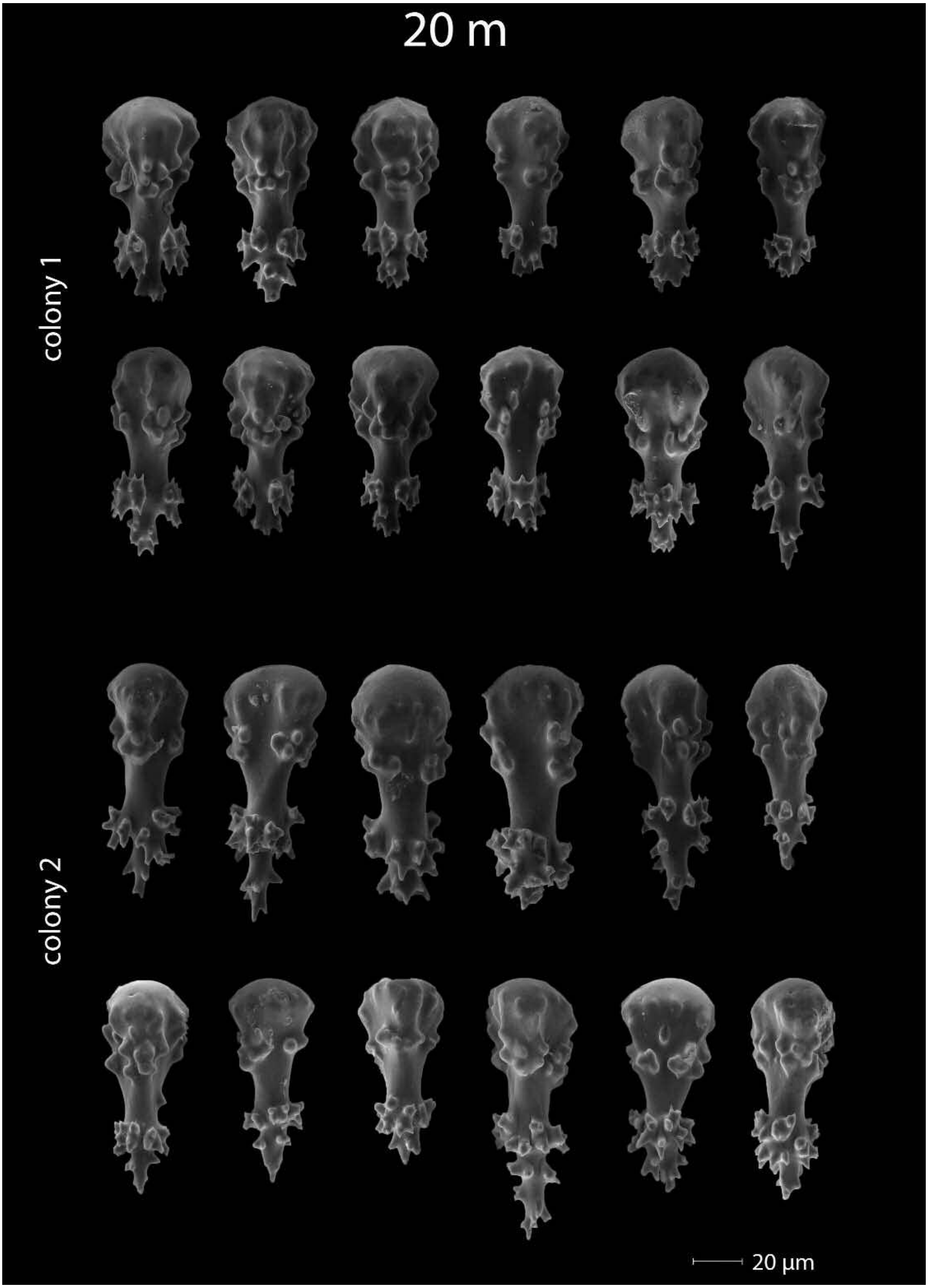

Fig. 9 - Balloon clubs from two *Eunicella singularis* colonies from 20 m depth.

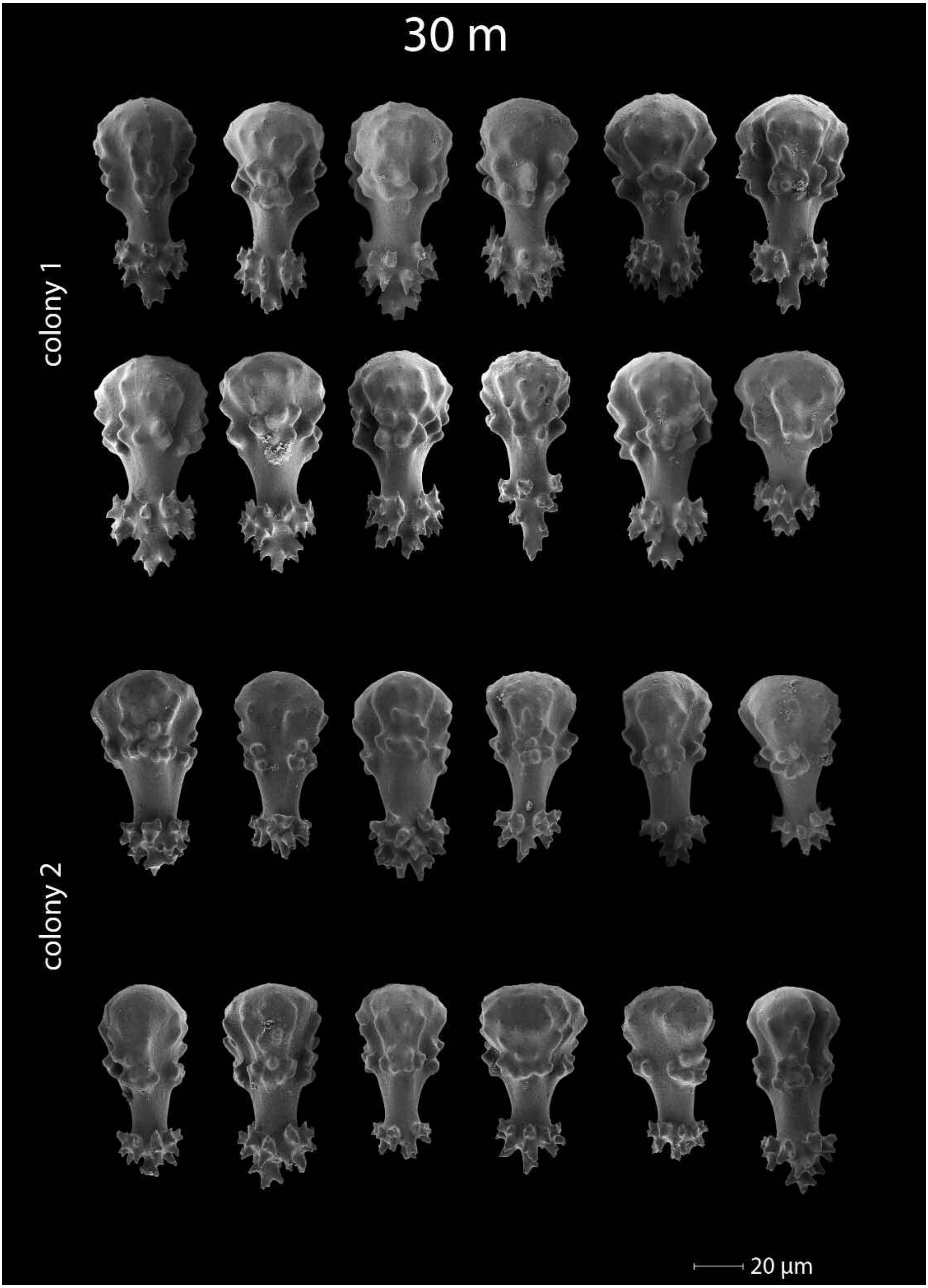

Fig. 10 - Balloon clubs from two *Eunicella singularis* colonies from 30 m depth.

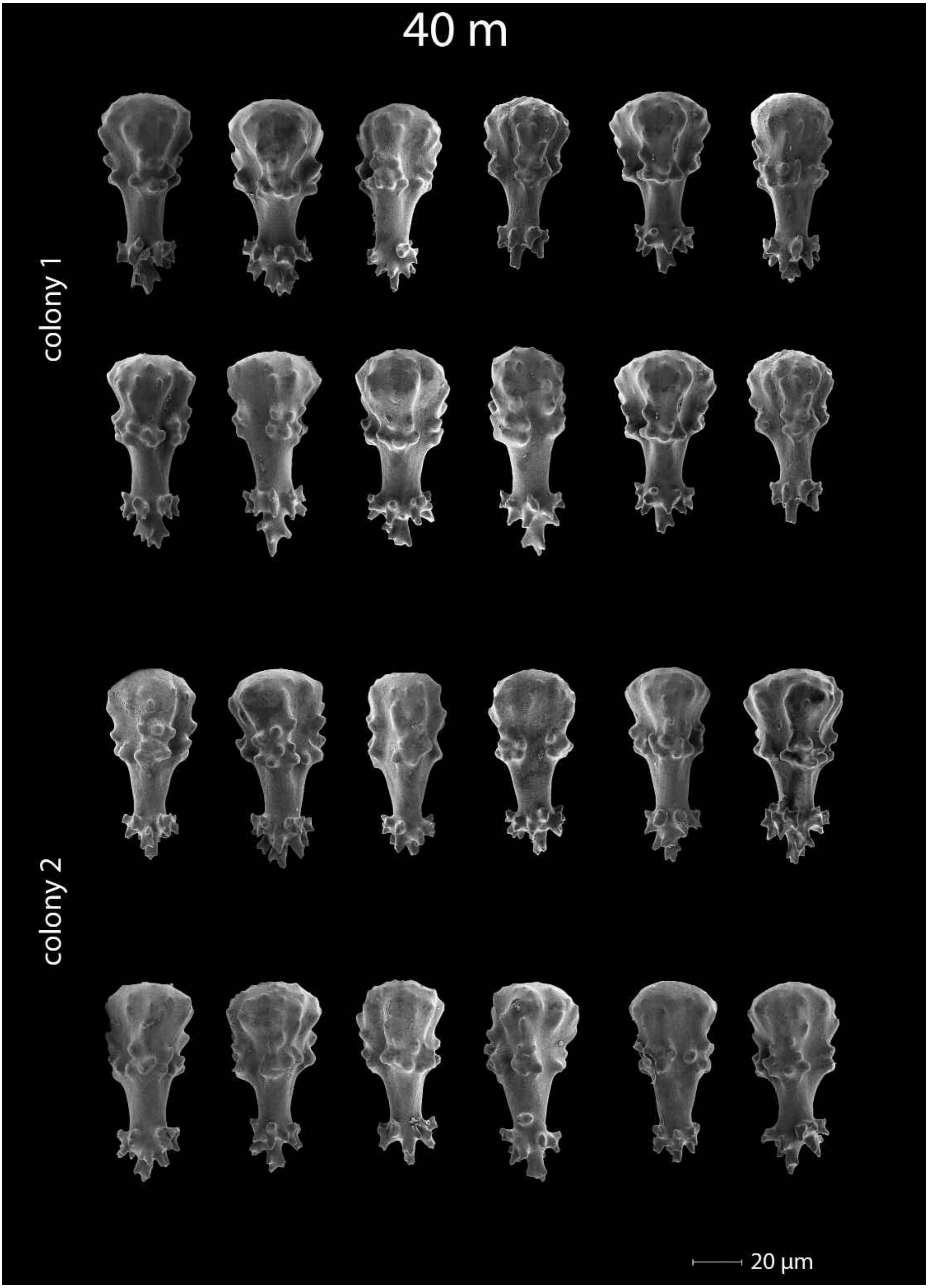

Fig. 11 - Balloon clubs from two *Eunicella singularis* colonies from 40 m depth.

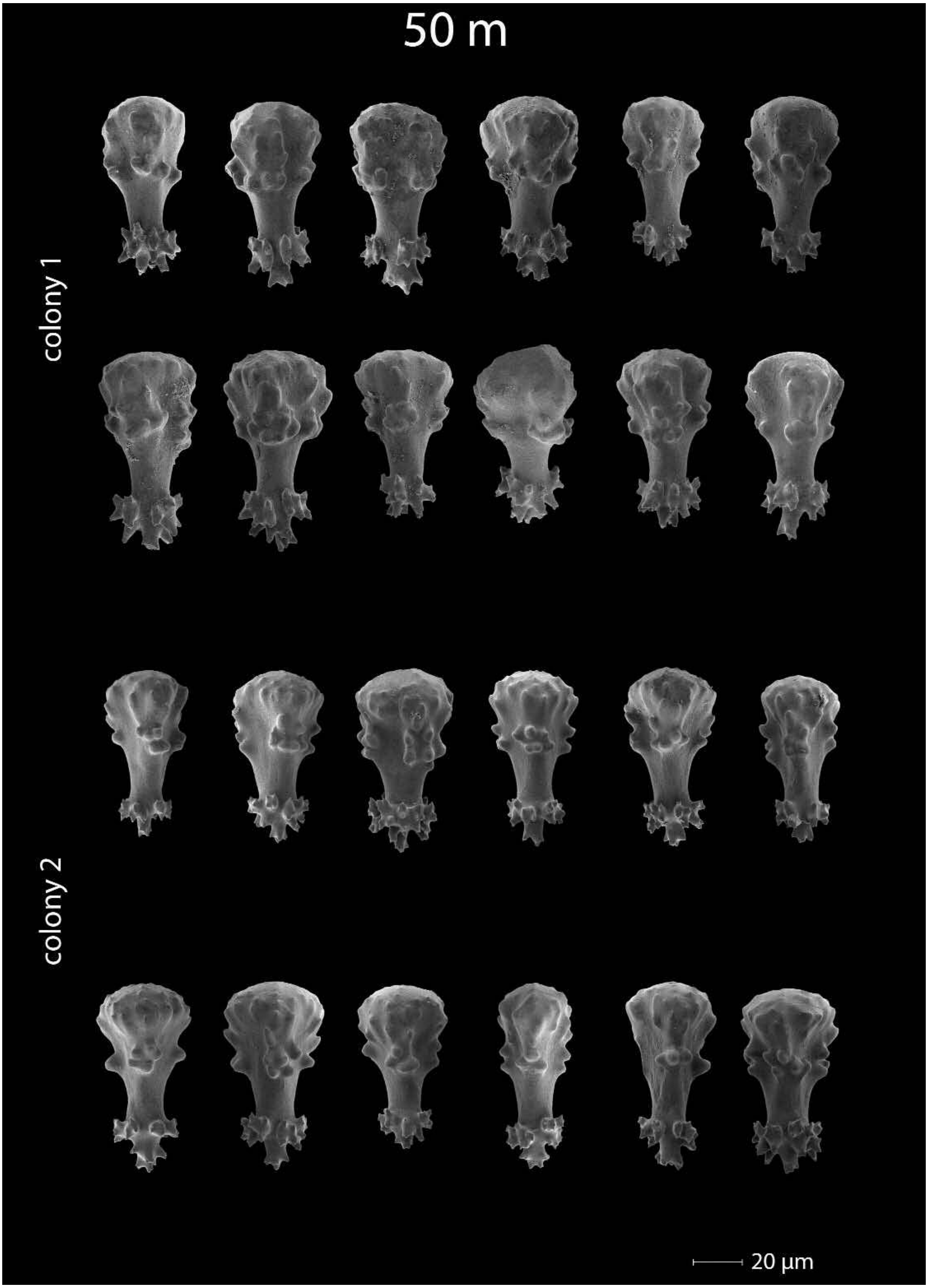

Fig. 12 - Balloon clubs from two *Eunicella singularis* colonies from 50 m depth.

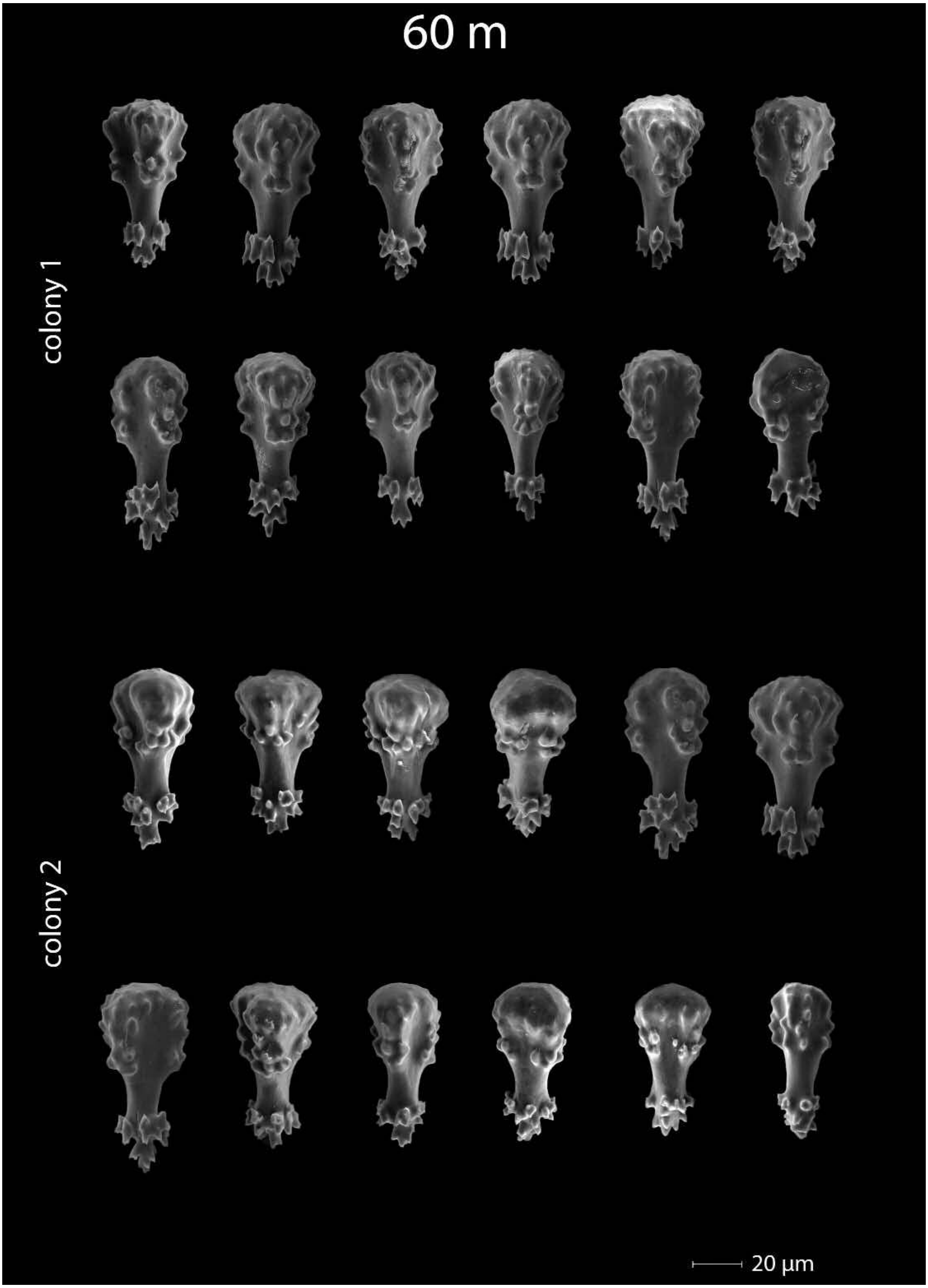

Fig. 13 - Balloon clubs from two *Eunicella singularis* colonies from 60 m depth.

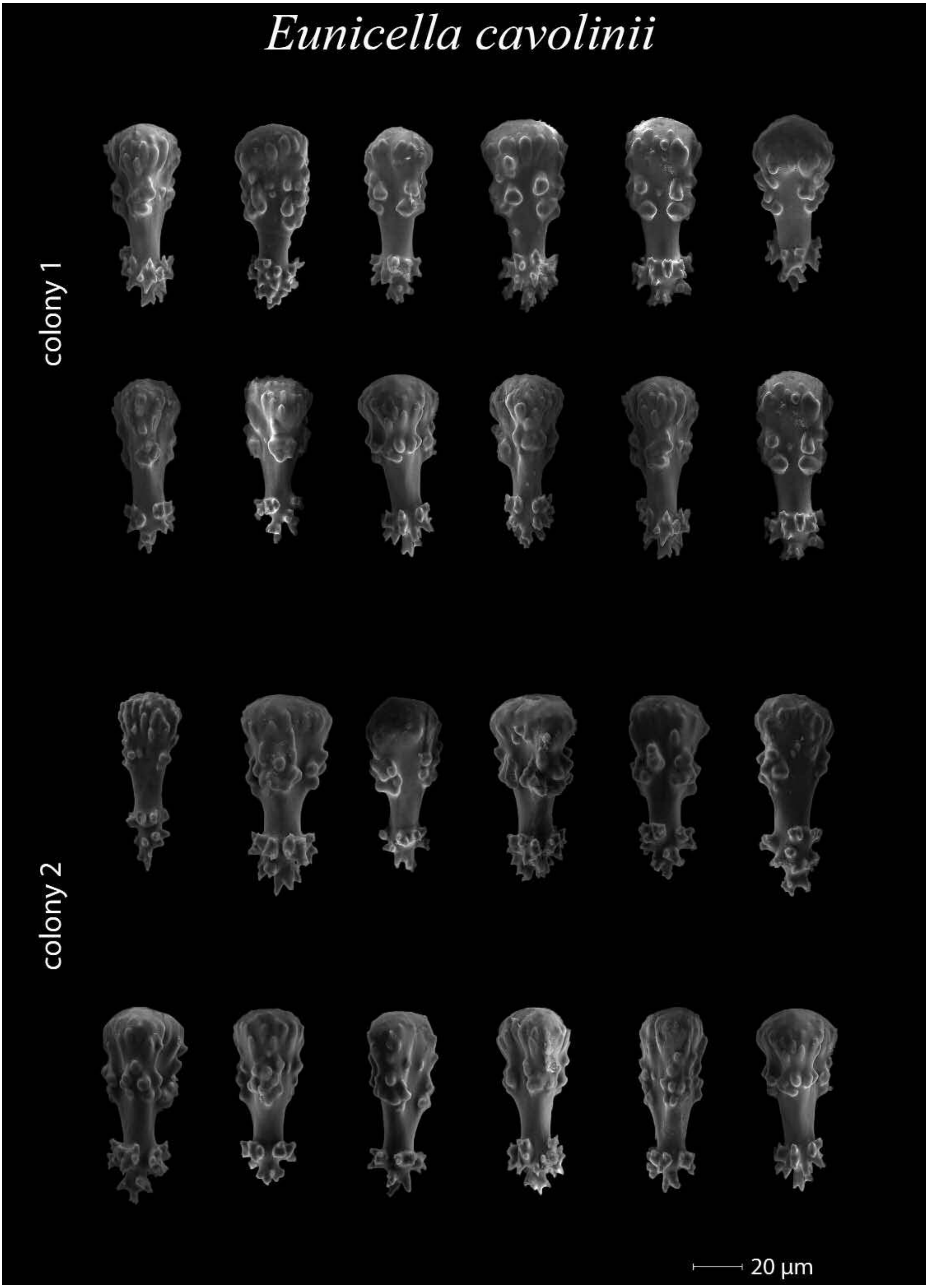

Fig. 14 - Baloon clubs from two *Eunicella cavolinii* colonies.

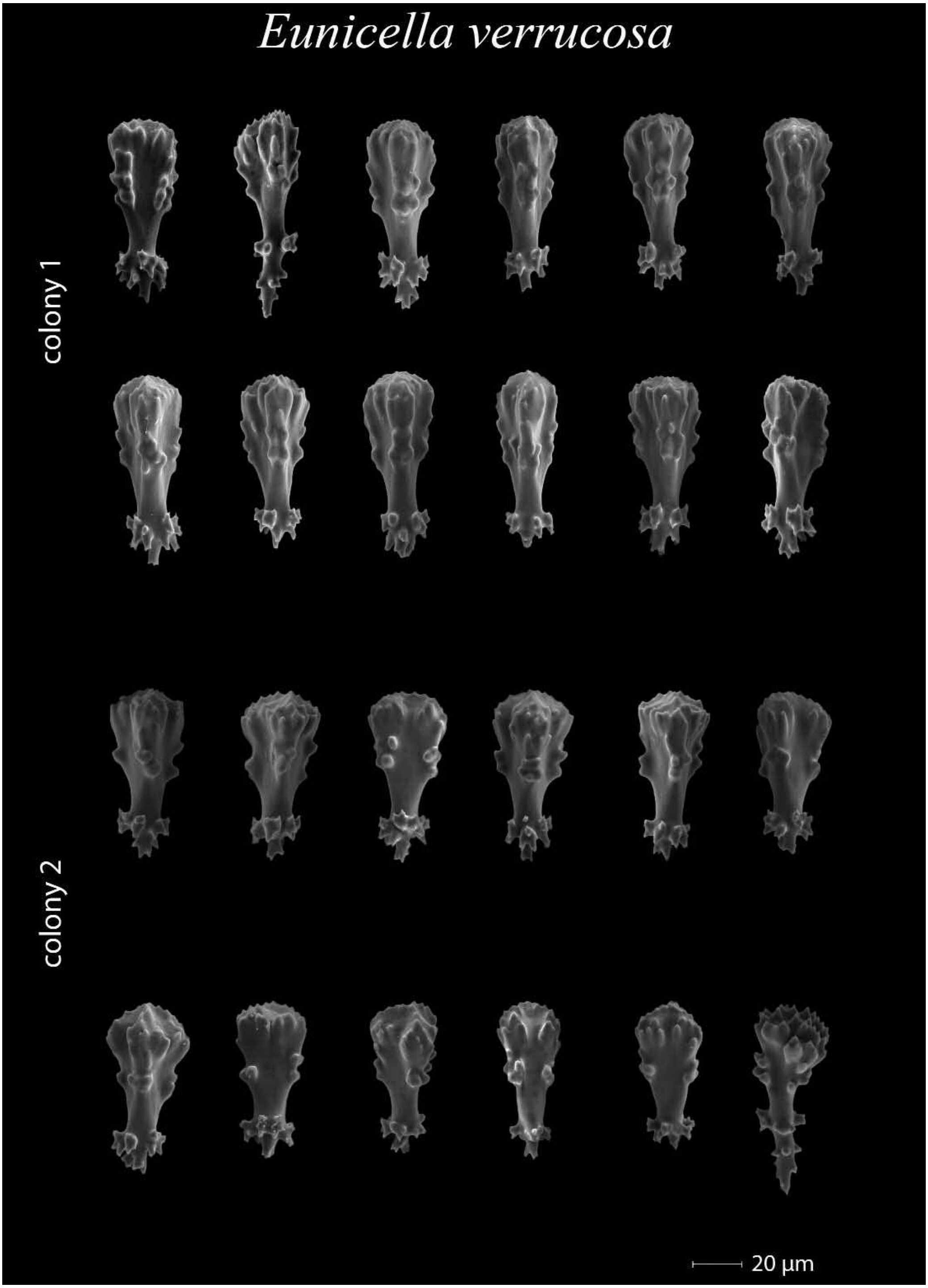

Fig. 15 - Balloon clubs from two *Eunicella verrucosa* colonies.

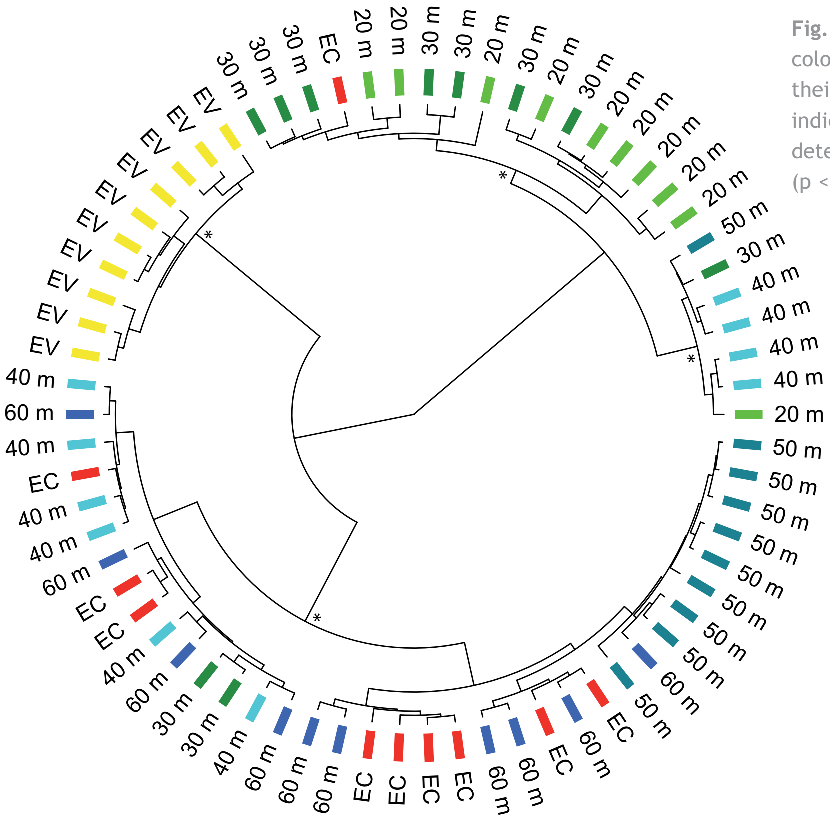

Fig. 16 - Cluster of the studied colonies aggregated regarding their sclerites size; *asterisk* indicates significant clusters determined by the simprof test ($p < 0.05$).

studies on *E. cavolinii* ITS2 sequences (AY827500-AY827535) and *E. singularis* sequence (AY827540) already deposited in GeneBank (Calderón et al. 2006), and relationship among *Eunicella* species or depths become unresolved. Such a great variability of ITS2 sequences in *E. cavolinii* makes, at this moment, this molecular marker an unhelpful source of phylogenetic information for the Mediterranean *Eunicella* species.

Presence of symbiotic algae

The analysis of the histological preparation showed that all the *E. singularis* colonies from 20 and 30 m depth presented zooxanthellae into their tissues, whereas all the *E. singularis* colonies form 40, 50 and 60 m depth did not present any zooxanthellae into their tissue (Fig. 17).

Environmental features

Both temperature and salinity were almost constant along the water column from October to February. In March the surface water was less saline than the deeper water, probably due to the strong rains that affected the study area during this month. Water column stratification began to develop in April with a thermocline forming around 30 m depth. Water column stratification was partially broken at the end of June due to strong winds, while the water column was fully stratified in July and August with a thermocline at 35 m depth. Finally, stratification was stretched out between 45 and 30 m depth in September (Fig. 18). PAR decreased exponentially with depth; over an annual average, if compared to 20 m depth, PAR was 37.8 ± 13.1 % at 30 m depth, 14.1 ± 7.1 % at 40 m depth, 4.6 ± 2.8 % at 50 m depth, and 1.4 ±1.0 % at 60 m depth.

DISCUSSION

The results from the comparison of the colony's shape and sclerite size and shape, and the presence of the symbiotic algae suggest the existence of two morphotypes of *Eunicella singularis*. A "shallow morphotype" (20 and 30 m depth colonies) correspond to the commonly known *E. singularis*. This morphotype has symbiotic algae in the tissue, and its colonies have a candlestick-like shape, being taller than wide, with a few ramifications and very large primary branches; the balloon clubs are large, they present a wide collar and spiny ends, and have very smooth heads. On the contrary, the "deep morphotype" of *E. singularis* (40 to 60 m depth colonies) lacks symbiotic algae in the tissue; its colonies are still taller than wide but they have more ramifications and shorter primary branches. All this colony shape features tend to accentuate with increasing depth from 40 to 60 m. In the same way, the sclerites features tend to change with increasing depth: sclerites show intermediate dimensions at 40 m depth, while they were much smaller in the 50 and 60 m depth colonies where they resemble those of *Eunicella cavolinii*. The results of the present study for the shallow *E. singularis* morphotype, the *E. cavolinii* and the *Eunicella verrucosa* colonies, agree with the previous knowledge on these species. *E. singularis* is the Mediterranean gorgonian species with the longest and thickest branches, the lowest ramification density and the greatest height to width ratio (Rossi 1959; Carpine and Grasshoff 1975; Weinberg 1976; Weinbauer and Velimirov 1998). *E. cavolinii* is characterized by colonies with a low height to width ratio, short and ramified branches (Carpine and Grasshoff 1975; Weinberg 1976), and its colony shape resemble much more the *Leptogorgia sarmentosa* or the *E. verrucosa* than the *E. singularis* (Rossi 1959; Weimbauer and Velimirov 1998). Balloon clubs have smooth heads in *E. singularis*, while they have much more rough heads in *E. cavolinii* (Rossi 1959, Carpine and Grasshoff 1975), and both balloon clubs and spindles were larger, and had wider collars and spiny ends in *E. singularis* than in *E. cavolinii* (Rossi 1959; Weinberg 1976). Conversely, *E. verrucosa* sclerites are very distinctive and cannot be confused with any of the other species (Rossi 1959; Carpine and Grasshoff 1975; Weinberg 1976).

In the Cap de Creus area, the limit in the distributions of the shallow and the deep morphotype

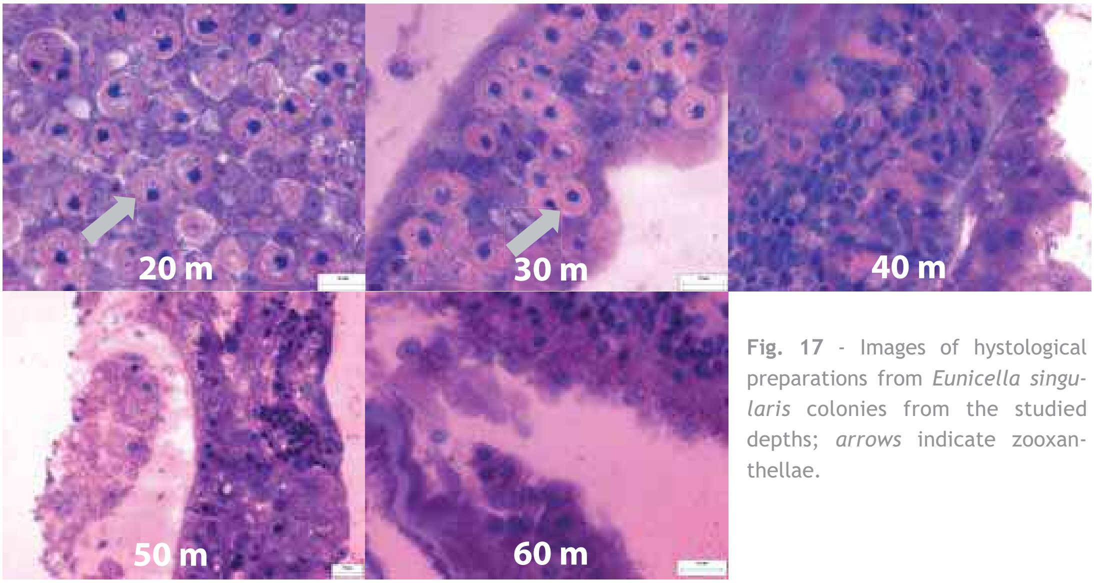

Fig. 17 - Images of hystological preparations from *Eunicella singularis* colonies from the studied depths; *arrows* indicate zooxanthellae.

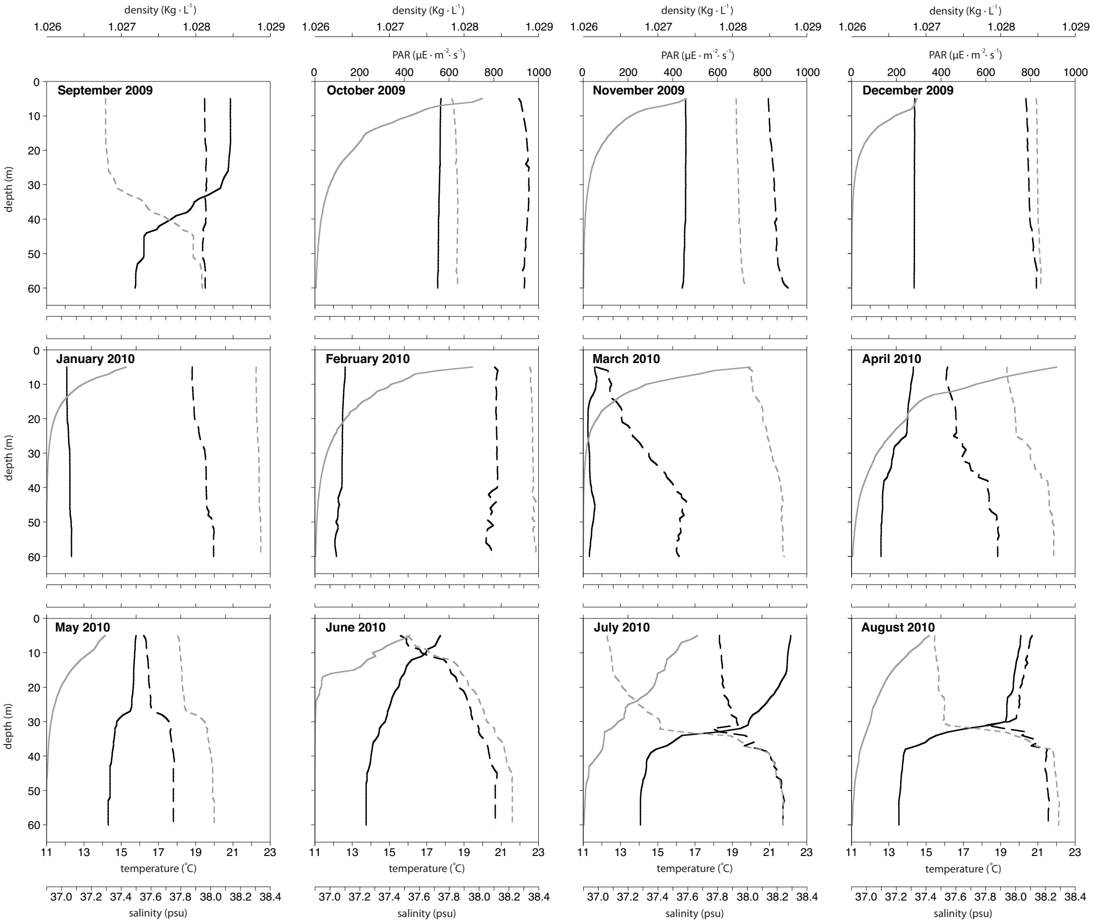

Fig. 18 - Water column characterization in the sampling location during the study period; *grey line* represents photosynthetically active radiation (PAR), *grey dotted line* represents water density, *black line* represents water temperature, and *black dotted line* represents salinity.

of *E. singularis* was located around 35 m depth. It is probable that the changing with depth of the main environmental features plays a major role in the differentiation and in the distribution of the two morphotypes. Shallow bottoms are subject to strong hydrodynamic forces (Hiscock 1983) and mainly exposed to bidirectional currents (Riedl 1971), whereas deeper nearshore bottoms are more affected by unidirectional water movements (Riedl 1971; Hiscock 1983) and largely sheltered from the direct physical damage that may be caused by strong storm-induced waves (Bongaerts et al. 2010). Shallow *E. singularis* colonies mainly inhabit horizontal and subhorizontal rocky bottoms where a candlestick-like shape with a few ramification and very large primary branches are more readily bent and thus reduce hydrodinamic forces (Velimirov 1976; Weinbauer and Velimirov 1998) allowing the colonies to withstand strong water movement. In particular, strong waves often transport many detached algae that could easily get entangled in more branching colony shapes increasing disproportionally the perceived hydrodynamic forces, while a morphotype with long and vertical branches could make entangling much more difficult. No differences in the *E. singularis* main architectural parameters were previously reported between shallow (20 m) and deep (30 m) colonies (Weinbauer and Velimirov 1998). However, over the larger bathymetrical extent considered in the present study, significant differences related to depth appeared, and the decrease of water movement induced by wave action with depth (Hiscock 1983) could explain the more ramified colony shape of the “deep morphotype”. Differences in water turbulence were also reported as the main cause of variability in shallow *E. singularis* colony shape (Théodor 1963; Skoufas et al. 2000), and architectural features and growth and branching patterns were shown to be an adaptation to the water flow in *E. cavolinii* colonies (Velimirov 1976; Weinbauer and Velimirov 1995). Branching network was narrower, and primary branch length was significantly lower in sheltered than in exposed *E. cavolinii* colonies, whereas the ramification density was significantly higher in sheltered colonies as well as in colonies from 70-80 m depth (Velimirov 1976). However, in another location, sheltered colonies of *E. cavolinii* exhibit a greater degree of porosity, longer primary branches and lower ramification density than colonies from exposed sites (Weinbauer and Velimirov 1995). Only colony height to width ratio did not vary significantly between sheltered and exposed sites in *E. cavolinii* (Weinbauer and Velimirov 1995). Water velocity and direction of movement can change rapidly in response of wave action, so the mechanical properties of the colonies must be specifically tuned to their environment, and sclerites are a major determinant of overall mechanical properties of an octocoral colony (Lewis and Von Wallis 1991). Drag forces on an erect basely-attached structure will cause bending in the direction of the water flow (Lewis and Von Wallis 1991). Before forces become high enough to cause damage, colony bends and relieves pressure (Jeyasuria and Lewis 1987); the elastic properties of the axial skeleton returns the colony to the erect position, and overall the sclerites limit the extent of these movements (Lewis and Von Wallis 1991). In *Eunicella* species, balloon clubs form a single layer that comprises the outer cortex, with clubs orientated perpendicularly to the colony axis with the heads exposed to the surface of the colony; immediately subjacent to the outer cortex there is a layer of large, thick spindles in close contact with each other. Clubs and spindles form a mechanism that limits compression effects (Lewis and Von Wallis 1991). In the same way as “shallow morphotype” of *E. singularis* had larger sclerites than the “deep morphotype”, *E. cavolinii* colonies exposed to strong hydrodynamic conditions had larger balloon clubs than colonies located in sheltered sites (Velimirov 1976), and shallow water *E. singularis* colonies had bigger spindles in exposed than sheltered sites (Skoufas 2006). It is interesting to highlight that the values of colony shape and slcerite features observed in exposed *E. cavolinii* colonies where similar to those observed in the present study in the shallow colonies of *E. singularis* from 20 and 30 m depth (Velimirov 1976). This observation emphasizes the strong effects that environmental conditions can play on the gorgonian macro- and micro-architectural features.

Morphological adaptations to different light intensities are well known for stony corals, and in Caribbean gorgonian species a variation of the morphology with depth and light intensity have been observed (Brazeau and Lasker 1988; West et al. 1993). In the case of the two morphotypes of *E. singularis*, an annual average reduction of the PAR values to less than the 15 % of the values at 20 m depth, was associated to the lack of symbiotic zooxanthellae. It is interesting to note that the depth threshold between the shallow and the deep morphotype also corresponds to the depth of the summer thermocline in the study area. Since *E. singularis* has phylopatric larvae (Théodor 1967; Weinberg and Weinberg 1979) and reproduces in late May-June when the water column is fully stratified (Ribes et al. 2007; Gori et al. 2007), it is possible that larvae from the “shallow morphotype” colonies are mostly relegated above the thermocline, whereas larvae from the “deep morphotype” colonies remain below the thermocline. However the thermocline can oscillate and sometimes partially break out, depending on the specific summer conditions of each year (Rossi et al. 2011), as it happened at the end of June 2010 (Fig. 18). Consequently there could be some years when larvae from the "shallow morphotype" are able to settle down at deeper bottoms, and/or larvae from the "deep morphotype" are able to settle down at shallower bottoms. Since a direct transmission of symbiotic algae from the female parent to newly released larvae is thus far suggested for *E. singularis* (Théodor 1969; Weinberg and Weinberg 1979), this variable year to year situation would result in a partial overlap in the distribution of the shallow and deep morphotypes of *E. singularis* between 30 and 40 m depth, and could explain the observation of both the colony and sclerite shapes in the 40 m depth colonies (Fig. 6 and 16), as well as the observation made by Théodor of colonies of *E. singularis* with and without zooxanthellae one near to the other at at 35 m depth off Cap Cerbère, and at 38 m depth off Cap d'Abeille (both locations are located a few km north of Cap de Creus) (Théodor 1969). Moreover, since gorgonians are long-lived species and display slow growth and low natural mortality rates (Weinberg and Weinberg 1979; Coma et al. 2004; Linares et al. 2007), morphological variation can originate as a consequence of adaptation to different environmental features experimented during the entire colony life, leading to the coexistence of different morphotypes in the zones of intermediate and most variable environmental features.

When described by Théodor, the "deep morphotype" of *E. singularis* was proposed as a subspecies (*E. singularis aphyta*) (Théodor 1969; Weinberg 1976). However, due to the coexistence of the two forms, other authors did not agree with this differentiation into subspecies preferring a differentiation into two varieties (Carpine and Grasshoff 1975). On the basis of the results of this work, we propose the existence of two different morphotypes of the species *E. singularis* with a gradual variation of colony shape and sclerite features with depth. Actually there is no genetic marker that can discriminate among the Mediterranean species of the genus *Eunicella*, and consequently that could quantify the degree of genetic differentiation and the gene flow between the two morphotypes. Further analyses using other genetic markers are necessary to assess the taxonomic status of these morphotypes and their eventual incipient process of speciation (Carlon and Budd 2002; Hirose et al. 2009). Apart from the differences in colony shape and sclerite features, the discriminating characteristic of the two morphotypes is the presence/absence of the symbiotic algae, which is a determinant difference that strongly affects the physiology and the ecology of the two morphotypes since symbiotic algae play a relevant role in processes such as growth and reproduction in cnidarian species (Sebens 1987).

Phenotypic plasticity in coral and gorgonian species leads to morphological variations as a response to the main environmental conditions (Shaish et al. 2007; Todd 2008). Depth-related differences in colony shape were already reported in the tropical gorgonians *Plexaura homomalla* and *Plexaura flexuosa*, where deep colonies (7-10 m) had longer branches and lower tributary to source ratios than shallower ones (1-4 m) (Brazeau and Lasker 1988), and branches and internode lenghts were significantly longer in the deep morphotypes of the gorgonian *Pseudopterogorgia bipinnata* complex, with a clear gap among morphotypes variances (Sánchez et al. 2007). As it was observed in the *E. singularis* colonies located at 40 m depth, *P. bipinnata* had a characteristic morphotype at certain depths that can co-exist with a morphotype that was not expected to occur in these conditions (Sánchez et al. 2007). The gorgonian *Briareum asbestinum* was morphologically variable among habitats and depths with a larger width to length ratio in the shallow (8 m) than in the deep (33m) colonies, but contrary to the result of the present study, *B. asbestinum* showed shorter sclerites in the shallow than in the deep colonies (West et al. 1993). In general, phenotypic plasticity confers broad adaptability to the range of environmental conditions encountered by sessile organisms (Bradshaw 1965, Smith et al. 2007), providing the capacity for the colony to grow into the most suitable shape for that particular environment (Warner 1996; Marfenin 1997).

The present study is the first one to focus on the "deep morphotype" of *E. singularis* without symbiotic algae since it was described for the first time (Théodor 1969). This previous lack of knowledge was probably due to the bathymetric distribution of this aposymbiontic form mainly concentrated below 35-40 m depth, which made the observation by scuba divers difficult. However, when quantitative remotely operated observations were conducted in the depth range from 40 to 70 m depth, it was found that the "deep morphotype" of *E. singularis* without zooxanthellae was very frequent and abundant, at least in some areas of the Western Mediterranean Sea where it can reach densities to up to 27.5 colonies m-2 (Gori et al. 2011).

COMMENTS

In the submitted version of this work Figures 2, 5, 9, 10, 11, 12, 13, 14, 15 and 17 correspond respectively to the Online Resource 1 to 10. Consequently, Figures 3, 4, 6, 7, 8, 16 and 18 of the present work correspond respectively to Figures 2 to 8 in the submitted version.

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Chapter 3

Size and spatial structure in deep *versus* shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea)

Additional Resource 7

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RESUM

A la Mediterrània occidental, la gorgònia *Eunicella singularis* (Esper, 1791) es troba en altes densitats en els fons sublitorals a profunditats des de 10 fins 70 m. Les colònies superficials d'aquesta espècie tenen zooxanteles simbiòtiques mentre que les gorgònies profundes no. Tot i que el coneixement de l'ecologia de les poblacions de poca profunditat s'ha incrementat durant les últimes dècades, gairebé no hi ha informació sobre l'ecologia de les poblacions del sublitoral profund. Durant l'octubre i el novembre del 2004 al Cap de Creus (42 ° 19'12 "N, 03 ° 19'34" E), una anàlisi dels transectes de vídeo fet per un vehicle operat per control remot va mostrar que a les poblacions de poca profunditat (10-25 m de profunditat) dominen les petites colònies no-reproductives, mentre que a les poblacions sublitoral profundes (50-67 m de profunditat) hi dominen les de grandària mitjana. L'alçada mitjana i màxima de les colònies és major en les poblacions més profundes que també presenten parcel·les de colònies més grans i àrees de cobertura contínua del substrat més amples. La distribució de les colònies és agrupada a vàries escales tant a les poblacions superficials com en les profundes. Aquests resultats suggereixen que els hàbitats poc profunds són adequats per a *E. singularis*, com ho demostra l'alta taxa de reclutament, però les pertorbacions poden limitar o retardar el desenvolupament d'aquestes poblacions en una etapa de maduresa. Això contrasta amb els hàbitats sublitorals profunds on una major estabilitat del medi ambient podria permetre el desenvolupament de poblacions madures dominades per colònies més grans i sexualment madures.

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ABSTRACT

In the Western Mediterranean Sea, the gorgonian *Eunicella singularis* (Esper, 1791) is found at high densities on sublittoral bottoms at depths from 10-70 m. Shallow colonies have symbiotic zooxanthellae which deeper colonies lack. While knowledge of the ecology of the shallow populations has increased during the last decades, there is almost no information on the ecology of the deep sublittoral populations. In October and November 2004 at Cap de Creus (42°19'12" N; 03°19'34" E), an analysis of video transects made by a remotely operated vehicle showed that shallow populations (10-25 m depth) were dominated by small, non-reproductive colonies, while deep sublittoral populations (50-67 m depth) were dominated by medium-sized colonies. Average and maximum colony heights were greater in the deeper populations, with these deeper populations also forming larger patch sizes and more extensive regions of continuous substrate coverage. These results suggest that shallow habitats are suitable for *E. singularis*, as shown by the high recruitment rate, but perturbations may limit or delay the development of these populations into a mature stage. This contrasts with the deep sublittoral habitats where higher environmental stability may allow the development of mature populations dominated by larger, sexually mature colonies.

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INTRODUCTION

In the study of marine hard-bottom communities, limited information is currently available regarding deep nearshore rocky bottoms (Virgilio et al. 2006). One of the main reasons for the scarcity of such studies is that nearshore rocky bottoms have been primarily explored by scuba diving; therefore, sampling effort has mainly focused from surface to a depth of 40 m (Sink et al. 2006; Menza et al. 2008). Furthermore, surveys using remotely operated vehicles (ROVs) and manned submersible vehicles have mostly been carried out below a depth of 150 m (Sink et al. 2006). Consequently, this has led to a significant lack of studies focusing on rocky bottoms at intermediate depths (Sink et al. 2006).

Recently, a few studies using ROVs have provided quantitative information on rocky-bottom communities located at 40-150 m depth. These studies have reported high abundances of many invertebrate species, including corals and gorgonians (Rossi et al. 2008; Bo et al. 2009). In tropical coral communities, it has been reported that many coral species exhibit distributions that encompass both shallow and deep coral reefs (Bongaerts et al. 2010), with live coral cover sometimes greater at depths of 30–60 m than in shallower areas (Menza et al. 2008). Similarly, in the Mediterranean Sea some gorgonian species have been observed to develop dense populations from shallow waters down to 70-90 m depth (Bo et al. 2009; Gori et al. 2011). While knowledge of the ecology of the shallow populations of Mediterranean gorgonians has increased considerably during the last two decades (e.g. Gili and Coma 1998; Rossi et al. 2006; Ribes et al. 2007; Linares et al. 2008), there is almost no information on the ecology of the deep sublittoral gorgonian populations located below 40 m depth.

Gorgonian corals play an important role as ecosystem engineers (Jones et al. 1994) in Mediterranean sublittoral communities, providing biomass and structural complexity (Gili and Coma 1998; Ballesteros 2006). Among Mediterranean gorgonians, the white gorgonian *Eunicella singularis* (Esper, 1791) is one of the most common and abundant species in the Western basin (Carpine and Grasshoff 1975; Weinberg 1976; Gori et al. 2011). The species is long-lived, gonochoric, and reproduces annually in late May-June (Ribes et al. 2007; Gori et al. 2007); it has internal fertilization followed by holoblastic segmentation of the zygote inside the body cavity of the polyps of female colonies (Weinberg and Weinberg 1979). The released planula larvae are on average 2.5 x 0.5 mm in size, and settle within a few days, generally near the parental colony (Théodor 1967; Weinberg and Weinberg 1979). In recent years, climate-induced mass mortalities have affected this species in several locations along the Western Mediterranean Sea but to date these disturbances have not affected the study area, Cap de Creus (Coma et al. 2006; Garrabou et al. 2009). *E. singularis* is widely distributed along the coast of Cap de Creus, where it can be considered the most common and abundant gorgonian species (Gori et al 2011). Along the northern coast of the cape the species occurs in high densities in sublittoral waters ranging from shallow to deep (15-70 m depth, Gori et al. 2011). The species presents two different morphotypes: a “shallow morphotype”, with colonies typically having a candlestick-like shape and a dirty greyish-white colour from the presence of symbiotic algae (Carpine and Grasshoff 1975; Weinberg 1976); and a “deep morphotype” in colonies at > 30-35 m depth, with a more variable colony shape and a bright white colour, as they lack symbiotic algae (Théodor 1969; Weinberg 1976). Research thus far indicates direct transmission of symbiotic algae from the female parent to newly released larvae (Théodor 1969; Weinberg and Weinberg 1979). The concentration of symbiotic algae in colony tissue is lower in *E. singularis* colonies at 35 m than 15 m depths (Ferrier-Pagès et al. 2009), with those deeper than 40 m not containing any symbiotic algae (Gori unpubl data).

Abiotic factors change considerably with depth in the Mediterranean Sea (Garrabou et al. 2002), so shallow and deep sublittoral gorgonian populations will be exposed to different environmental conditions. Shallow habitats are subject to strong hydrodynamic forces (Hiscock 1983; Weinbauer and Velimirov 1996a) and mainly exposed to bidirectional currents (Riedl 1971), whereas deeper nearshore habitats are more affected by unidirectional water movement (Riedl 1971; Hiscock 1983) and largely sheltered from the direct physical damage that may be caused by strong storm-induced waves (Bongaerts et al. 2010). Light intensity decreases exponentially with depth (Drew 1983; Mass et al. 2007), and high irradiance in the summer is conducive to strong stratification of the water column, which can result in severe depletion of suspended materials in shallower waters during the Mediterranean summer (Coma et al. 2000). Overall, variability in environmental conditions is dampened at greater depth in the nearshore bottoms of the Mediterranean Sea (Garrabou et al. 2002) because temperature, currents, and other water column features are more constant below the summer thermocline (Cebrián et al. 1996; Ballesteros 2006; Rossi et al. 2008).

Such differences in the main environmental features could result in differences in the suitability and stability of habitats, thus inducing differences among gorgonian populations at different depths. Both the environmental suitability and the stability of a habitat may be reflected in the size structure of a gorgonian population because the size structure reflects the factors affecting recruitment, growth and mortality rates in a particular habitat for a period of time equal to the longevity of the population (Grigg 1975; Gilmour 2004; Alvarado-Chacón and Acosta 2009). Perturbations, such as severe storms or mass mortalities, may alter the size structure of gorgonian populations, reducing density and causing the loss of large colonies (Lasker 1991; Weinbauer and Velimirov 1996a; Cerrano et al. 2005). These perturbations result in an increase in the proportion of small individuals in the population (Weinbauer and Velimirov 1996a; Gilmour 2004; Cerrano et al. 2005; Bramanti et al. 2009). Differences in maximum gorgonian colony heights in different habitats may be related to storm forces (Lasker and Coffroth 1983; Mitchell et al. 1993; Harmelin and Marinopoulus 1994) since toppling or detachment by strong currents or wave action is considered one of the main causes of mortality of large gorgonian colonies (Grigg 1977; Weinbauer and Velimirov 1996b; Weinberg and Weinberg 1979).

Differences in the main environmental features are also reflected in the spatial structure of sessile organisms such as gorgonian corals. Spatial structure patterns could be used to assess successional stages during the primary succession of sessile organism populations from the colonization of a clear substrate to development into a mature stage (Kershaw 1958; Dale and Blundon 1990). In the early stages of succession, dispersal mechanisms and the availability of free substrata lead to a clumped or random distribution of recruits (Szwagrzyk and Czerwczak 1993; Haase et al. 1997). As succession progresses, reproduction with a phylopatric dispersion of new recruits promotes a clumped distribution of individuals centered around the parents (Kershaw 1959). Intraspecific competition can alter this pattern to a random and ultimately overdispersed distribution of individuals in a mature stage of succession (King and Woodell 1973; Haase et al. 1997). The resultant nonrandom spatial distribution of individuals is defined as spatial pattern; when this nonrandomness exhibits a certain periodicity, the pattern has a scale that corresponds to the average size of regions of high density (patches) and regions of low density (gaps) (Dale and Blundon 1991; Dale 1999). Early stages of primary succession are expected to exhibit spatial pattern on small scales; as succession advances and clumps of individuals grow larger and eventually coalesce, the smallest scales disappear, so that the spatial pattern will occur on increasingly large scales (Yarranton and Morrison 1973; Schaefer 1993).

In this study, for the first time, we compared shallow (10-25 m depth) versus deep (50-67 m depth) sublittoral populations of the two morphotypes of the Mediterranean gorgonian *E. singularis* to address the following questions: (1) Are there differences in the size structure between shallow and deep populations? (2) Are there differences in the spatial structure between shallow and deep populations?

MATERIALS AND METHODS

Study area and sampling procedure

The study area lies along the northern coast of the Cap de Creus (42°19'12'' N; 03°19'34'' E) in the northwestern Mediterranean Sea (Fig. 1a, b). This is a metamorphic rocky coast, with a very articulated morphology showing an alternation of vertical cliffs with smooth ramps. Water temperature in the study area ranges from 10.1ºC to 24.3ºC at 20 m depth, and from 10.7ºC to 21.1ºC at 60 m depth (Gori unpubl data). Due to the general circulation pattern dominated by the Northern current (Millot 1990), the study area is directly exposed to a strong near-bottom current coming from the north (DeGeest et al. 2008), as well as to the main winds and wave action (Ulses et al. 2008). Between October and November 2004, a total of 15 video transects were performed in the study area as part of a larger sampling for characterization of the benthic communities of the Cap de Creus (for details see Gori et al. 2011, the present study area corresponds to sub-areas E and F in that paper). Transects were video-recorded with the ROV Phantom XTL equipped with a Sony FCB S3000P 3CCD (700 horizontal lines) camera, a depth sensor, a compass, and two parallel laser beams, which provided a scale defining the fixed width of the transects (0.5 m) for the subsequent video analysis. Videos were recorded on video tapes in DV format. In each sampled location, the seabed video recording started at the deepest point of the transect (between 29 and 71 in m depth) and proceeded at a constant speed of 0.4 knots toward the shallows until the ROV surfaced close to shore. The videos were transferred from tapes to hard disk, and video analysis was performed with the Final Cut software package (Apple). Sections of these video transects were selected depending on the analysis to be performed: size structure analysis was performed on transect sections with *Eunicella singularis* colonies all perfectly visible and perpendicular to the ROV camera, whereas spatial structure analysis was performed on transect sections where the ROV moved at a constant speed over a continuous rocky bottom and with all the *E. singularis* colony positions clearly identifiable in the video.

Size structure

The size structure was studied by measuring colony heights in 14 different sections of the video transects (1-14 in Fig. 1c, Table 1); seven from shallow waters (11-25 m in depth), and seven from deep sublittoral waters (50-67 m in depth). Colony height was measured with the Carnoy software package (Schols and Smets 2001) using still images extracted from the videos. Since the distance between the two laser beams was used to calibrate extracted images, measurements were performed on still images in which the laser beams were in the same plane as the colony to be measured. A total of 1384 colonies were measured: 636 from the shallow populations and 748 from the deep sublittoral populations.

Spatial structure

The spatial structure was studied in 10 sections of the video transects (A-J in Fig.1c, Table 1); five from shallow waters (14-22 m in depth), and five from deep sublittoral waters (50-60 m in depth). In these sections, the ROV moved over a continuous rocky bottom for approximately 100 or 120 s at a constant speed of 0.4 knots (corresponding to distances of 20 and 25 m respectively). In each of these video transect sections, time spent from the beginning of the section to the crossing of the two laser beams with the base of each gorgonian colony appearing within the central 0.5 m of the video transect was logged. Therefore, time was converted into distance from the beginning of each section by means of the known ROV speed. The positions of 1206 colonies were recorded: 572 from the shallow populations and 634 from the deep sublittoral populations.

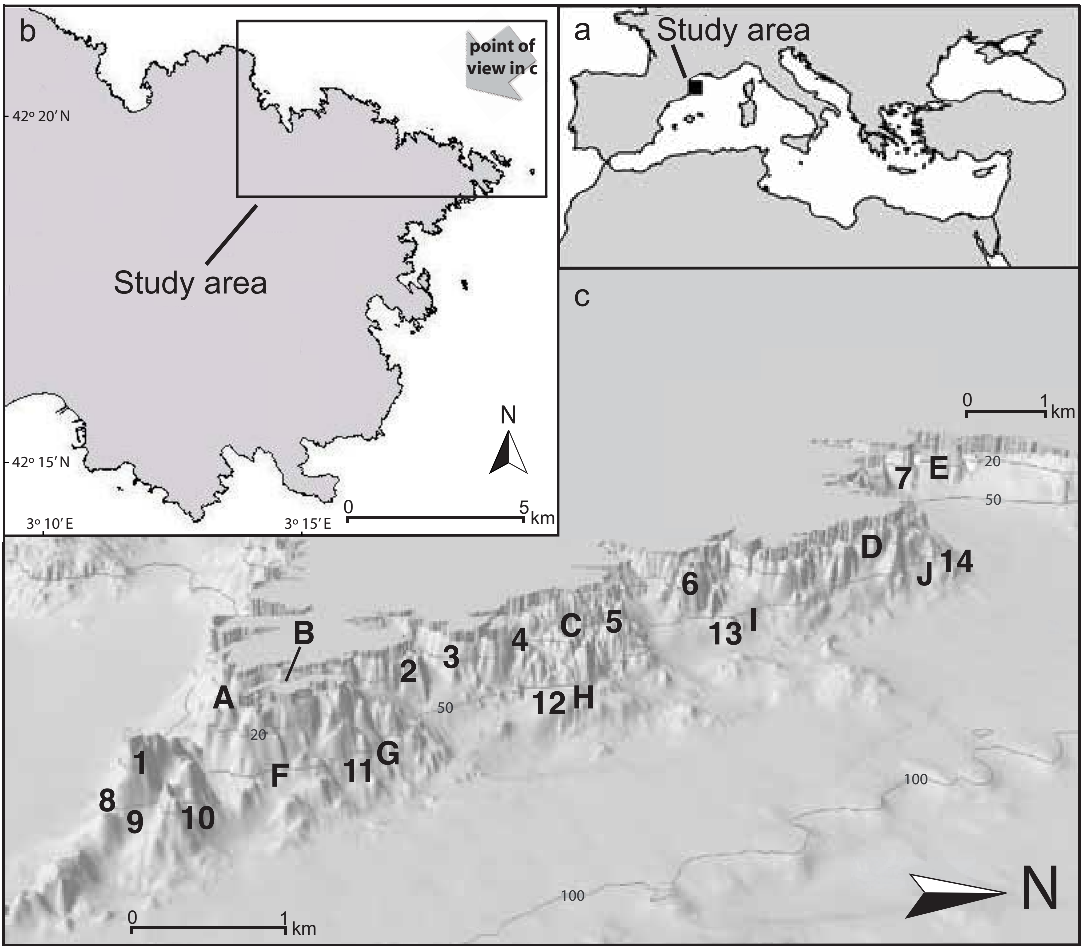

Fig. 1 - Map of study area. *Numbers* (1-14) indicate positions of video transects for size structure analysis; *letters* (A-J) indicate positions of video transects for spatial structure analysis of *Eunicella singularis* shallow and deep populations.

Data analysis

The size structure was analyzed in terms of descriptive statistics using distribution parameters such as skewness and kurtosis. Skewness (g1) is a measure of the symmetry of a distribution using its mean; if skewness is significant, the distribution is asymmetric. Positive skewness indicates the prevalence of small size classes in the population, whereas negative skewness indicates the dominance of large size classes. Kurtosis (g2) is a measure of the peakedness of a distribution near its central mode. A significant value of kurtosis indicates that the variable has longer tails than would be expected for a normal distribution and therefore, the prevalence of a particular size class in the population. Skewness and kurtosis coefficients are significant if g1 per standard error of skewness (SES) or g2 per standard error of kurtosis (SEK) is > 2 (Sokal and Rohlf 1995). Differences in the mean colony height between shallow and deep sublittoral populations were tested using the non-parametric Mann-Whitney U Test as our data did not follow the normality assumptions of parametric tests. To test for differences in the percentage of young colonies

Table 1 - Location, length, area and depth of the analyzed transect sections.

		Transect section	Position		Lenght (m)	Area (m^2)	Depth (m)	
			Start	End			Start	End
Size structure	**Shallow**	1	42°19'16.64"N, 3°19'54.73"E	42°19'17.19"N, 3°19'55.05"E	21	10.5	20	25
		2	42°19'30.03"N, 3°19'10.76"E	42°19'31.94"N, 3°19'12.76"E	77	38.5	16	23
		3	42°19'27.45"N, 3°18'35.19"E	42°19'30.47"N, 3°18'34.26"E	65	32.5	15	21
		4	42°19'44.08"N, 3°18'21.97"E	42°19'44.56"N, 3°18'22.38"E	19	9.5	20	21
		5	42°19'46.86"N, 3°17'52.93"E	42°19'47.60"N, 3°17'54.13"E	47	23.5	15	25
		6	42°20'01.37"N, 3°17'10.77"E	42°20'00.72"N, 3°17'09.97"E	33	16.5	11	15
		7	42°20'09.98"N, 3°15'03.98"E	42°20'10.72"N, 3°15'04.41"E	26	13	18	24
	Deep	8	42°19'05.72"N, 3°19'53.89"E	42°19'05.09"N, 3°19'53.92"E	19	9.5	50	50
		9	42°19'14.93"N, 3°20'00.29"E	42°19'16.15"N, 3°20'03.37"E	81	40.5	57	67
		10	42°19'18.11"N, 3°19'55.56"E	42°19'19.45"N, 3°19'56.40"E	46	23	52	57
		11	42°19'32.80"N, 3°19'13.71"E	42°19'33.32"N, 3°19'14.21"E	17	8.5	53	56
		12	42°19'52.67"N, 3°18'29.03"E	42°19'55.36"N, 3°18'31.21"E	92	46	54	59
		13	42°20'07.45"N, 3°17'19.00"E	42°20'09.14"N, 3°17'21.26"E	79	39.5	53	64
		14	42°20'34.27"N, 3°15'36.50"E	42°20'36.33"N, 3°15'35.70"E	65	32.5	50	60
Spatial structure	**Shallow**	A	42°19'16.44"N, 3°19'35.59"E	42°19'16.85"N, 3°19'36.62"E	25	12.5	19	21
		B	42°19'19.18"N, 3°19'11.21"E	42°19'19.72"N, 3°19'10.47"E	25	12.5	16	18
		C	42°19'46.70"N, 3°17'52.72"E	42°19'47.24"N, 3°17'53.51"E	25	12.5	14	19
		D	42°20'26.92"N, 3°16'04.82"E	42°20'27.35"N, 3°16'05.28"E	20	10	15	18
		E	42°20'10.12"N, 3°15'04.01"E	42°20'10.82"N, 3°15'04.43"E	25	12.5	15	22
	Deep	F	42°19'24.00"N, 3°19'42.20"E	42°19'24.10"N, 3°19'43.27"E	25	12.5	58	60
		G	42°19'32.89"N, 3°19'13.69"E	42°19'33.32"N, 3°19'14.20"E	20	10	50	51
		H	42°19'52.65"N, 3°18'29.04"E	42°19'53.23"N, 3°18'29.48"E	20	10	55	56
		I	42°20'07.68"N, 3°17'19.31"E	42°20'08.26"N, 3°17'20.11"E	25	12.5	57	60
		J	42°20'35.81"N, 3°15'35.90"E	42°20'36.56"N, 3°15'35.62"E	25	12.5	57	60

between shallow and deep sublittoral populations, a non-parametric Kolmogorov-Smirnov two-sample test was applied. We considered as young colonies the smallest colonies that could be distinguished using the video analysis (2-5 cm in height). These are non-reproductive colonies (Ribes et al. 2007) that still have no ramifications and are <10% of the maximum height documented for the species (Weinberg 1976; Weinberg and Weinberg 1979). These statistical analyses were performed using Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA).

The spatial structure was analyzed by applying spatial statistics with the Passage 2.0 software package (Rosenberg 2008). Adult and young colony distributions along each transect section were displayed in density plots. Each transect section was transformed into a string of contiguous quadrats (0.2 x 0.5 m), and the number of adult and young colonies inside each quadrat counted. The mean colony density along each transect, the percentage of occupied quadrats (occupancy), and the colony density in the occupied quadrats were tested for differences between shallow and deep sublittoral populations with a non-parametric Kolmogorov-Smirnov two-sample test. The significance of deviation from a random distribution of the gorgonian colonies was analyzed with the one-dimensional version of Ripley's K-function second-order spatial statistic, which was plotted as an L-function ($L(t) = t – K(t) / 2$) (Ripley 1976;

Table 2 - Size structure characteristics of studied populations of *Eunicella singularis*: colony height, skewness (g1) and kurtosis (g2).

	Population	Colonies	Height (cm)			Skewness			Kurtosis		
			Mean	SD	Max	g1	SE g1	Sig g1 (>2)	g2	SE g2	Sig g2 (>2)
Shallow	1	93	7.3	4.7	27.2	1.17	0.25	4.66	2.18	0.50	4.41
	2	119	14.9	7.9	37.6	0.30	0.22	1.35	-0.41	0.44	-0.93
	3	74	11.6	5.7	26.4	0.41	0.28	1.48	-0.42	0.55	-0.76
	4	104	9.8	5.7	26.2	0.73	0.24	3.10	0.06	0.47	0.14
	5	52	8.0	6.7	29.3	1.42	0.33	4.29	1.63	0.65	2.51
	6	128	9.5	6.8	30.4	0.92	0.21	4.28	0.14	0.42	0.32
	7	66	7.5	8.3	30.7	1.37	0.29	4.64	0.64	0.58	1.10
Deep	8	111	15.2	7.4	32.4	0.27	0.23	1.16	-0.63	0.46	-1.39
	9	114	13.2	5.3	26.2	0.07	0.23	0.30	-0.70	0.45	-1.55
	10	71	11.7	7.3	31.2	0.32	0.28	1.13	-0.62	0.56	-1.10
	11	114	9.6	6.8	47.1	2.09	0.23	9.22	7.89	0.45	17.57
	12	106	12.7	6.5	27.0	0.12	0.23	0.53	-0.81	0.47	-1.75
	13	134	14.7	8.4	32.2	0.00	0.21	0.01	-0.99	0.42	-2.38
	14	98	9.3	6.6	32.9	1.09	0.24	4.47	0.93	0.48	1.92

Haase et al. 1996; Fortin and Dale 2005). In Ripley's K-function, the number of neighboring colonies within a distance (t) of each coral colony was counted, and an edge correction was applied to colonies near the transect border (Fortin and Dale 2005). To test the null hypothesis that there was a complete spatial randomness in gorgonian colony distributions along the transect sections, a number of randomized transects were generated for comparison by randomly repositioning all of the observed colonies along each transect. For statistical significance (95% confidence interval) the values at the limit of 2.5% tails of 999 of these randomizations were used. If the sample statistic was found within the bounds of the confidence interval at any point, then the null hypothesis could not be rejected; a significant positive deviation of the sample statistic indicates overdispersion of the colonies, whereas a significant negative deviation indicates a clumped distribution (Fortin and Dale 2005). To determine the scale of the spatial pattern, three-term local quadrat variance (3TLQV) was calculated (Hill 1973; Dale 1999). The 3TLQV examines the average of squared differences among trios of adjacent block size of quadrats, by subtracting twice the total of the middle block from the sum of the two on either side. The resulting variance is plotted as a function of block size, and peaks are indicative of a pattern at that scale (Dale 1999).

RESULTS

Size structure

The mean (± SD) and maximum colony heights in the shallow populations (10.2 ± 7.1 cm and 37 cm, respectively) were significantly less than in the deep populations (12.5 ± 7.3 cm and 47 cm, respectively) (Mann-Whitney U test, $Z = 6.387$, $p < 0.05$). Analysis of the size structure showed two types of size frequency distributions. Most shallow populations showed an asymmetric distribution, with a high proportion of small colonies (<10 cm) and a lower proportion of larger size classes. Indeed, the skewness coefficients for shallow populations indicated that most of the size distributions were positively skewed (5 out of 7), displaying the largest peak in the first size class (0-10 cm). In contrast, most of the deep populations were symmetric (5 out of 7), displaying a unimodal and bell-shaped distribution with a peak in the 10-20 cm

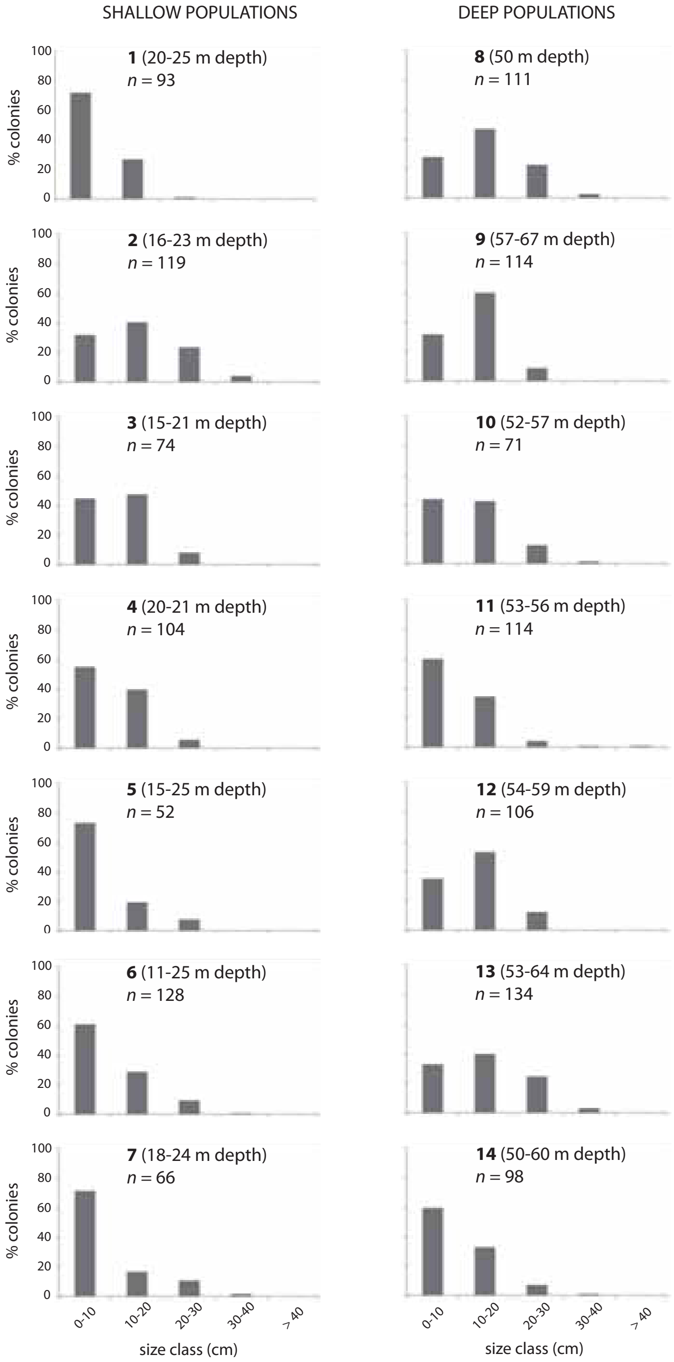

Fig. 2 - Size-frequency distribution of shallow (1-7) and deep (8-14) *Eunicella singularis* populations (*n* = number of colonies).

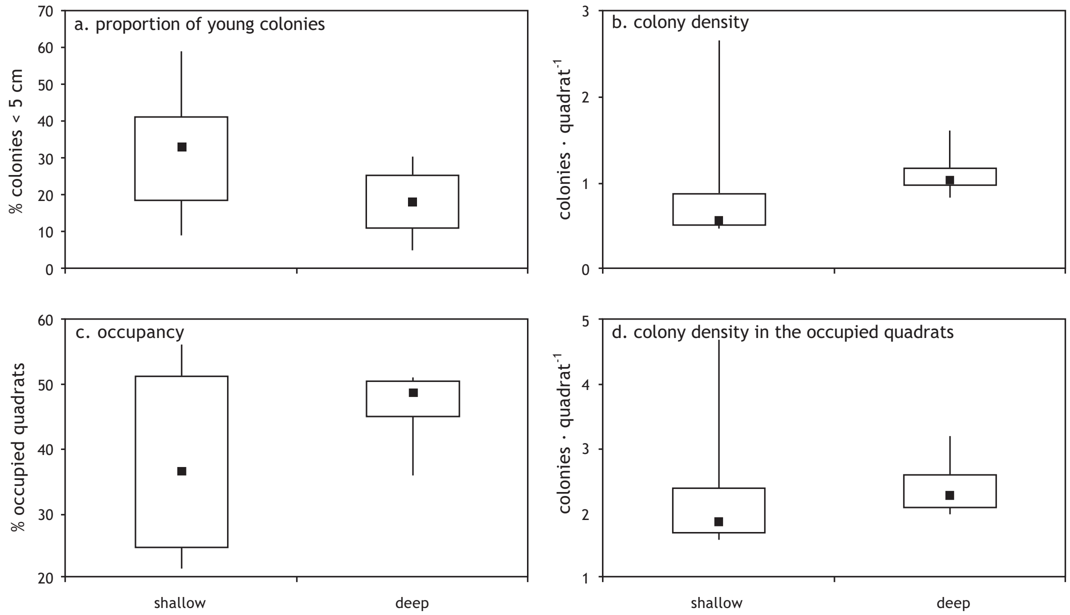

Fig. 3 - Comparison between shallow and deep *Eunicella singularis* populations. *Black square* indicates median value, *box* indicates first and third quartiles, and *line* indicates range between minimum and maximum values.

size class, and the rest (2 out of 7) were positively skewed (Fig. 2, Table 2). Most of the shallow and deep populations showed no significant kurtosis (10 out of 14). Among the rest of the populations, two shallow populations (1 and 5 in Fig. 2) displayed positive kurtosis (leptokurtic), indicating that they were slightly more peaked or overcentralized than normal distributions, while one deep population was leptokurtic (11 in Fig. 2) and another (13 in Fig. 2) was platykurtic (with a negative kurtosis coefficient), displaying a lower and wider peak around the mean (Table 2). The percentage of young colonies in the shallow populations was higher than in the deep populations. However, the results were not significantly different due to the high variability among shallow populations (Kolmogorov-Smirnov test, $p > 0.10$, Fig. 3a).

Spatial structure

The mean colony density along each transect, the percentage of occupied quadrats (occupancy), and the colony density in the occupied quadrats was highly variable in the shallow populations. These parameters were more constant in the deep populations, with higher values compared to the shallow ones. However, due to the high variability in the shallow populations, the comparison between shallow and deep populations were not significantly different (Kolmogorov-Smirnov test, $p > 0.10$, Fig. 3b, c and d, Fig. 4). Ripley's K analysis revealed a significantly clumped distribution of *Eunicella singularis* colonies at all scales in all of the transects with the exception of transect J, in which the distribution changed from clumped to random above a 1.1 m scale (Fig. 5). The 3TLQV analyses showed that all of the populations (except C) displayed spatial pattern on a small scale, between 0.4 and 1.0 m in shallow populations and between 0.4 and 1.4 m in deep sublittoral populations (Fig. 6). Spatial pattern on a second and sometimes a third main scale was found in shallow populations between 3.8 and 7.6 m (A, B, D and E in Fig. 6); only one shallow population displayed spatial pattern on a second scale at a distance greater than the maxi-

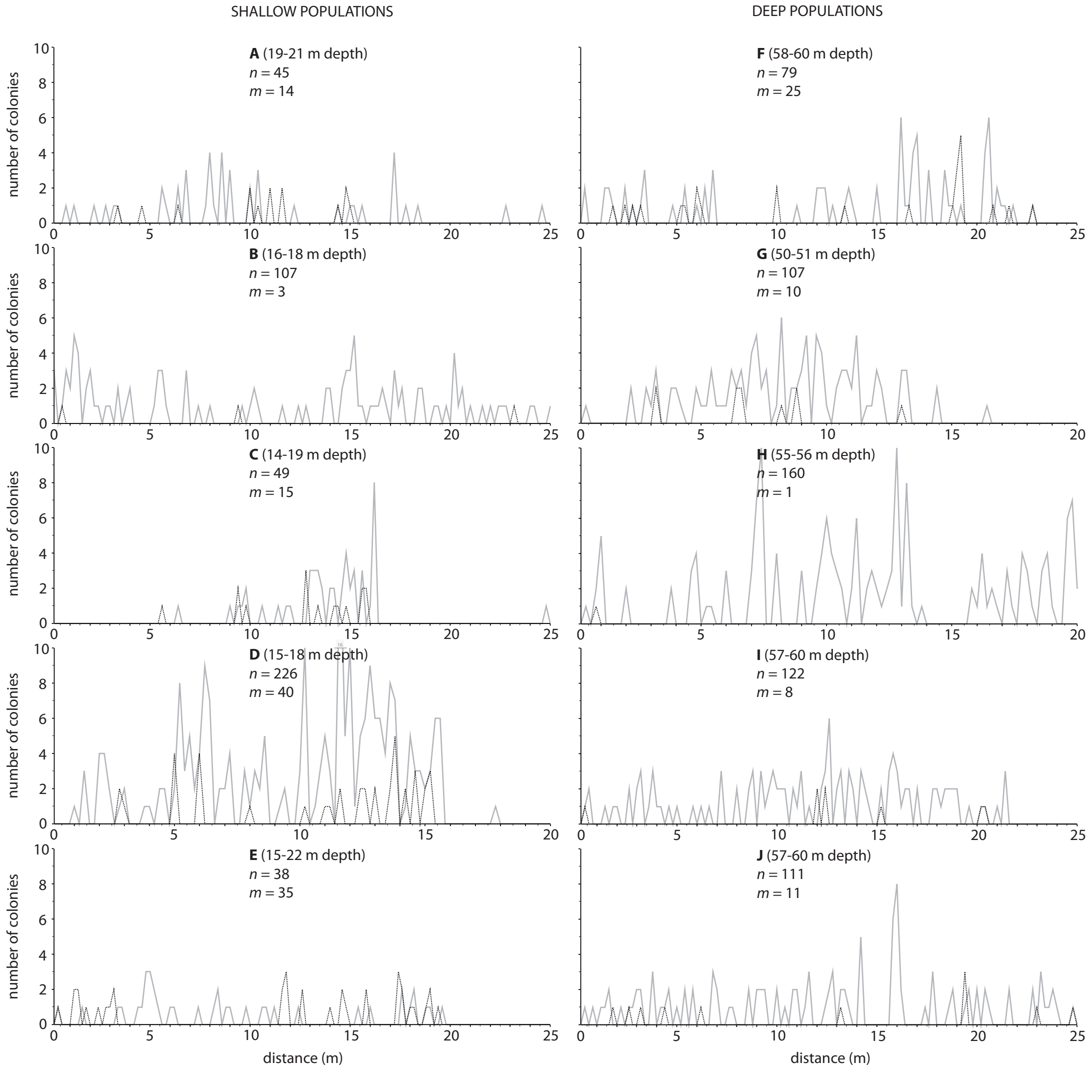

Fig. 4 - Density plots of shallow (A-E) and deep (F-J) *Eunicella singularis* populations. *Grey line* refers to adult colonies, and *black line* refers to young colonies (colonies <5 cm in height) (*n* = number of adult colonies, *m* = number of young colonies).

mum explored scale (C in Fig. 6). Conversely, spatial pattern on a second main scale was found in only one deep population at 7 m (F in Fig. 6), while in all of the other deep populations, spatial pattern on a second scale was always found at a distance geater than the maximum explored scale (G, H, I and J in Fig. 6).

DISCUSSION

Shallow *Eunicella singularis* populations had lower average and maximum colony heights and a higher percentage of young colonies than deep sublittoral populations. Shallow populations were dominated by the smallest size class (0-10 cm), which contrasted with the predominance of

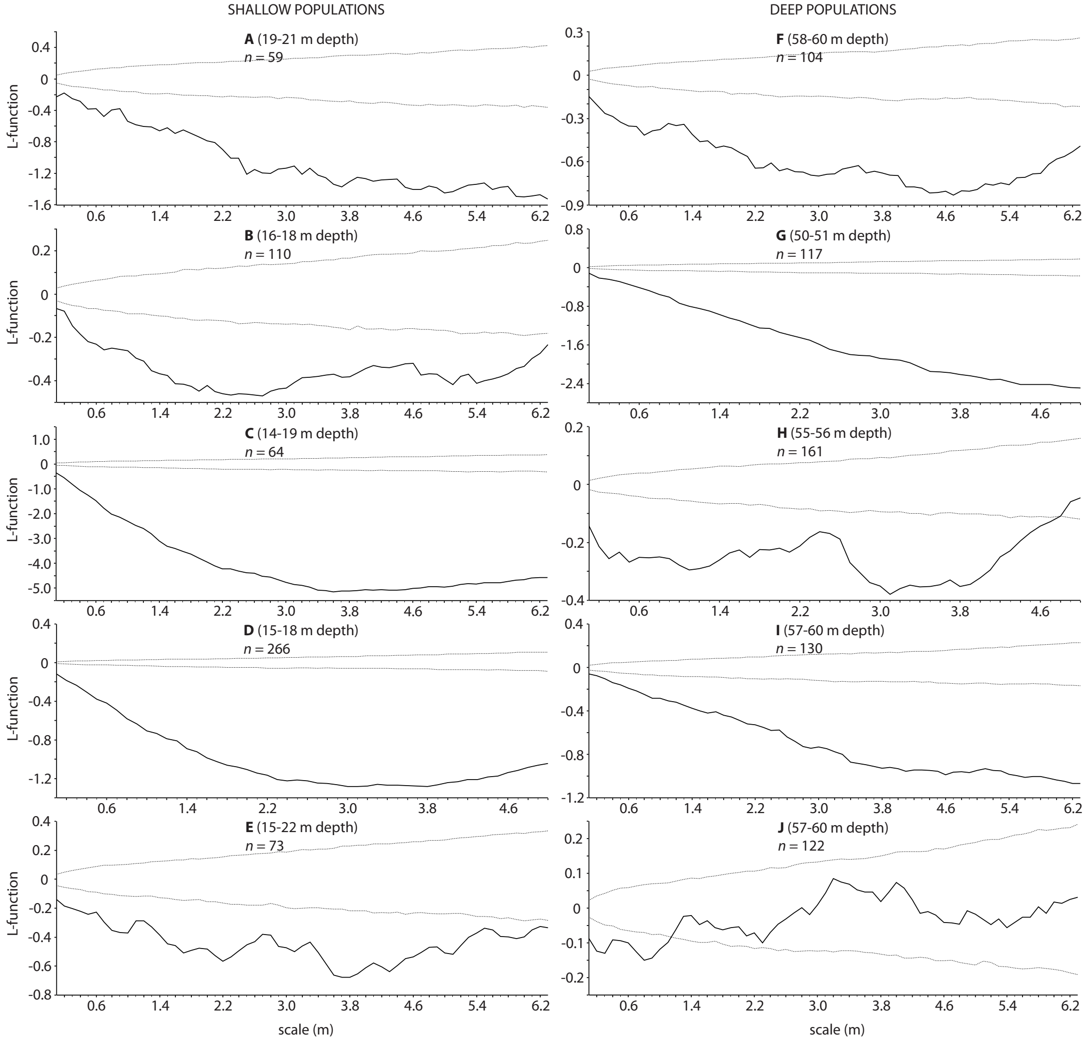

Fig. 5 - L-function (Ripley's K-function) of shallow (A-E) and deep (F-J) *Eunicella singularis* populations. Values below 95% confidence interval (*dotted lines*) indicate a statistically significant clumped distribution of colonies; values within the confidence interval indicate a random distribution of colonies; values over the confidence interval indicate a statistically significant overdispersed distribution of colonies (*n* = number of colonies).

the second size class (10-20 cm) in deep populations. The video analysis method allowed extensive sampling over the entire bathymetrical distribution of the species, but could have entailed a loss of accuracy in the measurements. However, this methodological constraint equally affected shallow and deep populations, so would not affect their comparison. The size structure of the shallow populations in this study agrees with the results of a previous investigation of shallow populations of this gorgonian species (Linares et al. 2008). The previous study showed a size structure dominated by small and non-reproductive colonies in almost all of the studied populations (21) along more than 600 km of coast line. This sugests that most shallow populations of this gorgonian are not recruitment limited (Linares et al. 2008). A continuous high

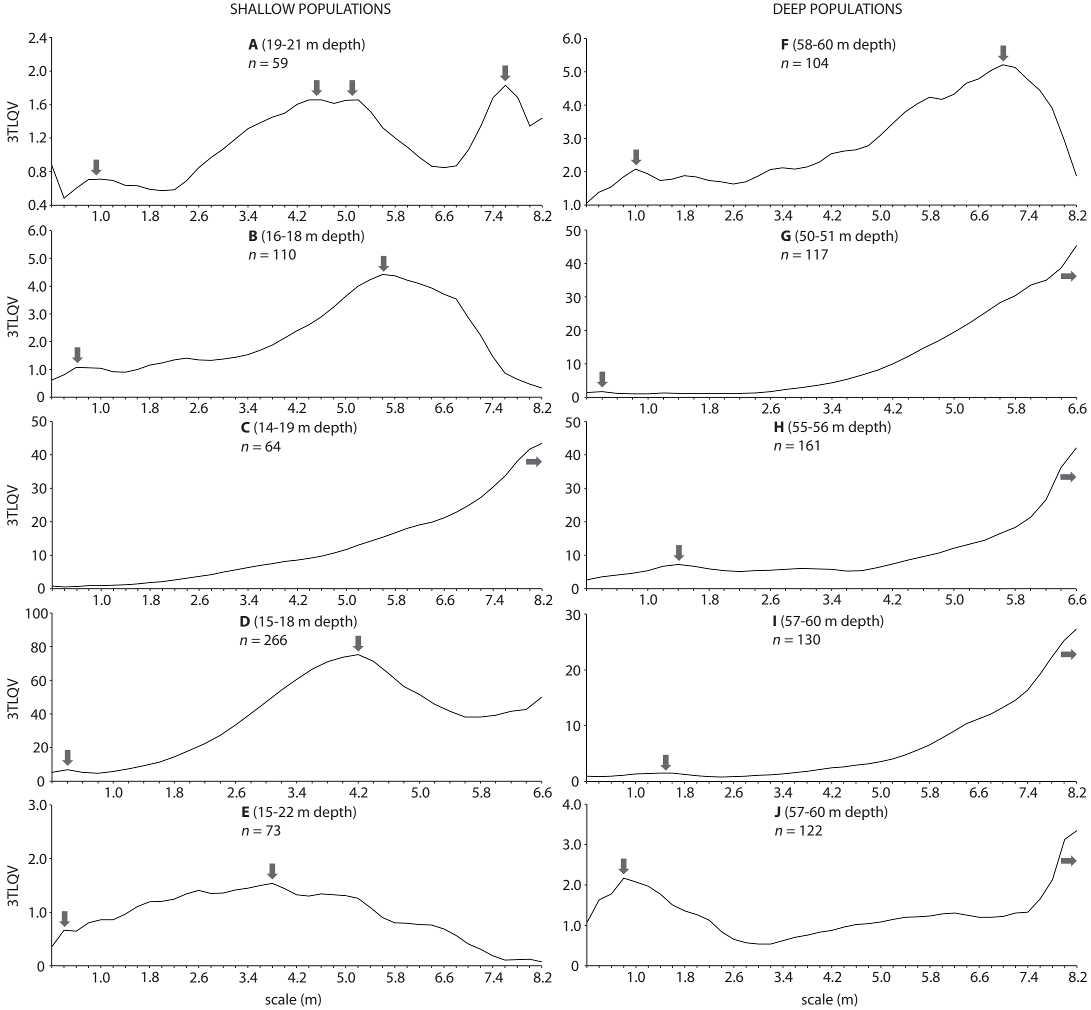

Fig. 6 - Three-term local quadrat variance (3TLQV) of shallow (A-E) and deep (F-J) *Eunicella singularis* populations as a function of scale (block size). *Arrows* indicate scales of spatial pattern (*n* = number of colonies).

recruitment rate over time is considered characteristic of a suitable habitat and will result in a population size structure dominated by small colonies, thus indicating expanding populations (Grigg 1975). In contrast, the deep sublittoral populations of the "deep morphotype" of *E. singularis* were mainly in a more mature stage of development, with dominance of medium-sized colonies and a higher presence of large colonies compared to the shallow populations. This size structure is considered the result of past pulses in recruitment (Grigg 1977; Lasker 1991) and has commonly been reported for gorgonian species from tropical waters (Jordán-Dahlgren 1989; Yoshioka 1994; Toledo-Hernández et al. 2007), deep waters (Mortensen and Buhl-Mortensen 2005; Watanabe et al. 2009), and for other Mediterranean gorgonian species, such as *Paramuricea clavata* (Harmelin and Garrabou 2005; Cupido et al. 2008; Linares et al. 2008) and *Eunicella cavolinii* (Weinbauer and Velimirov 1996a, 1996b). Changes in the fitness of the organisms over time or among habitats could determine differences in larval production and/or the survival of recruits (Brazeau and Lasker 1992; Yoshioka 1994; Weinbauer and Velimirov 1996b). The consequences of these changes may include high recruitment variability in time and space (Lasker 1990; Yoshioka 1996; Dunstan and Johnson 1998). However, long-lived gorgonian species have been shown to be buffered against such fluctuations in recruitment, even when normally low rates alternate with sporadic high peaks (Garrabou and Harmelin 2002; Linares et al. 2007). In long-lived species, recruitment variation is of limited importance, while the survival of large colonies is a key factor for population persistence (Gotelli 1991; Lasker 1991; Linares et al. 2007). Large gorgonian colonies contribute disproportionally to gamete production (Coma et al. 1995; Ribes et al. 2007), thus determining how long a population will be persist between recruitment events (Lasker 1990; Garrabou and Harmelin 2002; Linares et al. 2007).

Water movement induced by wave action decreases by one order of magnitude going from 20 m to 60 m depth (Hiscock 1983; Garrabou et al. 2002). This decrease could explain the larger colony sizes in the deep populations of *E. singularis* in comparison to shallow ones (Weinberg and Weinberg 1979), as has been previously reported for tropical gorgonian species (Birkeland 1974; Jordán-Dahlgren 1989) and for the other Mediterranean gorgonian species *P. clavata* (Harmelin and Marinopoulos 1994; Linares et al. 2008). Very strong water movements represent the most important cause of mortality in large gorgonian colonies, causing colony detachment or toppling (Birkeland 1974; Yoshioka and Yoshioka 1991; Weinbauer and Velimirov 1996b). Up to a certain size, colonies can withstand strong water movement. Whenever this size is exceeded, very strong currents or heavy swells may detach these larger colonies (Kinzie 1974; Grigg 1977; Weinberg and Weinberg 1979).

The occupancy of substrata was very variable from one shallow population to another, and was more constant and generally higher in the deep sublittoral populations. Spatial pattern on a small scale was present in all the populations independent of depth. The presence of this scale was probably due to the short larval dispersion of *E. singularis* (Théodor 1967; Weinberg and Weinberg 1979) and the resulting phylopatric distribution of recruits. Spatial pattern on a second and sometimes a third scale appeared in all of the shallow populations, whereas in the deep populations it was almost always greater than the maximum studied spatial scale. This second scale could be the result of the coalescence of patches of colonies, with higher values indicating larger patches of gorgonian colonies and a more continuous coverage of the rocky bottoms in the deep populations compared to the shallow populations. In contradiction to coral and gorgonian species that showed an overdispersed (Stimson 1974; Endean et al. 1997) or random distribution of their colonies (Abel et al. 1983; Mistri 1995), we found *E. singularis* colonies to be clumped at all scales in both shallow and deep populations, thus indicating no competition among colonies at the spatial scales examined (Dale 1999; Mortensen and Buhl-Mortensen 2004; Orejas et al. 2009). Such a clumped distribution is in agreement with the results from several studies on tropical and deep-sea gorgonian species (Yoshioka and Yoshioka 1989; Orejas et al. 2002; Mortensen and Buhl-Mortensen 2004). It might be assumed that from an initial random distribution of new propagules that invade an opening homogeneous substrata (Kershaw 1958), a clumped distribution of colonies could originate from the phylopatric dispersion of the newly produced larvae, especially for brooder species such as *E. singularis* (Weinberg and Weinberg 1979). Spatial aggregation caused by seed distribution close to the parents seems to be the rule in many vegetation types (Kershaw 1958; Lee 1993), and phylopatric distribution of larvae has been reported for many benthic invertebrate species (Jackson 1986; Miller 1998). In the shallow populations, possible competition with fast growing algae and mortality induced by strong water movements may influence the spatial structure, causing less continuity of the gorgonian coverage of the substrate and smaller gorgonian patches, which result in smaller secondary scales in the spatial pattern. Conversely, in deep sublittoral populations the absence of fast growing algae and strong water movement may provide enough time for the gorgonian populations to develop a more uniform coverage of the substrate.

In the Mediterranean Sea, water stratification during the summer clearly distinguishes shallow waters with low food availability from deeper waters with higher food availability for suspension feeders (Coma et al. 2000; Rossi and Gili 2005). It has been demonstrated that the Mediterranean *Corallium rubrum* colonies below the summer thermocline display higher and more continuous annual prey capture rates than shallow colonies (Tsounis et al. 2006), which results in greater energy storage in deeper populations than in shallower ones (Rossi and Tsounis 2007). However, the symbiosis of the “shallow morphotype” colonies of *E. singularis* with algae may alter this situation, enabling the development of dense populations in shallow habitats relying mainly on the symbiotic relationship with zooxanthellae during summer stratification of the water column, and captured zooplankton and particulate organic matter (POM) from fall to spring. Despite the lack of symbiotic algae in the “deep morphotype” colonies, this species can also develop dense populations in deeper habitats in which the colonies may rely on more continuous zooplankton and POM availability throughout the year.

In summary, *E. singularis* showed differences in both size and spatial structure between shallow and deep sublittoral populations. This result highlights the ability of this gorgonian species to establish and develop dense populations under different environmental conditions. Shallow habitats seem to be particularly suitable for this gorgonian species as evidenced by the high recruitment rate, but strong perturbations may limit or delay the development of the shallow populations into a more mature stage. In contrast, deep sublittoral habitats seem to be more stable, allowing the development of mature populations, mainly composed of sexually mature colonies.

COMMENTS

In the published version of this work, Table 1 and Figure 4 correspond respectively to the Online Resource 1 and 2. Consequently, Table 2, Figures 5 and 6 of the present work correspond respectively to Table 1, Figures 4 and 5 in the published version.

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Chapter 4

Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea)

Coral Reef (submitted)

RESUM

Les diferències experimentades en les condicions ambientals per les poblacions de gorgònies localitzades per sobre i per sota de la termoclina estival, poden afectar el seu cicle reproductiu i la seva ecològia tròfica.Pero, tot i les marcades diferències en la temperatura de l'aigua, els cicles reproductius d'una població superficial (20 m) i una profunda (60 m) de la gorgonian *Eunicella singularis* (Esper, 1791) resulten estar gairebé sincronitzats. Això suggereix que altres factors, a més de la temperatura, juguen un paper determinant en el control del cicle reproductiu d'aquesta espècie. L'emmagatzematge d'energia sota forma de lipids va mostrar una marcada estacionalitat en la població de poca profunditat amb valors més alts durant l'estiu; per contra, la concentració de lípids va ser menor i més constant en la població de profunditat. L'anàlisi dels marcadors tròfics també mostra una forta estacionalitat en la població de poca profunditat, mentre que apunta a una major uniformitat en la població profunda. La composició isotòpica de les colònies de poca profunditat és similar als valors observats en espècies de suspensívors passius amb algues simbiòtiques, mentre que les poblacions profundes han mostrat valors més semblants als observats en filtradors suspensívors asimbiòtics. Aquests resultats sugereixen que l'*E. singularis* depèn gairebé únicament, en les poblacions de poca profunditat, de la seva font energètica autotròfica, almenys durant l'estiu; mentre que les poblacions més profundes semblen adaptar-se a fonts d'alimentció heteròtrofiques.

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ABSTRACT

Differences in environmental conditions experienced by gorgonian populations located above and below the summer thermocline, may affect their reproductive cycle and trophic ecology. However, the reproductive cycle of a shallow (20 m depth) and a deep (60 m depth) population of the gorgonian *Eunicella singularis* (Esper, 1791) turned out to be almost synchronized despite the marked differences in water temperature. This suggests that other factors besides temperature play a role in determining the timing of the reproductive cycle of this species. The energy storage showed a marked seasonality in the shallow population, with highest values during the summer. Conversely, it was lower and more constant in the deep population. The analysis of trophic markers also showed a seasonality in the shallow population and a major uniformity in the deep one. Isotopic composition of the shallow colonies was similar to values observed for passive suspension feeders with symbiotic algae, whereas the deep colonies showed values close to asymbiotic passive suspension feeders that mainly feed on microzooplankton and particulate organic matter. These result suggested that *E. singularis* rely almost solely on its autotrophic source of energy in the shallow populations, at least during summer, whereas the deeper colonies rely on a heterotrophic food.

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INTRODUCTION

The study of spatial variability in the ecology of sessile benthic suspension feeders is becoming increasingly important to understand the potential adaptability of species to local conditions, and all in all, to achieve a complete understanding of their ecology and the role they play in marine benthic ecosystems. Both the ecology and physiology of these organisms are strongly dependent upon the environmental features, such as water temperature, currents, light and quantity and quality of available food (Gili and Coma 1998; Gardner 2000; Rossi et al. 2006). In coastal areas, these factors may change considerably with depth, even among close locations (Garrabou et al. 2002; Rossi et al. 2003). Shallow bottoms are highly influenced by strong hydrodynamic forces and bidirectional currents, whereas deeper sublittoral bottoms are more affected by unidirectional water movements (Riedl 1971) and are largely sheltered from the direct physical damage that may be caused by strong storm-induced waves (Hiscock 1983; Bongaerts et al. 2010). Light intensity decreases exponentially with depth (Drew 1983; Mass et al. 2007), and in temperate seas high irradiance during summer originate a strong water column stratification, which can result in severe depletion of suspended material in shallower waters (Coma et al. 2000; Rossi and Gili 2009a). Such depth related differences in main environmental features may strongly influence feeding (Lasker and Coffroth 1983; Tsounis et al. 2006a), reproduction (Grigg 1977; Weinber and Weinber 1979; Benayahu and Loya 1983, 1986; Rinkevich and Loya 1987; Tsounis et al. 2006b), growth, mortality and energy storage capabilities (Rossi and Tsounis 2007) of benthic suspension feeders, even over the tight depth range within the surface and 40 m depth. Conversely, the study of the variabilty of the biological processes of benthic suspension feeders over larger depth ranges has been rather neglected.

Marine hard-bottom communities located at intermediate depths, particularly coastal rocky bottoms in the 40-150 m depth range, have received relatively little attention because they lie below scuba depth (Menza et al. 2008; Rooney et al. 2010), and most submersible-based research was traditionally conducted at depths below 150 m (Sink et al. 2006; Virgilio et al. 2006; Hinderstein et al. 2010). Research in tropical costal areas has been mainly focused on coral reefs within the depth range of traditional scuba diving (Menza et al. 2008; Rooney et al. 2010), and much of our understanding of coral reef ecology in general is based on these relatively shallow depths (Menza et al. 2008). Far less is known about the mesophotic zone (Menza et al. 2008), defined as the deeper part of the photic zone in which light-dependent coral communities develop (Ginsburg 2007). This zone ranges between 30-40 m depth to the deeper part of the photic zone, which varies by location and extends to over 150 m in some regions (Hinderstein et al. 2010). Overall, this depth range accounts for two-thirds of the total depth range of zooxanthellate coral environments (Pyle 1996, 2000; Feitoza et al. 2005), but to this date it has remained largely unexplored (Bongaerts et al. 2010). Enabled by advanced technologies, mesophotic coral ecosystems studies are revealing extensive, productive and rich communities, which differ significantly from their shallow-water counterparts (Bongaerts et al. 2010; Kahng et al. 2010), and the processes occurring in the mesophotic zone might have a global relevance that has yet to be understood (Buesseler et al. 2007; Hinderstein et al. 2010). In a similar way, research on Mediterranean sublittoral hard-bottom communities has been mainly focused on bottoms within scuba diving depth (Virgilio et al. 2006; Rossi et al. 2008), and only recently, a few studies using Remotely Operated Vehicles (ROVs) have provided quantitative information on rocky-bottom communities located 40-150 m deep. These studies have reported dense populations of corals and gorgonian dwelling on sublittoral bottoms as deep as 100 m (Rossi et al. 2008; Bo et al. 2009, 2011; Gori et al 2011a).

Gorgonians are among the main structural species (*sensu* Jones et al. 1994) of the

Mediterranean coralligenous and precoralligenous communities (Gili and Ros 1985; Harmelin 1995; Ballesteros 2006), the diversity and richness in animal species of which have been compared to those of the tropical coral reefs (Ros et al. 1985; Ballesteros 2006). Gorgonians represent the most erect stratum of this community (Gili and Ros 1984), where they can develop very dense populations (Harmelin and Garrabou 2005) providing biomass and structural complexity (True 1970). Among Mediterranean gorgonians, the white gorgonian *Eunicella singularis* (Esper, 1791) is one of the most common and abundant species in the Western Mediterranean (Carpine and Grasshoff 1975; Weinberg 1976; Gori et al. 2011a). This species is long-lived, gonochoric, and its shallow populations reproduce annually in late May and June (Ribes et al. 2007; Gori et al. 2007); it is an internal brooder species (Weinberg and Weinberg 1979), and the planula larvae settle within a few days, generally near the parental colony (Théodor 1967; Weinberg and Weinberg 1979). It has been recently documented that the shallow populations of this species are dominated by small non reproductive colonies (Linares et al. 2008; Gori et al. 2011b), which contrasted with the dominance of medium-sized colonies and the presence of larger gorgonian patches in the populations located at 60 m depth (Gori et al. 2011b). Depth related morphological variation has been described wherein two morphotypes exist above and below the summer thermocline (Gori et al. submitted). These morphotypes differ not only in colony shape and sclerites features, indeed the most important discriminating characteristic between the two morphotypes is the presence of zooxanthellae in the shallow morphotype, and its absence in the deep morphotype (Théodor 1969; Gori et al. submitted). This is a significant difference that strongly affects their physiology and ecology since the presence of symbiotic algae play a relevant role in the trophic ecology of passive suspension feeders (Fitt and Pardy 1981; Mass et al. 2007; Einbinder et al 2009), and strongly influences processes such as growth and reproduction of cnidarian species (Sebens 1987). A comprehensive study of the reproduction and trophic ecology of *Eunicella singularis* in shallow versus deep sublittoral populations, involve the combination of several approaches and methodologies. In this work, we combined the study of the reproductive cycle (Coma et al. 1995, Ribes et al. 2007; Gori et al. 2007; Rossi and Gili 2009b), the biochemical balance (Rossi et al. 2006; Rossi and Tsounis 2007), the trophic markers such as fatty acids (Rossi et al. 2008b; Soler-Membrives et al. 2011; Tolosa et al. 2011) and stable isotope composition (Carlier et al 2007a; Treignier et al. 2009), in *E. singularis* shallow (20 m depth) and deep (60m depth) sublittoral populations in order to ascertain similarities and differences in: (1) their reproductive timing and gonadal output, (2) their energetic balance, and (3) their main food sources.

MATERIALS AND METHODS

Study area and sampling procedure

Two populations of *Eunicella singularis* were studied on the eastern side of the Cap de Creus peninsula (42º18’44’’ N; 003º19’05’’ E), in the north-western Mediterranean Sea (Fig. 1). The first population was located between 18 and 20 m depth, while the second one was located between 55 and 60 m depth. Gorgonian colonies were randomly sampled by scuba diving every month from June 2009 to July 2010; due to bad weather conditions samples lack from October and December 2009, and from February and March 2010. *E. singularis* colonies between 20 and 30 cm (± 0.5 cm) of maximum height (distance from the base to the farthest point) were sampled (sexually mature colonies according to Ribes et al. 2007). During each sampling a fragment of a primary branch from each of 15 different colonies from each population were randomly collected. A total of 150 branches from each population were collected. Branches were divided in two portions, one fixed in formalin (10%) to study the reproductive cycles, and the other portion frozen for biochemical analysis. Once in laboratory the portions stored for biochemical testing were lyophilized (16 hours at -110 ºC with a 100 mbar pressure) and stored at -20 ºC until analysis were undertaken.

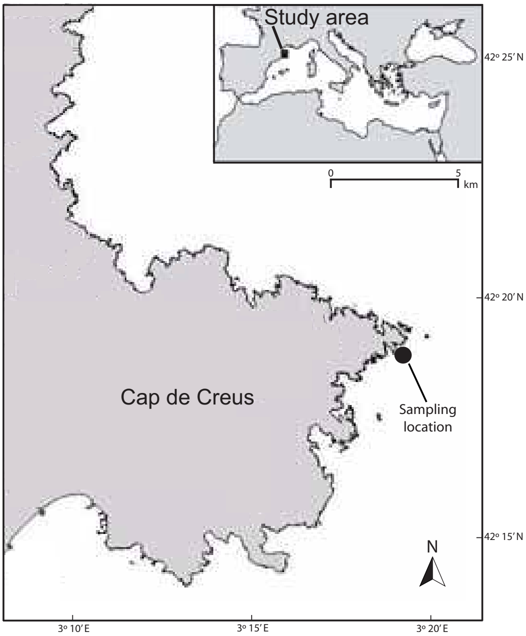

Fig. 1 - Map of study area with the indication of the position of the sampling location along the Cap de Creus coast.

Gonadal development cycle

The reproductive state of the two studied populations' colonies was determined monthly by observing the presence of mature gonads inside the gorgonian polyps. The sex of each sample was determined by observing gonadal color and appearance according to Ribes et al. (2007), while colonies where no gonads were found inside the dissected polyps were considered as indeterminate. When colonies contained gonads, but the determination of the sex was doubtful, it was confirmed by means of histological analysis. Samples for histological analysis were rinsed in distilled water and dehydrated in a set of ethanol solutions which gradually increased in concentration: 70%, 96% and 100% ethanol. The samples were then submerged in a mixture of 50% ethanol (100%) and 50% resin (Technovit 7100) for 2 hours and then embedded in a bifasic resin (Technovit 7100) and stored in the dark at 4ºC for 48 hours. After dehydration, samples were included in resin (Technovit 7100) and left to harden for 3 days at room temperature. Longitudinal sections of the branches were cut at a thickness of 3 µm, stained with haematoxylin and eosin, and sections were observed under a microscope to determine their sex.

The timing of gonadal development cycles of both populations was assessed by counting and measuring under a stereo microscope the diameter of all oocytes and spermaries contained in 5 polyps of each mature sampled colony, avoiding apical-ends where gonadal output may be affected by annual growth (Coma et al. 1995). The diameters of the gonads were measured with an eyepiece micrometer (+/- 10 µm). When the shape of gonads looked like an ellipsis, both minor (a) and major (b) diameters were measured and the diameter of the sphere with equivalent volume was calculated ($d = 2\ ((a/2)^2 \cdot b/2)^{1/3}$). To estimate the gonadal volume per polyp, diameters (d) were transformed into volume ($V = 4/3\pi(d/2)^3$). A total of 750 polyps from each population were examined, and more than 1500 and 900 gonads were measured from the shallow and the deep populations, respectively.

Biochemical balance

Organic matter in the coenenchyma of the gorgonians was assessed using monthly samples of 7 colonies from each population and month. Approximately 12 mg (± 0.01 mg) of coenenchyma dry weight from each sample were reduced to ash for 4 hours at 500ºC in a muffle furnace, and the difference among coenenchyma dry weight and ash weight was the organic matter (OM) (Slattery and McClintock 1995).

Biochemical analyses to determine the organic matter composition in carbohydrates, proteins and lipids were carried out using monthly samples of 5 colonies from each population and month. Approximately 6 mg (± 0.01 mg) of coenenchyma dry weight from each sample were homogenized in 3 ml of double distilled water, and carbohydrates were quantified colorimetrically according to the method of Dubois et al. (1956), with glucose as a standard. Approximately 6 mg (± 0.01 mg) of coenenchyma dry weight from each sample were homogenized in 2 ml of 1 N NaOH, and proteins were quantified colorimetrically according to the method of Lowry et al. (1951), with albumine as a standard. Finally, approximately 10 mg (± 0.01 mg) of coenenchyma dry weight from each sample were homogenized in 3 ml of chloroform-methanol (2:1), and total lipids quantified colorimetrically according to the method of Barnes and Blastock (1973), with cholesterol as a standard. Results are presented in µg carbohydrate–protein–lipid mg^{-1} of OM.

Trophic markers

Analyses to determine the fatty acids composition were carried out using monthly samples of 3 colonies from each population and month. Approximately 12 mg (± 0.01 mg) of coenenchyma dry weight from each sample were dissolved in 3:1 DCM:MeOH (dichloromethane-methanol), spiked with an internal standard (2-octyldodecanoic acid and the 5β-cholanic acid) and extracted using microwave assisted extraction (5 min at 70ºC). After centrifugation the extract was taken to near dryness in a centrifugal vacuum concentrator at a constant temperature, and fractionated by solid phase extraction according to Ruiz et al. (2004). The sample was redissolved in 0.5 ml of chloroform and eluted through a 500 mg aminopropyl glass column (previously activated with 4 mL of *n*-hexane). The first fraction was eluted with 3 ml of chloroform:2-propanol (2:1) and the fatty acids recovered with 8.5 ml of diethyl ether:acetic acid (98:2). The fatty acids fraction was methylated using a solution of 20% MeOH/BF3 heated at 90ºC for 1 h. The reaction was quenched with 4 ml of NaCl saturated water. The methyl esters of fatty acids were recovered by a double extraction with 3 ml of n-hexane. The combined extracts were taken to near dryness, redissolved with 1.5 ml of chloroform and eluted through a glass column filled with Na_2SO_4 to remove residual water; after removal of the chloroform with nitrogen, the extracted sample was stored at -20ºC until analysis by gas chromatography were carried out. Samples were redissolved in 40 µl of isooctane, and gas chromatography analysis performed with a Thermo Finnigan Trace gas chromatography ultra instrument equipped with a flame ionization detector, a splitless injector and a DB–5 Agilent column (30 m length, 0.25 mm internal diameter and 0.25 µm phase thickness). Helium was used as a carrier gas at 33 cm s^{-1}. The oven temperature was programmed to increase from 50ºC to 320ºC at 10ºC min^{-1}. Injector and detector temperatures were 300ºC and 320ºC, respectively. Methyl esters of fatty acids were identified by comparing their retention times with those of standard fatty acids (37 FAME compounds, Supelco® Mix C4-C24). Fatty acids were quantified by integrating areas under peaks in the gas chromatograph traces (Chromquest 4.1 software), with calibrations derived from internal standards.

The carbon:nitrogen (C:N) ratio and stable isotops ($\delta^{13}C$ and $\delta^{15}N$) composition of the gorgonian tissue were assessed using monthly samples of 3 colonies from each population and month. Approximately 6 mg (± 0.01 mg) of coenenchyma dry weight from each sample was fumed with concentrated HCl for 48 h in order to eliminate the inorganic fraction, and the C:N ratio and stable isotops composition were determined by using a Thermo Flash EA 1112 analyzer and a Thermo Delta V Advantage spectrometer.

Environmental features

Environmental features at the sampling location were monitored monthly from September 2009 to August 2010. Water density, fluorescence, turbidity, and photosynthetically active radiation (PAR, 400 to 700 nm) were measured at 1 m depth intervals from 5 to 60 m depth with a Seabird 25 and Seabird 19 CTDs equipped with a Seapoin Chlorophyll Fluorometer, a Seapoint Turbidity Meter, and a Biospherical Instruments Inc QSP-2300 and a Li-Cor underwater spherical quantum sensor LI-193. Recorded turbidity values expressed in formazin turbidity units (FTU) were subsequently converted into suspended sediment concentration (SSC, mg/L) (SSC = 1.21 FTU + 0.43, Guillen et al. 2000). Sea water temperature at 20 and 60 m depth were recorded every hour from August 2009 to August 2010 by

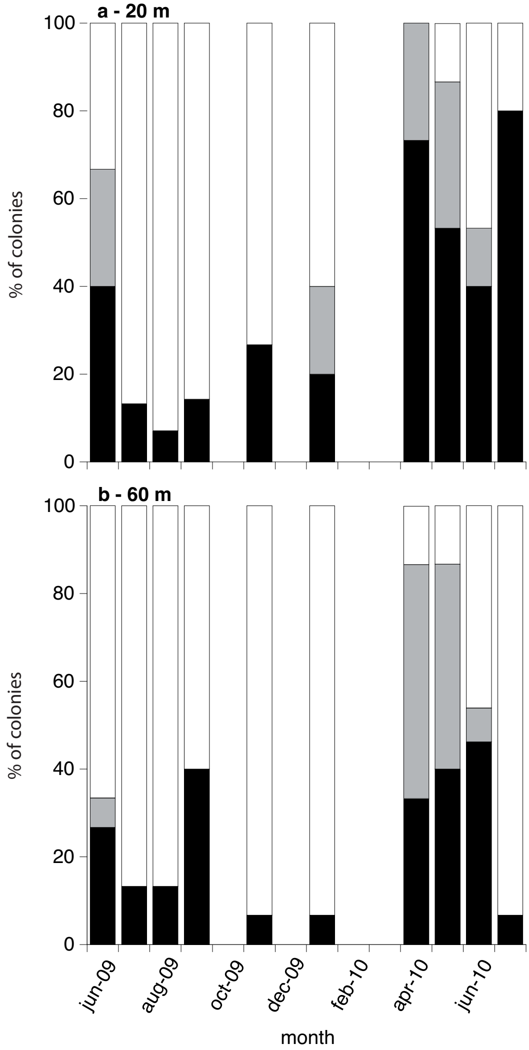

Fig. 2 - Reproductive state of the *Eunicella singularis* colonies from the shallow (a) and deep (b) population; in *black* are indicated the female colonies, in *grey* the male colonies and in *white* the indeterminate colonies.

means of HOBO PRO V2 temperature data loggers placed in the middle of each studied gorgonian population. Unfortunately, data loggers were repeatedly stolen during the study period causing a partial loose of data recorded in May, June and August 2010 at 20 m depth, as well as of data recorded in July 2010 at 60 m depth.

Data treatment

Differences between populations in gonad number and diameter, gonadal volume per polyp, organic matter in the coenenchyma, and content in carbohydrates, protein, lipid, and carbon/nitrogen ratio, were tested for each month using the non-parametric Wilcoxon-Mann-Whitney test, as data did not follow the normality assumptions. Test was performed with the R-language function Wilcox.test of the R software platform (R Development Core Team 2007). An ordination of the analyzed colonies based on their fatty acids composition (*n* = 53) was obtained with a principal component analysis (PCA) performed on transformed data ($p' = \arcsin(p)^{1/2}$) with the R-language function Princomp which is available in the Vegan library (Oksanen et al. 2005) of the R software platform (R Development Core Team 2007).

RESULTS

Gonadal development cycle

Female colonies were found in both populations all over the studied period, being much more frequent between April and July (Fig. 2). Conversely, male colonies were present in both populations from April to June, being present also in January only in the shallow population (Fig. 2). In both shallow and deep studied populations *Eunicella singularis* female colonies showed two overlapping cohorts of oocyte: the first formed by gonads with diameters between 50 and 300 μm, and the second one with diameters between 300 and 900 μm approximately; only eggs from the second cohort were mature during the summer, and were released as planulae between May and July in the shallow population and between May and June in the deep population (Fig. 3). In the shallow population an average of 0.03 ± 0.18 and 0.30 ± 0.48 larvae per female polyp were found in June and July 2009 respectively, while an average of 0.93 ± 1.31 and 0.45 ± 0.77 larvae per female polyp were found in June and July 2010 respectively. Conversely, in the deep population an average of 0.10 ± 0.31 larvae per female polyp was

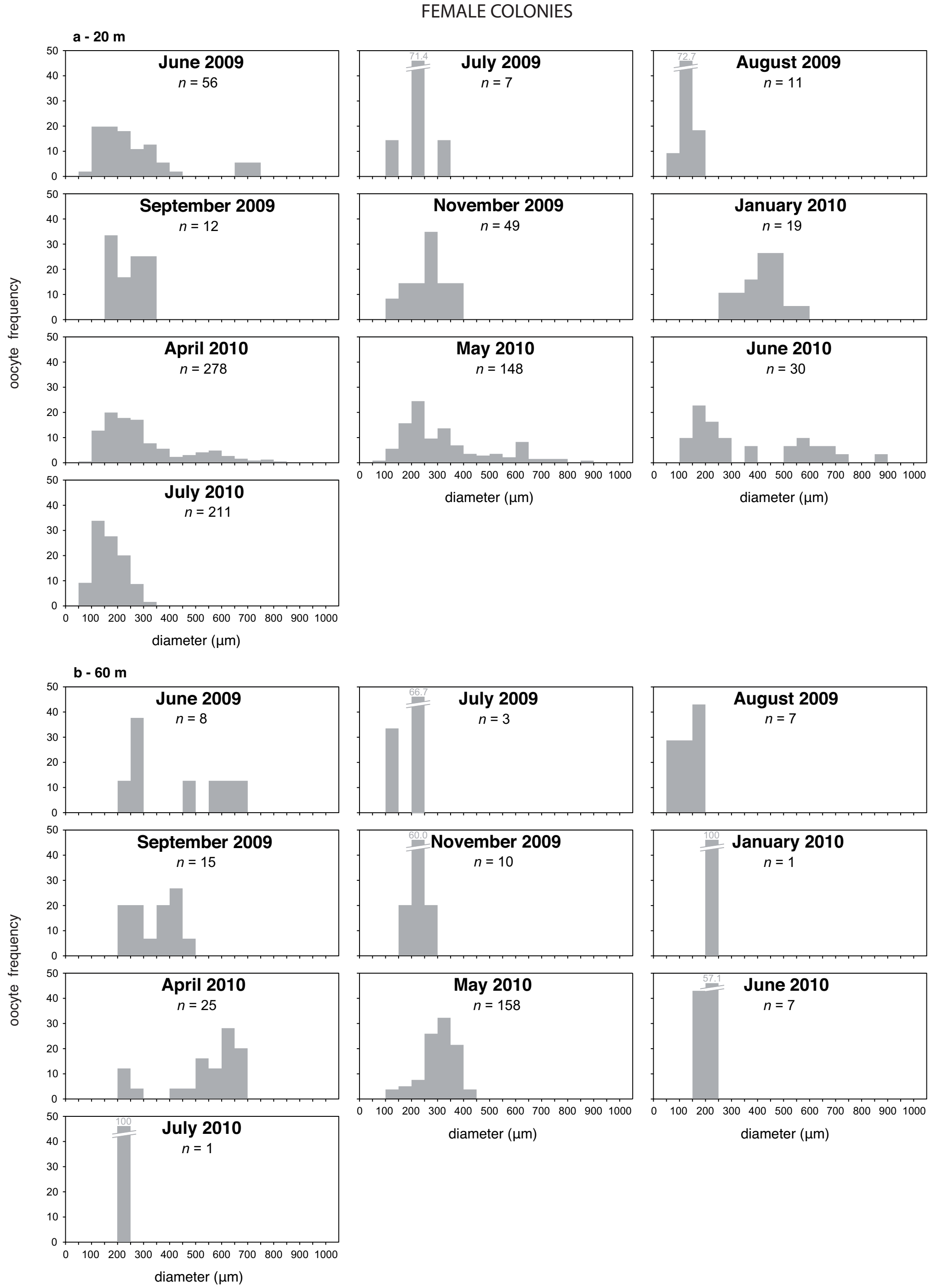

Fig. 3 - Distribution of gonadal diameter frequency (µm) in female *Eunicella singularis* colonies from the shallow (a) and deep (b) population (*n* = gonads number).

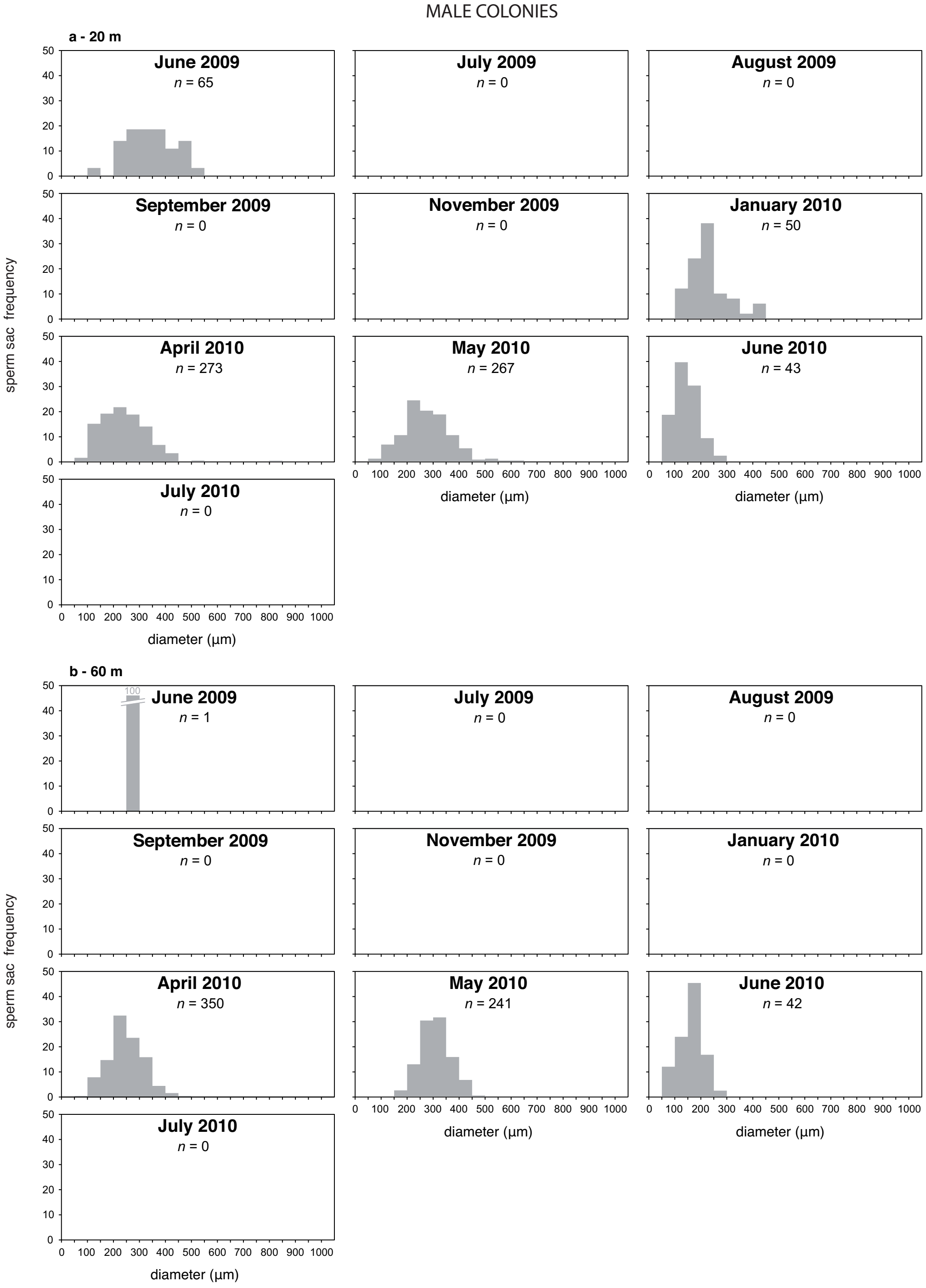

Fig. 4 - Distribution of gonadal diameter frequency (µm) in male *Eunicella singularis* colonies from the shallow (a) and deep (b) population (*n* = gonads number).

Table 1 - Monthly changes in diameter and number of *Eunicella singularis* gonads in the studied populations (mean ± SE).

Month	Gonad diameter (µm)		Gonad number/polyp	
	20 m	60 m	20 m	60 m
female colonies				
June 2009	266 ± 23	423 ± 65	1.9 ± 0.3	0.4 ± 0.2
July 2009	215 ± 21	176 ± 23	0.7 ± 0.3	0.3 ± 0.2
August 2009	131 ± 8	129 ± 14	2.2 ± 0.4	0.6 ± 0.4
September 2009	247 ± 18	338 ± 23	1.2 ± 0.5	0.5 ± 0.1
November 09	255 ± 11	221 ± 9	2.5 ± 0.4	2.5 ± 0.6
January 2010	414 ± 19	200 ± 0	1.3 ± 0.3	0.2 ± 0.2
April 2010	285 ± 10	534 ± 30	5.1 ± 0.5	1.0 ± 0.2
May 2010	320 ± 14	300 ± 5	3.7 ± 0.7	5.3 ± 0.9
June 2010	351 ± 40	187 ± 8	0.9 ± 0.2	0.3 ± 0.3
July 2010	163 ± 4	200 ± 0	3.5 ± 0.7	0.2 ± 0.2
male colonies				
June 2009	340 ± 11	250 ± 0	3.3 ± 0.7	0.2 ± 0.2
July 2009				
August 2009				
September 2009				
November 09				
January 2010	217 ± 11		3.3 ± 0.6	
April 2010	234 ± 5	244 ± 4	13.7 ± 1.5	8.8 ± 1.0
May 2010	270 ± 5	303 ± 4	10.7 ± 1.7	6.9 ± 1.1
June 2010	142 ± 8	158 ± 7	4.3 ± 2.0	4.2 ± 1.0
July 2010				

found only in June 2009, and an average of 0.30 ± 0.47 larvae per female polyp was found only in June 2010. Male gonadal development cycle began earlier in the shallow than in the deep population (Figs. 2 and 4), and showed only a cohort of spermaries being all the male gametes spawned in both populations between May and June (the larger amount) and June and July (the remaining ones) (Fig. 4). Shallow population showedalmost always higest values of gonadal volume, with statistically significative differences in June 2009, January and June 2010 in the female colonies (Fig. 5a), whereas significative differences were observed in June 2009 and April 2010 in male colonies (Fig. 5b). These diferences were due both to differences in the gonadal diameters and gonadal number per polyp (Table 1).

Biochemical balance

On annual average, organic matter was 26.2 ± 4.7 % of the coenenchyma dry weight in the shallow population, and 21.9 ± 4.5 % in the deep population. The percentage of organic matter in the coenenchyma was quite variable throughout the studied period in both populations, and significantly higher in the shallow population in January and from May to July 2010 (Fig. 6). Carbohydrate concentration was significantly higher in shallow than in deep population almost throughout all the studied period (Fig. 7a). Protein concentration showed a seasonal changes in the deep population with higher values in August 2009 and July 2010 respect to the shallow population (Fig. 7b). Lipid concentration showed seasonal changes in the shallow population with values in August 2009, January 2010, and from May to July 2010 significantly higher than in the

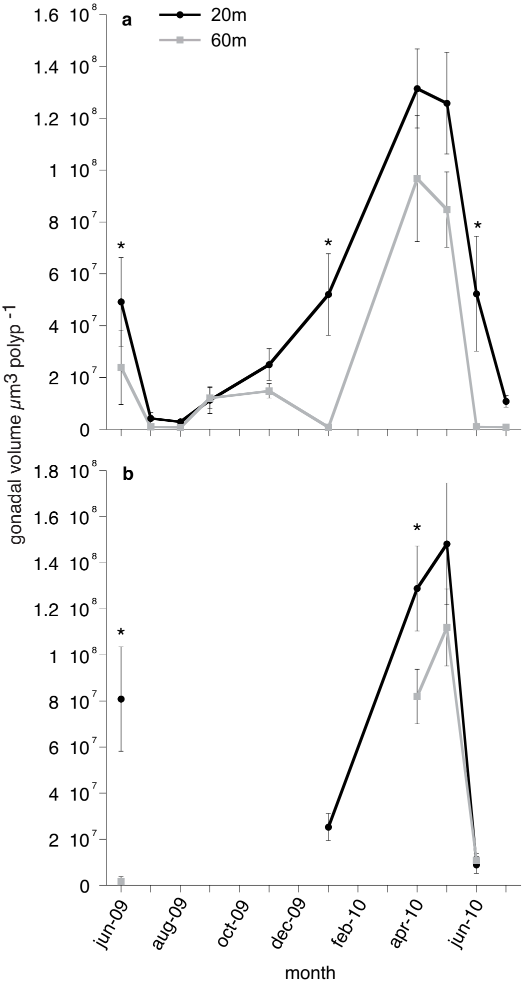

Fig. 5 - Monthly changes in mean polyp volume of female (a) and male (b) gonads (μm^3 polyp^{-1}) in the *Eunicella singularis* colonies from the shallow and deep population (mean ± SE); *asterisk* indicate significant differences between the two populations (Wilcoxon-Mann-Whitney test, $p < 0.05$).

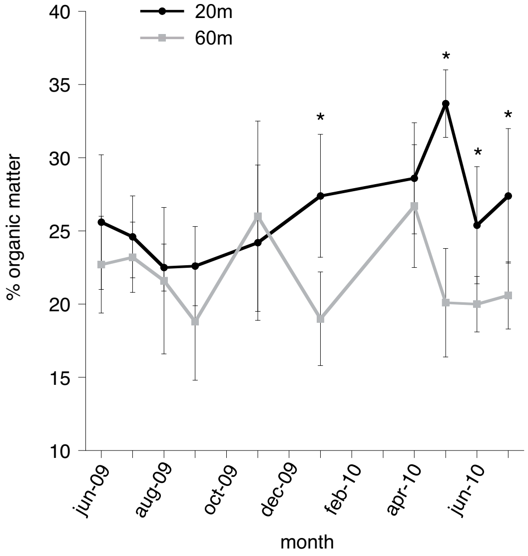

Fig. 6 - Annual cycle (June 2009 - July 2010) of organic matter in the coenenchyma of *Eunicella singularis* colonies (*n* = 7) from the shallow and deep population (mean ± SD); *asterisk* indicate significant differences between the two populations (Wilcoxon-Mann-Whitney test, $p < 0.05$).

deep population, which conversely showed lower and more constant values (Fig. 7c).

Trophic markers

A total of 36 fatty acids were identified in colonies from both populations (Table 2). The first two principal components of the PCA explained the 72.4 % of the data variance in the fatty acids composition of the studied colonies; the first axis explained 41.8 %. The PCA showed two groups formed by respectively shallow and deep colonies sampled during spring and summer. Conversely colonies from both populations sampled during winther were mixed in a third weaker group (Fig. 8). There was a clear covariance between the C20:4(n-6) and the C20:5(n-3) from one side, and among the C18:1(n-7), the C18:0 and the C24:1(n-9) from the other side. A weaker covariance was observed among the C18:2(n-6), the C16:1(n-7), the C22:6(n-3) and the C16:0 (Fig. 8).

The C:N ratio showed a strong seasonality in the shallow population with summer values significative higher than in the deep population (Fig. 9), while the samples from the deep and the shallow population resulted to be clearly separated in the stable isotops composition analysis, with the deep population colonies showing always higher $\delta^{15}N$ values respect to the shallow colonies (Fig 10).

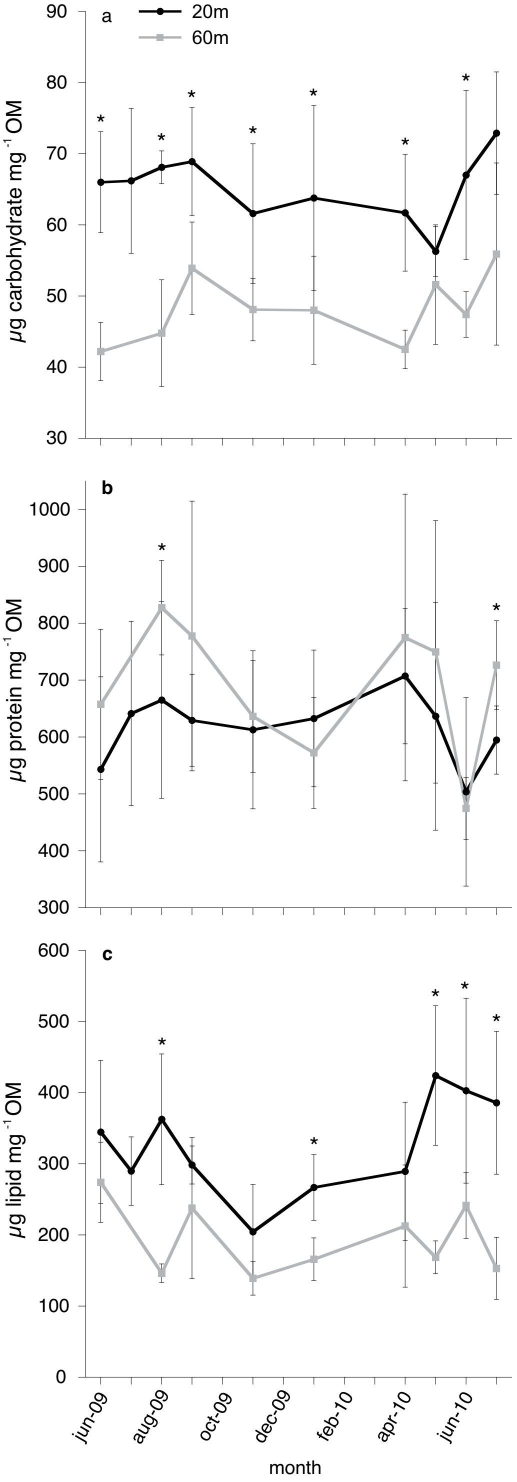

Fig. 7 - Annual cycle (June 2009 - July 2010) of carbohydrate (a), protein (b) and lipid (c) concentration (µg · mg^{-1} OM) in tissue of *Eunicella singularis* colonies (*n* = 5) from the shallow and deep population (mean ± SD); *asterisk* indicates significant differences between the two populations (Wilcoxon-Mann-Whitney test, $p < 0.05$).

Environmental features

Water density was almost constant along the water column from October to February. In March the surface water was less dense than the deeper water, probably due to the strong rains that affected the study area during this month. Water column stratification began to develop in April with a thermocline forming around 30 m depth; it was partially broken at the end of June due to strong winds, while the water column was fully stratified in July and August with a thermocline at 35 m depth. Finally, stratification was stretched out between 45 and 30 m depth in September (Fig. 11). PAR decreased exponentially with depth (Fig. 11); over an annual average, if compared to 20 m depth, PAR was 37.8 ± 13.1 % at 30 m depth, 14.1 ± 7.1 % at 40 m depth, 4.6 ± 2.8 % at 50 m depth, and 1.4 ±1.0 % at 60 m depth. Fluorescence tended to be higher below the summer thermocline during the water column stratification period from March to September; the suspended sediment concentration (SSC) was much more variable, showing high values in shallow waters between April and June, and high values in deeper water in May, June, August and September (Fig. 11).

DISCUSSION

Reproductive cycle

Significant differences in the reproductive cycle between the shallow and the deep studied populations of *Eunicella singularis* were pointed out in this study. Both male and female gonadal volume, as well as tha maximum gonadal diameters were higher in the shallow then in the deep population. A similar difference was already observed in the tropical gorgonian *Briareum asbestinum* whose female colonies from shallow water had more and larger oocytes than those from deep water (West et al. 1993)

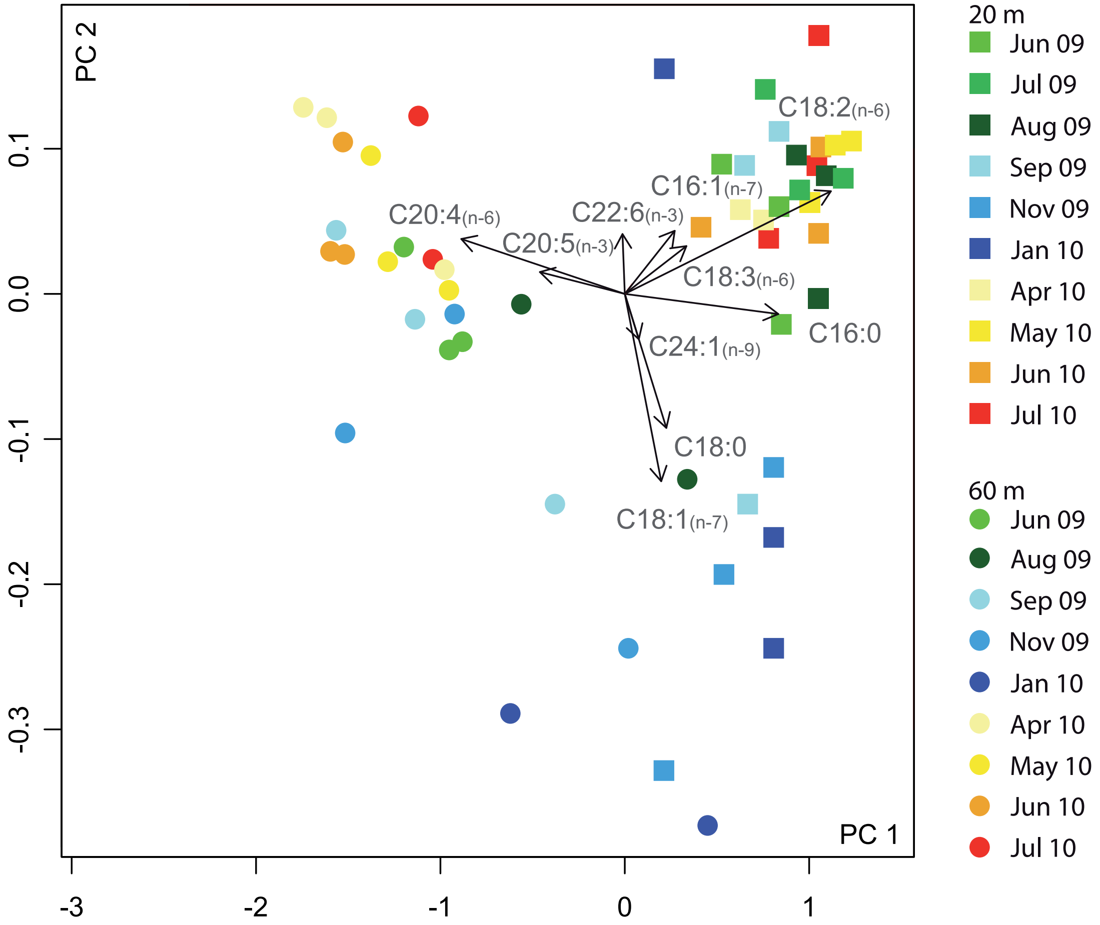

Fig. 8 - Principal component analysis (PCA) biplot showing the ordination of the studied colonies regarding their composition in fatty acids, and the roles of the first 10 fatty acids sorted according to the explained variance.

and the differences were reported to be linked to a greater productivity in shallow compared to deep water (McCloskey and Muscatine 1984; West et al. 1993). In a similar way, the tropical corals *Acropora palifera* and *Stylophora pistilata* showed smaller numbers of planulae produced at greater depths (Kojis and Quinn 1981; Rinkevich and Loya 1987). In *E. singularis* the male reproductive cycle started earlier in the shallow than in the deep population, whereas male gamets spawning occured majoritary between May and June in both populations. In the same way, the main decrease in female gonadal volume occurred between May and June in both populations, although the release of larvae from the female colonies was more prolonged in the shallow (June and July) than in the deep population (June). Although reproduction is synchronized, it was already reported to do not occur simultaneously within a soft coral population where, after a first spawning was observed, the majority of the colonies from both sexes had not yet spawned, or had only partially spawn their gametes (Benayahu and Loya 1983).

Overall, the reproductive cycle of *E. singularis* turned out to be almost synchronized between the shallow and the deep populations, despite the marked differences in water temperature,

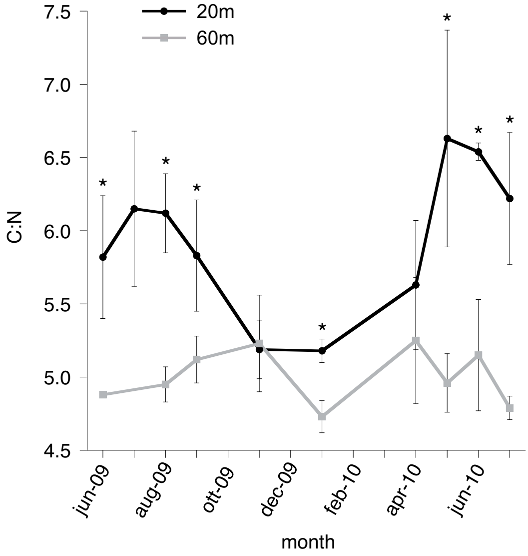

Fig. 9 - Annual cycle (June 2009 – July 2010) of C:N in tissue of *Eunicella singularis* colonies (*n* = 3) from the shallow and deep population (mean ± SD); *asterisk* indicates significant differences between the two populations (Wilcoxon-Mann-Whitney test, $p < 0.05$).

suggesting that other factors besides temperature may play a role in determining the timing of the reproductive cycle of this species. This is surprising, because previous studies reported a delay in the release of sexual products with increasing depth in the same species (Weinberg and Weinberg 1979; Gori et al. 2007), as well as in other gorgonian species from the Mediterranean Sea (Santangelo et al. 2003; Tsounis et al 2006b; Rossi and Gili 2009) and in gorgonians and soft corals species from tropical areas (Grigg 1977; Benayahu and Loya 1983, 1986; West et al. 1993). However, these studies compared populations living within the first 30-45 m depth, where very strong temperature oscillations have been reported to occur in very short elapsed times during the reproductive period (Rossi et al. 2009b, 2011). It could be possible that being sheltered from such great temperature variations during summer, make the 60 m depth populations to be more independent from the shallower environmental conditions (i.e. temperature strong oscillations). Temperature has been argued to be one of the major factors for the determination of the gonadal development and sexual product release cycles in many corals and gorgonians (Fautin 2002; Ribes et al. 2007; Harrison 2011), and time lags in spawning at greater depths have been attributed to differences in time of the peak water temperature along the depth gradient (Grigg 1977; Benayahu and Loya 1983, 1986). Despite the differences that exist between depths in terms of both light and temperature, and the well-characterized responses of corals to these factors, *E. singularis* released gametes simultaneously in deep and shallow populations. In a similar way in the corals *Montastrea cavernosa*, *Montastrea franksi*, and *Diploria strigosa* colonies located at 33-42 m depth were observed to broadcast spawn in synchrony with shallower colonies located at 16 m depth (Vize 2006). Temperature alone may not be the only factor affecting the spawning behavior of gorgonians (Rossi and Gili 2009b), since reproduction may also be related to seasonal fluctuations in food availability for suspension feeders (Hartnoll 1975; Szmant-Froelich and Pilson 1984; Ben-David-Zaslow and Benahayu 1999), as well as to lunar and solar light cues (Vize 2006; Alino and Coll 1989; Benayahu et al. 1990; Jokiel et al. 1995; Levitan

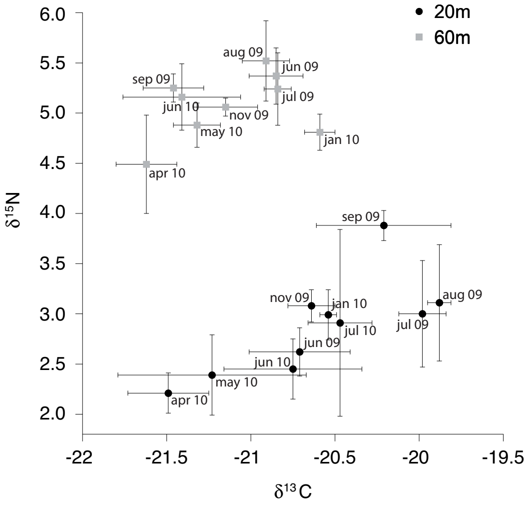

Fig. 10 - Stable isotops ($\delta^{13}C$ to $\delta^{15}N$) composition of *Eunicella singularis* colonies (*n* = 3) from the shallow and deep population (mean ± SD).

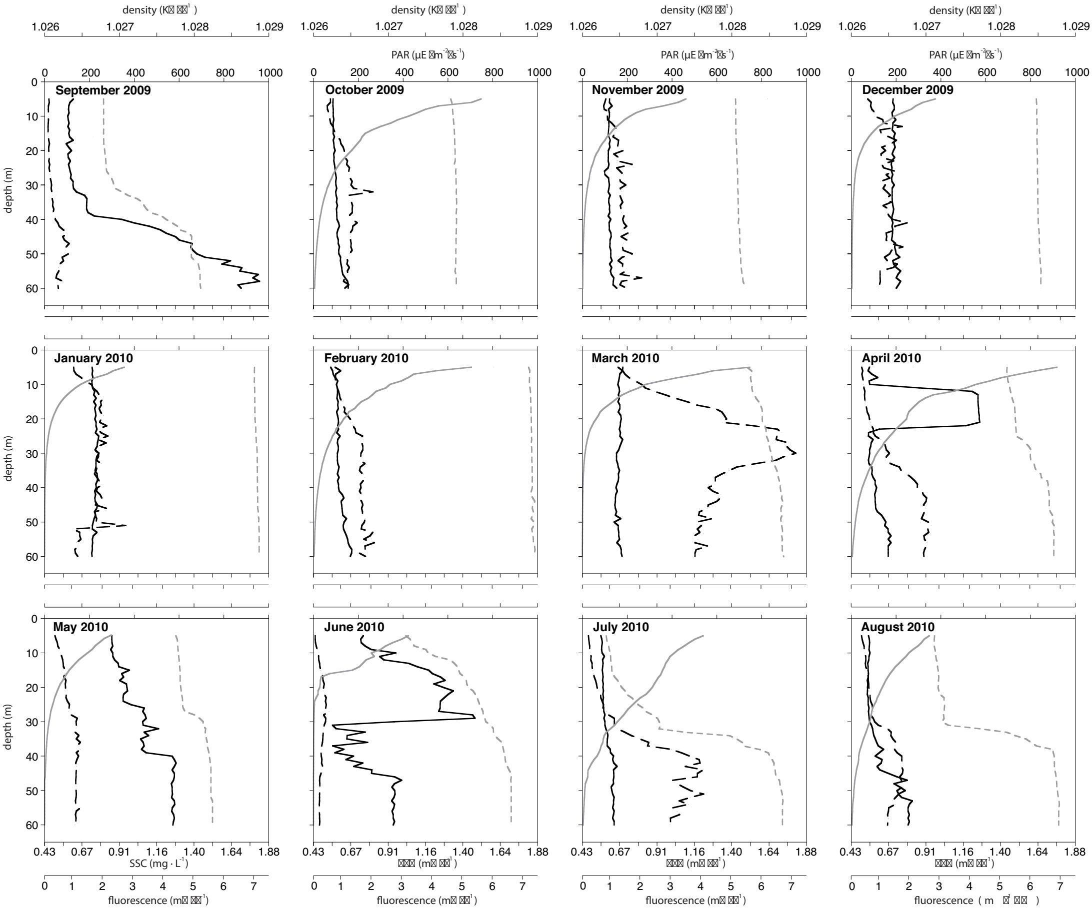

Fig. 11 - Water column characterization in the sampling location during the study period; *grey line* represents photosynthetically active radiation (PAR), *grey dotted line* represents water density, *black line* represents suspended sediment concentration (SSC), and *black dotted line* represents fluorescence.

et al. 2004). Planulation in the gorgonian *Acabaria biserialis* occurred following the major seasonal bloom of its main food source (Zeevi Ben-Yosef and Benayahu 1999), and the year-round gamete release observed in the azooxanthellate soft coral *Dendronephthya hemprichi* was related to the relatively persistent availability of phytoplankton, which is the main food source of this species (Fabricius et al. 1995a, 1995b) and supply the metabolic demands for the gametogenesis (Dahan and Benayahu 1997). Since reproduction involves a major energy investment (Coma et al. 1998), it is likely that investment in gonad development appears to be related to resource availability (Ribes et al. 2007), and oocyte maturation is controlled by availability of energy rich food in asymbiotic species (Dahan and Benayahu 1997; Rossi and Gili 2009b), and/or by high light levels in symbiotic ones (Ben-David-Zaslow et al. 1999)

If compared to previous studies on the reproduction of shallow *E. singularis* populations, this study also highlighted significant differences in the gonadal output among shallow populations located along almost 700 km of Mediterranean coast (Ribes et al. 2007; Gori et al. 2007), with the Cap de Creus population displaying lower gonadal volume respect to the other populations. Such a spatial variability in gonadal output has been already reported for coral species (Fan and Dai 1995; Rinkevich and Loya 1987; Kruger et al. 1998), and have been explained as a result in different allocation of resources to gametes production in response to instable environmental conditions experienced at higher latitudes or shallow waters (Sier and Olive 1994). Differences in gonadal output could be due to differences in nutritional state of gorgonians as a consequence of differences in food quality and availability between different geographical areas, depths or years (Stimson 1987; Harland et al. 1992; Rossi et al. 2003; Ben-David-Zaslow et al. 1999). Year-to-year variations in reproductive cycles are known for several alcyonacean and scleractinian species (Rinkevich and Loya 1982, 1987; Brazaeu and Lasker 1989; Weil 1990; Dahan and Benayahu 1997; Ben-David-Zaslow et al. 1999), and this variability may also affect the differences in time and gonadal output between deep and shallow populations. For example, it was observed that the Mediterranean *Corallium rubrum* released gamets simultaneously in deep and shallow populations in 2002, whereas in 2003 the release was depth staggered, with the shallow colonies releasing earlier than the deep ones (Tsounis et al. 2006b).

Biochemical balance

The energy storage was also different between the shallow and the deep studied populations, following the same pattern observed for their reproductive output.

Lipid concentration showed a marked seasonality in the shallow population, with highest values during the summer, while it was lower and more constant in the deep population. Shallow colonies had similar lipid content respect to the *E. singularis* colonies located at the same depth in the Medes Islands (30 km apart from the study area, Gori et al. 2007), being significantly higher that the deeper colonies. Interestingly, colonies settled at 30-35 meters depth in Cabo de Palos, had more lipid that shallower colonies both from Medes islands and Cap de Creus (Gori et al. 2007). However, it is unlikely that Cabo de Palos showed higher availability of plankton and/or Particulate Organic Matter (POM) compared to Cap de Creus, which is an interception point of strong northern currents coming from the Gulf of Lion, forming a complex eddies system strongly enriched in nutrient and POM (Rubio et al. 2009). Conversely, it is possible that the differences in summer concentration of lipids observed among these three shallow populations could be related to a greater amount of light in the southernmost population of Cabo de Palos, which stimulates a greater lipid production by the symbiotic algae, whereas the lack of symbiotic zooxantellae (Gori et al. submitted) could explain the lower and more constant lipid levels observed in the deep population. Harland et al. (1992) demonstrated differences in lipid stored by symbiotic corals between depths of 3-6 and 13 m, indicative of a light constraint with increasing depth, and Lasker et al. (1983) showed that depth may have an influence on capture rates and food availability for gorgonians, but also stressed the fact that the same genus but different species may show slight differences due to morphological and metabolic cues. Different studies highlight the importance of zooxantellae in the traslocation of lipids from the algal cells to the cnidarian tissue. Patton et al. (1983) showed the immediate translocation of lipids from the zooxantellae to the host in *S. pistillata*, and Stimson (1987) related this potential production of lipids with the reproductive output of the reef coral *Pocillopora* spp. More recently, Tolosa et al. (2011) showed the importance of temperature in the lipid transference from the zooxantellae to the host in *Turbinaria reniformis*, and Treignier et al. (2008) demostrated that both light and feeding determine the lipid composition of this species. The present results of lipid storage in *E. singularis* point to a an important role played by the zooxantellae (and light) in the shallower colonies, at least during summer.

Trophic difference in *Eunicella singularis*: heterotrophy versus autotrophy

The recorded environmental factors were, in general, more constant in the deeper area (50-60

Table 2 - Monthly fatty acid composition (% of total fatty acids) in the two *Eunicella singularis* studied populations (mean ± SD); *n* = number of analysed colonies.

Fatty Acids	Jun 09	Jul 09	Aug 09	Sep 09	Nov 09	Jan 10	Apr 10	May 10	Jun 10	Jul 10
20m	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 2)	(*n* = 3)	(*n* = 3)	(*n* = 3)
C12:0	0.2 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.3
C13:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0.1	0.1 ± 0.1	0 ± 0
C14:0	0.9 ± 0.1	0.7 ± 0.1	1.1 ± 0.7	1.3 ± 0.7	2.2 ± 0.7	1.5 ± 0.2	1.2 ± 0.0	0.8 ± 0.2	1.1 ± 0.2	0.6 ± 0.6
C14:1(n-5)	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.5	0.1 ± 0.1	0.1 ± 0.1	0 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0 ± 0
C15:0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.1
C15:1(n-5)	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.0	0.2 ± 0.1	0 ± 0
C16:0	14.9 ± 1.0	14.7 ± 1.5	16.6 ± 0.4	17.6 ± 4.2	9.6 ± 8.4	17.7 ± 3.6	16.1 ± 0.3	17.3 ± 0.7	20.1 ± 3.9	12.6 ± 2.3
C16:1(n-7)	8.9 ± 0.7	10.7 ± 1.4	9.0 ± 1.0	7.1 ± 2.9	2.6 ± 1.4	5.3 ± 2.5	9.6 ± 0.7	11.9 ± 0.1	11.9 ± 2.6	8.2 ± 1.2
C16:2(n-6)	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.5	0 ± 0	0 ± 0	0 ± 0
C17:0	0.3 ± 0.2	0 ± 0.1	0.1 ± 0.1	0.5 ± 0.7	0.9 ± 0.6	0.9 ± 0.6	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.6 ± 0.1
C17:1(n-7)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:0	1.2 ± 1.5	0.2 ± 0.2	1.3 ± 1.1	0 ± 0	13.3 ± 9.3	9.5 ± 8.4	0.4 ± 0.2	0.2 ± 0.4	0.6 ± 1.0	0 ± 0
C18:1(n-7)	6.8 ± 3.1	3.6 ± 2.3	4.5 ± 3.0	12.8 ± 16.4	25.9 ± 15.0	16.0 ± 14.2	5.4 ± 1.3	3.9 ± 0.4	4.2 ± 0.7	3.9 ± 3.9
C18:1(n-9 trans)	4.0 ± 0.5	4.0 ± 0.4	4.3 ± 0.8	4.5 ± 0.4	7.8 ± 3.0	5.0 ± 0.7	5.9 ± 0.4	4.8 ± 0.2	4.3 ± 0.6	6.0 ± 0.3
C18:2(n-4)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:2(n-6cis)	23.6 ± 2.6	30.6 ± 2.4	29.0 ± 2.6	21.0 ± 10.1	9.8 ± 6.0	13.1 ± 6.1	22.2 ± 1.3	30.3 ± 2.7	19.8 ± 14.8	30.6 ± 3.9
C18:3(n-3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:3(n-6)	4.7 ± 1.9	4.0 ± 0.7	5.9 ± 1.2	4.2 ± 1.6	3.9 ± 2.9	2.5 ± 1.0	2.6 ± 0.5	3.5 ± 0.2	4.3 ± 1.7	8.3 ± 3.4
C18:4(n-3)	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	2.4 ± 3.4	0.7 ± 0.3
C20:0	0.4 ± 0.1	0 ± 0	0.2 ± 0.3	0.3 ± 0.3	0.8 ± 0.4	0.4 ± 0.4	0.2 ± 0.2	0.3 ± 0.0	0.5 ± 0.2	0.9 ± 0.2
C20:1(n-9)	0.3 ± 0.3	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.2	0 ± 0	0 ± 0	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.1	0.2 ± 0.4
C20:2(n-6)	0.9 ± 0.5	1.2 ± 0.4	0.9 ± 0.1	0.8 ± 0.3	0.3 ± 0.3	0.4 ± 0.1	1.0 ± 0.5	1.2 ± 0.3	1.1 ± 0.3	1.3 ± 0.6
C20:3(n-3)	0.6 ± 0.7	0.6 ± 0.1	0.9 ± 0.5	0.9 ± 0.4	3.9 ± 5.8	0.1 ± 0.1	1.2 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.6
C20:3(n-6)	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0 ± 0	0.1 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.2
C20:4(n-6)	15.6 ± 5.5	12.8 ± 3.5	11.4 ± 1.1	13.6 ± 4.2	6.9 ± 3.1	13.8 ± 9.2	13.8 ± 2.1	8.8 ± 0.6	10.3 ± 3.5	8.4 ± 2.4
C20:5(n-3)	2.1 ± 0.3	2.0 ± 0.5	1.9 ± 0.4	2.1 ± 0.7	1.5 ± 0.4	2.4 ± 1.9	3.7 ± 1.4	1.8 ± 0.6	1.5 ± 1.3	2.2 ± 0.9
C21:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.2
C22:0	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.8 ± 0.2
C22:1(n-9)	0.2 ± 0.3	0 ± 0	0.1 ± 0.1	0.2 ± 0.3	1.3 ± 1.5	0.2 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.4
C22:2(n-6)	0.1 ± 0.1	0.2 ± 0.1	0 ± 0	0.1 ± 0.1	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.2
C22:6(n-3)	10.6 ± 2.1	10.7 ± 2.1	8.7 ± 2.3	8.9 ± 3.3	5.1 ± 2.9	7.1 ± 4.8	12.0 ± 2.0	10.7 ± 0.1	13.4 ± 2.2	10.9 ± 2.9
C23:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C24:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C24:1(n-9)	0.2 ± 0.4	0 ± 0	0.3 ± 0.6	0.4 ± 0.7	2.2 ± 1.7	0.7 ± 0.8	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.8
C24:4(n-6)	1.4 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.6	0.4 ± 0.3	1.0 ± 0.7	1.0 ± 0.6	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.3
C24:5(n-3)	0.4 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.4 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
60m	(n = 3)		(n = 2)	(n = 3)	(n = 3)	(n = 2)	(n = 3)	(n = 3)	(n = 3)	(n = 2)
C12:0	0.1 ± 0.1		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.6 ± 1.0	0 ± 0	0 ± 0
C13:0	0.1 ± 0.2		0.3 ± 0.5	0.6 ± 0.4	0 ± 0	0.1 ± 0.1	0.2 ± 0.3	0.2 ± 0.3	0.8 ± 0.7	1.0 ± 1.4
C14:0	1.6 ± 0.0		8.3 ± 3.7	2.9 ± 0.2	2.8 ± 0.1	2.1 ± 0.1	1.1 ± 0.9	1.0 ± 0.9	2.3 ± 0.6	1.4 ± 2.0
C14:1(n-5)	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.3	0.7 ± 0.6	0 ± 0
C15:0	1.1 ± 0.1		4.5 ± 2.6	1.8 ± 0.6	2.0 ± 0.8	1.0 ± 0.4	1.2 ± 0.1	1.2 ± 0.1	1.9 ± 0.4	3.0 ± 0.5
C15:1(n-5)	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.4	0.4 ± 0.4	0 ± 0	0 ± 0
C16:0	7.4 ± 0.9		27.2 ± 7.6	4.9 ± 4.9	8.8 ± 8.7	13.5 ± 8.6	1.9 ± 3.3	5.7 ± 2.5	0 ± 0	0 ± 0
C16:1(n-7)	3.0 ± 0.0		2.3 ± 0.7	4.1 ± 0.9	3.3 ± 1.2	0.9 ± 0.8	4.2 ± 0.0	2.7 ± 0.6	4.4 ± 1.1	7.8 ± 1.5
C16:2(n-6)	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C17:0	1.4 ± 0.1		3.0 ± 1.2	1.0 ± 0.1	0.9 ± 0.8	1.3 ± 0.3	0.6 ± 0.1	1.7 ± 0.3	2.0 ± 0.3	3.7 ± 0.3
C17:1(n-7)	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:0	1.6 ± 1.4		3.6 ± 5.1	0.8 ± 1.3	4.2 ± 7.3	13.2 ± 10.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:1(n-7)	5.7 ± 1.6		4.2 ± 5.9	10.4 ± 7.3	17.7 ± 9.5	38.7 ± 4.1	2.2 ± 3.8	3.9 ± 3.6	3.5 ± 3.0	1.6 ± 2.2
C18:1(n-9 trans)	5.3 ± 0.2		6.5 ± 1.2	7.6 ± 1.3	7.2 ± 0.7	6.5 ± 2.8	7.7 ± 0.1	5.6 ± 1.2	7.4 ± 0.7	12.7 ± 0.9
C18:2(n-4)	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:2(n-6cis)	1.9 ± 0.3		3.1 ± 4.4	2.5 ± 1.2	0.9 ± 0.9	0.6 ± 0.9	1.7 ± 0.4	1.5 ± 0.3	1.7 ± 0.1	3.4 ± 0.8
C18:3(n-3)	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C18:3(n-6)	0.9 ± 0.1		3.6 ± 5.1	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0.0	1.6 ± 0.4	1.8 ± 0.5	3.2 ± 0.4
C18:4(n-3)	0.1 ± 0.1		0 ± 0	0 ± 0	0.0 ± 0.1	0 ± 0	0.5 ± 0.3	0.2 ± 0.1	0.8 ± 0.4	0.2 ± 0.3
C20:0	0.9 ± 0.1		0.8 ± 1.2	0.7 ± 0.6	1.5 ± 1.5	1.2 ± 0.8	1.4 ± 0.2	1.7 ± 0.3	2.2 ± 0.5	3.8 ± 0.9
C20:1(n-9)	1.3 ± 0.3		0 ± 0	1.3 ± 0.8	0.4 ± 0.7	0 ± 0	1.2 ± 0.3	1.4 ± 0.3	1.3 ± 1.2	0 ± 0
C20:2(n-6)	0.6 ± 0.1		0.4 ± 0.6	0.9 ± 0.3	0.9 ± 0.5	0.2 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.7	0.8 ± 1.1
C20:3(n-3)	2.4 ± 0.0		0.5 ± 0.6	2.4 ± 1.0	3.2 ± 1.6	1.6 ± 0.7	1.9 ± 0.9	1.6 ± 0.4	2.4 ± 0.7	1.6 ± 2.2
C20:3(n-6)	0.9 ± 0.1		0.7 ± 1.0	1.2 ± 0.6	0.4 ± 0.4	0 ± 0	1.2 ± 0.4	1.0 ± 0.2	0.7 ± 0.6	0 ± 0
C20:4(n-6)	38.0 ± 1.8		15.5 ± 4.8	32.8 ± 8.1	22.2 ± 10.8	10.9 ± 9.3	41.3 ± 10.1	39.1 ± 5.5	38.7 ± 2.3	19.2 ± 0.2
C20:5(n-3)	9.4 ± 0.8		3.5 ± 1.2	5.8 ± 1.7	6.2 ± 1.8	2.4 ± 1.9	9.6 ± 1.3	10.3 ± 1.3	9.6 ± 2.6	9.7 ± 1.8
C21:0	0.3 ± 0.1		1.2 ± 0.6	0.2 ± 0.3	0 ± 0	0.2 ± 0.3	0.2 ± 0.3	0.6 ± 0.1	0 ± 0	0.7 ± 1.1
C22:0	0.6 ± 0.1		2.6 ± 1.7	1.1 ± 0.7	1.3 ± 1.0	0.9 ± 0.7	1.1 ± 0.2	1.2 ± 0.2	1.9 ± 0.4	3.3 ± 0.7
C22:1(n-9)	0.2 ± 0.2		0.6 ± 0.8	0.8 ± 1.5	0.1 ± 0.2	0.5 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0.9 ± 1.2
C22:2(n-6)	0.4 ± 0.1		0 ± 0	0.7 ± 0.6	1.0 ± 0.6	0 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.6	0.8 ± 1.2
C22:6(n-3)	9.2 ± 0.5		4.8 ± 1.3	9.2 ± 1.9	11.1 ± 3.1	1.9 ± 1.2	12.2 ± 1.7	7.1 ± 3.1	8.2 ± 3.3	16.0 ± 4.7
C23:0	0 ± 0		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3.4 ± 6.0	0 ± 0	0 ± 0
C24:0	0 ± 0		0 ± 0	0 ± 0	0.1 ± 0.2	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
C24:1(n-9)	0 ± 0		1.0 ± 1.3	1.2 ± 2.1	0.1 ± 0.3	1.2 ± 1.7	0 ± 0	0 ± 0	0 ± 0	0.7 ± 1.1
C24:4(n-6)	3.6 ± 0.2		1.8 ± 0.5	3.8 ± 1.1	2.3 ± 1.0	0.6 ± 0.6	3.7 ± 0.6	2.8 ± 0.2	3.4 ± 0.8	2.7 ± 0.1
C24:5(n-3)	2.0 ± 0.1		0 ± 0	1.4 ± 1.2	1.3 ± 0.5	0.3 ± 0.2	1.6 ± 0.5	1.9 ± 0.3	2.6 ± 0.9	1.7 ± 0.2

meters depth) than in the shallower one (20-30 meters depth), a factor previously reported by the model showed by Garrabou et al. (2002). Although the environmental features were assessed with only once a month CTD monitoring, the obtained data show more stable conditions but also a non neglectable quantity of suspended material and phytoplankton in the deeper zone. It has been demonstrated that the *C. rubrum* colonies located above the summer thermocline display lower and more variable annual prey capture rates than deeper colonies (Tsounis et al. 2006a), which reverts in lower lipid concentration in shallower populations than in deeper ones (Rossi and Tsounis 2007) that are not stressed by the summer constraints occurring above the thermocline (Gili and Coma 1998; Rossi et al. 2006).

Similarly to the lipid concentration, also the fatty acid and stable isotope composition exhibited little detectable seasonality in the deep sublittoral population compared to the shallower one. The trophic markers of microalgal origin (Dalsgaard et al. 2003), including the 16:1(n-7) and 22:6(n-3), were more abundant and follow a seasonal trend in the shallow population, whereas 60 m depth colonies presented a significantly lower proportion without following any clear seasonality (Table 2). In the same way, the $\delta^{13}C$ had a larger range of annual variability at 20 m depth than at 60 m, suggesting a more stable source for carbon at 60 m during the whole year, and a more variable origin throughout the year (from more pelagic to more benthic) at 20 m. These results suggested a marked divergence in the trophic strategy of both populations: at 20 m depth, the dominant fatty acid marker was the 18:2(n-3), which is considered to proceed from macroalgal origin (detritus) in the Mediterranean Sea (Pond et al. 2002; Soler-Membrives et al. 2011), whereas the 20:4 (n-3), which indicates ciliate and flagellate (heterotrophic) origin (Zhukova and Kharlamenko 199; Broglio et al. 2003; Soler-Membrives et al. 2011), was less abundant. Conversely, in the deep population, the 20:4(n-3) was clearly dominant, being the 18:2(n-3) less abundant. Long chained fatty acids, that seem to be synthesized *ex novo* by *E. singularis* (e.g. the 24:4) are more abundant in the specimens from the deep population than in the colonies from the shallow one. Treignier et al. (2008) showed that non stressed corals with zooxantellae had a higher lipid transference due to the optimum fitness of the symbiont algae. Higher photosynthetic production from zooxanthellae during the summer and the transfer of lipids to the host may account for the seasonality in the C:N ratio in the shallow population. The trend suggested by the fatty acid analyses was corroborated by the stable isotope analyses, with the deep population showing high $\delta^{15}N$ values close to other asymbiotic passive suspension feeder species, that mainly feed on microzooplankton and particulate organic matter (Jacob et al. 2006; Carlier et al. 2007a, Carlier et al. 2007b). Whereas the lower $\delta^{15}N$ values observed in the shallow population throughout the year were similar to those obtained for passive suspension feeder species with endosymbiotic photosynthetic organisms (Carlier et al 2007a).

The significant transfer of $\delta^{13}C$ from zooxanthellae to the coral host in high light conditions, as well as the lack of stomach contents of shallow *E. singularis* colonies reported during spring (Rossi 2002), suggest that *E. singularis* rely almost only on its autotrophic source of energy in the shallow populations, at least during spring and summer, whereas the deeper colonies that lack symbiotic algae seems to rely to heterotrophic food source, being microzooplankton the most predominant prey group as already reported for other asymbiotic Mediterranean gorgonian sepecies (Coma et al. 1994; Ribes et al. 1999, 2003; Rossi et al. 2004; Tsounis et al. 2006a; Picciano and Ferrier-Pagès 2007).

The importance of deep sublittoral communities in the Mediterranean

In a warm temperate sea like the Mediterranean, seasonal cycles of primary production strongly control the bentho-pelagic coupling operated by suspension feeder species such as gorgonians. However the results obtained in this study pointed to a marked difference between shallow and deep sublittoral gorgonian populations. Deep populations of *E.*

singularis deal in a more stable environment, which revert in a lower but more constant energy storage capabilities, whereas shallower colonies were clearly more stressed by heterotrophic food constrains during summer (Coma and Gili 1998; Coma et al. 2000), and its dependence on the autotrophic metabolism of its symbiotic algae may be a key factor to understand how temperature and low food availability influence colony fitness (Ferrier-Pagés et al. 2009).

COMMENTS

In the submitted version of this work Table 2 correspond to the Online Resource 1.

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General Discussion

SYNTHESIS OF RESULTS AND GENERAL DISCUSSION

This thesis addressed a first description of the ecology of deep sublittoral populations of Mediterranean gorgonians by means of a multidisciplinary approach dealing with (1) the evaluation of spatial distribution patterns, (2) the analysis of the morphological variation of the gorgonian colonies, (3) the study of the population size and spatial structure, and (4) the analysis of the reproductive cycles and the trophic ecology. To approach these topics, several different methodologies (in the sampling procedures, as well as in the laboratory and in data analysis) have been applied to address old and new questions and challenges on the ecology of Mediterranean gorgonians. The first chapter presented the results of applying quantitative analysis to underwater video transects performed with a Remotely Operated Vehicle (ROV), in order to address the spatial distribution patterns of gorgonian populations. The second chapter combined the analysis of photographic images obtained by ROV, with scanning electronic microscope (SEM) analysis of gorgonian sclerites and histological and genetic analysis, all in order to investigate the morphological variability driven by depth. The third chapter offered a combination of classical methods for population size and spatial structure analysis that have been innovatively applied to ROV video transects to address the study of gorgonian populations over a larger bathymetrical scale. Finally, in the fourth chapter the microscopy and histology techniques for the study of gorgonian reproductive cycle were coupled with biochemical analysis of tissue composition (carbohydrate, protein and lipid) in order to determine the energetic state of gorgonian colonies and its relation to the reproductive cycle, as well as the analyses of the fatty acids and stable isotopes composition to get some insight into the trophic ecology of the gorgonians throughout the year and in a depth comparison. In this section we present an overview of the main results obtained in the four chapters mentioned above as well as the links between chapters.

Spatial distribution patterns of Mediterranean gorgonians over a large spatial and bathymetrical extent

By using a ROV for the exploration of the northwestern Mediterranean coastal benthic communities from shallow waters down to the beginning of the continental shelf, high abundances of gorgonians were identified to dwell on rocky bottoms located below the depth of conventional scuba diving (approximately 40 m depth). Gorgonians were identified as a frequent and conspicuous elements of the precoraligenous and coraligenous communities, were they play a paramount role as structural species, reaching densities up to 30 colonies m^{-2}. In contrast with the high diversity of species in tropical gorgonian communities (Lasker and Coffroth 1983; Yoshioka and Yoshioka 1989; Chiappone et al.

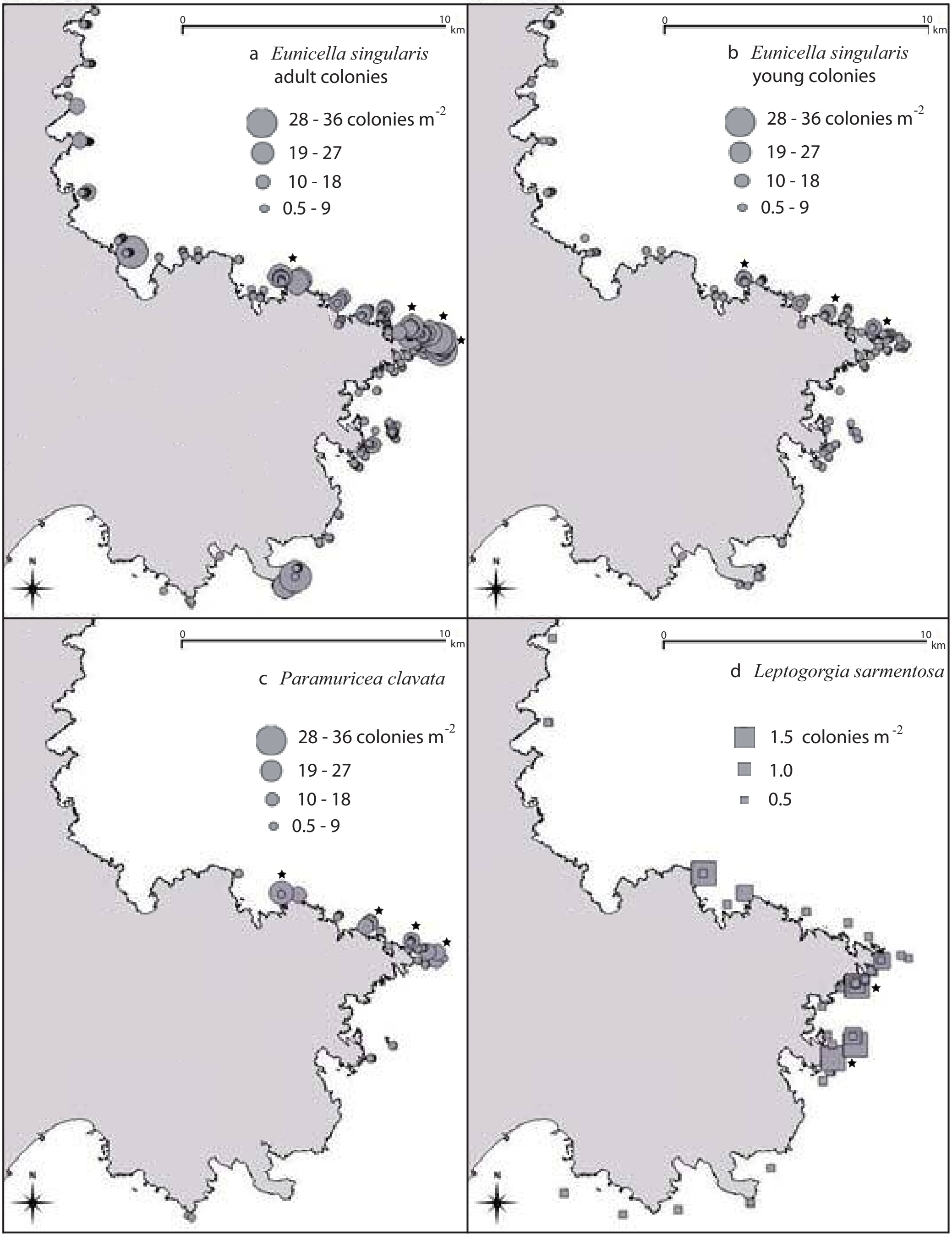

Fig. 1 - Spatial distribution of *Eunicella singularis* adult (a) and young (b) colonies, *Paramuricea clavata* (c), and *Leptogorgia sarmentosa* (d) in the study area. *Squares* are used instead of *bubbles* in Fig. 1d to highlight the lower magnitude of the density values. *Black stars* indicate hot spots of species abundance. The spatial distribution of each species can be displayed on Google Earth by means of Additional Resources 3 to 6.

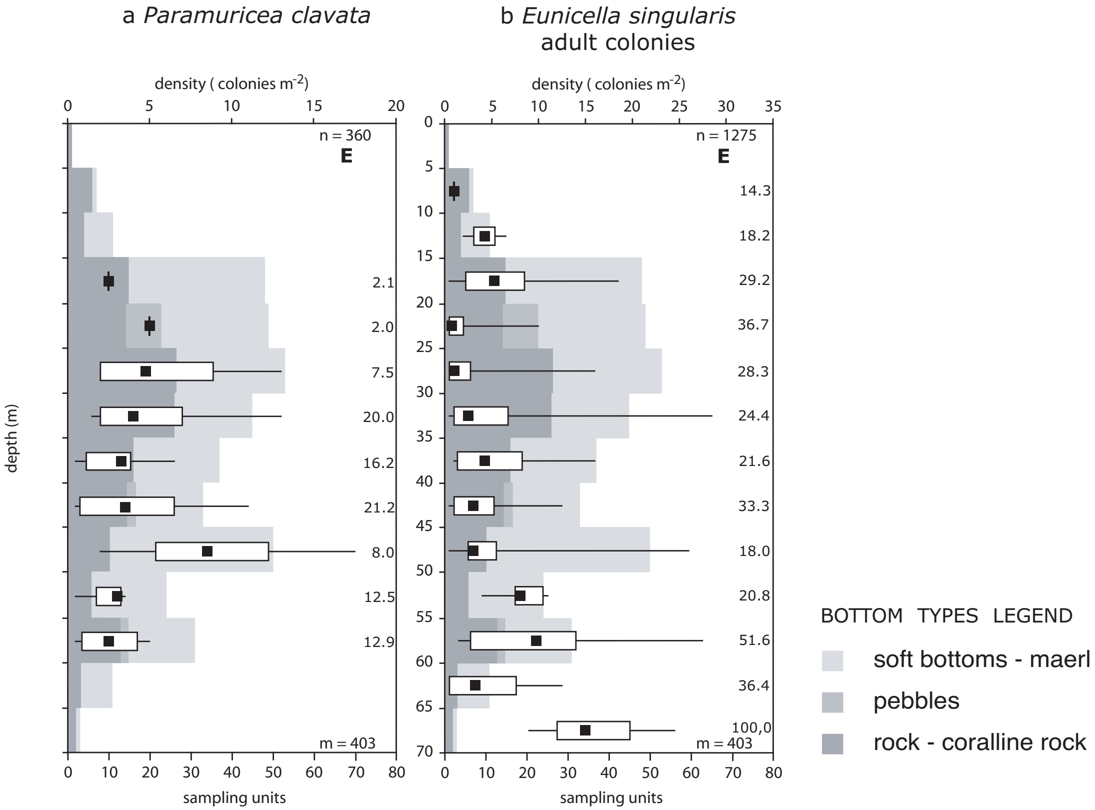

Fig. 2 - Bathymetrical distribution of the density of *Eunicella singularis* adult colonies and *Paramuricea clavata* colonies in the northern coast of the Cap de Creus. *Black square* indicates the median value; the *box* indicates the first and third quartiles; and the *line* indicates the range between minimum and maximum values. *Grey-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* on the right indicate the percentage of sampling units with presence of the species. Total number of colonies (*n*) and sampling units (*m*) are indicated.

2003), the Mediterranean gorgonian assemblages were mostly monospecific, and the coexistence of several species was rarely observed. *Eunicella singularis* was reported to be the most common and abundant gorgonian species, being widely distributed throughout the studied area (Figs. 1a and b) as well as in other locations in the Western Mediterranean Sea (Weinberg 1979, 1980; Linares et al. 2008). Its distribution is mainly driven by the requirement of hard bottom substrates, without a slope angle preference, thus high densities of *E. singularis* were also observed on vertical walls independently of light exposure. Conversely, *Paramuricea clavata* showed a very patchy distribution (Fig. 1c) that was strongly associated with vertical rocky walls, and this strong association could be the main explanation for the observed confinement of populations of this species to a few sites (Carpine and Grasshoff 1975; Weinberg 1976; Linares et al. 2008). The gorgonian species *Leptogorgia sarmentosa* has only been documented as isolated colonies (Fig. 1d), mainly on soft bottoms and maerl. The highest occurrence and abundances of *E. singularis* and *P. clavata* were concentrated in areas that were directly exposed to strong near-bottom currents; in contrast, *L. sarmentosa* was mainly found along the sheltered eastern side of the cape, where there is a turbulent circulation but no strong near-bottom currents. High abundances of *P. clavata* were identified on vertical and horizontal bottoms at depths from 25 to 50 m (Fig. 2a), whereas high densities of *E. singularis* were observed from 15 to 70 m depth (Fig. 2b). This highlighted that the research on Mediterranean gorgonians to this date was only focused on the fraction of the species populations located within the bathymetric range up to 40 meters. The finding of high density deep sublittoral gorgonian populations demonstrates the importance of studying the distribution of benthic species over a large spatial and bathymetrical range as well as that ROV studies can provide complementary information to the data obtained by scuba diving.

Morphological variation of a gorgonian species along a depth gradient

E. singularis is the only Mediterranean gorgonian species hosting symbiotic algae. Colonies inhabiting at depths shallower than 30–35 m displayed the common candlestick-like colony morphology, whereas colonies deeper than 30–35 m displayed a more variable colony morphology and brighter white color, and were identified as the subspecies without symbiotic algae (*E. singularis aphyta*) described by Théodor (1969). Since this aposymbiontic form is rare in the 0-40 m depth range, no study focused on it until today. The combination of scuba sampling and ROV observations allowed a detailed examination of *E. singularis* colonies located from 20 to 60 m depth. The analysis of colony's shape, sclerite variability, genetic markers and presence of symbiotic algae, confirmed the existence of two different morphotypes: a "shallow morphotype" (colonies from 20 to 30 m depth) with symbiotic algae that correspond to the commonly known *E. singularis*, and a "deep morphotype" (colonies from 40 to 60 m) displaying more ramified colonies and lacking symbiotic algae, corresponding to the aphyta variety. All this colony shape features tend to accentuate with increasing depth from 40 to 60 m (Fig. 3). In the same way, the sclerite features tend to change with increasing depth: sclerites were larger in the "shallow morphotype", showed intermediate dimensions at 40 m depth and, finally, they were clearly smaller in the "deep morphotype", where they resembled those of *Eunicella cavolinii* (Fig. 4). It is probable that the changes with depth of

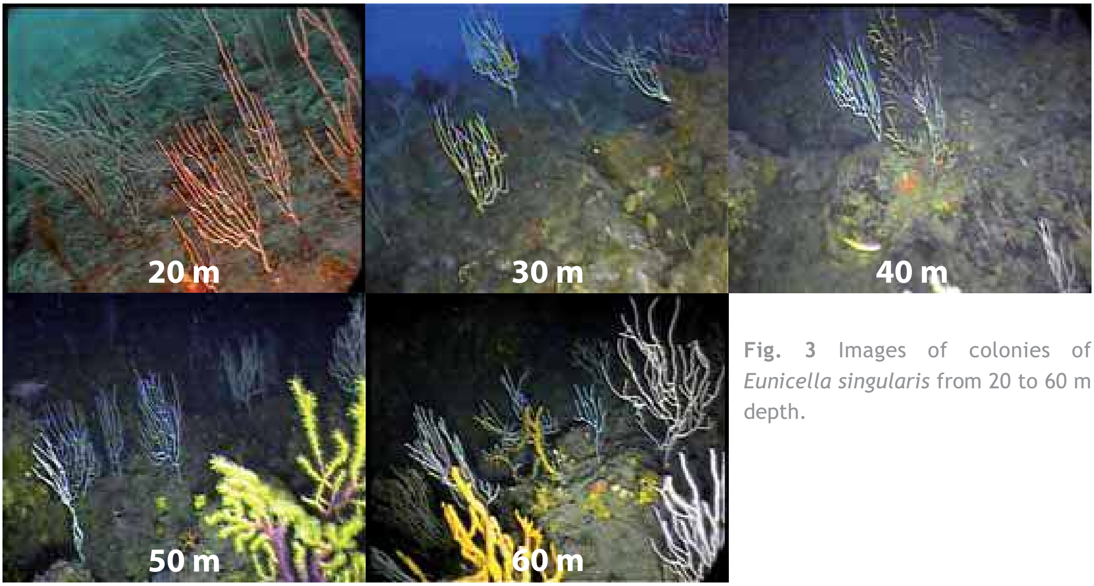

Fig. 3 Images of colonies of *Eunicella singularis* from 20 to 60 m depth.

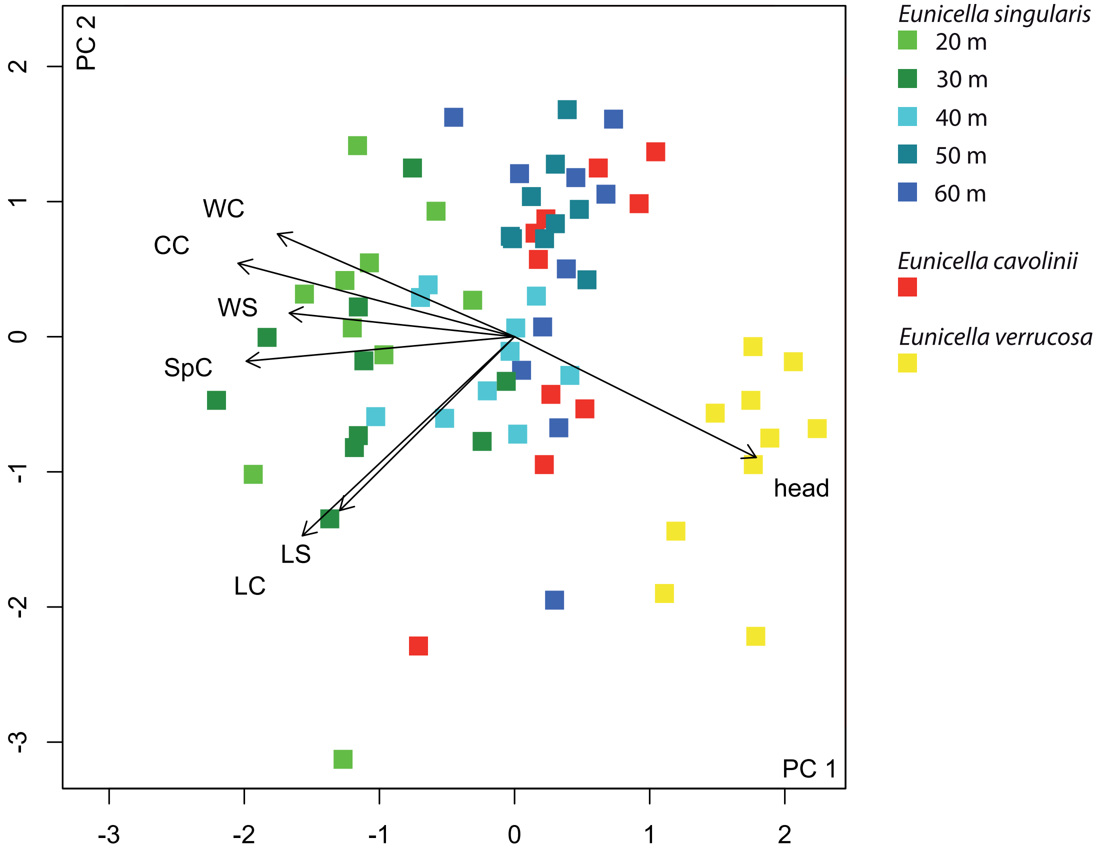

Fig. 4 Principal component analysis (PCA) biplot showing the ordination of the studied colonies regarding their spicule, and the roles of the analyzed features; LC = mean length of the balloon club, WC = mean width of the balloon club, SpC = mean width of the balloon club spiny end, CC = mean width of the balloon club collar, head = mean roughness of the balloon club head, LS = mean length of the spindles, WS = mean width of the spindles.

the main environmental features could play a major role in the differentiation and depth distribution of the two morphotypes. The candlestick-like shape with little ramification and very large primary branches are more readily bent and thus reduce hydrodynamic forces allowing the colonies to withstand the strong water movements characteristic of shallow water (Hiscock 1983), whereas the decrease with depth of water movement induced by wave action (Hiscock 1983) could explain the more ramified colony shape of the "deep morphotype" and the differences in the sclerites since both clubs and spindles are part of the mechanism that determine the mechanical properties of a gorgonian colony (Lewis and Von Wallis 1991). Phenotypic plasticity confers broad adaptability to the range of environmental conditions encountered by sessile organisms (Bradshaw 1965; Smith et al. 2007), providing the capacity for the colony to grow into the most suitable shape for that particular environment (Warner 1996; Marfenin 1997). Apart from differences in colony shape and spicules features, the discriminating characteristic of the two *E. singularis* morphotypes is the presence/absence of the symbiotic algae, which strongly affects the physiology and ecology of the two morphotypes, since symbiotic algae play a relevant role in processes such as growth and reproduction (Sebens 1987).

Size and spatial structure in deep versus shallow gorgonian populations

Shallow *E. singularis* populations (20 m) had lower colony heights (Table 1) as well as a higher percentage of young colonies than deep sublittoral populations (60 m) (Fig. 5). Size structure of shallow populations were dominated by small colonies (Fig. 6), thus indicating expanding populations and shallow habitats to be particularly suitable for this gorgonian species, as evidenced by the high recruitment rate. In contrast, deep sublittoral populations were dominated by medium-sized colonies (Fig. 6), and showed a more continuous coverage of the rocky bottom (Fig. 7). Since very strong water movements represent the most important cause of mortality in large gorgonian colonies, causing colony detachment or toppling (Birkeland 1974; Yoshioka and Yoshioka 1991; Weinbauer and Velimirov 1996), it is probable that the decrease with depth of the hydrodynamic forces (Hiscock 1983; Garrabou et al. 2002) could explain the larger colony sizes recorded in the deep populations. Further possible competition with fast growing algae and mortality induced by strong water movements may be the reason for the more patchy gorgonian coverage in the shallow populations. Conversely, in deep sublittoral populations, the absence of both fast growing algae and strong water movements, may provide enough time for the populations to develop a more continuous distribution.

Table 1 - Colony height in shallow and deep populations of *Eunicella singularis*.

	Population	Colonies	Height (cm)		
			Mean	SD	Max
Shallow	1	93	7.3	4.7	27.2
	2	119	14.9	7.9	37.6
	3	74	11.6	5.7	26.4
	4	104	9.8	5.7	26.2
	5	52	8.0	6.7	29.3
	6	128	9.5	6.8	30.4
	7	66	7.5	8.3	30.7
Deep	8	111	15.2	7.4	32.4
	9	114	13.2	5.3	26.2
	10	71	11.7	7.3	31.2
	11	114	9.6	6.8	47.1
	12	106	12.7	6.5	27.0
	13	134	14.7	8.4	32.2
	14	98	9.3	6.6	32.9

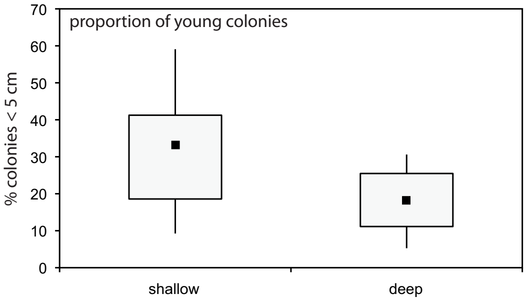

Fig. 5 - Comparison between shallow and deep *Eunicella singularis* populations. *Black square* indicates median value, *box* indicates first and third quartiles, and *line* indicates range between minimum and maximum values.

Reproductive cycle and trophic ecology in deep versus shallow gorgonian populations

The reproductive cycle turned out to be almost synchronized between shallow (20 m) and the deep populations (60 m) (Fig. 8), despite the marked differences in water temperature. This suggests that other factors besides temperature play a role in determining the timing of the reproductive cycle of this species. The energy storage (quantified as lipid concentration in the tissues) showed a marked seasonality in the shallow population, with highest values during the summer. Conversely, lipid concentration was lower and more constant in the deep population (Fig. 9). It could be argued that this estival energy surplus observed in the shallow colonies could lead to the higher gonadal output observed and to the more prolonged release of larvae observed in the shallow population. The analysis

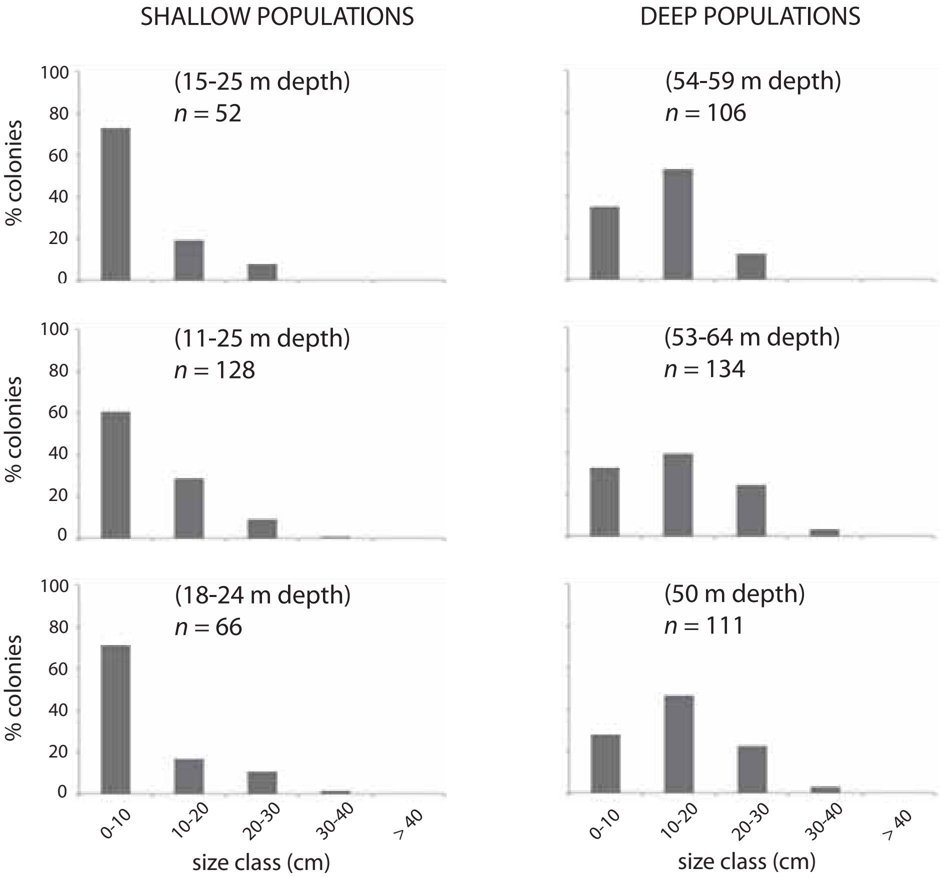

Fig. 6 - Examples of size-frequency distribution of shallow and deep *Eunicella singularis* populations (*n* = number of colonies).

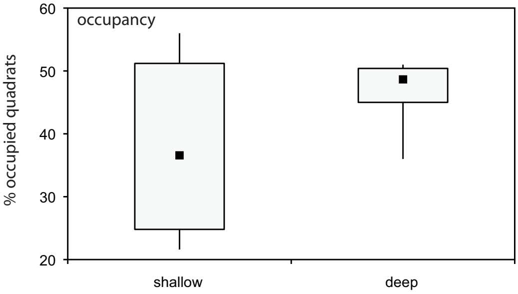

Fig. 7 - Comparison between shallow and deep *Eunicella singularis* populations. *Black square* indicates median value, *box* indicates first and third quartiles, and *line* indicates range between minimum and maximum values.

of trophic markers also showed a strong seasonality in the shallow population, whereas they point out to a major uniformity in the deep one. Isotopic composition of the shallow colonies was similar to values observed for passive suspension feeders with symbiotic algae (Carlier et al. 2007a), whereas the deep colonies showed high values close to other asymbiotic passive suspension feeders that mainly feed on microzooplankton and particulate organic matter (Fig. 10) (Jacob et al. 2006; Carlier et al. 2007a; Carlier et al. 2007b). During spring and summer the shallow colonies mainly depend upon the symbiotic algae and the capture of detritus of macroalgal origin, whereas in fall and winter they prey particles of animal origin. Conversely,

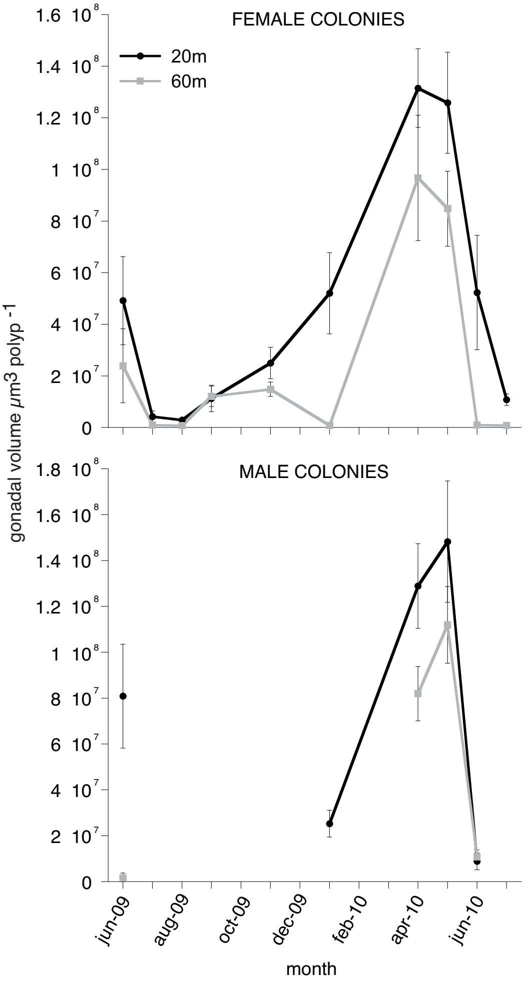

Fig. 8 - Annual cycle (June 2009 - July 2010) of mean polyp volume of female and male gonads (µm^3 polyp^{-1}) in the *Eunicella singularis* colonies from the shallow and deep population (mean ± SE).

deep colonies mainly feed on unicellular animals (Soler-Membrives et al 2011). Treignier et al. (2009) showed a significant transfer of δ^{13}C from zooxanthellae to the host, thus we suggest that *E. singularis* rely almost solely on its autotrophic source of energy in the shallow populations, at least during summer, whereas the deeper populations seem to be adapted to a heterotrophic food source, being microzooplankton the most predominant prey group.

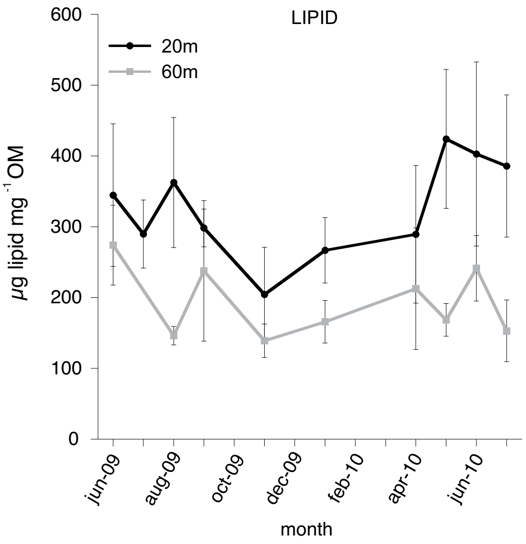

Fig. 9 - Annual cycle (June 2009 - July 2010) of lipid concentration (µg · mg^{-1} organic matter) in tissue of *Eunicella singularis* colonies from the shallow and deep population (mean ± SD).

CONCLUDING REMARKS AND FURTHER PROGRESS

The understanding of Mediterranean gorgonian ecology increased significantly in the last decades (Mistri and Ceccherelli 1993; Coma et al. 1995; Linares et al. 2007; Ribes et al. 2007). However, although Mediterranean sublittoral gorgonians are known to be present from shallow to deep waters (Rossi 1959; Laborel et al. 1961; Carpine and Grasshoff 1975; Weinberg 1976), the research focused, up to this date, almost only on the shallow gorgonian populations located from the surface to approximately 40 m depth. Intermediate depths, particularly coastal rocky bottoms in the 40-150 m depth range, has remained mostly unexplored until now. In this scenario, any effort intended to expand our knowledge on the ecology of these deep sublittoral gorgonian populations is of great interest to achieve a comprehensive understanding of their ecology, which is at the base of the development of any management and conservation plan.

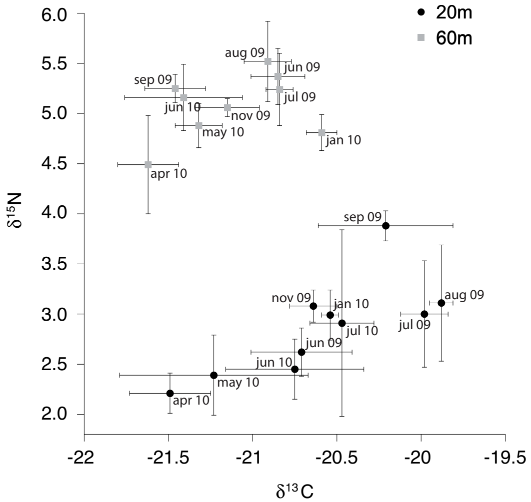

Fig. 10 - Stable isotops ($\delta^{13}C$ to $\delta^{15}N$) composition of *Eunicella singularis* colonies (*n* = 3) from the shallow and deep population (mean ± SD).

In this thesis, we applied a multidisciplinary approach in an attempt to begin to explore the ecology of the deep sublittoral populations of Mediterranean gorgonians, combining classical methods, new technologies and modern genetic and biochemical approaches. This combination made possible to highlight similarities and differences related to depth, and allowed to progress in the study of the variability of the ecology of these organisms as well as in the understanding of the ecological role that they play in the dynamics of the marine benthos. We are aware, however, that this thesis has generated many new questions that need to be addressed in future research. We identified questions that remain currently unresolved, as well as a few aspects that could enhance our understanding of the Mediterranean gorgonian ecology:

(1) An extensive exploration of Mediterranean rocky bottoms located in the depth range from 40 to 150 m should be undertaken to identify the location of dense populations of gorgonians and other rich benthic communities. This is of particular importance and urgency, given that these communities are nowadays suffering the destructive effects of different benthic fishing gears such as bottom trawling and benthic long-line.

(2) An effort to identify genetic markers that are able to distinguish among the species of the genus *Eunicella* should be undertaken, and this probably requires to focus on the study of DNA regions such as the D-loop, as well as to take into account the strong variability of such genetic markers.

(3) The use of fast-evolving genetic markers such as microsatellites could be a useful tool for the study of the connectivity between shallow and deep gorgonian populations, in order to explore the properties of these spatially structured population systems.

(4) A study focusing on the transmission of symbiotic algae from the parental colonies to the larvae and/or on the capacity of *Eunicella singularis* larvae to later acquire symbiotic algae, could provide useful information about the degree of differentiation of the two morphotypes of this species.

(5) Long term monitoring of marked deep sublittoral populations will provide data on recruitment, growth and mortality rates that could be used for the construction of life history tables of these deep populations. On the basis of the life history tables, demographic models can be developed in order to project population trends overtime. Results obtained can be compared with similar models developed for shallow population to get some insight of the dynamics of these possible metapopulation systems.

(6) An analysis of biochemical, stable isotops and fatty acids composition performed separately for the symbiotic algae and the gorgonian tissue could provide more detailed information on the role played by the zooxanthellae in the symbiosis with the gorgonian.

(7) Finally, the study of the main eco-physiological response of *E. singularis* colonies with and

without symbiotic algae could also provide useful information on the role played by the zooxanthellae in the physiology of this species.

Although scientific research often leads to few answers and to much more new questions, we hope that the results of this thesis may serve as a stimulus for future studies focused on hard-bottom benthic communities located at intermediate depths, which have remained fairly ignored for a long time.

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Resum

INTRODUCCIÓ

Els ecosistemes marins sublitorals, que s'estenen des de la línia costanera fins als marges de la plataforma continental, presenten moltes comunitats de fons tous i comunitats de fons rocoses amb una alta biodiversitat. La presència d'espècies sèssils que creen estructures en tres dimensions és un dels factors que contribueix més significativament a la riquesa de les comunitats d'aquests fons durs. L'heterogeneïtat de les estructures generades proporciona refugi i hàbitat a una rica fauna associada. A part de les praderies d'algues i de fanerògames marines, els invertebrats sèssils com els coralls i les gorgònies, són algunes de les espècies estructurals més representatives en el bentos marí.

CORALLS I GORGÒNIES

Els coralls i les gorgònies pertanyen a ordres diferents dins de la classe Anthozoa, phylum Cnidaria (excepte els coralls de foc, que pertanyen a l'ordre Milleporina, classe Hydrozoa). Les espècies de corals durs (Hexacorallia, Scleractinia) presenten formes molt diferents, que van des de pòlips solitaris fins a colònies de grans dimensions formades per centenars de milers de pòlips. Mentre que els coralls tous, les gorgònies (Octocorallia, Alcyonacea) i les espècies de corall negre (Hexacorallia, Antipatharia) sempre són organismes colonials.

Tots els corals i totes les gorgònies mostren un esquelet que, en funció de la seva composició en carbonat de calci i/o proteïnes, pot ser més o menys flexible. Tenir un esquelet dur permet a la majoria dels corals i de les gorgònies mantenir una posició erecte i conseqüentment participar en la construcció de complexes estructures tridimensionals, cosa que els dóna un rol molt important com a enginyers de l'ecosistema (Jones et al. 1994). Els esculls de corall són coneguts per estar principalment en aigües càlides, tot i que també en trobem en zones d'aigües temperades i fredes (Roberts et al. 2009; Wild 2011); les gorgònies són membres comuns i visibles de la majoria d'aquests esculls (Fig. 1) (Lasker i Coffroth 1983; Brazaeu i Lasker 1989; Mortensen et al. 1995; Ben-David-Zaslow i Benayahu 1999). En els esculls, les espècies de corall creixen formant complexes estructures tridimensionals que proporcionen diferents hàbitats per a centenars d'organismes associats. Els factors ambientals com la corrent de l'aigua, la disponibilitat d'aliments i la resuspensió de sediments varien molt dins dels hàbitats generats en un mateix escull de coral (Connell 1978; Kim i Lasker 1997; Wild 2011). L'heterogeneïtat de l'estructura generada pels coralls i per les gorgònies augmenta l'abundància i la diversitat funcional de la fauna associada (Mortensen et al. 1995; Witherell i Coon 2000; Roberts et al. 2002; Henry i Roberts 2007) fent dels esculls de coral un dels ecosistemes amb més biodiversitat del planeta (Connell 1978). Els coralls i les gorgònies cobreixen les seves demandes energètiques a través de la simbiosi amb les zooxanteles i/o filtrant la matèria orgànica particulada (POM) i capturant plàncton (Wildish i Kristmanson 1997). D'aquesta manera, els coralls i les gorgònies sovint inicien els cicles dels elements a través de l'alliberament de matèria orgànica, sobretot de moc (Wild et al. 2004; Crossland 1987), mentre que la captura de plàncton i POM determina un important fluxe d'energia i matèria entre el sistema pelàgic i el bentònic (Gili i Coma 1998).

GORGÒNIES MEDITERRÀNIES

De les aproximadament 20 espècies de gorgònies que habiten en el Mar Mediterrani (Carpine i Grasshoff 1975), l'*Eunicella singularis* (Esper, 1791), l'*Eunicella cavolinii* (Koch, 1887), la *Paramuricea clavata* (Risso, 1826), la *Leptogorgia sarmentosa* (Esper, 1789) i el *Corallium rubrum* (Linnaeus, 1758) són les més freqüents a la zona sublitoral: des de la superfície fins a la vora de la plataforma continental (Harmelin 1995; Gili et al. 1989). L'*E. singularis*, l'*E. cavolinii* i la *P. clavata* es troben entre les espècies estructurals més visibles i abundants en els fons rocosos sublitorals caracteritzats per les comunitats mediterrànies del coral·ligen i del precoral·ligen (Gili i Ros 1985; Harmelin 1995; Ballesteros 2006). En aquestes comunitats, aquestes gorgònies poden desenvolupar poblacions molt denses (Harmelin i Garrabou 2005) i jugar un paper important com a enginyers de l'ecosistema (Jones et al. 1994) proporcionant biomassa i complexitat estructural (Fig. 2) (True 1970). Les gorgònies representen l'estrat més elevat d'aquesta comunitat (Gili i Ros 1985), la diversitat i riquesa de la qual ha estat comparat amb la dels esculls de corall tropicals (Ros et al. 1985; Ballesteros 2006). L'alta biodiversitat de la comunitat de coral·ligen entre les comunitats bentòniques de la Mediterrània es deu principalment a la gran heterogeneïtat i diversitat dels microhàbitats originats per la seva complexa estructura tridimensional (Laborel et al 1961; Gili i Ros 1985; Harmelin 1995). *C. rubrum* és una espècie de gorgònia que es troba sovint a la comunitat del coral·ligen; tot i que també és una espècie característica de la comunitat de les coves semi-fosques (Gili i Ros 1985). Contràriament, la *L. sarmentosa* és una gorgònia que es troba comunment als fons tous i de maerl on pot desenvolupar poblacions molt denses (Weinberg 1979; Mistri 1995).


Fig. 1 - Esculls de corall tropical al Mar Roig.

Les diferents espècies de gorgònies de la Mediterrània comparteixen trets comuns en la seva ecologia reproductiva: colònies gonocòriques, un cicle de reproducció sexual anual amb l'ovogènesi de major durada que l'espermatogènesi (Coma et al. 1995; Ribes et al. 2007; Rossi i Gili 2009). L'*E. singularis* i el *C. rubrum* són espècies amb incubació interna en què l'embriogènesi es porta a terme dins dels pòlips de les colònies femelles i les larves plànules madures s'alliberen a través la boca del pòlip (Weinberg i Weinberg 1979; Vighi 1970). En canvi, la *P. clavata* és una espècie en la qual l'embriogènesi es porta a terme a la superfície de les colònies femelles (Coma et al. 1995). Fins ara, no hi ha informació clara sobre la modalitat de reproducció de l'*E. cavolinii,* tot i que s'ha suggerit que sigui una espècie amb incubació interna (Rossi 1959). La *L. sarmentosa* se suposa que allibera els gamets a la columna d'aigua, ja que no han estat mai observades larves dins dels pòlips o a la superfície de les colònies femelles (Rossi i Gili 2009). *E. singularis* és la única gorgònia mediterrània que mostra la simbiosi amb les zooxanteles (Rossi 1959; Carpine i Grasshoff 1975; Weinberg 1976), mentre que totes les altres espècies són completament heteròtrofes i s'alimenten d'una àmplia gamma de preses que va des dels nanoeukaryotes fins als copèpodes, constituint el microplàncton i la matèria orgànica particulada, la major part de la seva dieta (Coma et al. 1994; Ribes et al. 1999, 2003; Rossi et al. 2004; Tsounis et al. 2006a; Picciano i Ferrier-Pagès 2007). Les taxes de captura registrades suggereixen que l'impacte de les gorgònies sobre les comunitats planctòniques dóna lloc a una disminució substancial del plàncton en la columna d'aigua (Ribes et al. 1999, 2003). Conseqüentment, les gorgònies tenen un paper fonamental en les xarxes alimentàries del sublitoral ja que determinent un important fluxe d'energia i matèria des del sistema plànctonic fins al sistema bentònic (Gili i Coma 1998). Les gorgònies de la Mediterrània són espècies que viuen molts anys amb unes taxes de creixement lent i una baixa mortalitat natural (Weinberg i Weinberg 1979; Mistri i Ceccherelli 1993; Harmelin 1995; Coma et al. 1998, 2004; Linares et al. 2007; Bramanti et al. 2009). Aquesta lenta dinàmica de població fa que les gorgònies siguin especialment vulnerables a l'augment de les pertorbacions antropogèniques (Dayton et al 1995; Garrabou i Harmelin 2002; Santangelo et al. 2007; Linares i Doakes 2010) com els anclatges, les xarxes de pesca, el busseig i la contaminació (Harmelin i Marinopoulos 1994; Bavestrello et al. 1997; Coma et al. 2004) a més de les recents mortalitats en massa (Linares et al. 2005; Coma et al. 2006; Garrabou et al. 2009) probablement relacionades amb anomalies climàtiques indirectament vinculade a les activitats humanes.

Fig. 2 - Població de gorgònies del Mediterrani (*Paramunicea clavata*) al Cap de Creus.

HIPOTESIS DE PARTIDA

Les hipòtesis que han donat origen a aquesta tesi es basen en els resultats d'investigacions recents sobre la variabilitat espacial en l'ecologia de les gorgònies de la Mediterrània, així com en la identificació d'una important llacuna en els coneixements d'aquests organismes.

Variabilitat espacial

Els principals factors ambientals que influeixen en l'ecologia dels suspensívolrs bentònics a les zones costaneres de la Mediterrània són molt variables en una escala espacial petita (Rossi et al. 2003). Aquesta variablitat pot tenir repercussions en l'alimentació, reproducció, creixement, mortalitat i en les capacitats d'emmagatzematge d'energia de les diferents espècies de gorgònies. Les comparacions espacials són cada vegada més importants per entendre la variabilitat potencial i la capacitat d'adaptació de les espècies a les condicions locals, i en definitiva, per aconseguir una comprensió global de la seva ecologia. Un estudi anterior (inclòs en aquesta tesi com a annex, pàg. 133) que se centra en la variabilitat espacial en les característiques reproductives de l'*Eunicella singularis* i de la *Paramuricea clavata* va informar de fortes diferències pel que fa a l'emmagatzematge d'energia i d'un decalatge en els cicles reproductius entre dues poblacions de gorgònies situades a 600 km de distància (Gori et al. 2007). Comparacions espacials a gran escala van mostrar fortes variacions en l'estructura de talles de les poblacions de *P. clavata*, *E. singularis* i *Corallium rubrum* entre diferents localitats de la Mediterrània Occidental (Santangelo et al. 2003; Tsounis et al. 2006b; Linares et al. 2008; Bramanti et al. 2009). De la mateixa manera, es va veure que la distribució vertical de la *P. clavata* canvia significativament al llarg d'un gradient latitudinal com a resposta als canvis en la temperatura de l'aigua i en la llum (Linares et al. 2008).

La manca de coneixement en les profunditats intermèdies

En l'estudi de les comunitats marines de fons durs, les profunditat intermitges, i de manera particular els fons rocosos costaners situats entre 40 i 150 m de profunditat, han rebut relativament poca atenció ja que es troben per sota de la profunditat de busseig (Menza et al. 2008; Rooney et al. 2010) i la majoria de la investigació basada en submergibles es va dur a terme tradicionalment a profunditats per sota de 150 m (Sink et al. 2006; Virgilio et al. 2006; Hinderstein et al. 2010).

La investigació sobre l'ecologia de coralls i de gorgònies tropicals s'ha centrat principalment en els esculls situats en el rang de profunditat de busseig tradicional (Menza et al. 2008; Rooney et al. 2010), i conseqüentment gran part de la nostra comprensió de l'ecologia dels esculls de corall es basa en aquestes profunditats relativament superficials (Menza et al. 2008). Es coneix molt menys sobre els esculls de corall situats a la zona mesofòtica (Menza et al. 2008), definida com la part més profunda de la zona fòtica on encara es desenvolupen comunitats de coralls que depenen de la llum (Ginsburg 2007). Aquesta zona s'estén des de els 30-40 m de profunditat fins a la part inferior de la zona fòtica, que varia segons la ubicació i s'estén a més de 150 m en algunes regions (Hinderstein et al. 2010). Els esculls de corall dels ecosistemes mesofòtics alberguen espècies que també es troben en les seves parts més superficials, però també poden presentar un nombre d'espècies exclusives d'aquestes profunditats (Hinderstein et al. 2010). En general, la zona mesofòtica correspon a dues terceres parts de la distribució batimètrica de les espècies de coralls amb algues simbiòtiques (Pyle 1996, 2000), però tot i això fins ara s'ha mantingut en gran part inexplorada (Bongaerts et al. 2010). La investigació sobre els esculls de coralls mesophòtics, feta possible gràcies als recents avenços tecnològics, està revelant la presència de comunitats productives i riques que divergeixen significativament de les comunitats més superficials (Kahng et al. 2010). I més important encara es que els processos que tenen lloc a la zona mesophotica poden tenir una importància a nivell global que encara s'ha de quantificar (Buesseler et al. 2007; Hinderstein et al. 2010).

De manera similar, tot i que se sap que les gorgònies sublitorals de la Mediterrània estan presents fins a 100 m de profunditat (Rossi 1959; Laborel et al 1961; Carpine i Grasshoff 1975; Weinberg 1976), la gran majoria dels estudis realitzats fins avui s'han centrat en les poblacions d'aigües poc profundes. Les característiques ambientals canvien considerablement amb la profunditat en el Mar Mediterrani (Garrabou et al. 2002; Rossi i Gili 2009), afectant diferentment les poblacions de gorgònies superficials i profundes. Els hàbitats poc profunds estan subjectes a fort hidrodinamisme (Hiscock 1983; Weinbauer i Velimirov 1996) i estan exposades a corrents bidireccionals (Riedl

1971), mentre que els hàbitats més profunds es veuen més afectats per corrents unidireccionals (Riedl 1971; Hiscock 1983) i són en gran part a l'abric dels danys físics directes que poden ser causats per l'onatge generat per fortes tempestes (Bongaerts et al. 2010). La intensitat de la llum disminueix exponencialment amb la profunditat (Drew 1983); l'alta irradiació de l'estiu és la causa per a una forta estratificació de la columna d'aigua, la qual cosa pot resultar en un greu esgotament de les matèries en suspensió en aigües poc profundes durant l'estiu (Coma et al 2000; Rossi i Gili 2009). En general, la variabilitat en les condicions ambientals és menor a major profunditat en el mar Mediterrani perquè la temperatura, els corrents, i altres característiques de la columna d'aigua són més constants per sota de la termoclina d'estiu (Cebrián et al. 1996; Garrabou et al. 2002; Ballesteros 2006; Rossi et al. 2008). Aquestes diferències en les característiques principals del medi ambient podrien induir a diferències entre les poblacions de gorgònies situades a diverses profunditats. Per exemple, un decalatge temporal del cicle reproductor del *C. rubrum* i una diferència significativa en la quantitat de gamets produits es va reportar entre les poblacions situades a 18 i 40 m de profunditat (Tsounis et al. 2006c). De la mateixa manera es va observar un canvi significatiu amb la profunditat de l'ecologia tròfica del *C. rubrum* (Tsounis et al 2006a; Rossi i Tsounis 2007). Tant la *P. clavata* com el *C. rubrum* presenten estructures de talles dominades per colònies més grans en les poblacions més profundes que en les poblacions superficials (Harmelin i Marinopoulos 1994; Tsounis et al 2006b; Rossi et al. 2008).

Aquesta observació d'una gran variabilitat espacial en l'ecologia de les espècies de gorgònies de la Mediterrània, tant en les comparacions horitzontals com en les verticals, fa de l'investigació de les poblacions de gorgònies del sublitoral més profund, situades entre els 40 i 150 m de profunditat, un important avenç en l'estudi de les gorgònies de la Mediterrània, amb l'objectiu d'aconseguir una comprensió global de la seva ecologia.

OBJECTIUS DE LA TESI

Aquesta tesi aborda una primera descripció de l'ecologia de les poblacions profundes de gorgònies del sublitoral de la Mediterrània a través de quatre capítols que contenen noves dades i resultats, seguit d'un cinquè capítol que conté una discussió general.

CAPÍTOL I - PATRONS DE DISTRIBUCIÓ ESPECIAL DE LES GORGÒNIES *Eunicella singularis, Paramuricea clavata* I *Leptogorgia sarmentosa* (CAP DE CREUS, MAR MEDITERRANI NORD-OCCIDENTAL

Aquest capítol se centra en l'exploració de la zona sublitoral del Cap de Creus (Mar Mediterrani nord-occidental), per tal d'investigar la presència i els patrons de distribució espacial de les poblacions profundes de gorgònies del sublitoral de la Mediterrània. Aquest estudi va ser motivat per la manca de dades quantitatives sobre la presència de les espècies *Eunicella singularis*, *Paramuricea clavata*, i *Leptogorgia sarmentosa* en tota la seva àrea de distribució batimètrica. Mitjançant un vehicle operat per control remot (ROV), van ser identificades altes abundàncies de gorgònies que habiten en els fons rocosos situats per sota de la profunditat de busseig convencional (aproximadament 40 m de profunditat). Les gorgònies es van identificar com a elements freqüents i visibles de les comunitats del coralígen i del precoralígen on juguen un paper primordial com a espècies estructurals tot arribant a densitats de fins a 30 colònies per m^{-2}. En contrast amb l'alta diversitat d'espècies de les comunitats de gorgònies tropicals (Lasker i Coffroth 1983; Yoshioka i Yoshioka 1989; Chiappone et al. 2003), les associacions de gorgònies de la Mediterrània són majoritàriament monoespecífiques i la coexistència de diverses espècies s'ha observat en rares ocasions. L'*E. singularis* ha resultat ser una de les espècies de gorgònies més comuns i abundants que es distribueix àmpliament per tota l'àrea d'estudi (Figs. 3a i b), així com en altres indrets de la Mediterrània

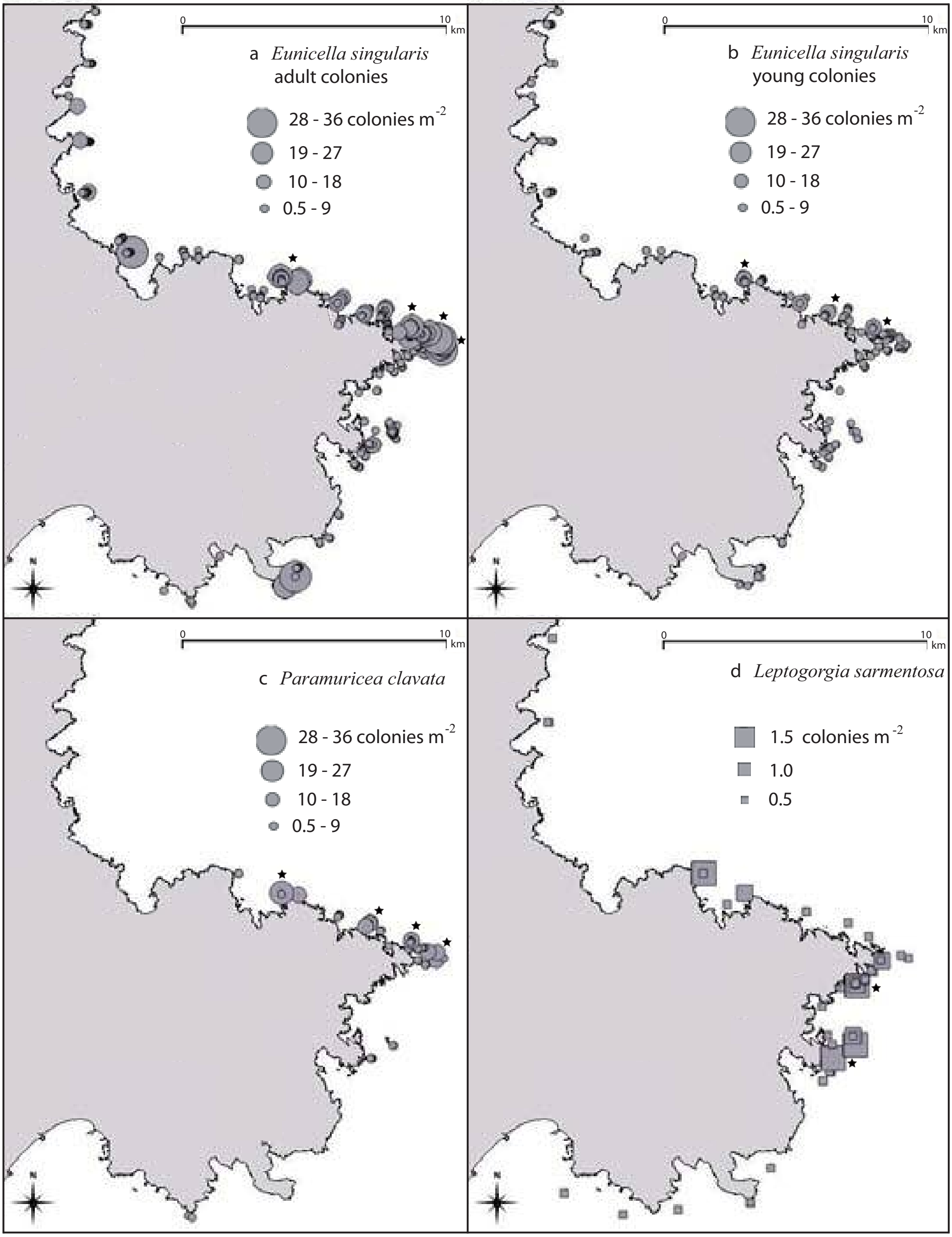

Fig. 3 - Distribució espacial de colònies adultes (a) i de colònies joves (b) d'*Eunicella singularis*, *Paramunicea clavata* (c) i *Leptogorgia sarmentosa* (d) a l'àrea d'estudi. Els *quadrats* van ser utlitizat enlloc dels *cercles* a la Fig. 3d per evidenciar la menor magnitud dels valors de densitat. Les *estrelles negres* indiquen els punts d'alta densitat. La distribució espacial de casa espècie es pot visualitzar al Google Earth mitjançant els recursos adicionals del 3 al 6.

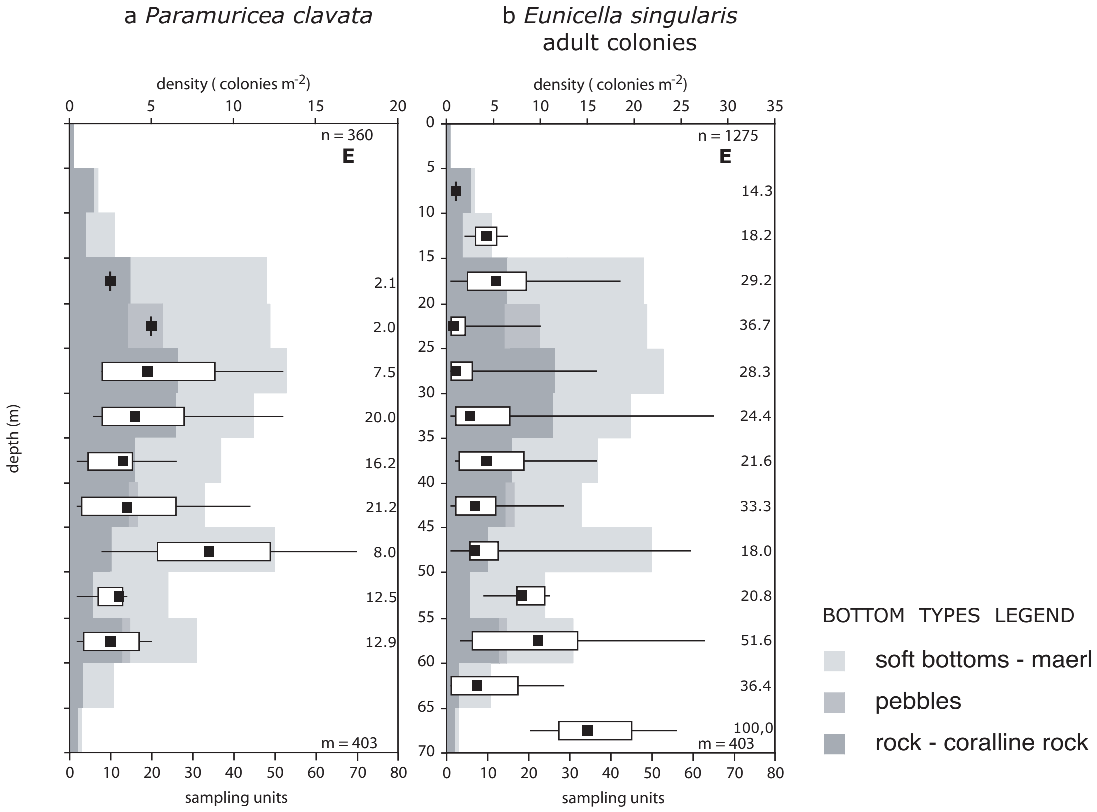

Fig. 4 - Distribució batimètrica de la densitat de colònies adultes d'*Eunicella singularis* i *Paramunicea clavata* a la costa nord del Cap de Creus. Els *quadrats negres* indiquen la mediana; la *caixa* indica el primer i el tercer quartil; i la *línia* indica l'interval entre el valor mínim i el màxim. Els *histogrames en tonalitat de grisos* representen el nombre total d'unitats de mostreig per a cada tipus de substrat (vegeu la llegenda) al llarg del rang batimètric estudiat. Els *números* a la dreta indiquen el porcentatge de unitat de mostreig on s'ha detectat la presència d'aquestes espècies. El nombre total de colònies (*n*) i de unitats de mostreig (*m*) estan també indicats.

Occidental (Weinberg, 1979, 1980; Linares et al. 2008). La seva distribució depèn principalment de la presència de substrats de fons dur sense una preferència en l'angle del pendent. En canvi, *P. clavata* ha mostrat una distribució molt més irregular (Fig. 3c) i fortament associada amb parets rocoses verticals. Aquesta forta associació podria ser la principal explicació per la presència de poblacions d'aquesta espècie a pocs llocs (Carpine i Grasshoff 1975; Weinberg 1976; Linares et al. 2008). L'espècies de gorgònia *L. sarmentosa* s'ha documentat només com a colònies aïllades (Fig. 3d), principalment en fons tous i de maërl. La major presència i abundància d'*E. singularis* i *P. clavata* es concentra en les zones que estant exposades directament a les fortes corrents a prop del fons, en canvi la *L. sarmentosa* es troba principalment en el costat est del Cap de Creus, on hi ha una circulació turbulenta, però no forts corrents a prop del fons. Altes abundàncies de *P. clavata* es van identificar en els fons rocosos verticals i horitzontals a profunditats des de 25 fins 50 m (Fig. 4a), mentre que altes densitats d'*E. singularis* es van observar a partir de 15 m de profunditat fins a 70 (Fig. 4b). Això posa en relleu que la investigació sobre les gorgònies de la Mediterrània fins a avui s'ha centrat només en la fracció de les poblacions que es troben en el rang batimètric de fins a 40 metres; mentre que la troballa de poblacions molt denses de gorgònies al sublitoral profund demostra la importància d'estudiar la distribució de les espècies bentòniques sobre una gran extensió espacial i batimètrica, així com que els estudis fets amb ROV poden proporcionar una informació complementària a les dades obtingudes pel busseig.

CAPÍTOL 2 - VARIACIÓ MORFOLÒGICA AL LLARG D'UN GRADIENT BATIMÈTRIC: REDESCOBRIMENT I DESCRIPCIÓ DELS DOS MORFOTIPUS DE LA GORGÒNIA MEDITERRANIA *Eunicella singularis*

L'*Eunicella singularis* es troba amb altes densitats en aigües sublitorals que van des de 15 a 70 m de profunditat. És l'única espècie de gorgònia mediterrània que té algues simbiòtiques, però l'aspecte de les seves colònies sembla canviar amb la profunditat: les colònies més superficials de 30-35 m mostren la morfologia a canelobre i un color blanc grisenc degut a la presència de les algues simbiòtiques; mentre que les colònies situades a més profunditat de 30-35 m mostren una morfologia més variable, són d'un color blanc brillant i es van identificar com la subespècie sense algues simbiòtiques (*E. singularis aphyta*) descrita per Théodor (1969). Com que aquesta forma aposymbiontica és rara entre 0 i 40 m de profunditat, cap estudi s'ha centrat en ella fins avui. Mitjançant la combinació de la recollida de mostres bussejant i les observacions per control remot, aquest capítol se centra en un examen detallat de les colònies d'*E. singularis* que es troben entre 20 i 60 m de profunditat. L'anàlisi de la forma de colònia, la variabilitat de les espícules, els marcadors genètics i la presència d'algues simbiòtiques, han suggerit l'existència de dos morfotipus diferents: un "morfotipus superficial" (colònies de 20 a 30 m de profunditat) amb algues simbiòtiques que correspon a la clàssicament coneguda *E. singularis*; i un "morfotipus profund" (colònies de 40 a 60 m) que mostra colònies més ramificades i sense algues simbiòtiques que correspon a la varietat aphyta. Totes aquestes característiques de la forma de les colònies tendeixen a accentuar-se amb l'augment de profunditat entre 40 i 60 m (Fig. 5). De la mateixa manera, les característiques de les espícules tendeixen a canviar amb la profunditat: són més grans en el "morfotipus superficial";

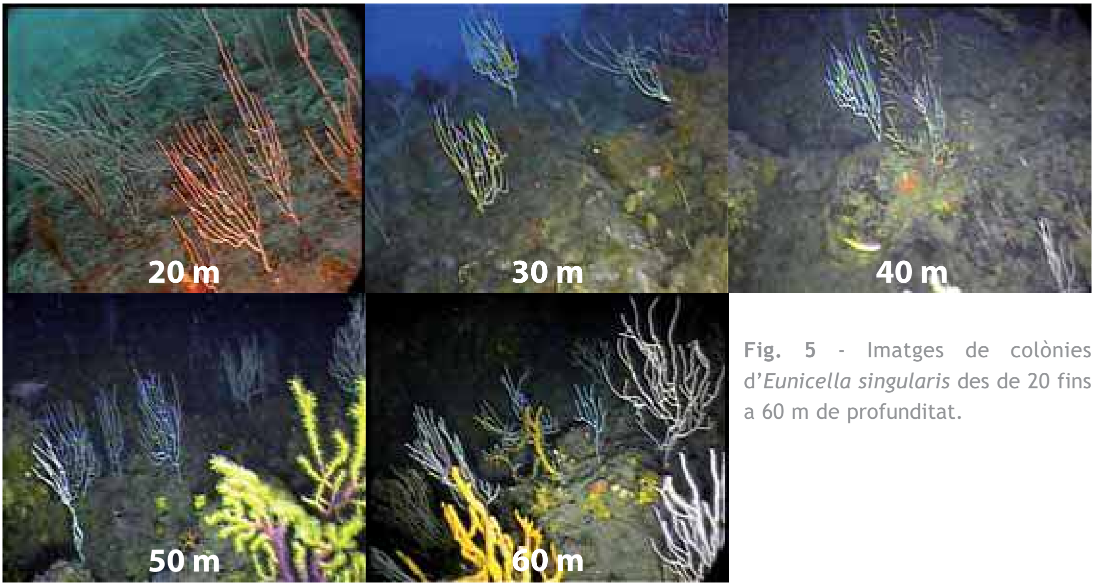

Fig. 5 - Imatges de colònies d'*Eunicella singularis* des de 20 fins a 60 m de profunditat.

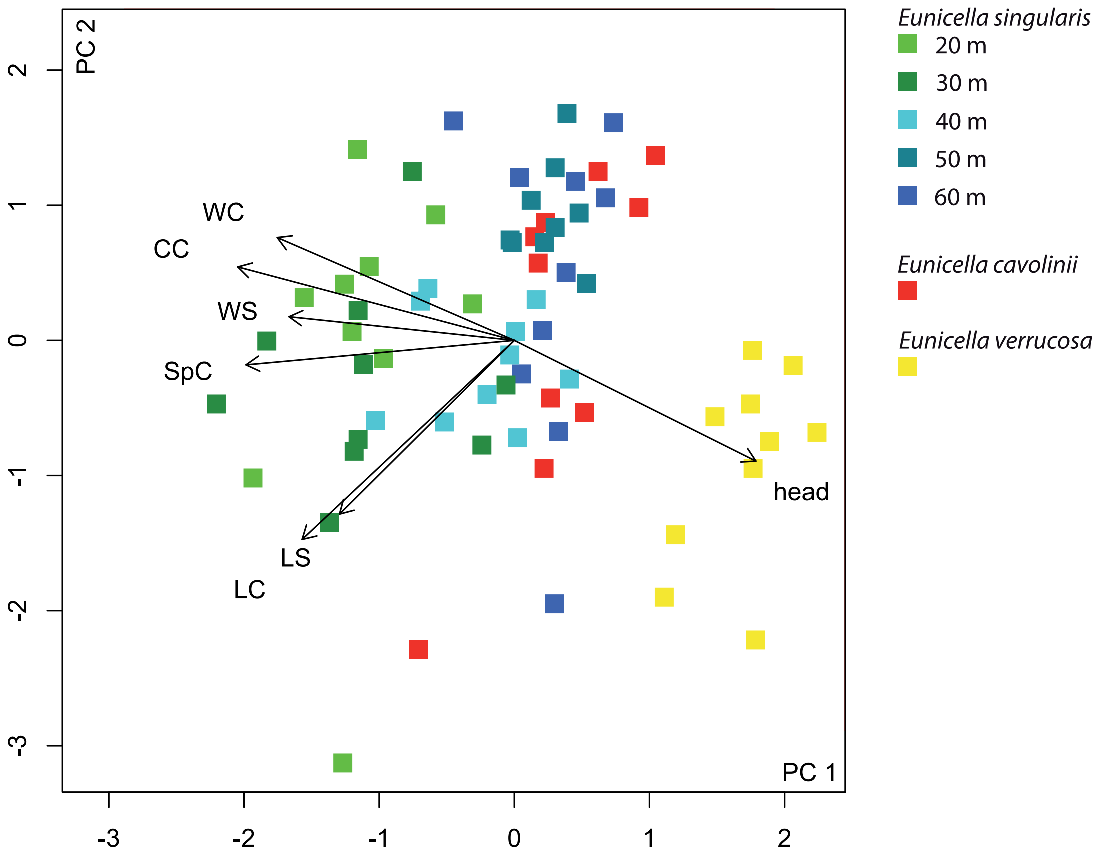

Fig. 6 - Anàlisi dels components principals (PCA) que mostra com s'ordenen les colònies estudiades respecte les característiques de les seves espícules, i el paper d'aquestes carecterístiques; LC = llargada mitjana de les espícules amb forma de maça, WC = amplada mitjana de les espícules amb forma de maça, SpC = amplada mitjana del peu espinós de les espícules amb forma de maça, CC = amplada mitjana del coll de les espícules en forma de maça, head = nivell mitjà de rugositat del cap de les espícues amb forma de maça, LS = llargada mitjana de les espícules amb forma de fus, WS = amplada mitjana de les espícules amb forma de fus.

mostren dimensions intermèdies en les colònies de 40 m de profunditat i finalment són clarament més petites en el "morfotipus profund", on s'assemblen a les d'*Eunicella cavolinii* (Fig. 6). És probable que els canvis en la profunditat de les principals característiques ambientals poden jugar un paper important en la diferenciació i la distribució en profunditat dels dos morfotipus d'*E. singularis*. Les colònies amb forma de canelobre amb poques ramificacions i branques primàries molt llargues són més fàcilment doblades per les forces hidrodinàmiques, d'aquesta manera les colònies resisteixen als forts moviments d'aigua característics d'aigües poc profundes (Hiscock 1983). La disminució amb la profunditat dels moviments de l'aigua causats per l'onatge (Hiscock 1983) podria explicar la forma de les colònies més ramificades del "morfotipus profund" i les diferències en les espícules, ja que aquestes formen part d'el mecanisme que determina les propietats mecàniques d'una colònia de gorgònies (Lewis i Von Wallis 1991). La plasticitat fenotípica confereix una àmplia adaptabilitat a la gamma de condicions ambientals trobades pels organismes sèssils (Bradshaw 1965; Smith et al. 2007) proporcionant la capacitat de la colònia per assumir la forma més adequada per a cada ambient particular (Warner 1996; Marfenin 1997). A part de les diferències en la forma de la colònia i en les característiques de les espícules, el principal caràcter discriminatori dels dos morfotipus d'*E. singularis* és la presència o l'absència de les algues simbiòtiques: aquestes afecten en gran mesura la fisiologia i l'ecologia dels dos morfotipus ja que juguen un paper rellevant en processos com el creixement i la reproducció (Sebens 1987).

CAPÍTOL 3 – ESTRUCTURA DE TALLA I ESTRUCTURA ESPACIAL DE POBLACIONS PROFUNDES VERSUS SUPERFICIALS DE LA GORGÒNIA MEDITERRANIA *Eunicella singularis* (CAP DE CREUS, MAR MEDITERRANI NORD-OCCIDENTAL)

Les poblacions poc profundes d'*Eunicella singularis* (20 m) tenen colònies de menor alçada, així com un major percentatge de colònies joves que en les poblacions sublitorals profundes (60 m) (Fig. 7). L'estructura de talles de les poblacions superficials està dominada per colònies petites (Fig. 8), el que indica que es tracta de poblacions en expansió i que els hàbitats poc profunds són particularment favorables per aquesta espècie, tal i com ho demostra l'alta taxa de reclutament. En contrast, les poblacions del sublitoral profund estan dominades per colònies de mida mitjana (Fig. 8), i mostren una cobertura més contínua del fons rocós. Els forts moviments d'aigua representen la causa més important de mortalitat en les grans colònies de gorgònies, provocant el desprendiment de les colònies (Birkeland 1974; Yoshioka i Yoshioka 1991; Weinbauer i Velimirov 1996). És probable que la disminució amb la profunditat de les forces hidrodinàmiques (Hiscock 1983; Garrabou et al. 2002) pugui explicar la mida de colònies més gran registrada a les poblacions de profunditat. La possible competència amb algues de ràpid creixement i la mortalitat induïda pels forts moviments d'aigua pot ser la raó de la menor continuïtat de la cobertura de les poblacions de gorgònies de poca profunditat. En canvi, en les poblacions del sublitoral profund, l'absència d'algues de creixement ràpid i l'absència de forts moviments d'aigua pot proporcionar el temps suficient a les poblacions per desenvolupar una distribució més contínua (Fig. 9).

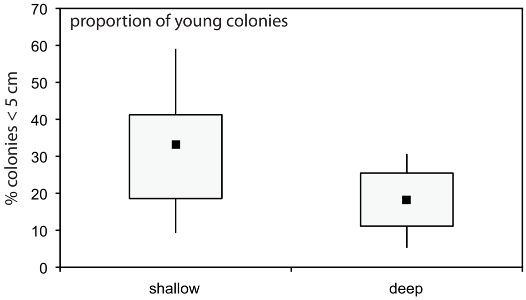

Fig. 7 - Comparació entre les poblacions profundes i superficials d'*Eunicella singularis*. Els *quadrats negres* indiquen la mediana, les *caixes* indiquen el primer i el tercer quartil, i les *línies* indiquen el rang entre els valors mínims i màxims.

CAPÍTOL 4 - CICLE REPRODUCTIU I ECOLOGIA TRÒFICA EN POBLACIONS PROFUNDES VERSUS SUPERFICIALS DE LA GORGÒNIA MEDITERRANIA *Eunicella singularis* (CAP DE CREUS, MEDITERRANI NORD-OCCIDENTAL)

Aquest capítol se centra en l'estudi del cicle reproductiu de dues poblacions de *Eunicella singularis*, situades respectivament a 20 i 60 m de profunditat i en l'estudi de la seva ecologia tròfica a través de l'examen de múltiples marcadors tròfics indirectes. Tot i les marcades diferències en la temperatura de l'aigua, els cicles reproductius resulten estar gairebé sincronitzats entre les poblacions superficials i les profundes (Fig. 10). Això suggereix que altres factors, a més de la temperatura, juguen un paper determinant en el control del cicle reproductiu d'aquesta espècie. L'emmagatzematge d'energia (quantificat com la concentració de lípids en els teixits) va mostrar una marcada

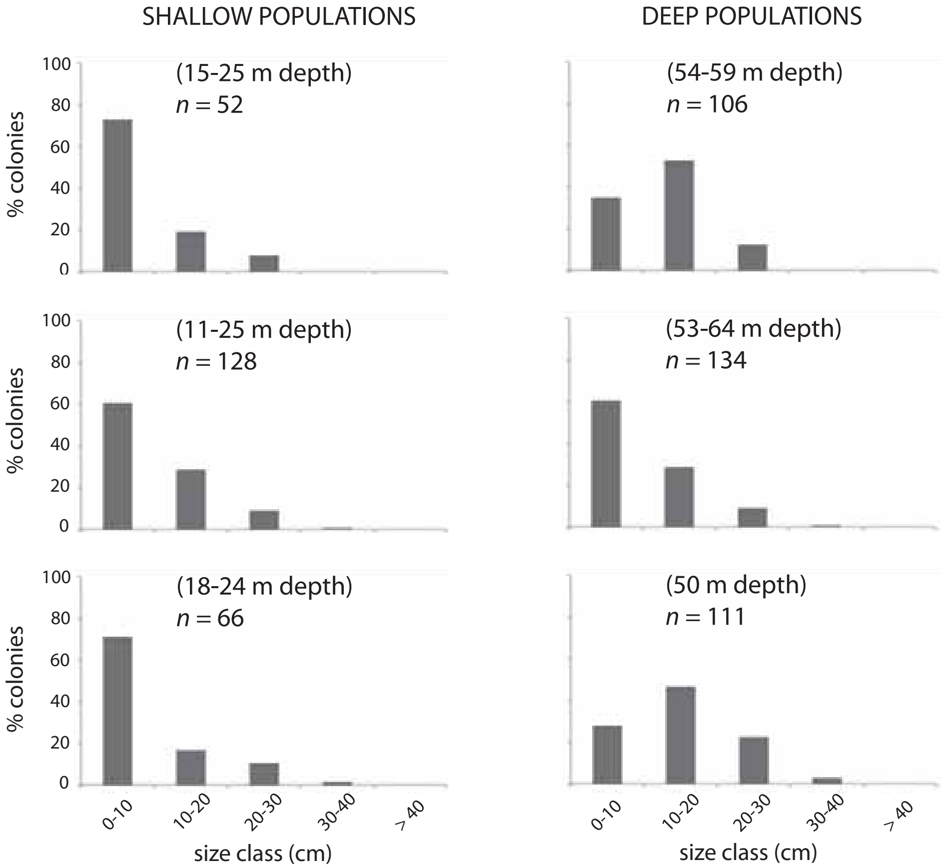

Fig. 8 - Exemples de distribució de talles de poblacions superficials i profundes d'*Eunicella singulars* (*n* = nombre de colònies).

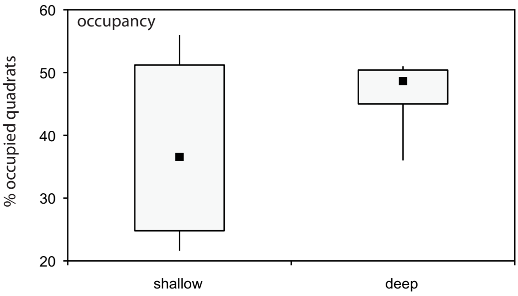

Fig. 9 - Comparació entre les poblacions profundes i superficials d'*Eunicella singularis*. Els *quadrats negres* indiquen la mediana, les *caixes* indiquen el primer i el tercer quartil i les *línies* indiquen el rang entre els valors mínims i màxims.

estacionalitat en la població de poca profunditat amb valors més alts durant l'estiu; per contra, la concentració de lípids va ser menor i més constant en la població de profunditat (Fig. 11). Es podria argumentar que aquest plus d'energia estival observat en les colònies superficials podria ser la causa de la major producció gonadal observada i de l'alliberament més prolongat de les larves observades en la població de poca profunditat. L'anàlisi dels marcadors tròfics també mostra una forta estacionalitat en la població de poca profunditat, mentre que apunta a una major uniformitat en la població profunda. La composició isotòpica de les colònies de poca profunditat és similar als valors observats en altres espècies de suspensívors pas-

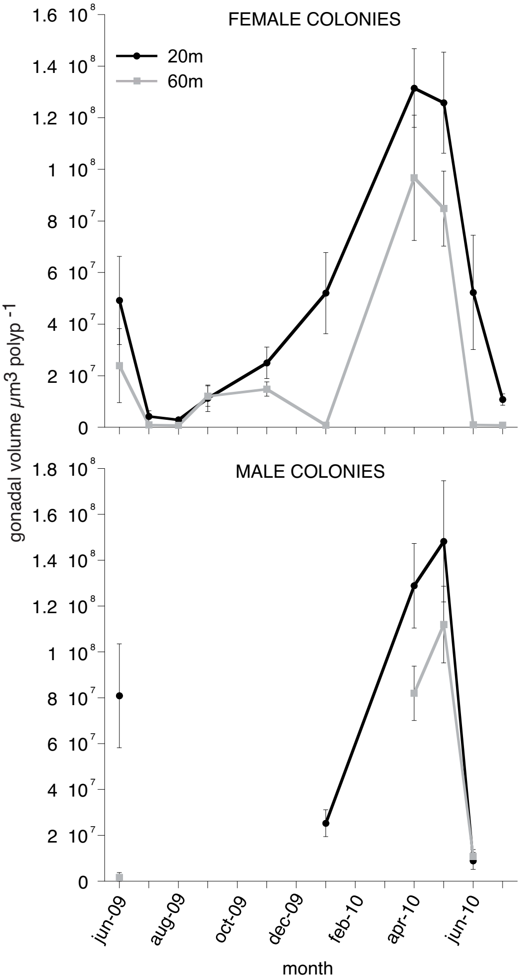

Fig. 10 - Cicle anual (juny 2009 - juliol 2010) del volum mitjà de gònades femenines i masculines (µm^3 polyp^{-1}) en les poblacions superficials i profundes d'*Eunicella singularis* (mitjana ± SE).

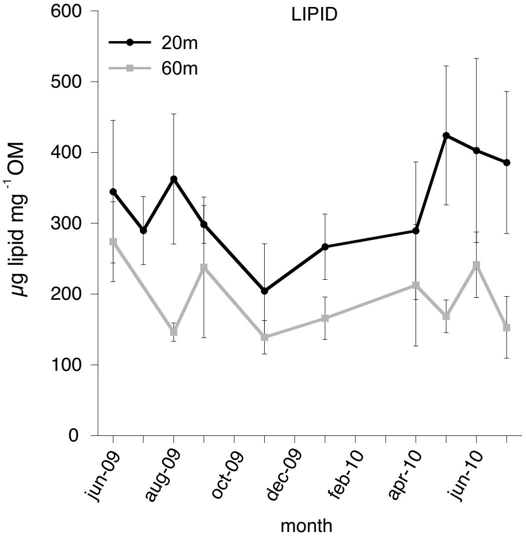

Fig. 11 - Cicle anual (juny 2009 - juliol 2010) de la concentració de lípids (µg · mg^{-1} materia organica) en el teixit de les colònies de les poblacions superficials i profundes d'*Eunicella singularis* (mitjana ± SD).

sius amb algues simbiòtiques (Carlier et al. 2007a), mentre que les poblacions profundes han mostrat valors més alts semblants als observats en altres filtradors suspensívors asimbiòtics (Fig. 12) (Jacob et al. 2006; Carlier et al 2007a, 2007b). Durant la primavera i l'estiu les colònies poc profundes depenen principalment de les algues simbiòtiques i de la captura de detritus originats per la descomposició de macroalgues, mentre que a la tardor i a l'hivern s'alimenten de partícules d'origen animal. Per contra, les colònies de profunditat s'alimenten principalment d'animals unicel·lulars (Soler-Membrives et al. 2011). Treignier et al. (2009) van mostrar una transeferència significativa de δ^{13}C des de les zooxantelas a l'hoste, per això creiem que les poblacions de poca profunditat d'*E. singularis* depènen gairebé únicament de la seva font energètica autotròfica, almenys durant l'estiu; mentre que les poblacions més profundes semblen adaptar-se a fonts d'alimentció heteròtrofiques, sent el microzooplancton la presa més predominant.

SÍNTESI DELS RESULTATS I DISCUSSIÓ GENERAL

Aquesta tesi afronta una primera descripció de l'ecologia de les poblacions sublitorals profundes de les gorgònies mediterrànies mitjançant una aproximació multidisciplinar basada en (1) l'avaluació dels patrons de distribució espacial, (2) l'anàlisi de la variació morfològica de les colònies de gorgònies, (3) l'estudi de l'estructura de talla de les poblacions i (4) l'anàlisi dels cicles reproductius i l'ecologia tròfica. Per abor-

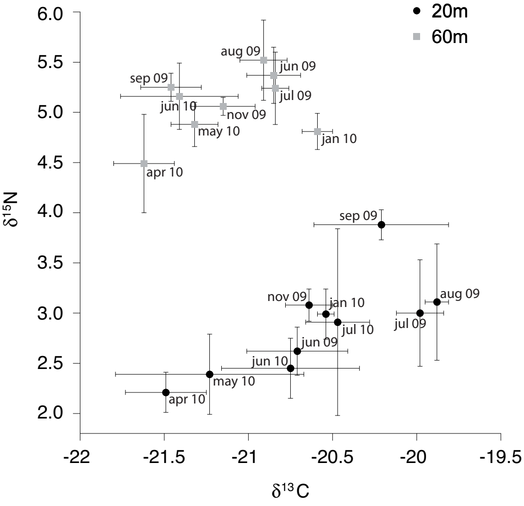

Fig. 12 - Composició en isòtops estables ($\delta^{13}C$ i $\delta^{15}N$) de poblacions superficials i profundes d'*Eunicella singularis* (*n* = 3).

dar aquests temes s'han utilitzat diverses metodologies (tant en el mostreig com en el laboratori i en les anàlisis de dades) per respondre a qüestions noves i velles sobre l'ecologia de les gorgònies mediterrànies. El primer capítol presenta els resultats de l'aplicació de l'anàlisi quantitativa dels transectes de vídeo realitzats amb vehicles d'operació remota (ROV) per tal d'identificar els patrons de distribució espacial de les poblacions de gorgònies. El segon capítol combina l'anàlisi d'imatges fotogràfiques obtingudes pel ROV, amb anàlisis de les espícules mitjançant el microscopi electrònic d'escombrat (SEM) i anàlisis genetics i histològics. El tercer capítol ofereix una combinació dels mètodes clàssics per a l'anàlisi de l'estrucutra de talla i l'estructura espacial de les poblacions de gorgònies aplicades, però, de manera innovadora a l'anàlisi de transectes vídeo obtinguts amb ROV, per abordar l'estudi de les poblacions de gorgònies en una escala batimètrica major. Finalment, en el quart capítol l'aplicació de tècniques de microscòpia i histologia per a l'estudi del cicle reproductiu de les gorgònies ha sigut complementada amb l'anàlisi bioquímic de la composició del teixit (carbohidrats, proteïnes i lípids) per tal de determinar l'estat energètic de les poblacions de gorgònies i la seva relació amb el cicle reproductiu, així com amb anàlisis dels isòtops estables i de la composició d'àcids grassos per obtenir una visió de l'ecologia tròfica de les gorgònies al llarg de l'any.

CONCLUSIONS I ESTUDIS FUTURS

La comprensió de l'ecologia de les gorgònies de la Mediterrània ha augmentat significativament en les últimes dècades (Mistri i Ceccherelli 1993; Coma et al. 1995; Linares et al. 2007; Ribes et al. 2007). Pero, no obstant se sap que les gorgònies de la Mediterrània estant presents des d'aigües superficials fins a aigües profundes (Rossi 1959; Laborel et al. 1961; Carpine i Grasshoff 1975; Weinberg 1976), la investigació sobre la seva ecologia ha estat centrada gairebé exclusivament en les poblacions que es troben per sobre dels 40 m de profunditat. Les profunditats intermitges, i de particular manera els fons rocosos costaners entre 40 i 150 m de profunditat, s'han mantingut majoritàriament sense explorar. En aquest escenari, qualsevol esforç destinat a ampliar els nostres coneixements sobre l'ecologia d'aquestes poblacions és de gran interès per aconseguir una comprensió global de la seva ecologia, que és a la base del desenvolupament de qualsevol pla de maneig i conservació.

En aquesta tesi s'ha aplicat un enfocament multidisciplinari en una primera aproximació a l'estudi de l'ecologia de les poblacions de gorgònies del sublitoral profunt de la Mediterrània. Amb aquesta finalitat s'han combinat els mètodes clàssics amb les noves tecnologies (ROV) i amb moderns enfocaments genètics i bioquímics. Aquesta combinació de diferents enfocaments ha fet possible posar de relleu les similituds i les diferències relacionades amb la profunditat, permetent avançar en l'estudi de la variabilitat de l'ecologia d'aquests organismes, així com en la comprensió del paper ecològic que tenen en la dinàmica del bentos marí. Som conscients, però, que aquesta tesi ha generat moltes noves preguntes que han de ser ateses en futures investigacions. S'han identificat preguntes interessants que no queden resoltes, així com alguns aspectes que podrien millorar la nostra comprensió de l'ecologia de les gorgònies de la Mediterrània:

(1) Una exploració extensa dels fons rocosos del Mediterrani situats en el rang de profunditat de 40 a 150 metres s'ha de dut a terme per identificar la ubicació de denses poblacions de gorgònies i riques comunitats bentòniques. Això és de vital importància i urgència, ja que aquestes comunitats pateixen avui dia els efectes de mètodes de pesca bentònica destructius com l'arrossegament i els palangres de fons.

(2) S'ha de fer un esforç per identificar marcadors genètics capaços de distingir entre les espècies del gènere *Eunicella*, i és probable que això impliqui centrar-se en l'estudi de regions d'ADN com el D-loop, així com també tenir en compte la forta variabilitat d'aquests marcadors genètics.

(3) L'ús de marcadors genètics de ràpida evolució, com els de microsatèl·lits, podrien ser una eina útil per a l'estudi de la connectivitat entre les poblacions de gorgònies superficials i profundes amb la finalitat d'explorar les propietats d'aquests sistemes de població estructurades espacialment.

(4) Un detallat estudi centrat en els mecanismes de transmissió de les algues simbiòtiques des de les colònies a les larves i/o sobre la capacitat de les larves d'*Eunciella singularis* d'assumir més tard algues simbiòtiques, podrien proporcionar informació útil sobre el grau de diferenciació dels ambdós morfotipus d'aquesta espècie.

(5) El seguiment temporal d'algunes parcel·les marcades de poblacions sublitorals profundes podria proporcionar dades sobre les taxes de reclutament, creixement i mortalitat que són essencials per al desenvolupament de models demogràfics aplicables a aquestes poblacions. La comparació dels models així obtinguts amb els models ja desenvolupats per les poblacions de poca profunditat, permetria explorar la dinàmica d'aquests possibles sistemes metapoblacionals.

(6) Una anàlisi de la composció bioquímica en isòtops estables i en àcids grassos feta per separat a les algues simbiòtiques i al teixit de les gorgònies podria proporcionar informacions més detallades sobre el rol jugat per les zooxanteles en la simbiosi amb la gorgònia.

(7) Finalment, l'estudi de les principals respostes ecofisiològiques de colònies d'*E. singularis* amb i sense algues simbiòtiques podrien proporcionar informació útil sobre el paper exercit per la zooxantela en la fisiologia d'aquesta espècie.

Tot i que la recerca científica sovint acaba amb alguna resposta i amb moltes més noves preguntes, esperem que els resultats d'aquesta tesi puguin servir d'estímul per a futurs estudis centrats en les comunitats bentòniques de fons durs situat a profunditats intermèdies que han estat fins ara força ignorates.

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Annex

Spatial variability in reproductive cycle of the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Anthozoa, Octocorallia) in the Western Mediterranean Sea

Additional Resource 1

Marine Biology (2007) 151:1571-1584

RESUM

Paramuricea clavata (Risso, 1826) i *Eunicella singularis* (Esper, 1791) són les espècies de gorgònies més representatives de les comunitats del sublitoral de fons durs al Mar Mediterrani Occidental. Els cicles reproductius de dues poblacions d'ambdues espècies van ser estudiats a dos llocs diferents situats a uns 600 km de distància (Illes Medes i el Cabo de Palos). La variació estacional dels nivells de concentració dels lípids en el teixit de les gorgònies va ser utilitzat com a eina per quantificar l'emmagatzematge d'energia per cada població estudiada i d'aquesta manera poder explicar les possibles diferències entre poblacions en la producció gonadal. La proporció de sexes en les poblacions de les Illes Medes d'ambdues espècies va ser de 1:1, mentre que a Cabo de Palos, la proporció de sexes va ser significativament desviada a favor dels mascles (1:7) en *P. clavata* i a favor de les femelles (1,7:1) en l'*E. singularis*. L'alliberació dels gamets es va produir coincidint amb un augment de la temperatura de l'aigua de mar durant la primavera, però comparant les dues localitats s'ha observat un decalatge en el temps de l'aliberació dels gamets que suggereix que la temperatura és el factor principal de la sincronització del desenvolupament gonadal en aquestes espècies. En les dues espècies es van trobar diferències significatives en el volum de les gònades per pòlip, degudes principalment a diferències en el nombre de gònades per pòlip, amb valors més alts observats en les poblacions de Cabo de Palos. Però els patrons observats en les concentracions de lípids no permeten concloure que els nivells de lípids puguin explicar les diferències en els volums gonadals.

Coautors: Cristina Linares, Sergio Rossi, Rafel Coma, Josep-Maria Gili

ABSTRACT

Paramuricea clavata (Risso, 1826) and *Eunicella singularis* (Esper, 1791) are the most representative gorgonian species in hard bottoms sublittoral communities in the Western Mediterranean Sea. Reproductive cycles of two populations of both species were studied in two distinct locations approximately 600 km apart (Illes Medes and Cabo de Palos). Seasonal variation of lipids concentration levels in the gorgonian tissue was used as a tool to quantify energy storage by each studied population in order to explain possible interpopulation differences in gonadal output. Sex ratio in Illes Medes populations of both species was 1:1, while in Cabo de Palos sex ratio was significantly male biased (1:7) in *P. clavata*, and female biased (1.7:1) in *E. singularis* populations. Spawning timing occurred in all cases coinciding with a marked increase in sea-water temperature in spring, but comparing localities there was a temporal shift in the time of gametes release, appearing the temperature to be the main synchronizing factor of gonadal development within these populations. Significant differences in gonadal volume per polyp were found in both species owing mainly to differences in the number of gonads per polyp, with Cabo de Palos populations displaying higher values in both studied species; however, the observed patterns in lipids concentrations levels, disable to conclude that lipid levels explain the observed differences in gonadal output.

Co-authors: Cristina Linares, Sergio Rossi, Rafel Coma, Josep-Maria Gili

INTRODUCTION

In sessile marine invertebrates success in sexual reproduction is highly dependent on synchronization in reproductive cycles. Individuals that do not spawn synchronically are clearly disadvantaged by a substantial reduction in the probability of successful fertilization of their gametes (Oliver and Babcock 1992). Therefore, in several species, sexual reproductive cycles are seasonal, and individual cycles are usually synchronous within a species' breeding range (Beauchamp 1993). Between the more common sessile invertebrates, cnidarians is one of the most studied group, specially corals where seasonality in reproduction has been well documented (Babcock et al. 1986; Alino and Coll 1989; Harrison and Wallace 1990).

Hypotheses on the major factors which may influence the synchronization of gametogenesis and spawning time in coral species have been discussed in several studies (Harrison and Wallace 1990; Richmond and Hunter 1990; Van Veghel 1994). The timing of reproductive cycles has been related to different environmental factors that could serve as cues to regulate reproduction through an endogenous system (Giese and Pearse 1974). The main factors involved in synchronic spawning suggested by different authors are: the increase in sea-water temperature for gamete maturation, the lunar phase for spawning date, and the day-night cycle for timing of release (Kojis and Quinn 1981; Babcock et al. 1986; Harrison and Wallace 1990; Richmond and Hunter 1990; Hayashibara et al. 1993). Seasonal fluctuations of sea-water temperature have been suggested as the most important environmental factor controlling the annual cycle of reproduction of coral species that live in areas with seasonal changes (Giese and Pearse 1974; Grigg 1977; Babcock et al. 1986; Beauchamp 1993).

Since long, many studies have demonstrated that reproductive traits vary with latitudinal location (Rinkevich and Loya 1979; Kojis 1986; Fan and Dai 1995). However geographic variability in those reproductive traits affected by environmental conditions is still poorly understood. The same coral species may reveal changes in its mode of reproduction in different geographical areas, as well as populations of the same species may display local shifts in reproductive timing and output (Richmond and Hunter 1990). A geographic comparison of reproductive output and timing of gamete release between localities may reveal population responses to different environmental conditions in coral species (Babcock et al. 1994; Goffredo et al. 2002).

Paramuricea clavata (Risso, 1826) and *Eunicella singularis* (Esper, 1791) are among the most representative gorgonian species in the Western Mediterranean sublittoral communities (Weinberg 1979), and the importance of these species on the plankton-benthos coupling has been described in several studies (Coma et al. 1994; Gili and Coma 1998). While the first species is typically found in sciaphilic rocky walls (True 1970; Weinberg 1979), the second one is a zooxanthellate species abundant on horizontal and subhorizontal rocky bottoms (Weinberg 1979). Both species share common reproductive traits as the annual sexual reproduction with longer duration of oogenesis as compared to spermatogenesis, and gonochoric colonies (Weinberg and Weinberg 1979; Coma et al. 1995; Ribes et al. in press). However *P. clavata* is a surface brooder and embryogenesis takes place on the surface of female colonies (Coma et al. 1995), and *E. singularis* is a brooder and embryogenesis takes place inside female polyps being the mature planulae larvae released from the polyp mouth (Weinberg and Weinberg 1979).

The aim of this study was the spatial comparison, on a relevant geographic scale, of reproductive timing and output between populations of the two species in the Western Mediterranean Sea, regarding the local sea-water temperature cycle, and the nutritional state of gorgonians in each population. Both kinds of factors have been rarely studied together taking into consideration more than one species and population. In this study we hypothesize that the spawning periods

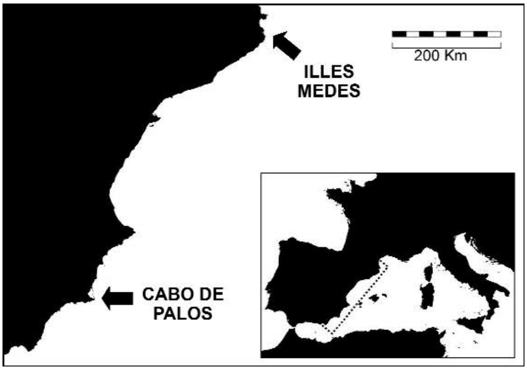

Fig. 1 - Map showing study locations along the Mediterranean spanish coast.

coincide with sea-water temperature peak, but also after the most successful feeding season. According to precedent studies, we used the seasonal variation of concentration levels of lipids in gorgonian tissue as a tool to quantify energy storage by each studied population due to the fact that a relationship between lipid concentration levels and food (and/or light) availability has been reported in coral species (Szmant-Froelich and Pilson 1980; Ben-Davied-Zaslow and Benayahu 1999; Harland et al. 1992; Rossi et al. 2006a).

MATERIALS AND METHODS

Two populations of *Paramuricea clavata* and *Eunicella singularis* were studied in two areas separated by approximately 600 km along the Spanish coast in the Western Mediterranean Sea (Figure 1). In the first area (Illes Medes: 43° 2’ 30” N, 3° 13’ 30” E) the sampled populations were located between 18 and 20 m depth while in the second area (Cabo de Palos: 37° 38’ 01” N, 0° 41’ 04” W) the populations were located between 30 and 35 m depth. Both studied populations were situated in the upper limit of bathymetric distribution of the species in each area.

Sampling

Monthly sampling was carried out during 14 months from October 2002 to November 2003; due to logistic reasons it was not possible to sample in January 2003 at Cabo de Palos, and in February and August 2003 at the Illes Medes. *P. clavata* colonies between 30 and 40 cm (± 0.5 cm) of maximum height (distance from the base to the farthest point) and *E. singularis* colonies between 20 and 30 cm (± 0.5 cm) were sampled (sexually mature colonies according to Coma et al. 1995 and Ribes et al. in press). During each sampling a fragment of primary branch from each of 30 different colonies of each population were randomly collected by scuba divers (in order to obtain at least 10 female and 10 male colonies based on a sex ratio 1:1 described in Coma et al. (1995) and Ribes et al. in press). A total of 750 branches of each species from both areas were collected. Branches were divided in two portions, one fixed in formalin (10%) in order to study population sex ratio and gonadal development cycles, and the other one stored in liquid nitrogen for biochemical analysis. Once in laboratory the portions stored for biochemical testing were lyophilized (16 hours at -110 ºC with a pressure of 100 mbar) and stored at -20 ºC until analysis (following the protocol of Rossi et al. 2006b).

Population sex ratio

In order to quantify population sex ratio we have only considered branches sampled from March to the spawning moment (in each population). This is when the sex of each branch can be unequivocally determined observing gonadal colour and appearance with a binocular stereomicroscope according to Coma et al. (1995) and Ribes et al. (in press). Branches in which no gonads were found inside 10 polyps were not considered.

Gonadal development cycles

To examine the timing of gonadal development cycles, 10 male and 10 female branches were randomly chosen each month from March to September. The diameter of all oocytes and spermaries present in 10 polyps were measured with an eyepiece micrometer (+/- 20 μm), avoiding apical-ends where gonadal output may be affected by annual growth (Coma et al. 1995). After spawning, branches in which no gonads were found inside 10 polyps were considered as males, according to the fact that only female colonies present gonads during all the year (Coma et al. 1995, Ribes et al. in press). When the shape of gonads was like an ellipsis, both minor (a) and major (b) diameters were measured and the diameter of the sphere with equivalent volume calculated ($d = 2((a/2)^{2*} b/2)^{1/3}$). In *E. singularis*, planulae found inside polyps were also counted. Totally 2150 polyps from *P. clavata* and 2410 polyps from *E. singularis* were examined and more than 22000 and 12000 gonads were measured from *P. clavata* and *E. singularis*, respectively.

Biochemical analysis

Biochemical analysis was carried out using monthly samples of 10 branches for each species in each population. Male and female colonies were not considered separately owing to the random collection of the samples. Approximately 20 mg (± 0.01 mg) of coenenchyma dry weight from each sample was homogenized in 7.5 ml of chloroform-methanol (2:1) and total lipids quantified colorimetrically according to the method of Barnes and Blastock (1973), with cholesterol as a standard. To quantify the percentage of tissue in coenenchyma dry weight, approximately 30 mg (± 0.01 mg) of coenenchyma dry weight from each sample were ashed for 4 hours at 500ºC in a muffle furnace. The difference between dry weight and ash weight was the Organic Matter (OM) (Slattery and McClintock 1995). Results are presented in µg of lipid mg^{-1} of gorgonian OM.

Data treatment

The sex ratio was tested by means of a chi-square test. To estimate the gonadal volume per polyp (average ± SE), diameters (d) were previously transformed into volume ($V= 4/3\pi(d/2)^3$). To assess significant differences between populations in volume, diameter and number of gonads for each species, we only compared the months that displayed the maximum volume in each population using a one-way ANOVA test, with colonies nested to site factor. The gonadal diameter frequency distribution during the period studied is shown using data from 5 female colonies (50 polyps dissected) and 5 male colonies (50 polyps dissected) randomly chosen each month. Seasonal variability in lipid concentration levels and significant differences between populations of the same specie were assessed using two-way ANOVA test (Sokal and Rohlf 1995).

RESULTS

Population sex ratio

Paramuricea clavata population in Illes Medes showed a sex ratio (59 female and 56 male colonies) not significantly different from 1:1 (χ^2=0.078; p=0.780); in contrast Cabo de Palos population showed a sex ratio (17 female and 120 male colonies) completely biased in favor of male colonies 1:7 (χ^2=77.438; p<0.01). Hermaphroditic colonies with polyps containing both spermaries and oocytes were very uncommon in both populations, Illes Medes (0.86%) and Cape of Palos (0.72%). In *Eunicella singularis*, Medes population showed a sex ratio (27 female and 26 male colonies) not significantly different from 1:1 (χ^2=0.019; p=0.891); while Cabo de Palos population showed a sex ratio (91 female and 54 male colonies) significantly biased in favor of female colonies 1.7:1 (χ^2=9.441; p<0.01). Similar to *P. clavata*, *E. singularis* showed few hermaphroditic colonies in both populations, Illes Medes (1.85%) and Cabo de Palos (0.65%).

Paramuricea clavata gonadal development cycles

In female colonies of both populations, only eggs showed diameters between 200 and 550 µm were spawned, while gonads displayed diameters between 50 and 200 µm were retained, thus showing that two overlapping cohorts of oocytes exist although they could not be distinguished before spawning (Figure 2a, b). Spawning took place between May and July in Illes Medes population. The lack of female colonies samples during July 2003 did not allowed us to determine whether spawning occurred between May and July or May and August in Cabo de Palos population (Figure 2a, b). Male gonadal development cycle showed a single cohort of spermaries that

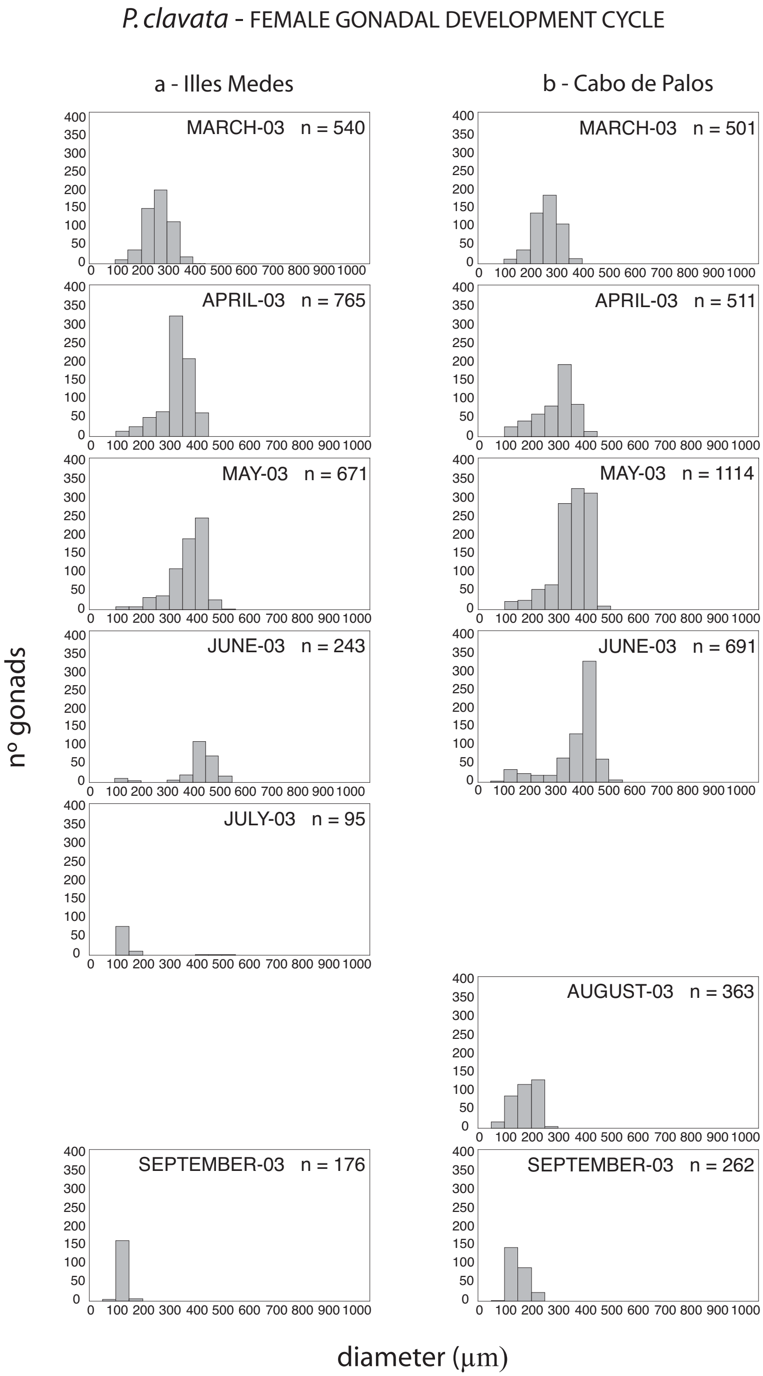

Fig. 2 - *Paramuricea clavata*. Distribution of gonadal diameter frequency (μm) of 50 female polyps (except in Cabo de Palos May and August, see Table 1); Illes Medes (a), Cabo de Palos (b) (*n* = gonads number).

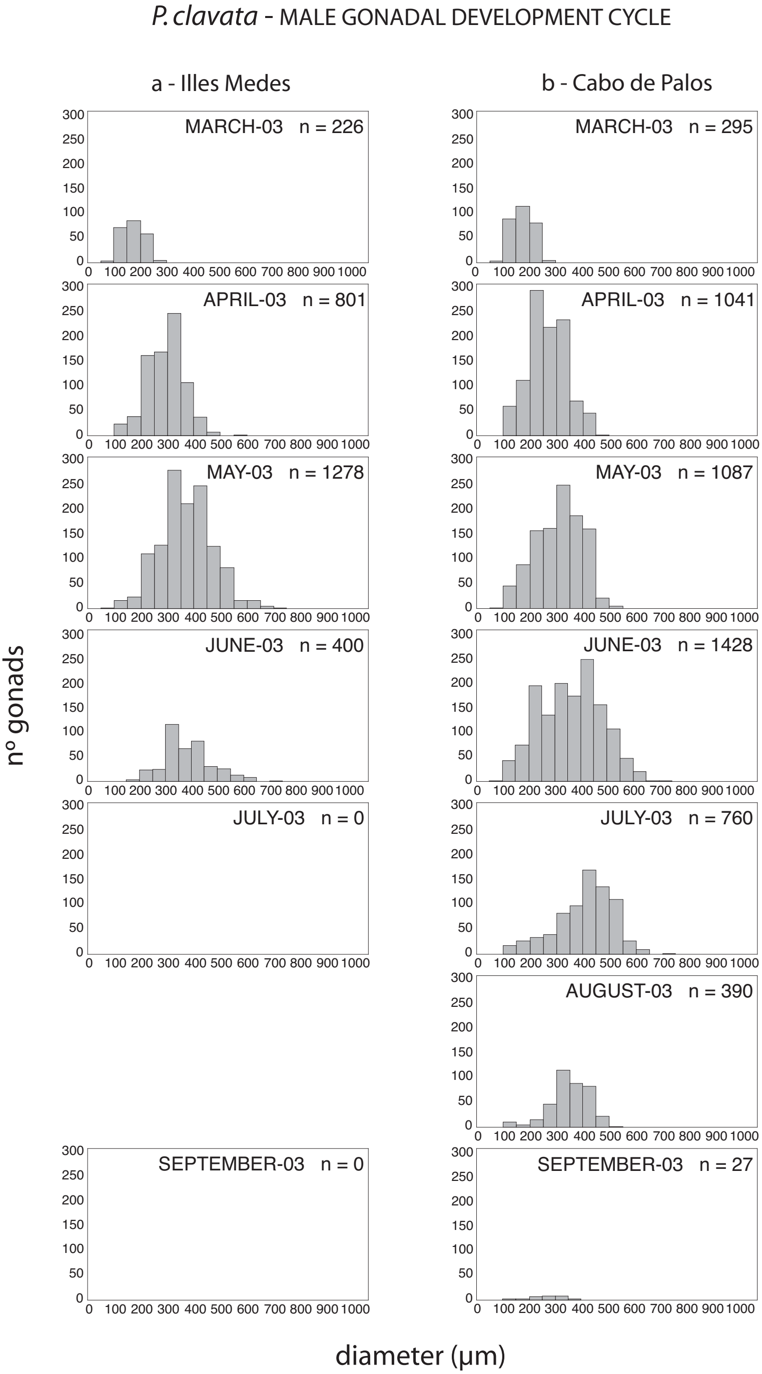

Fig. 3 - *Paramuricea clavata*. Distribution of gonadal diameter frequency (µm) of 50 male polyps; Illes Medes (a), Cabo de Palos (b) (*n* = gonads number).

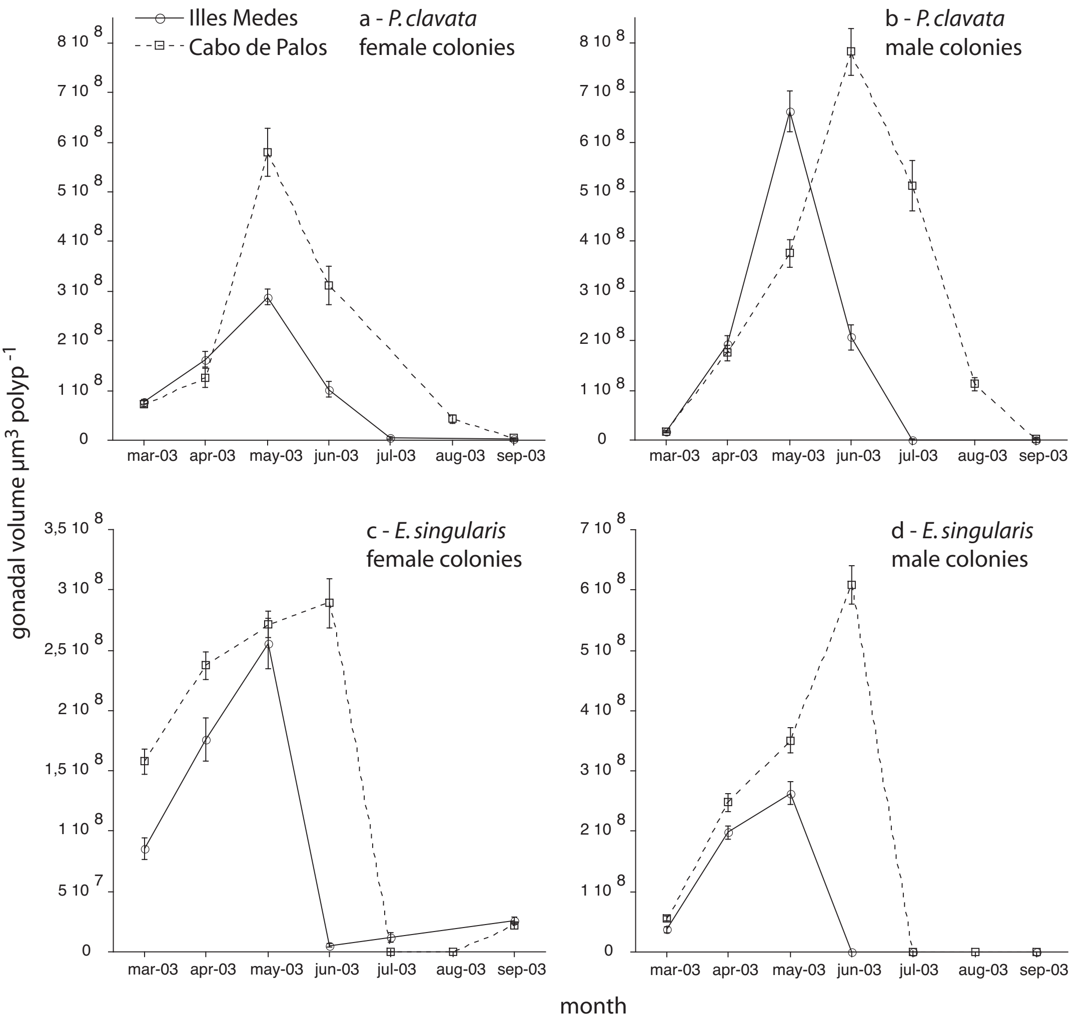

Fig. 4 - Monthly changes in mean polyp volume of gonads (µm^3 polyp $^{-1}$); *Paramuricea clavata* female colonies (a), *Paramuricea clavata* male colonies (b), *Eunicella singularis* female colonies (c) and *Eunicella singularis* male colonies (d) (mean ± SE).

increased in number and diameter until spawning between May and July in Illes Medes population and between June and September in Cabo de Palos population (Figure 3a, b). Female average gonadal volume per polyp differed significantly between population, being higher in Cabo de Palos population (one-way ANOVA, $F_{1,126}=8.965$, $p<0.05$). This difference was owing to significant differences in average number of gonads per female polyp (one-way ANOVA, $F_{1,126}=16.637$, $p<0.01$), while no significant differences appeared in average diameter of female gonads (one-way ANOVA, $F_{1,2260}=2.666$, $p=0.129$) (Figure 4a and Table 1a). Both populations did not differ in male gonadal volume per polyp (one-way ANOVA, $F_{1,180}=0.562$, $p=0.463$), although significant differences appeared in average number of gonads per male polyp (one-way ANOVA, $F_{1,180}=6.439$, $p<0.05$), but not in diameter of male gonads (one-way ANOVA, $F_{1,5280}=0.146$, $p=0.707$) (Figure 4b and Table 1b).

Table 1 - Monthly changes of number and diameter of *Paramuricea clavata* gonads; female colonies (**a**), male colonies (**b**) (mean ± SE).

Month	Gonad diameter (µm) Mean	SE	Gonad number /polyp Mean	SE	Total number of polyps
a - female colonies					
Illes Medes					
mar-03	237	1.6	9.8	0.6	100
abr-03	292	1.9	11.0	1.0	100
may-03	349	2.1	11.6	0.6	100
jun-03	398	4.7	2.8	0.4	100
jul-03	117	5.3	2.1	0.3	80
aug-03	-	-	-	-	-
sep-03	96	0.9	3.3	0.4	100
Cabo de Palos					
mar-03	221	1.7	8.7	0.9	60
abr-03	269	3.2	10.2	1.3	50
may-03	329	2.0	27.9	2.0	40
jun-03	352	3.3	11.7	1.3	60
jul-03	-	-	-	-	-
aug-03	152	2.4	18.2	2.8	20
sep-03	126	1.5	3.7	0.4	100
b - male colonies					
Illes Medes					
mar-03	165	2.1	5.4	0.5	100
abr-03	251	1.6	19.1	1.2	100
may-03	348	2.0	24.3	1.3	100
jun-03	361	3.3	7.1	0.7	100
jul-03	271	6.8	0.1	0.1	100
aug-03	-	-	-	-	-
sep-03	0	0	0	0	100
Cabo de Palos					
mar-03	161	1.9	4.2	0.7	100
abr-03	254	1.9	16.3	1.1	100
may-03	298	1.9	21.4	1.3	100
jun-03	339	2.1	28.9	1.3	100
jul-03	383	2.7	14.4	1.2	100
aug-03	312	4.0	5.7	0.6	100
sep-03	251	10.6	0.3	0.1	100

Eunicella singularis gonadal development cycles

E. singularis female colonies showed in both populations two overlapping cohorts of oocyte: the first formed by gonads with diameters between 50 and 300 µm, and the second one with diameters between 300 and 900 µm approximately (Figure 5a, b). Only eggs from the second cohort were mature during the summer, and were released as planulae between May and June in Illes Medes population and between June and July in Cabo de Palos population (Figure 5a, b). In Illes Medes population an average of 1.01 ± 0.94 larvae polyp^{-1} was found in June, while in Cabo de Palos population an average of 1.35 ± 1.25 and 1.24 ± 1.30 larvae polyp^{-1} were found in July and August respectively. Male gonadal development cycle showed only a cohort of spermaries being all male gametes spawned between May and June in Illes Medes population,

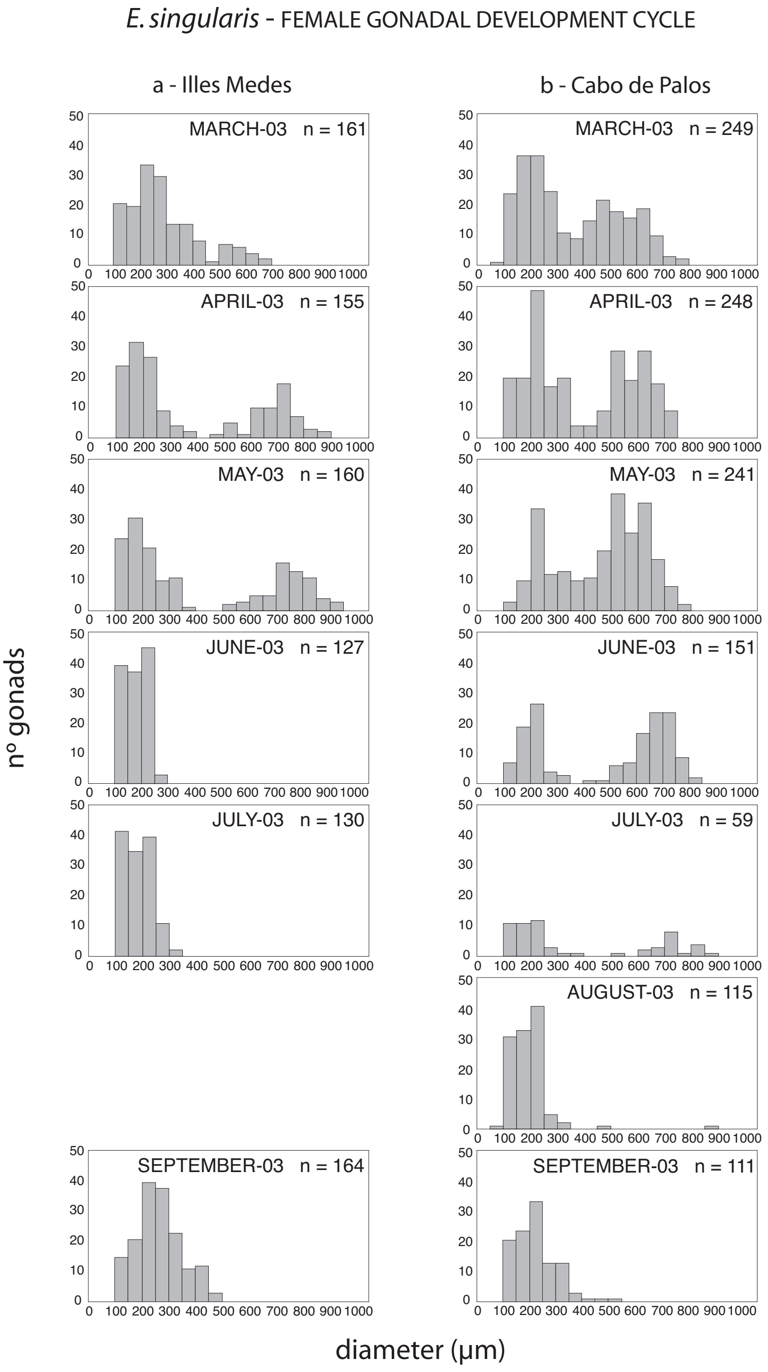

Fig. 5 - *Eunicella singularis*. Distribution of gonadal diameter frequency (µm) of 50 female polyps; Illes Medes (a), Cabo de Palos (b) (*n* = gonads number).

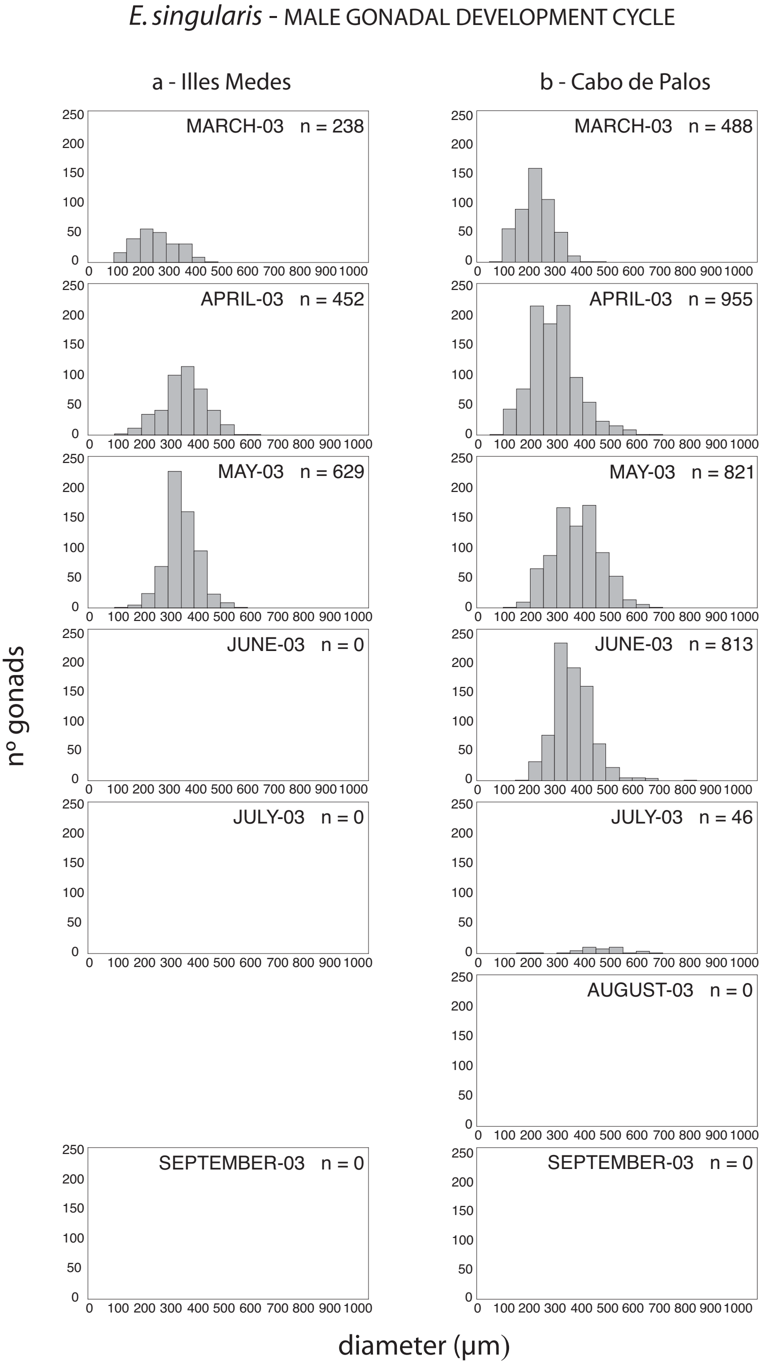

Fig. 6 - *Eunicella singularis*. Distribution of gonadal diameter frequency (µm) of 50 male polyps; Illes Medes (a), Cabo de Palos (b) (*n* = gonads number).

Table 2 - Monthly changes of number and diameter of *Eunicella singularis* gonads; female colonies (**a**), male colonies (**b**) (mean ± SE).

Month	Gonad diameter (µm)		Gonad number /polyp		Total number of polyps
	Mean	SE	Mean	SE	
a - female colonies					
Illes Medes					
mar-03	315	10.3	2.7	0.2	100
abr-03	354	14.9	2.9	0.2	90
may-03	413	18.1	2.9	0.2	80
jun-03	157	3.6	1.9	0.2	100
jul-03	161	2.8	2.8	0.2	100
aug-03	-	-	-	-	-
sep-03	219	4.5	3.2	0.2	100
Cabo de Palos					
mar-03	335	8.5	4.3	0.2	100
abr-03	384	9.4	4.5	0.2	100
may-03	459	8.9	3.8	0.2	100
jun-03	465	13.5	3.1	0.2	100
jul-03	310	16.7	1.7	0.2	100
aug-03	188	7.5	2.6	0.2	100
sep-03	212	4.9	2.9	0.2	100
b - male colonies					
Illes Medes					
mar-03	231	3.6	4.4	0.5	90
abr-03	307	3.2	10.7	0.6	70
may-03	317	1.9	13.6	0.8	100
jun-03	0	0	0	0	80
jul-03	0	0	0	0	90
aug-03	-	-	-	-	-
sep-03	0	0	0	0	90
Cabo de Palos					
mar-03	209	2.2	9.0	0.7	100
abr-03	271	2.0	18.1	0.9	100
may-03	338	2.5	14.0	0.7	100
jun-03	360	2.0	20.8	0.8	100
jul-03	426	17.2	0.7	0.2	70
aug-03	200	0	0.01	0.0	100
sep-03	0	0	0	0	100

and between June and July in Cabo de Palos population, showing synchronization with female gonadal cycle (Figure 6a, b). In female colonies, no significant differences appeared in average gonadal volume per polyp (one-way ANOVA, $F_{1,162}$=0.267, p=0.613) (Figure 4c), as well as in average diameter of female gonads (one-way ANOVA, $F_{1,527}$=1.537, p=0.229), and in average number of gonads per female polyps (one-way ANOVA, $F_{1,162}$=0.216, p=0.647) (Table 2a). On the contrary, in male colonies the gonadal volum was higher in Cabo de Palos population (one-way ANOVA, $F_{1,180}$=11.612, p<0.01) (Figure 4d). These divergences were due to differences both in average diameter of male gonads (one-way ANOVA, $F_{1,3431}$=9.655, p<0.01), and in average number of gonads per male polyps (one-way ANOVA, $F_{1,180}$=161.850, p<0.01) (Table 2b).

Table 3a - Two-way ANOVA comparing lipids levels for *Paramuricea clavata* among sites and seasons. Probability values of < 0.05 were considered significant. Abbreviations: df, degrees of freedom; MS, mean square; F, F ratio; p, probability.

a - *P. clavata*	**df**	**MS**	**F**	**p**
Season	3	93486	142.298	<0.05
Site	1	19366	29.477	0.087279
Season*Site	3	11437	17.409	0.159225
Error	242	6570		

b - Scheffé's contrast test				
Lipids	104.55	160.94	187.27	131.88
Source	1	2	3	4
Autumm 1				
Winter 2	<0.05			
Spring 3	<0.05	0.471		
Summer 4	0.288	0.416	<0.05	

Table 3b - Two-way ANOVA comparing lipids levels for *Eunicella singularis* among sites and seasons. Probability values of < 0.05 were considered significant. Abbreviations: df, degrees of freedom; MS, mean square; F, F ratio; p, probability.

a - *E. singularis*	**df**	**MS**	**F**	**p**
Season	3	389395	24.295	<0.05
Site	1	317420	19.804	<0.05
Season*Site	3	37566	2.344	0.073662
Error	242	16028		

b - Scheffé's contrast test				
Lipids	261.58	254.26	404.15	401.93
Source	1	2	3	4
Autumm 1				
Winter 2	0.992			
Spring 3	<0.05	<0.05		
Summer 4	<0.05	<0.05	0.9998	

Seasonal variation of tissue concentration levels of Lipids

In *P. clavata* lipid levels were significantly different between seasons, although did not show significant differences between populations (Tabla 3a). During the spring and summer Cabo de Palos population showed constantly the highest values, while in Illes Medes population lipids showed a significant decrease in July followed by a second increase in September (Figure 7a). Significant differences were found in *E. singularis* between sites and seasons. Lipid levels were higher during the spring and summer than in the rest of the year (Table 3b), although a decrease did occur during July in Illes Medes population. Moreover, Cabo de Palos population displayed the highest values during the gametogenesis period (Figure 7b).

DISCUSSION

A spatial variability in reproductive traits of *Paramuricea clavata* and *Eunicella singularis* is shown in this study. The sex ratio for both species in Illes Medes (1:1) was similar to that documented in previous studies in the same locality (Coma et al. 1995; Ribes et al. in press), while sex ratio is male biased (1:7) in *P. clavata*, and female biased (1.7:1) in *E. singularis* in Cabo de Palos. Differences in population sex ratio between localities or depths have been reported in several anthozoan species (Benayahu and Loya 1983; Soong 1991). In the Mediterranean Sea, a similar trend is reported for the octocoral *Corallium rubrum* were sex ratio is biased to female colonies near Livorno (Italy) (Santangelo et al. 2003), while is 1:1 in Costa Brava (Spain) populations (Tsounis et al. 2006). Other temperate and tropical octocoral species always present a skewed sex ratio that has been explained by taking into consideration that a male biased sex ratio may increase fertilization success, resulting advantageous for a sessile gonochoric coral which posses internal or surface fertilization (Benayahu and Loya 1984; Farrant 1986; Brazeau and Lasker 1990). Our results, however, do not fit with this explanation, since both species studied show an inverse biased sex ratio in Cabo de Palos populations and probably sex ratio can be much more variable between populations than has been previously thought. In *P. clavata* a male biased sex ratio has been reported in Portofino (Italy) as a result of a mass mortality episode and explained by considering that possibly females may not be healthy enough after spawning to survive a serious thermal stress (Cerrano et al. 2005). Further studies are needed to investigate this aspect of species plasticity along a relevant geographic and bathymetric scale in order to determine its eventual relationships with mortality events or a possible adaptative signification.

The study shows large differences in reproductive output between both species. *P. clavata* female colonies produce a larger mean number of gonads per polyp than *E. singularis* despite little differences in mean female gonads diameter (Table 1a, 2a). This variation resulted in a larger female gonadal volume produced by *P. clavata*. This difference can be related to the differences in reproductive strategy of the two species, *P. clavata* is a surface brooder while *E. singularis* is a brooder in which the smaller number of female gonads per polyp may be due to limitations in brooding space (Szmant 1986). This last strategy may contribute to the reduction of the mortality during the first stages of the development due to a higher protection of the larvae inside polyps. Similar evidence points to the fact that brooding species produce fewer gonads per polyp than broadcasting species and was indeed reported for scleractinian corals (Shlesinger et al. 1998).

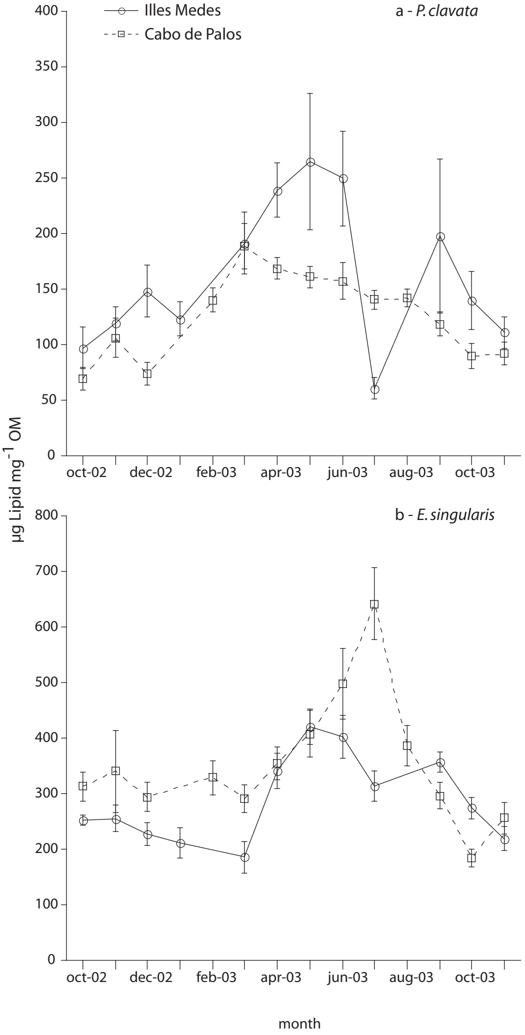

Fig. 7 - Annual cycle (October 2002 – November 2003) of lipids concentrations (µg mg^{-1} OM) in tissue of *Paramuricea clavata* (a) and *Eunicella singularis* (b) colonies (*n*=10) at Illes Medes and Cabo de Palos (mean ± SE).

In both species spawning fits well with a marked increase in sea-water temperature in spring. In the populations of Illes Medes, the observed spawning period agree with previous studies (Coma et al. 1995, Ribes et al. in press). However, comparing localities, in both species there was a clear temporal shift in the time of gametes release. This shift appeared well linked to the shift in sea-water temperature rising in spring in both sites at the depth where the populations are placed (Figure 8). Therefore the temperature seems to be the main factor synchronizing gonadal development within these populations. Several anthozoan species, both octocorallia and hexacorallia, showed a similar evidence when geographically separated populations were compared (Harrison and Wallace 1990; Richmond and Hunter 1990; Gutiérrez-Rodríguez and Lasker 2004). In particular, the hexacoral *Goniastrea australiensis* showed a two months shift in spawning time related to latitudinal differences (Kojis and Quinn 1981), as well as, *Pocillopora verrucosa* spawning time varied several months along a latitudinal gradient (Kruger and Schleyer 1998). In several coral species one month difference in spawning time

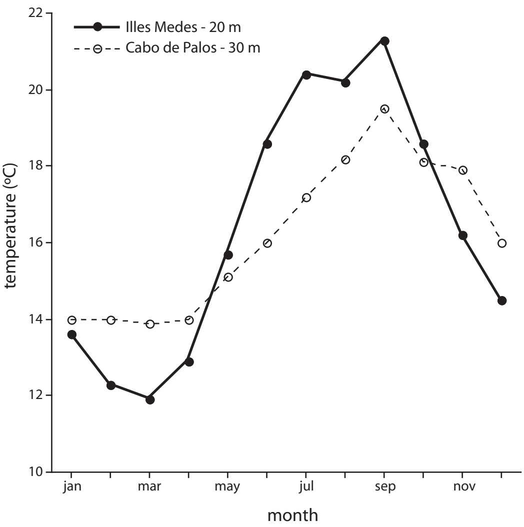

Fig. 8 - Mean monthly sea-water temperatures (ºC at the depth where populations were situated in each location; Illes Medes (from J. Pascual, unpublished data), Cabo de Palos (from Instituto Hidrográfico de la Marina, unpublished data).

has been reported between inshore and offshore reefs in the GBR and has been related to differences in sea-water temperature rising (Babcock et al. 1986). Other studies showed differences in time of octocoral reproduction along a depth gradient (Grigg 1977; Benayahu and Loya 1983, 1986) as found before for *E. singularis* (Weinberg and Weinberg 1979). In all cases, the earlier spawning was attributed to earlier rising of sea-water temperature at lower latitudes or at shallow waters. As we found in *P. clavata* and *E. singularis*, interpopulation differences in spawning time were reported in the hexacoral *Echinopora lamellosa*, with a northern shallow population spawning earlier than a southern deeper one (Fan and Dai 1995).

More differences between localities appear in gonadal output. In *P. clavata* female gonadal volume, and in *E. singularis* male gonadal volume were highest in Cabo de Palos population, being these divergences owing mainly to differences in the number of gonads per polyp. Considering the total population, the total volume of male and female gametes produced within a population is more relevant than the average volume of male and female gametes produced per colony (Jürgens et al. 2002). Taking into account the sex ratio of each studied population, a compensation of the differences in gonadal output seems to appear. In fact, in *P. clavata,* the highest volume of female gonads produced by Cabo de Palos population coincides with a greater presence of male colonies within this population. In the same way, the highest volume of male gonads produced by *E. singularis* in Cabo de Palos population is in concordance with a female-biased sex ratio. Although that, differences in gonadal output also remain at population level, suggesting that the exposition to different local conditions may be reverted in a different gonadal output in both species studied. Spatial variability in gonadal output has been reported for other coral species (Harrison and Wallace 1990) due to differences in gonadal diameters (Sier and Olive 1994; Fan and Dai 1995) or in gonadal number produced per polyp (Rinkevich and Loya 1987; Kruger et al. 1998), and have been explained as a result in different allocation of resources to eggs production in response to inestable environmental conditions experienced at higher latitudes or shallow waters (Sier and Olive 1994).

Differences in gonadal output could be due to differences in nutritional state of gorgonians as a consequence of differences in food quality and availability between different geographical areas or depths (Stimson 1987; Harland et al. 1992; Rossi et al. 2003). During our study, the comparison between reproductive output and lipids concentrations levels in each population did not show a significant relationship. *P. clavata* showed higher lipid concentration values in Illes Medes than in Cabo de Palos population despite a highest gonadal output in Cabo de Palos. This evidence may be due to the male biased sex ratio observed in Cabo de Palos population, since lower lipid concentration levels have been reported in male with respect to female colonies (Rossi et al. 2006b). In *E. singularis* we found higher lipid concentrations in the Cabo de Palos in contrast to the Illes Medes population during the period of gametogenesis, but also in this case the female biased sex ratio of the Cabo de Palos population may have caused the highest values observed. The differences in sex ratio and the discrepancies found between species disable us to conclude that lipid concentration levels, as an indicator of the nutritional state of gorgonians, explain the observed differences in gonadal output between populations.

In Illes Medes the seasonal variation of tissue concentration levels of lipids in *P. clavata* shows a clear decrease during summer, coinciding with the trend reported before for the same population (Rossi et al. 2006b). During summer a little decrease in lipid concentration occurs also in *E. singularis* in the same locality, while do not occur in both species in Cabo de Palos. These results seem to agree with the hypothesis of a summer feeding constraints in gorgonian shallow populations due to water stratification, poor organic matter availability, and low frequency resuspension events (Coma and Ribes 2003; Rossi et al. 2006b). Coral species displaying zooxanthellae symbiosis obtain high energy compounds from the symbiotic algae (Patton et al. 1983; Muscatine et al. 1984). Moreover, during periods of low food availability, the presence of zooxanthellae can benefit the host trough the maintenance of the stored lipids for longer time periods (Szmant-Froelich and Pilson 1980; Fitt and Pardy 1981). Consequently, the smaller intensity of the lipid decrease observed in *E. singularis* could be due to the zooxanthellae symbiosis contribution during summer coinciding with feeding constraints, but in presence of high light intensity.

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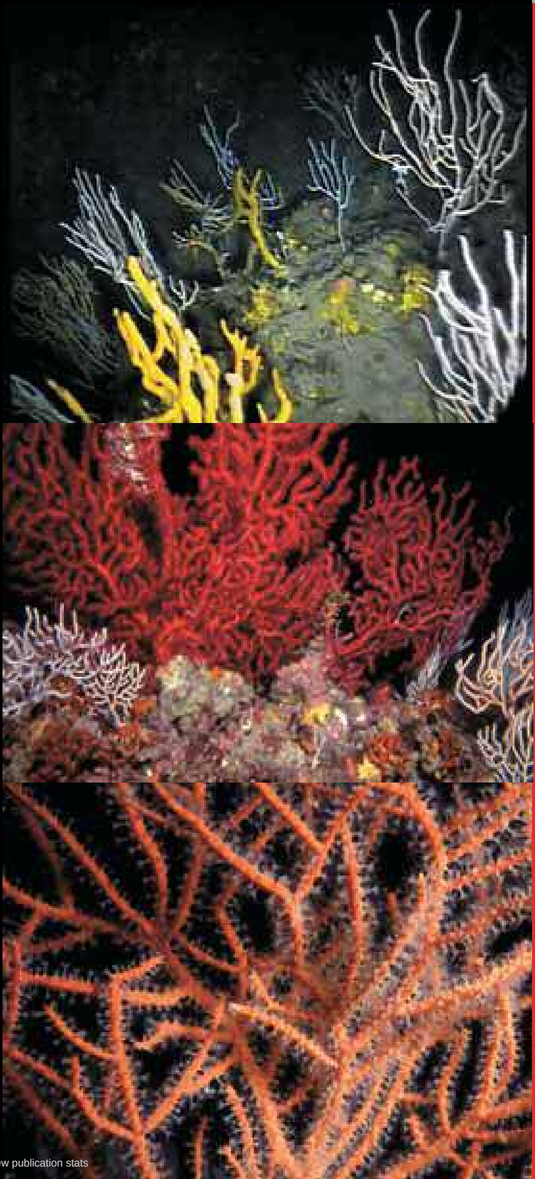

The understanding of Mediterranean gorgonian ecology increased significantly in the last decades. However, although Mediterranean sublittoral gorgonians are known to be present from shallow to deep waters, the research focused, up to this date, almost only on the shallow gorgonian populations located from the surface to approximately 40 m depth. Intermediate depths, particularly coastal rocky bottoms in the 40-150 m depth range, has remained mostly unexplored until now.
This thesis addressed a first description of the ecology of deep sublittoral populations of Mediterranean gorgonians by means of a multidisciplinary approach combining classical methods, new technologies and modern genetic and biochemical analyses. The first chapter presented the results of applying quantitative analysis to underwater video transects performed with Remotely Operated Vehicles (ROV), in order to address the spatial distribution patterns of gorgonian populations. The second chapter combined the analysis of photographic images obtained by ROV, with scanning electronic microscope (SEM) analysis of gorgonian sclerites and histological and genetic analysis, all in order to investigate the morphological variability of gorgonians along a depth gradient. The third chapter offered a combination of classical methods for population size and spatial structure analysis that have been innovatively applied to ROV video transects to address the study of gorgonian populations over a larger bathymetrical scale. Finally, in the fourth chapter the microscopy and histology techniques for the study of gorgonian reproductive cycle were coupled with biochemical analysis of tissue composition (carbohydrate, protein and lipid) in order to determine the energetic state of gorgonian colonies and its relation to the reproductive cycle, as well as the analyses of the stable isotopes and fatty acids composition to get some insight into the trophic ecology of the gorgonians throughout the year and in a depth comparison.
Although scientific research often leads to few answers and to much more new questions, we hope that the results of this thesis may serve as a stimulus for future studies focused on hard-bottom benthic communities located at intermediate depths, which have remained fairly ignored for a long time.