Ecology and conservation of the Mediterranean endemic coral *Cladocora caespitosa*

Ecología y conservación del coral endémico del Mediterráneo *Cladocora caespitosa*

Diego K. Kersting

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Programa de Doctorat en Ciències del Mar
Departament d'Ecologia
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Memoria presentada por Diego K. Kersting para optar al grado de doctor por la Universitat de Barcelona. Año 2013.

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Cladocora caespitosa Martí Kersting Molinos 2013

A Marta y a Martí

A mis padres

A las Islas Columbretes

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GENERAL INTRODUCTION

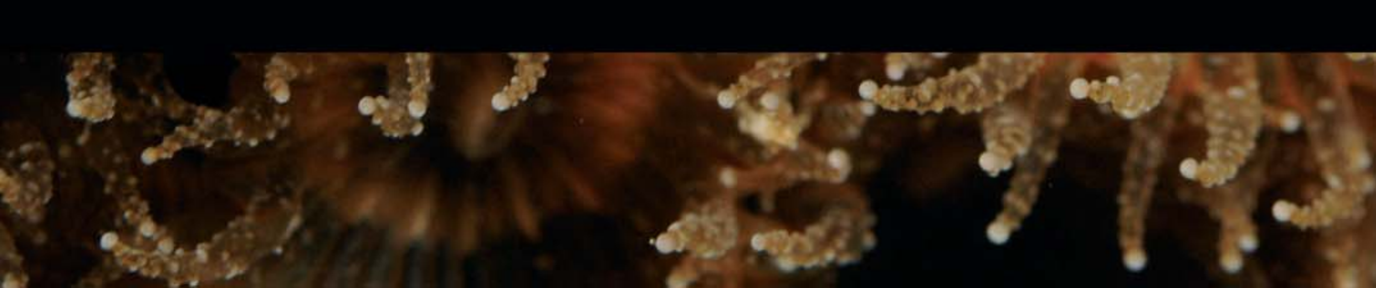

Photo: ***Cladocora caespitosa*** **polyps**

CONSERVATION OF MARINE BENTHIC COMMUNITIES

Marine ecosystems are declining worldwide threatened by overexploitation, pollution, invasive species, diseases, alteration and loss of habitat, and global climate change (Jackson 2001; Steneck and Carlton 2001; Harvell et al. 2002; Hughes et al. 2003; Kappel 2005; Harley et al. 2006). The rapid changes that are altering the structure of habitats and communities are causing the decline of many species, while urgently needed conservation measures are in most cases non-existent, partly due to the lack of information on the species and the level of threat it is subject to. Therefore, to assess the conservation status of species, knowledge of their biology, ecology and potential impacts that they are subjected to, is essential. However, the lack of this information is widespread, even for key or emblematic species. Gaining this knowledge is crucial to understanding the processes involved in species declines, and for the development of adequate conservation measures.

Marine protected areas provide one of the best examples of local or regional conservation measures that have been shown to be effective in protecting species from impacts such as overexploitation or habitat loss (Lubchenko et al. 2003; Lester et al. 2009). However, conservation efforts at local or regional scales loose their effectiveness when subject to additional *new threats* that act at a much broader geographic scale, i.e., climate change and invasive species (Allison et al. 1998; Lubchenko et al. 2003). Therefore, while these conservation measures are a powerful tool, they need to be complemented by other approaches; which in most cases are not easy to implement due to the global scale of the impacts that cause the disturbances. This is undoubtedly a new and difficult challenge for conservation science, especially if global measures to mitigate the causes of the disturbances are not taken (e.g., reducing CO_2 emissions to the atmosphere or controlling shipping activities in the case of species introductions). On the other hand, Marine Reserves, are serving as unique laboratories to study the response of species and communities to these *new threats* free from many additional anthropogenic effects which may confound the results of research.

Many *anonymous* marine species remain unstudied and unprotected so their decline (if happening) is likely to pass unnoticed; thus, conservation measures, even if developed, may arrive late. The species objective of this PhD thesis, the Mediterranean endemic coral *Cladocora caespitosa*, may serve as an example or case study of those species lacking essential information suitable to run conservation measures; especially on the key ecological processes involved in their conservation status and the responses to the rapid environmental changes that are happening globally.

Cladocora caespitosa
The endemic reef-builder of the Mediterranean Sea

Cladocora caespitosa (Linnaeus 1767) is the only colonial and zooxanthelate scleractinian coral native to the Mediterranean Sea (Zibrowius 1980). This endemic species may occur in a wide range of substratum, depth and hydrodynamic conditions (Zibrowius 1980; Schiller 1993).

The colonies of this coral display two types of colony distribution, for which Peirano et al. (1998) proposed the terms *bed* and *bank*; i.e., *bed*: a great number of distinct subspherical colonies 10 -30 cm in diameter and *bank*: large formations reaching several decimetres in height and covering several square meters in surface area.

Cladocora caespitosa colonies are phaceloid, i.e., the tubular corallites develop vertically with sub-parallel growth axes of the main branches, keeping corallites' walls independent from each other. Two density bands are deposited per year in its skeleton: a high density band in autumn and winter and a lower-density band in the summer (Peirano et al. 1999, 2005) (Fig. 1). These bands are detectable in x-radiographs, which is a quite effective method to estimate corallite growth, as done in several studies (Peirano et al. 1999, 2005, 2009; Kružić and Benković 2008; Kružić et al. 2012).

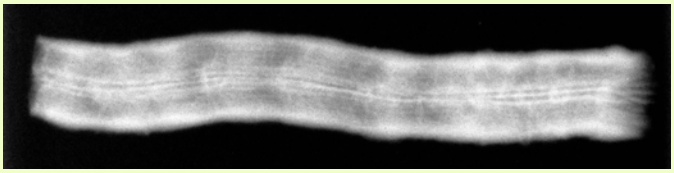

Figure 1. High and low density bands in an x-radiographed *Cladocora caespitosa* corallite from the Columbretes Islands.

Cladocora caespitosa has adapted to live in a diversity of very different environments, from shallow photophillic communities to deeper circalitoral assemblages (Fig. 2). This plasticity could be related to its ability to regulate heterotrophy in order to compensate lowered autotrophy in low light conditions. In general terms, autotrophy and heterotrophy are predominant in summer and winter respectively (Ferrier-Pagès et al. 2011). However, *C. caespitosa* has the ability to up-regulate heterotrophy and maintain symbiosis even under suboptimal conditions by altering feeding effort rapidly and strongly (Hoogenboom et al. 2010). The role of heterotrophy is different in low and in high light conditions (note that light conditions may be influenced by both seasonal changes or habitat); in the dark heterotrophy has a compensatory role for carbon acquisition (used for calcification), while in high light conditions food mainly provides a source of nitrogen and phosphorus, which enable tissue and colony growth through budding (Hoogenboom et al. 2010). These authors concluded that colonies of *C. caespitosa* can grow new biomass only when both light (for energy) and food (for nutrients) are available and that feeding plays a strong role in the energy budget of this species.

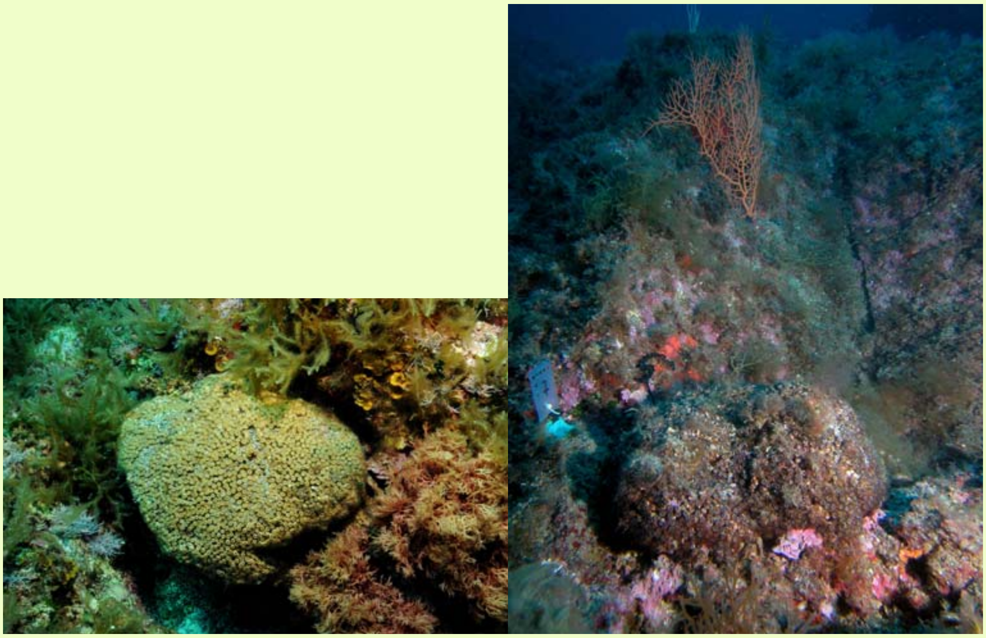

Figure 2. *Cladocora caespitosa* colonies in contrasting communities. Left among algae in a shallow photophilic community (Columbretes Islands) and right in circalitoral assemblages (Cap de Creus).

Cladocora caespitosa corallites, analyzed for trace elements using laser ablation, have provided an invaluable archive of the past climate conditions in the Mediterranean; which has allowed to obtain weekly resolution in temperature records in corallites 10 – 95 years old (Silenzi et al. 2005; Montagna et al. 2006, 2007).

This coral is among the benthic species that have been recurrently affected by climate-related mortalities in the Mediterranean Sea (Perez et al. 2000; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009; Kersting and Linares 2009). Aquaria thermal tolerance experiments have shown that prolonged, higher than average, temperatures may induce necrosis in the polyps (Rodolfo-Metalpa et al. 2005, 2006b). However, while polyps were affected by tissue necrosis (both in aquaria and *in situ*), bleaching (massive loss of zooxanthellae) was never detected (Rodolfo-Metalpa et al. 2005, 2006b). In fact, past studies have shown the resistance of zooxanthellae (described as *Symbiodinium* clade temperate-A, Visram et al. 2006) to thermal stress (Rodolfo-Metalpa et al. 2006a).

In addition to climate change, other impacts have been reported to threat *C. caespitosa*, including invasive algal species (Kružić and Benković 2008; Kružić et al. 2008b) and eutrophication (Kružić et al. 2007).

Columbretes Islands and the Illa Grossa Bay

Most of the work of this PhD has been performed in the Columbretes Islands Marine Reserve. As described in chapter I, this Marine Reserve hosts one of the few extensive fields of *Cladocora caespitosa* bioconstructions in the Mediterranean Sea, serving as an extraordinary and unique laboratory to study this emblematic species.

The Columbretes Islands are located 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean) within a 90 x 40 km volcanic field at 80 - 90 m water depth. These islands and associated submarine volcanic field are one of the rare examples of Quaternary volcanism in the Mediterranean (Muñoz et al. 2005).

The Columbretes archipelago consists of a main larger island (L'Illa Grossa), and three groups of islets. From north to south they are: L'Illa Grossa, La Ferrera, La Foradada and El Carallot (Fig. 3). Illa Grossa (Fig. 4, 39°53.825' N, 0°41.214'E; 14 ha), is a C-shaped, drowned Quaternary volcanic caldera that is opened to the NE from which it is exposed to winter storm waves (Aparicio and García 1995; Sánchez-Arcilla et al. 2008). The island forms a bay in the shape of a horse shoe, Illa Grossa Bay (Fig. 5), that has a total surface of 150,000 m^2 and an average depth of 15 m. Within the bay the sea bed is covered mainly by rocky substrata and biogenic sand. The archipelago is located within a 5500 ha marine reserve, created in 1990 and which has been managed by the *Secretaría General de Pesca* (MAGRAMA) since its inception.

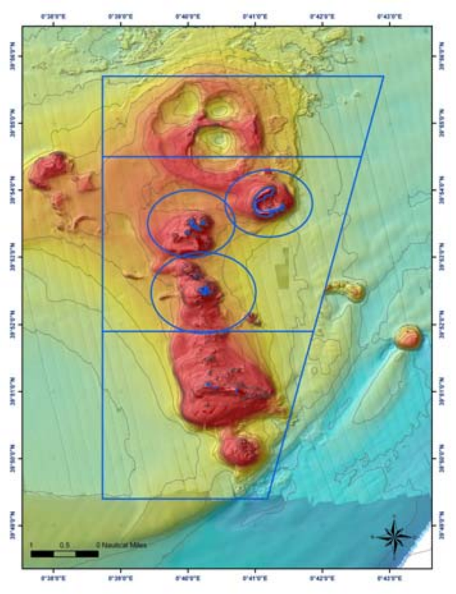

Figure 3. The Columbretes Islands Marine Reserve: bathymetry and marine reserve zonation. Islets from North to South: L'Illa Grossa, La Ferrera, La Foradada and El Carallot. Islets and zonation in blue. Source: Secretaría General de Pesca (MAGRAMA).

Figure 4. Illa Grossa (Photo: Silvia Revenga, Banco de fotos de reservas marinas SGP/MAGRAMA).

Figure 5. Illa Grossa Bay.

EVIDENCE-BASED CONSERVATION: WHY THIS SPECIES NEEDS TO BE PROTECTED?

From dominating reefal communities to relict microreefs: historical decline of an emblematic species

Tropical reef ecosystems disappeared from the Mediterranean Sea at the end of the Messinian (~ 5 million years ago) in the Late Miocene (Esteban 1996). The oldest *Cladocora caespitosa* fossil reef known to date is from the Late Pliocene (~ 3 million years ago), when this coral formed true monospecific reefs both in the eastern and western Mediterranean (Aguirre and Jiménez 1998; Dornbos and Wilson 1999). Aguirre and Jiménez (1998) considered these fossil banks as the continuation of past reefal ecosystems to the present, following the extinction of Mediterranean tropical reefs. Large fossils banks of *C. caespitosa* are also known from the Early Pleistocene (Bernasconi et al. 1997), from the Middle and Late Pleistocene and from the Holocene (see Peirano et al. 1998, for a review). Therefore, *C. caespitosa* was more abundant in past Mediterranean coasts, coinciding with a warmer climate, occurring mostly in environments with alluvial inputs and high turbidity (Peirano et al. 2004). However, more recent studies have shown that, although fossil *C. caespitosa* banks grew in a generally warmer subtropical sea, they did so in colder environments that were related to cold freshwater inputs in coastal lagoons (Peirano et al. 2009); comparable to the present environmental conditions in the largest continuous *C. caespitosa* reef in the Mediterranean in Mjlet National Park (Adriatic Sea, Kružić and Benković 2008). This is supported by the palaeontological record, which shows that extensive reefs of this coral occurred in such coastal environments (Fornos et al. 1996; Aguirre and Jiménez 1998; Mastronuzzi 2006). The results obtained by Peirano et al. (2009), showed that the studied fossil reef grew in an environmental mean temperature of approximately 17 °C, similar to present temperature records in the Northern Adriatic. In fact, the same authors found that the maximum growth rates of present *C. caespitosa* were attained with similar mean annual sea surface temperature (17.5 °C – 18 °C).

In current Mediterranean coastal environments, large *C. caespitosa* bioconstructions are scarce and populations are generally formed by small disperse colonies. Nevertheless, some micro-reefs of this coral are still to be found, the best examples occur in Mjlet National Park (Adriatic Sea, Kružić and Benković 2008) and in the Columbretes Islands (NW Mediterranean, this thesis) (Fig. 6). These uncommon reef formations could be considered as a relict or a “missing link” from the past Mediterranean marine ecosystems (Kühlman et al. 1991).

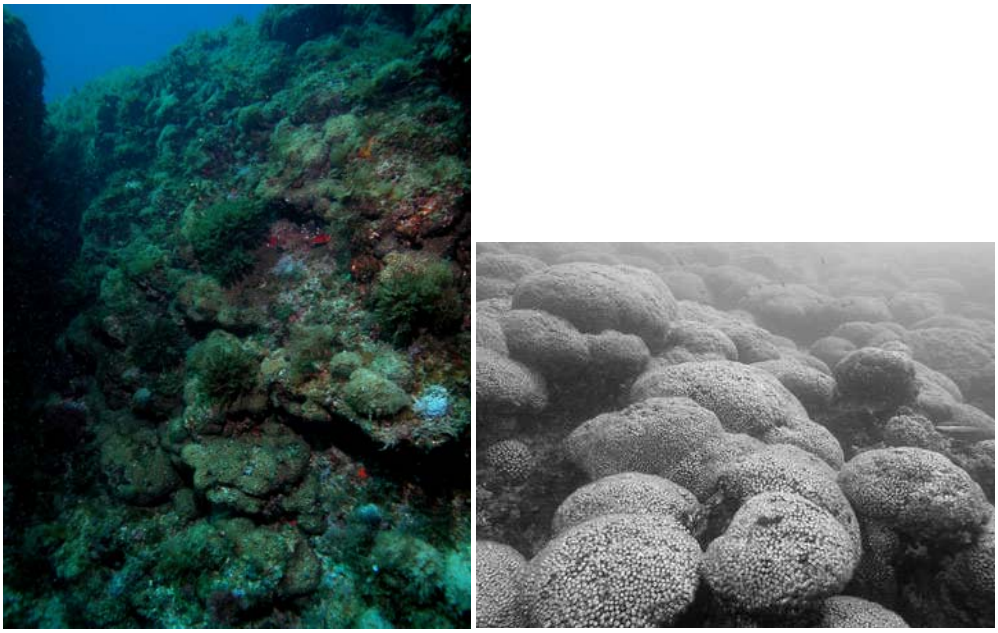

Figure 6. *Cladocora caespitosa* banks in the Columbretes Islands (left) and in Mjlet National Park (right, photo: Kružić and Benković 2008).

The fossil evidence demonstrates that *C. caespitosa* once grew at a much larger scale, forming large reefs, and thus on a geological time scale the species has declined (i.e., 10^6 yr order of magnitude). In recent history (i.e., the late 20th and early 21st century) studies have clearly stated concerns on the recent past decline, which is mostly related to anthropogenic disturbances. Augier (1982) already included *C. caespitosa* in a list of endangered species 3 decades ago. Currently climate change appears to be a major threat to the species and a major contributor to its continuing decline (Morri et al. 2000).

The extensive occurrence of *C. caespitosa* reefs in the subtropical Mediterranean may seem contradictory to the climate-related mortalities that this species is suffering nowadays (Rodolfo-Metalpa et al. 2000; Garrabou et al. 2009; this thesis). However, as stated above, fossil reefs of this coral seem to have occurred in coastal environments that showed lower temperatures than the open sea, and, in fact, one of the possible causes of the death proposed for some of these fossil reefs was a prolonged rise in seawater temperature (Peirano et al. 2009). These findings could partially explain this contradiction; however, the historical causes of the decline of the *C. caespitosa* reefs in the Mediterranean and the comparison between the present and past *C. caespitosa* is an exciting and understudied topic that needs further research.

Despite the differences in the temporal scales and the processes involved in the decline of *C. caespitosa* reefs in the Mediterranean, there is a common feature in both recent and ancient reduction of the populations and bioconstructions of this coral: uncertainty of the causes. Both its populations and their geographical range have undergone such reduction that this species and its reefs could meet the IUCN criteria to be classified under the Endangered category (IUCN 2012).

Long-lived and ecosystem engineer species

Cladocora caespitosa is an ecosystem engineer, i.e., it directly or indirectly modulates the availability of resources to other species, by causing physical changes in biotic or abiotic materials, modifying, maintaining and/or creating habitats (Jones et al. 1994). The most obvious ecological engineering is attributable to species with large per capita effects, living at high densities, over large areas for a long time (i.e., long-lived), and forming structures that persist throughout prolonged periods of time (Jones et al. 1994). Long-lived engineer species, such as *C. caespitosa*, play a main role in the biodiversity and structure of benthic communities, due to their trophic activity, biomass and perennial structures that shape the habitat (Dayton et al. 1974; Hughes and Jackson 1985; Sebens 1991).

Long-lived coral species are characterized by their slow dynamics (i.e., low growth and recruitment rates), which are buffered by their longevity and low natural mortality (Edmunds 2000; Hughes and Tanner 2000; Adjeroud et al. 2007; Coles and Brown 2007; Linares et al. 2007). Due to these traits, these species are highly vulnerable to catastrophic impacts (Hughes and Tanner 2000; Linares et al. 2007).

In the Mediterranean Sea long-lived ecosystem engineer species are well represented in circalitoral coralligenous outcrops. The red gorgonian *Paramuricea clavata* is one of the most studied long-lived ecosystem engineer coralligenous species in the, together with the red coral *Corallium rubrum* (Garrabou and Harmelin 2002; Linares et al. 2007). However, long-lived animal species with an important structural role are quite rare in Mediterranean shallow infralitoral communities. *Cladocora caespitosa* is one of the few examples of this second group inhabiting mostly in shallow communities (Zibrowius 1980), although it may also occur in greater depths, including circalitoral communities (Morri et al. 1994).

Heritage value

The concept of patrimony or heritage, traditionally applied to architecture and culture, is being applied to a wider scope of disciplines in the social sciences (from law to geography) and more recently it has entered into debates and actions related to nature and biodiversity (Cormier Salem et al. 2002, 2005). A heritage is a set of material or immaterial elements to which are attached specific values and rights that are linked to a social group and are inherited and transmitted from one generation to the next (see Michon et al. 2012, for this and other definitions).

Under this anthropocentric and social point of view of natural heritage, *Cladocora caespitosa* constitutes an invaluable natural patrimony due to its extensive ancient history, representing the remains of past Mediterranean ecosystems, together with its characteristics as a long-lived ecosystem engineer species capable of building sizeable, long-lasting structures and its fragility in the actual context of climate change.

Lack of knowledge for conservation measures

Until now, some *Cladocora caespitosa* bioconstructions have been described (See Fig. 8 in Chapter I), but only a few populations have been studied in depth (Schiller 1993; Peirano et al. 2001; Kružić and Benković 2008) and thus there is a general lack of knowledge on the ecology of this species.

Cladocora caespitosa was first included in a list of endangered species by Augier (1982), and it has been included in some protection lists (Table 1), as well as in several habitats lists at European, Mediterranean and Spanish levels (Table 2). However, its inclusion is limited and probable by default, most probably linked to the lack of knowledge on demographical characteristics and potential threats. Hence, there is an important need to increase our knowledge of this species in order to improve its management and protection.

A clear example of the lack of information on the species is the comment relating to this species that appears in the Red List of the IUCN (http://www.iucnredlist.org):

No population information known or information available about threats. Therefore this species is listed as Data Deficient. However, this species could fall into a threatened category if more information was known. Research on these aspects of this species' ecology is recommended. This assessment should be re-evaluated in 10 years to include addition information and to determine the effects of continued or increased threats from climate change and ocean acidification.

Organization	Status
CITES	Appendix II
IUCN	Red List of Threatened Species (Data Deficient)
Council of Europe Bern Convention	List of Threatened Species

Table 1. Legal protection status of *Cladocora caespitosa*.

Habitats list	Code	Name
EUNIS	A3.238	Facies with *Cladocora caespitosa*
NATURA 2000	1170	Reefs
Barcelona Convention RAC/SPA	III.6.1.14	Facies with *Cladocora caespitosa*
Lista Patrón de los Hábitats Marinos Presentes en España	301041408	Roca infralitoral medianamente iluminada sin fucales con *Cladocora caespitosa*
Lista Patrón de los Hábitats Marinos Presentes en España	301041607	Roca infralitoral de modo calmo, escasamente iluminada, dominada por invertebrados con *Cladocora caespitosa*

Table 2. *Cladocora caespitosa* in habitats lists.

TOWARDS THE CONSERVATION OF *CLADOCORA CAESPITOSA*: WHAT DO WE NEED TO KNOW?

Conservation Biology has emerged as a vital component of the social response to the contemporary wave of ecological destruction, and its main focuses and concerns are species, populations, communities and ecosystems that are threatened by habitat alteration, fragmentation and destruction (Soulé and Kohm 1989). The main task of this discipline is to provide tools and knowledge that will anticipate, prevent, minimize, and/or repair such ecological damage. When assessing an impacted population's viability, this knowledge has to be focused on the key processes involved in its maintenance and recovery. Therefore, the main traits of the species and its populations need to be known, and the main impacts and responses assessed.

Although some aspects of the biology of the subject of this thesis have been studied (see some examples in the species section above), there is a critical need for knowledge on certain topics of vital importance to the species conservation. In this context, information on population structure and distribution, reproduction, recruitment, natural and catastrophic mortality rates and long-term effects of global change-induced disturbances, is essential to understand the viability of the species and its populations and to establish adequate conservation and management plans.

BIOLOGICAL AND ECOLOGICAL TRAITS

Colonial corals are long-lived clonal organisms whose mode of construction greatly increases the complexity of their life histories and population dynamics, compared with those of aclonal (solitary) animals (Hughes and Jackson 1985; Hughes and Connell 1987). Colonial organisms are made up of repeated building blocks or modules (e.g., coral polyps) and after larval settlement, most of the growth of a colony is achieved by increasing the number of genetically identical modules (Jackson 1977). Individual colony growth is potentially indeterminate in many species and it can be inhibited by physical stress, local availability of resources or by the mortality of modules. This may lead to variation in size between colonies within an age cohort, which often makes it impossible to distinguish morphologically between a rejuvenating individual and a newly settled one (Hughes and Jackson 1980). Large coral colonies are estimated to be centuries old and the product of a long history of modular growth, partial mortalities, fissions and fusions (Hughes and Jackson 1985), a category into which the large *Cladocora caespitosa* colonies in the Illa Grossa Bay fall.

Distribution and population structure

Habitat, distribution and population structure, together with its interaction with environmental conditions is essential base knowledge to describe and understand further processes involved in the ecology of the species. The *Cladocora caespitosa* population in the Illa Grossa Bay is one of the few examples among the rare micro-reefs existing in the present-day Mediterranean. However, the basic information on its distribution, cover, population structure and growth rates, is completely missing. Thus, in **Chapter I** the knowledge gap is filled with a study of the characteristics of the *C. caespitosa* population in the Illa Grossa Bay. This information is needed to demonstrate the importance of this population at a Mediterranean scale and will form the baseline for further research.

Reproduction

Understanding reproductive biology is essential to comprehending the population dynamics of marine organisms (Fadlallah 1983); successful reproduction ensures the addition of new individuals to the populations, the colonization of new areas, and the recovery of populations after disturbances. Therefore, this knowledge is crucial for their management and preservation.

Despite the importance of this subject, little is known on most reproductive traits of *Cladocora caespitosa*. Spawning has been observed *in situ* in the Adriatic Sea (Schiller 1993; Kružić et al. 2008b) and Kružić et al. (2008b) reported this species to be hermaphroditic. The objective of **Chapter II** is to increase our knowledge on the reproductive biology of the species by studying its sexual condition and reproductive cycle. The results were compared with previous studies in the Adriatic Sea.

Recruitment, mortality and recovery

In corals, recovery after disturbances relies, to a great extent, on recruitment and survival (Connell et al. 1997; Hughes et al. 2000; Adjeroud et al. 2007; Coles and Brown 2007) and these processes become even more critical in long-lived species (Edmunds 2000; Hughes and Tanner 2000; Adjeroud et al. 2007; Coles and Brown 2007). Consequently, knowledge on these traits and, in general, on population dynamics, is of great importance if the viability of their populations has to be assessed.

Chapter III focuses on the study of the main life history traits of this population. As we are dealing with a long-lived ecosystem engineer species, recruitment and natural mortality rates are extremely important to assess the resilience of this population facing the actual context of impacts derived from global change, which are evaluated in the following sections.

GLOBAL CHANGE-RELATED DISTURBANCES

Global change is a term widely used to describe the effects of human activities on the Earth (National Research Council 2000) and it encompasses, among other components, climate change and biological invasions (Vitousek et al. 1997; Occhipinti-Ambrogi 2007). Worldwide scientific consensus on anthropogenic global climate change is a fact (Oreskes 2004; Cook et al. 2013) and its effects are being recorded in a wide variety of ecosystems, from forests (e.g., Dale et al.

2001; Allen et al. 2010) to coral reefs (e.g., Hoegh-Guldberg 1999; Hughes et al. 2003).

The world ocean has exhibited changes in heat content during the last decades, showing a net warming (Levitus et al. 2000; IPCC 2013, Fig. 7). As a result of sea warming, benthic communities have been altered worldwide through changes on species distribution and abundances, diseases and mass mortalities, as well as other changes in basic biological processes such as reproduction (Harrison and Wallace 1990; Harvell et al. 1999; Parmesan and Yohe 2003; Bianchi 2007; Garrabou et al. 2009).

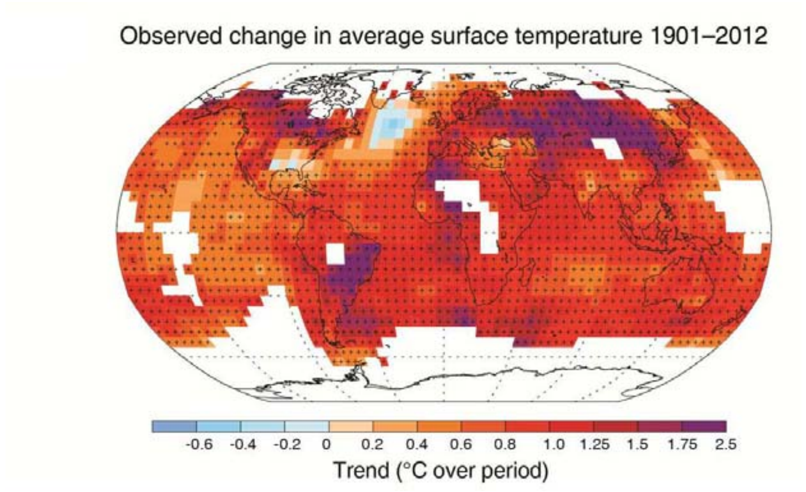

Figure 7. Change in average surface temperature 1901-2012 (IPCC 2013).

The Mediterranean Sea is a global change hot-spot (Occhipinti-Ambrogi 2007; Lejeusne et al. 2010; Zenetos et al. 2012). The frequency of abnormally warm summers has increased in this area, resulting in unprecedented mass-mortality events. Although some early mortalities were detected in the 1970s and 1980s (e.g., Harmelin and Marinopoulos 1994; Vacelet 1994), the first multispecies mass-mortality event was described in the NW Mediterranean in the summer of 1999 (Cerrano et al. 2000; Perez et al. 2000; Romano et al. 2000). A few years later, in the summer of 2003, a new mass-mortality episode occurred in NW Mediterranean coastal waters, this time over a larger geographic area (Garrabou et al. 2009). Both events affected over 30 species of benthic invertebrates, mostly cnidarians, sponges and bryozoans (Perez et al. 2000; Lejeusne et al. 2010).

In addition, non-indigenous species are widespread and abundant in the Mediterranean which hosts almost 1,000 introduced species (Zenetos et al. 2010, 2012), of which 128 are macrophytes (Zenetos et al. 2012). Marine corridors (e.g., the Suez Canal), shipping and aquaculture (Zenetos et al. 2012) have been reported to be the main vectors of these introductions.

Benthic communities in the Mediterranean Sea are being subjected to drastic changes related to the above mentioned global change disturbances. Emblematic and keystone species, such as the red gorgonian *Paramuricea clavata*, the red coral *Corallium rubrum* and *Cladocora caespitosa*, are threatened by these impacts (Garrabou et al. 2001; Linares et al. 2005; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009). These are slow growing, long-lived species that are being overwhelmed by recent rapid changes in environmental conditions and are subject to unprecedented mass-mortalities. Furthermore, the habitats and communities in which these species occur, among other emblematic ones like the *Posidonia oceanica* meadows, are being invaded by non-indigenous algal species. Effects of these invasions on native species and communities have been widely reported (Piazzi et al. 2001; Ballesteros et al. 2007; Baldacconi and Corriero 2009; Cabanellas-Reboredo et al. 2010; Deudero et al. 2010; Žuljević et al. 2011; Cebrian et al. 2012; Linares et al. 2012); however, since some of these invasions are relatively recent and the number of affected native species and communities is increasing, further research is needed to assess impacts.

Responses of *Cladocora caespitosa* to Mediterranean warming

As stated above, in the Mediterranean Sea climate change-related mortalities have recurrently impacted benthic communities during more than a decade. Until now, several studies have been undertaken and they can be classified in either field studies encompassing relative short periods of time or aquaria thermotolerance experiments. However, there was a lack of long-term studies on the responses of benthic organisms to these changes, which are required to evaluate the resilience of these populations and communities. In **Chapter IV**, we assessed the long-term response of *Cladocora caespitosa* to sea warming.

Invasive algal species and interaction with *Cladocora caespitosa*

Keeping with the impacts derived from global change, **Chapter V** focuses on to study the dispersal dynamics of two invasive algal species (*Lophocladia lallemandii* and *Caulerpa racemosa*) over the Illa Grossa Bay to assess their potential impact on the *Cladocora caespitosa* population and to detect possible coral defence mechanisms towards the invasive algae.

OBJECTIVES

The aim of this thesis was to study the ecology of the only reef-builder zooxanthellate coral of the Mediterranean, *Cladocora caespitosa*, in order to contribute to its conservation. Specific objectives covered from the characterization of the bioconstructions of *C. caespitosa* to the study of the life-history traits of the species and the responses of the species to major threats affecting coastal Mediterranean ecosystems, such as warming and biological invasions.

The thesis is structured in two parts and the specific objectives were:

Part I. Biological and ecological traits of *Cladocora caespitosa*.

- To describe the *Cladocora caespitosa* population of the Illa Grossa Bay in terms of spatial distribution, size structure, colony morphology and growth.
- To increase our knowledge on the reproductive biology of the species by studying its sexual condition and reproductive cycle.
- To study basic life-history traits (recruitment and natural mortality rates) in order to assess resilience

Part II. Responses to global change disturbances.

- To assess the long-term response of this coral to warming.
- To study the colonisation of the Illa Grossa Bay by two invasive algal species (*Lophocladia lallemandii* and *Caulerpa racemosa*) and their interactions with *Claodocora caespitosa*.

This PhD has been conceived as a whole; however, each chapter has been submitted as separate papers for publication in scientific journals (three chapters are already published while the other two are under review). Chapters I, II and IV have already been published, but format has been unified in order to keep it consistent throughout the document. Original text, figures and tables that appear in the published papers have been preserved. References have been unified in a single reference list at the end of the document.

ADVISOR'S REPORT

Dr. **Cristina Linares,** advisor of the PhD thesis entitled **"Ecology and conservation of the Mediterranean endemic coral *Cladocora caespitosa*"**, certifies that the dissertation presented here has been carried out by **Diego K. Kersting** in its totality, participating in all the tasks: conceiving and performing the experiments, processing the samples, analysing the data and writing the manuscripts. As adviser, I have participated in designing, guiding and correcting the chapters and manuscripts written by the PhD candidate.

Publication status of the chapters of this thesis:

Chapter I. Kersting DK, Linares C (2012) *Cladocora caespitosa* bioconstructions in the Columbretes Islands Marine Reserve (Spain, NW Mediterranean): distribution, size structure and growth. Marine Ecology 33: 427-436
5-Year Impact Factor (2012): 2.257

Chapter II. Kersting DK, Casado C, López-Legentil S, Linares C (2013) Unexpected divergent patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*. Marine Ecology Progress Series 486: 165-–171
5-Year Impact Factor (2012): 3.158

Chapter III. Kersting DK, Teixidó N, Linares C. Recruitment and natural mortality of the temperate coral *Cladocora caespitosa*: implications for the recovery of endangered populations.
Submitted to: Coral Reefs
5-Year Impact Factor (2012): 4.209

Chapter IV. Kersting DK, Bensoussan N, Linares C (2013) Long-term responses of the endemic reef-builder *Cladocora caespitosa* to Mediterranean warming. Plos One 8: e70820
5-Year Impact Factor (2012): 4.244

Chapter V. Kersting DK, Ballesteros E, De Caralt S, Linares C. Invasive macrophytes in a marine reserve (Columbretes Islands, NW Mediterranean): spread dynamics and interactions with the endemic scleractinian coral *Cladocora caespitosa*.
Submitted to: Biologicasl Invasions
5-Year Impact Factor (2012): 3.027

Contributions of the authors:

Chapter I. Conceived and designed the experiments: DKK CL. Performed the experiments: DKK CL. Analyzed the data: DKK. Contributed reagents/materials/analysis tools: DKK CL. Wrote the paper: DKK CL.

Chapter II. Conceived and designed the experiments: DKK SSL CL. Performed the experiments: DKK CC CL. Analyzed the data: DKK. Contributed reagents/materials/analysis tools: DKK SLL CL. Wrote the paper: DKK SSL CC CL.

Chapter III. Conceived and designed the experiments: DKK CL. Performed the experiments: DKK NT CL. Analyzed the data: DKK NT. Contributed reagents/materials/analysis tools: DKK CL. Wrote the paper: DKK NT CL

Chapter IV. Conceived and designed the experiments: DKK CL. Performed the experiments: DKK. Analyzed the data: DKK NB. Contributed reagents/materials/analysis tools: DKK CL. Wrote the paper: DKK NB CL.

Chapter V. Conceived and designed the experiments: DKK EB CL. Performed the experiments: DKK EB SDC. Analyzed the data: DKK SDC. Contributed reagents/materials/analysis tools: DKK EB SDC CL. Wrote the paper: DKK EB SDC CL.

Regarding to the authors of the different chapters, CC is the only that has not been awarded a PhD degree. I hereafter guarantee that none of the information contained in the corresponding chapter (II) will be used to elaborate part of her PhD thesis.

For all of the above, I consider that the contribution of the PhD candidate grants him the right to defend his thesis in front of a scientific committee.

Barcelona, October 19th 2013.

Dr. Cristina Linares

Cladocora caespitosa bioconstructions in the Columbretes Islands Marine Reserve: distribution, size structure and growth

Photo: *Cladocora caespitosa* bank in the Illa Grossa Bay (Columbretes Islands)

CHAPTER I

Cladocora caespitosa bioconstructions in the Columbretes Islands Marine Reserve (Spain, NW Mediterranean): distribution, size structure and growth

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Abstract

Today, living *Cladocora caespitosa* banks appear to be restricted to a few Mediterranean locations and are threatened by the escalating impacts affecting coastal areas. In this study the exceptional occurrence of the Mediterranean coral *Cladocora caespitosa* in the Columbretes Islands Marine Reserve (NW Mediterranean) is characterised in terms of spatial distribution, cover area, colony size and growth rates. The coral colonies form beds and banks in rocky bottoms within a semi-enclosed bay that offers both hydrodynamic protection and high water exchange. The spatial distribution of the *C. caespitosa* colonies, from 5 to 27 m depth, is highly aggregated, depending on seafloor morphology and showing up to 80% of substrate coverage. The annual corallite growth rates obtained through alizarin red staining method and x-ray image analysis are similar, and range between 2.55 ± 0.79 mm and 2.54 ± 0.81 mm, respectively. The exceptional nature of these bioconstructions is due to their cumulative cover area, which is comparable in size to the largest *C. caespitosa* bioconstructions described to date in Mljet National Park (Croatia, Adriatic Sea).

Keywords:
Cladocora caespitosa, Coral bioconstruction, Growth rate, Mediterranean Sea, Scleractinia , Spatial distribution.

Resumen

Actualmente, los arrecifes de *Cladocora caespitosa* en el Mediterráneo presentan una distribución muy restringida y están amenazados por el progresivo aumento de los impactos que afectan a las zonas costeras. La Reserva Marina de las Islas Columbretes (Mediterráneo Noroccidental, España) alberga arrecifes excepcionales de este coral mediterráneo. El objetivo principal del presente estudio es caracterizar estos arrecifes según su distribución, cobertura, tamaño de colonias y tasas de crecimiento. Se ha encontrado que este coral forma campos de colonias y arrecifes sobre el substrato rocoso de la Bahía de L'Illa Grossa, que ofrece protección frente al hidrodinamismo, a la vez que permite un elevado intercambio de agua con mar abierto. Las colonias de *C. caespitosa* se localizan entre los 5 y 27 m de profundidad y su distribución es altamente agregada y ligada a la morfología del fondo; llegándose a obtener coberturas de hasta el 80 % del substrato. Las tasas anuales de crecimiento de los coralitos se obtuvieron mediante dos métodos: tinción con rojo de alizarina y el análisis de radiografías. Se obtuvieron tasas de crecimiento similares con ambos métodos, oscilando entre los 2,55 ± 0,79 mm y 2,54 ± 0,81 mm, respectivamente. La excepcionalidad de estas bioconstrucciones radica en el área total ocupada, comparable a la del mayor arrecife de *C. caespitosa* descrito hasta la fecha en el Parque Nacional de Mjlet (Croacia, Mar Adriático).

Palabras clave:
Cladocora caespitosa, Bioconstrucciones, Crecimiento, Mar Mediterráneo, Scleractinia, Distribución espacial

INTRODUCTION

The scleractinian *Cladocora caespitosa* (Linnaeus, 1767) is the only Mediterranean endemic zooxanthellate coral with reef-forming capacity at the present time (Morri et al. 1994) and in the past (e.g., Aguirre and Jiménez 1998). The oldest fossil reef known to date is after Messinian Event (Late Pliocene, Aguirre and Jiménez 1998), following the extinction of ancient tropical reef ecosystems in the Mediterranean (e.g., Esteban 1996). Therefore, *C. caespitosa* banks may be considered the unique continuation of the reef ecosystems to the present day (Kühlman et al. 1991; Aguirre and Jiménez 1998).

This species occurs in a wide range of substratum, depth and hydrodynamic conditions (Zibrowius 1980; Schiller 1993). Schuhmacher and Zibrowius (1985) classified *C. caespitosa* as a constructional but ahermatipic coral, as it does not contribute significantly to the framework of reefs. In the last few years, the family to which the genus *Cladocora* belongs has been revised based on contrasting molecular investigations. The genus has been excluded from Faviidae and included first in Caryophylliidae (Romano and Cairns 2000) and afterwards in Oculinidae (Fukami *et al.* 2008).

The distribution of extant *C. caespitosa* colonies has decreased compared with the fossil distribution (Laborel 1987). The causes of this historic reduction are not clear but they could be associated with environmental changes. Such decreases seem to be continuing today (Morri et al. 2001). This decline is being reinforced by recurrent mass mortality events that were recorded for *C. caespitosa* during the last decade (Perez et al. 2000; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009; Kersting and Linares 2009), probably caused by climate warming (Lejeusne et al. 2010). Thus, global warming seems to be an important threat for this temperate coral, which was already included by Augier (1982) in a list of endangered marine species.

Although *C. caespitosa* is a conspicuous species, large bioconstructions of this coral are very rare at the present time, and the common *C. caespitosa* populations are built up of small, disperse colonies. Some *C. caespitosa* bioconstructions have been described, but only in a few have the distribution patterns and main population characteristics been intensively studied (Schiller 1993; Peirano et al. 2001; Kružić and Požar-Domac 2003; Kružić and Benković 2008).

In this study, we describe the *C. caespitosa* bioconstructions in the Illa Grossa Bay (Columbretes Islands, NW Mediterranean) in terms of spatial distribution, size structure and growth rates. The results are compared with published data from other areas providing new comparative information on biological and ecological features of different populations of this endangered coral species across the Mediterranean Sea.

MATERIALS AND METHODS

Study site

The Columbretes Islands emerge 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean) within a 90x40 km volcanic field at 80-90 m water depth (Muñoz et al. 2005). A marine reserve encircles the archipelago covering an area of 5500 ha., Illa Grossa (39°53.825'N, 0°41.214'E), the largest of the islets in Columbretes (14 ha), is a C-shaped drowned Quaternary volcanic caldera that is open to the NE in the main direction of winter storm waves (Fig.1) (Aparicio and García 1995, Sánchez-Arcilla et al. 2008). The bay formed by this islet has a total surface of 150,000 m^2 and hosts the studied *Cladocora caespitosa* population.

The sea bottom in the bay has an average depth of 15 m and is covered mainly by rocky substrata and biogenic sands in the central and deeper areas. A wide flat channel crosses the bay with the main storm direction (NE). In the bay, the rocky slopes of the islet sink abruptly, reaching at least 5 m depth in the shallowest areas. In the central part of the bay the seafloor becomes less steep, although eroded remnants of the successive volcanic eruptions, in form of crests (rock formations over 5 m in height and 10 m in length) and blocks, are frequent in the NW and SE borders (Aparicio and García 1995). The infralittoral photophilic algal community covers the illuminated parts of the crests and blocks and is mainly dominated by dense facies of *Dictyopteris polypodioides* starting at 5 m depth (Templado and Calvo 2002).

Sea surface temperature have (SST) been taken daily with a calibrated mercury-in-glass thermometer since 1991. The mean monthly SST in Illa Grossa Bay ranges from 13.16 ± 0.80 °C (February) to 26.19 ± 1.16 °C (August) (± SD) (average obtained from daily measures between 1991 to 2010; D K Kersting and C. Linares, unpublished observations).

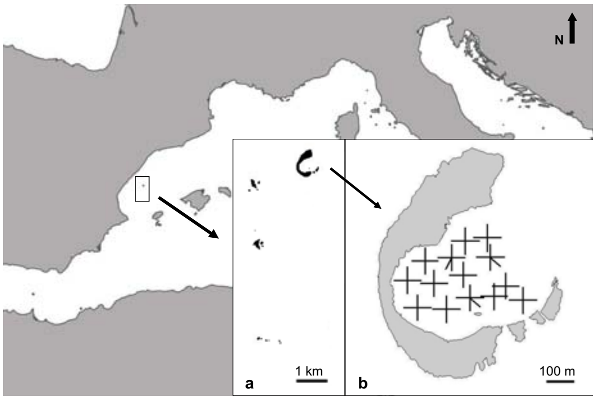

Figure 1. (a) Location of Columbretes Islands (NW Mediterranean Sea, Spain). (b) Illa Grossa islet and the surveyed transects.

Spatial distribution

Due to the size of the bay and the ubiquity of the *Cladocora caespitosa* colonies high resolution mapping techniques, such as used in Kružić and Benković (2008), had to be disregarded. Instead, an interpolation technique using transects was chosen and allowed to cover the whole bay. Therefore, Illa Grossa Bay was surveyed through radial transects starting from 14 homogeneously distributed points throughout the bay from 1 to 30 m depth (Fig. 1). There was at least four transects per point (North, East, South and West). Transects were 50 m long and 1 m wide (50 m^2) and each of them was subdivided into ten 5 m^2 areas to record: depth range, number of colonies and colony diameters (major axis D1 and minor axis D2 following Peirano et al. 2001). The colony diameters were measured to the nearest half centimeter with a 127 cm aluminium tree caliper. The *C. caespitosa* cover was obtained by approximating the colony base area to that of a circumference (D1 = D2) or an ellipse (D1 ≠ D2) depending on the shape of each colony.

The *C. caespitosa* cover in the bay was mapped through interpolation of the coral cover data obtained in transects with a gridding method (inverse distance to a power, Surfer version 9 software). In the mapping and estimations of coral cover, each of the 5 m^2 areas was considered as a single geographical location related to its coral cover data.

Spatial autocorrelation of coral cover at different distance classes was studied with spatial correlograms (Oden and Sokal 1986) using Moran's I coefficient (Moran 1950). The autocorrelation coefficient Moran's I evaluates whether the spatial pattern observed is clustered, dispersed or random. The distance classes used for the correlogram where 10, 20, 30, 40, 50, 100 and 200 m and the distance matrix was obtained from the geographical position of each 5 m^2 transect subunit. Significant positive autocorrelation means that within a particular distance class, the coral cover value is more similar (clustered) than obtained random from any distance class.

The spatial analysis was undertaken using Passage 2.0 software (Rosenberg and Anderson 2011; http://www.passagesoftware.net). Distance matrix used for the Moran's I correlogram was generated with Geographic Distance Matrix Generator 1.2.3 software (http://biodiversityinformatics.amnh.org/open_source/gdmg).

Relationships between the seafloor morphology and coral cover were searched by overlaying the bathymetry of the bay (authorized use by the Ministry of Environmental and Rural and Marine Affairs, Spanish General Secretariat for the Sea) and the coral cover map.

Size-frequency distribution and colony morphology

To choose a single size descriptor the correlation between diameter (D1, D2) and height (H) was studied in 115 colonies (Fig.2). D1 showed a positive and significant relationship with D2 ($r^2 = 0.8946$; $p < 0.01$; $n = 115$) and with H ($r^2 = 0.8134$, $p < 0.01$; $n = 115$). Hence, D1 was selected as the colony size descriptor, given also its easy measurement. D1, measured on 1511 colonies in the bay, was used to obtain the size-frequency distribution of the population, which was analysed in terms of descriptive statistics using skewness (Sokal and Rohlf 1995).

As no quantitative data are available on the local current regime in the bay, the relationships between the hydrodynamics of the bay and the shape of *Cladocora caespitosa* colonies were investigated through the sphericity Is-index (maximum height/maximum diameter of a colony) (Riedl 1966; Kružić and Benković 2008, Fig. 2) and the correlation between D1 and the depth of colony occurrence.

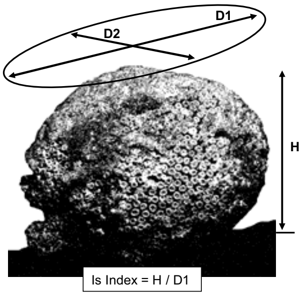

Figure 2. Size descriptors used in the biometry of *Cladocora caespitosa*.

Colony growth rates

The annual polyp growth rate was estimated through two methodologies: alizarin red staining technique and sclerochronology (x-ray image analysis of the corallites) (Fig. 3). The staining method was applied both in aquaria and *in situ* to colonies living between 14 and 16 m depth. The alizarin red concentration used in both cases was 10 mg·l^{-1}, and the staining lasted for 24 hours (Lamberts 1978, Schiller 1993, Rodolfo-Metalpa et al. 1999). The colonies stained in aquaria were re-installed in the bay after the staining treatment. This transplantation was done using underwater putty to fix the base of the colony to the rock at the same location and depth where it had been previously collected. The *in situ* staining was undertaken by covering each colony with a semi-spherical transparent plastic structure well fitted to the ground to avoid significant losses in the alizarin red solution (as described in Lamberts 1978). This structure was large enough to not interfere with the colony, thus avoiding polyp retraction. Five colonies were stained in 2008 (four in aquaria and one *in situ*) and seven in 2009 (six in aquaria and one *in situ*). The corallites of each colony were collected 12 months after the staining. The corallites were cleared of organic material and tissue by submersion in H_2O_2 (30 %) for 24 h and were then polished with an electric mini-borer with a diamond cutting wheel until the alizarin mark limit was clearly noticeable. A total of 540 corallites were used to measure the annual growth rate with this method. The measurement was performed with a caliper to the nearest 0.01 mm from the edge of the calyx to the upper limit of the staining (Schiller 1993; Rodolfo-Metalpa et al. 1999).

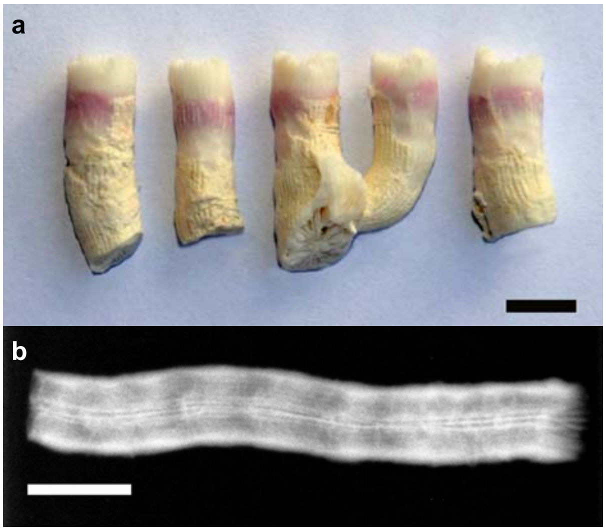

Figure 3. (a) Alizarin red stain in *Cladocora caespitosa* corallites. (b) X-ray image of a *C. caespitosa* corallite with annual high and low density bands. Scale bars: 0.5 cm.

Evaluation of mean growth rates through corallites x-ray analyses was conducted on 13 colonies of *Cladocora caespitosa*. Corallites were collected, cleaned in H_2O_2 (30 %), x-radiographed with a medical unit and growth rates calculated with CORAL XDS software (http://www.nova.edu/ocean/ncri//projects/coralxds/index.html) (Peirano et al. 2005, Kružić and Benković 2008).

Given that the goal of the study was to provide mean growth rates of *C. caespitosa* colonies in Columbretes in order for comparison with similar published references, only the differences in polyp growth obtained from both methodologies (alizarin red and x-ray) were tested through a Kolmogorov-Smirnov two-sample test. The low number of colonies stained with alizarin *in situ* and in the aquaria prevented us to analyze statistically the differences between both techniques as well as differences relating to depth and sites. All statistical analyses were performed using STATISTICA 8 software.

RESULTS

Spatial distribution

The depth distribution of *Cladocora caespitosa* colonies in the Illa Grossa Bay ranged from 5 to 27 m. The colonies occurred in rocky and small block (averaged diameter < 1 m) bottoms as well as in vertical, sub-vertical and horizontal substrata. The highest *C. caespitosa* cover was found between 10 and 20 m depth; about 85 % of the cumulative *C. caespitosa* colony area was concentrated at this depth range (Fig. 4). Coral cover and depth showed no linear correlation ($r = -0.24$, $p < 0.01$).

Although *C. caespitosa* colonies were present throughout the bay with an average cover of 1.9 %, some areas displayed remarkably higher colony concentrations with contagious distributions. In these areas, the coral cover reached values up to 80 % (in 5 m^2) and maximum colony densities of 5.5 colonies per m^2. Scattered banks of *C. caespitosa* (bioconstructions covering several square meters) were to be found within colony beds.

The Moran's I Correlogram indicates a significant ($p < 0.01$) positive autocorrelation of the coral cover data, in agreement with contagious distribution at distance classes of 10 to 50 m (Fig. 5).

The main colony concentration zones were located at the NW and SE areas of the bay, where the steepest crests occur (Fig. 6). The mean cover obtained in these areas ranged from 2.7 to 7 % in the NW and SE areas, respectively. The estimates of the overall surface covered by the colonies reached 240 m^2 in the NW area and

910 m^2 in the SE area. The cumulative cover area of *C. caespitosa* in the bay was estimated to be approximately 2900 m^2.

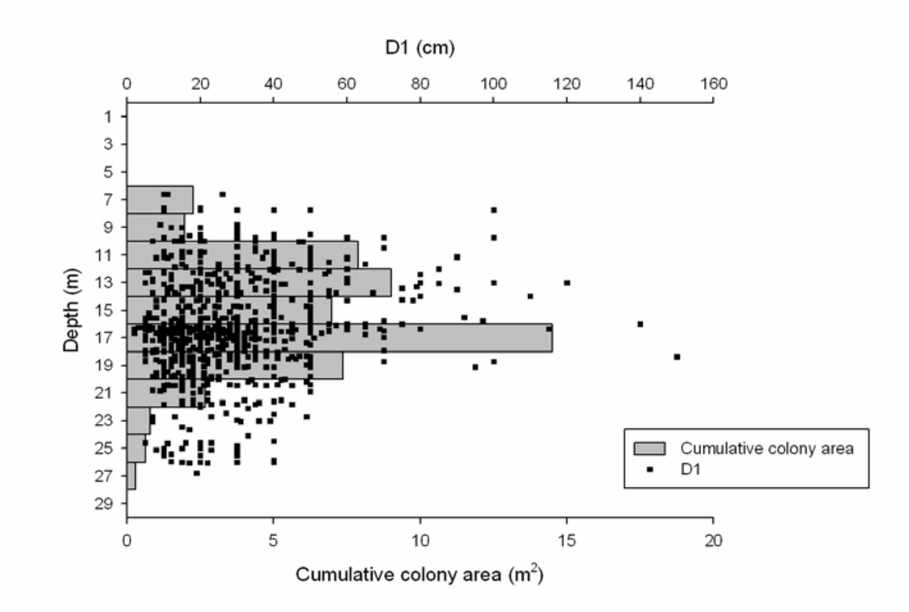

Figure 4. Depth distribution of D1 and cumulative colony area per depth in the surveyed transects.

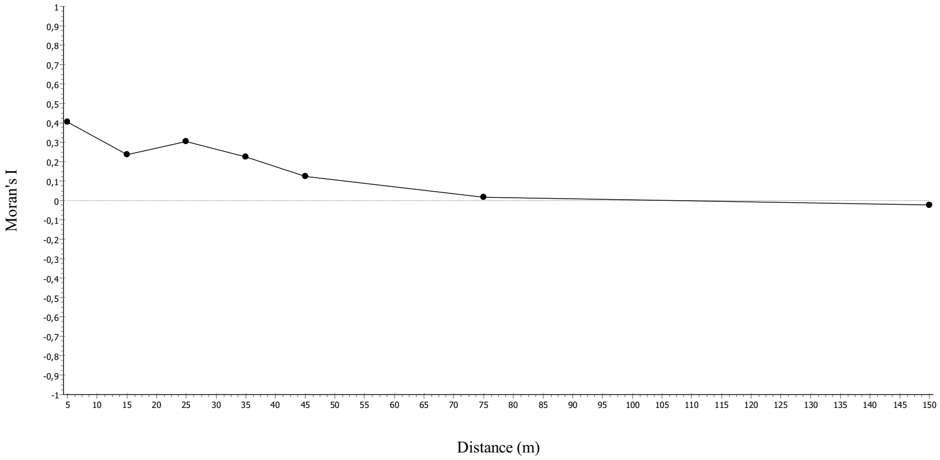

Figure 5. Spatial correlogram (Moran's I) for *Cladocora ca*espitosa cover in the Illa Grossa Bay. Solid dots show significance ($p < 0.01$) for the Moran's I coefficient in the distance classes.

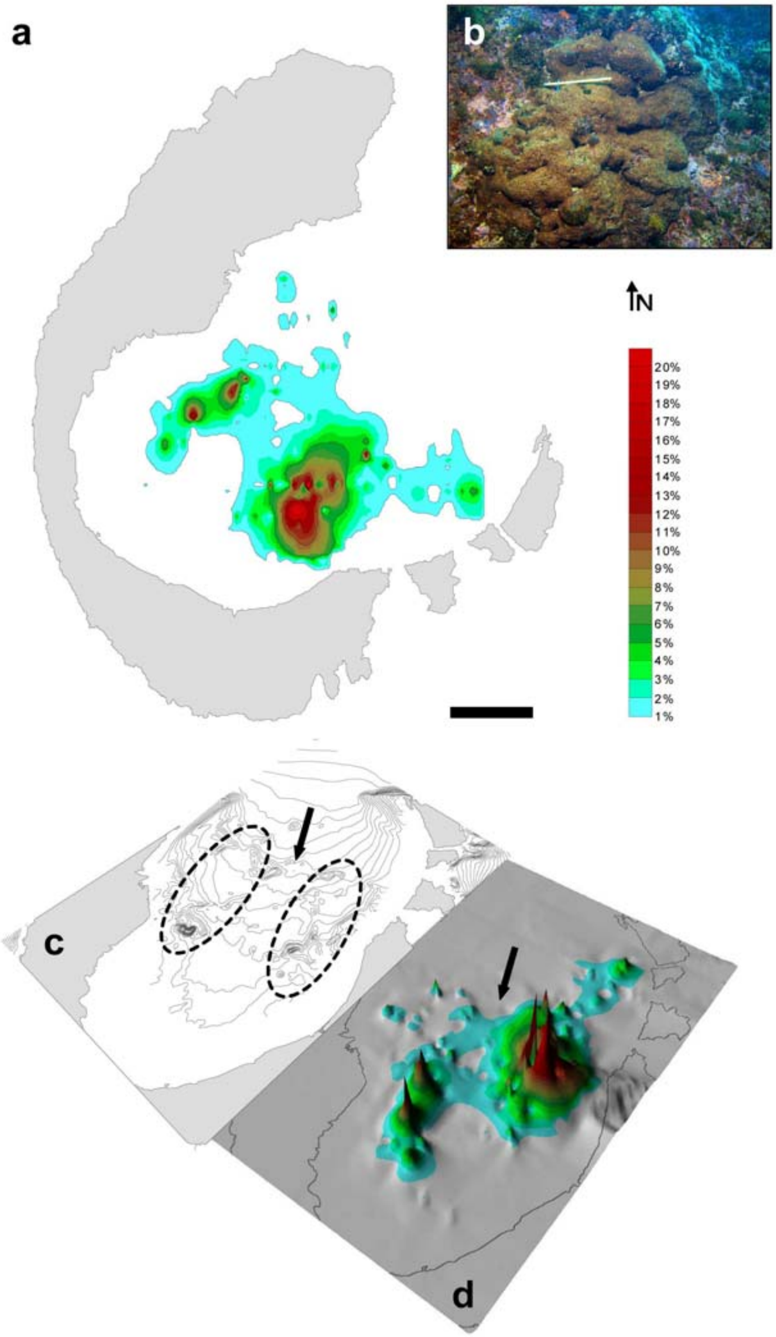

Figure 6. *Cladocora caespitosa* bioconstructions in the Illa Grossa Bay. (a) Coral cover (%) map of the bay. Scale bar: 100 m. (b) Detail of a *C. caespitosa* bank in the study area (the scale is 30 cm). (c) Bathymetric map of the study bay, dotted ovals show the zones where rock crests are predominant and arrows show the NE–SW central channel in the bay. (d) 3D coral cover map of the area (note that the relief in this map reflects the coral cover values, not the sea-floor morphology).

Size-frequency distribution and colony morphology

The size-frequency distribution of this population was unimodal and non-normal (K-S d = 0.127, $p < 0.01$, Fig. 7). The skewness of the distribution was significantly positive ($g_1 = 1.667$; Sokal and Rohlf 1995), which indicates the prevalence of small classes in the population. The mean colony diameter was 31.48 ± 21.02 cm (± SD), and the maximum and minimum diameters recorded within transects were 150 and 2 cm, respectively. Regarding colony morphology, the average Is-Index value obtained for this population was 0.55 ± 0.21 (± SD).

D1 and depth showed no correlation ($r = -0.19$, $p < 0.01$). All the size classes were represented in the middle depth range of distribution (10 - 20 m), whereas the largest colonies were absent in the upper and lower limits (Fig. 4).

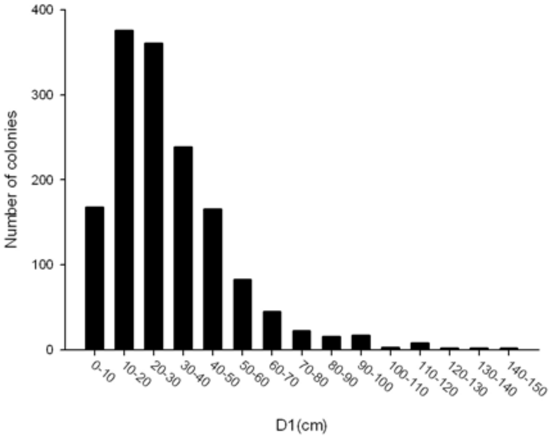

Figure 7. Size frequency distribution of *Cladocora caespitosa* colonies in Illa Grossa (n = 1511).

Colony growth rates

In the *Cladocora caespitosa* corallites, two bands are deposited annually: a high-density band (HD) in the winter and a low-density band (LD) in the summer, as previously found by Peirano et al. (1999, 2005). However, the annual HD and LD bands were not always noticeable in radiographed corallites and, consequently, many samples had to be disregarded. The image analysis of the 30 corallites resulted in 95 annual HD and LD bands.

Based on the alizarin staining method, the mean annual growth rate obtained for 2008 and 2009 was 2.55 ± 0.79 mm (± SD). The individual minimum and maximum growths were 0.49 and 5.49 mm, respectively. The mean annual growth rate obtained by means of the x-ray images was 2.54 ± 0.81 mm (± SD), and the minimum and maximum growth rates were 1.41 and 5.19 mm, respectively. While alizarin staining method provided growth rates from a unique year, x-ray method obtained growth data from different years. Nonetheless, growth rates obtained from both methodologies did not display significant differences (Kolmogorov-Smirnov two-sample test, p = 0.1).

DISCUSSION

The characteristics of the *Cladocora caespitosa* population in the Columbretes Islands Marine Reserve were exceptional in the current framework of this species in the Mediterranean Sea. Despite the common occurrence of colonies of *C. caespitosa*, barely 10 living banks and beds of this species have been described in different locations at the Western and Eastern Mediterranean Sea to date (Fig. 8). The mean colony diameter (D1) and coral cover values obtained in Columbretes were higher than those reported in other Mediterranean sites such as the Bay of Piran and La Spezia (Schiller 1993, Peirano et al. 2001).

Figure 8. Main living *Cladocora caespitosa* bioconstructions described in the literature. 1. Columbretes Islands (Present work). 2. Port-Cros (Laborel and Laborel-Deguen 1978). 3. La Spezia region (Morri et al. 1994, 2000; Peirano et al. 2001, 2005; Rodolfo-Metalpa et al. 2005). 4. Bay of Piran (Schiller 1993). 5. Rovinj (Zibrowius 1980). 6. Prvić (Zibrowius 1980; Kružić and Benković 2008). 7. Pag (Kružić and Benković 2008). 8. Mljet (Kružić and Požar-Domac 2003; Kružić and Benković 2008). 9. Eubée, Gulf of Atalanta (Laborel 1961). 10. Tunisia (Zibrowius 1980).

The overall surface covered by *Cladocora. caespitosa* was comparable in size to the largest described *C. caepitosa* bank reef known in the Mediterranean, at Veliko jezero in the Mljet National Park (Croacia) as reported by Kružić and Benković (2008). However, the type of colony distribution in these two sites differed widely: there is a continuous reef in Veliko jezero and a combination of banks and separate colonies in Columbretes (Fig. 6). By applying the terminology proposed by Peirano et al. (1998), that is, bed (a great number of distinct subspherical colonies 10 to 30 cm in diameter) or bank (large formations reaching several decimetres in height and covering several square meters in surface area), the *C. caespitosa* population of the Columbretes Islands can be considered a combination of both types of colony distribution. A veritable reef development is almost certainly limited by the hydrodynamic conditions within the bay. There are frequent remains of broken colonies throughout the bay, the result of the combined action of boring organisms and hydrodynamics. In fact, some of the detritic deposits within the bay have an important fraction of corallite fragments.

The contagious distribution pattern of *C. caespitosa* in the bay is probably related to at least two factors: reproduction strategies and sea bottom morphology. Clumped distributions have been reported for benthic species with philopatric dispersion (e.g., Gori et al. 2011), including *C. caespitosa* (Peirano et al. 2001). In the case of *C. caespitosa* the only study dealing with reproduction (Kružić et al. 2008a) shows the occurrence of mechanisms that force the eggs to stay near the parental colonies (e.g., eggs covered in mucus coating). These mechanisms reduce the dispersion of eggs and consequently of larvae, which will finally develop new colonies near the parental ones. On the other hand the distribution of *C. caespitosa* in the bay has shown to be associated with sea bottom morphology and hydrodynamic protection. The two areas in the bay displaying higher coral cover values occurred in sites with irregular bottom morphology, where rock crests and blocks are common. These features and the relative location of these sites in the bay, at both sides of the central NE-SW channel (Fig. 6), ensure relative protection during the strong NE storms occurring in fall and winter (Sánchez-Arcilla et al. 2008).

No correlation between depth and the colony diameter (D1) or coral cover was found. Most of the Illa Grossa Bay *C. caespitosa* population concentrates in the 10 m to 20 m depth range. The low coral cover and the absence of the bigger colony sizes in the shallowest limit of the distribution (5 - 10 m) are probably related to a higher exposure to waves. But this absence is as well noticeable in the deeper range (20 - 30 m), in this case the low cover and smaller sizes could be related to seafloor morphology and substrata, as detritic beds are more common in this depth range and rocks are rare.

The Is-index obtained for the Columbretes colonies showed a high degree of colony sphericity and is comparable to the minimum values obtained by Kružić and Benković (2008) in Mljet. These lower values were related by these authors to the influence of strong bottom sea currents. In spite of the protection given by the C-shaped L'Illa Grossa islet to waves and currents coming from the N, S and W, currents can be quite strong in the bay with E and NE winds. Hence, the combination of protection and elevated water exchange with the open sea seems to be a common factor in the development of large *C. caespitosa* bioconstructions; the reef described by Kružić and Benković (2008) in Mljet is a perfect example. Other similarities in the bioconstructions of Mljet and Columbretes were the depth range in which the colonies are found and the temperature regime.

High erect algal cover has been considered a limiting factor in *C. caespitosa* development (Peirano et al. 1998). The occurrence of shallow beds of this coral has been attributed to factors inhibiting algal growth, such as water turbidity or the grazing activity of sea urchins (Herndl and Velimirov 1986; Morri et al. 2001). It has even been hypothesised that the occurrence of *C. caespitosa* banks happens only below the compensation depth of photophilic algae (Rodolfo-Metalpa et al. 1999). The algal cover in Columbretes is significantly high (Templado and Calvo 2002), and in the Illa Grossa Bay, *C. caespitosa* often occurs within a high coverage of *Dictyopteris polypodioides* in the infralittoral photophilic algal community, although colonies in a sciaphilic habitat are also found. *Halimeda tuna*, *Cystoseira sauvageauana* and *C. compressa* frequently grow in the interstices between polyps of some of the colonies as well. Despite the high algal cover in the bay, only *Codium bursa* and *C. coralloides* have been occasionally observed overgrowing *C. caespitosa* colonies. Therefore, contrary to the previously found that large beds and banks are limited by high algal cover, an important *C. caespitosa* population has developed in Columbretes despite of the dense photophilic algal community, reinforcing the high level of ecological plasticity of this coral, which is capable of living in such contrasting environments as photophilic communities (e.g., Columbretes) or circalittoral coralligenous assemblages e.g., in Bonassola and Riomaggiore, Ligurian coast (Morri et al. 1994), or in Cap de Creus and Medes Islands, Catalan coast (D K Kersting and C Linares pers. obs.).

Alizarin staining technique and x-ray analysis showed no significant difference in the growth rates obtained. Kružić and Požar-Domac (2002) used both methodologies in parallel and with similar results. However, certain factors must be taken into account when considering both methodologies. As mentioned above, annual HD and LD banding are not always noticeable in radiographed corallites. The causes explaining this fact remain unknown and further research should investigate the factors which determine the pattern of calcium seasonal deposition. On the other hand, the staining was successful using both treatments, in aquaria and *in situ*. In this study, the *in situ* staining method was used to stain *C. caespitosa* corallites for the first time, and it was an effective and easily implemented method with minimal manipulation of the colonies. The only limitation was that the staining structure requires a tight fitting to the ground.

The annual growth rate obtained for *C. caespitosa* in Columbretes fits into the lower range of the results obtained by different authors using either alizarin staining or x-ray analysis on living colonies (Table 1). These rates demonstrate the slow growth of this species. Bearing in mind these growth rates, the mean age of the colonies in the bay of Columbretes Island could be roughly estimated at 50 years; almost 10 % of the colonies may be over 100 years old, and some colonies in Columbretes could reach ages up to 300 years.

The large *C. caespitosa* bioconstructions in the Mediterranean certainly have a high patrimonial value due to their rarity, their slow growth and the dynamics of this coral species. Moreover, their conservation is an important concern in the face of the increasing threats affecting these exceptional bioconstructions. Although the Columbretes Islands Marine Reserve protects *C. caespitosa* in the Illa Grossa Bay from direct human impacts, this population could be endangered by global change-related disturbances such as recurrent mortalities linked to positive thermal anomalies (Kersting and Linares 2009) or the presence of invasive algal species such as *Caulerpa racemosa* and *Lophocladia lallemandii*. Given that Columbretes Islands are isolated at the edge of the continental shelf 60 km from the nearest coast, and the main current regime from north to south (Font et al. 1990) in this area, the connectivity of the Columbretes *C. caespitosa* population with the nearest populations at the coast west of the islands is probably very low. To evaluate the viability of these endangered Mediterranean bioconstructions, further scientific studies are needed on topics such as population dynamics and connectivity; especially in the present context of the impacts from global change.

Locality	**Annual growth (mm.year^{-1})**	**Method**	**Authors**
Prvić (Adriatic)	3.2 ± 0.1	X-ray	Kružić and Benković (2008)
Pag (Adriatic)	3.1 ± 0.1	X-ray	Kružić and Benković (2008)
Mljet (Adriatic)	3.7 ± 1.3	X-ray	Kružić and Benković (2008)
Mljet (Adriatic)	4.7 ± 0.6 / 4.7 ± 0.6	Alizarin / X-ray	Kružić and Požar-Domac (2002)
Bay of Piran (Adriatic)	4.4 ± 0.6	Alizarin	Schiller (1993)
La Spezia (NW Med.)	3.01	X-ray	Peirano et al. (2005)
La Spezia (NW Med.)	1.3 ± 0.6 - 4.3 ± 1.4	X-ray	Peirano et al. (1999)
La Spezia (NW Med.)	4.8 ± 1.7	Alizarin	Rodolfo-Metalpa et al. (1999)
Mallorca (NW Med., Aquarium)	5	Direct measure	Oliver Valls (1989)
N to S Adriatic	2.6 ± 0.2 - 4.1 ± 0.6	X-ray	Peirano et al. (2009)
Ligurian Sea	3.7 ± 0.5 - 3.3 ± 0.4	X-ray	Peirano et al. (2009)
S Italy	3.1 ± 0.3 - 3.2 ± 0.3	X-ray	Peirano et al. (2009)
Tunisia	2.3 ± 0.2	X-ray	Peirano et al. (2009)
Illa Grossa (NW Med.)	2.5 ± 0.8 / 2.5 ± 0.8	Alizarin / X-ray	Present work

Table 1. *Cladocora caespitosa* annual growth rates obtained in this study and cited in the literature.

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Unexpected divergent patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*

Photo: Opened mouth (*Cladocora caespitosa*)

CHAPTER II

Unexpected divergent patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*

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Abstract

Knowledge of reproductive biology is essential to understanding population dynamics and ecological processes in corals. Sexual condition and the reproductive cycle of the Mediterranean endemic scleractinian *Cladocora caespitosa* was assessed through histological analyses. Our results showed that this species is gonochoric in the Western Mediterranean Sea. Oocytes and spermaries were detected annually from March to October, reaching their maximum size between July and August coincidentally with the highest seawater temperatures. A drastic decrease in gametes between August and October indicated that spawning occurred at the end of summer. These results differ from those obtained for the Adriatic Sea, where this species was described as hermaphroditic and spawning occurred at the beginning of summer. The unusual plasticity of this temperate coral and the endangered condition of *C. caespitosa* bioconstructions in the Mediterranean highlight the need for further research on this topic.

Keywords
Coral reefs, *Cladocora caespitosa*, Reproduction, Mediterranean Sea

Resumen

El conocimiento de la biología reproductora es esencial para entender la dinámica poblacional y los procesos ecológicos en corales. En el presente trabajo se han estudiado la condición sexual y el ciclo reproductor del coral escleractinio endémico del Mediterráneo *Cladocora caespitosa* mediante análisis histológicos. Los resultados obtenidos muestran que esta especie es gonocórica en el Mediterráneo Occidental. Los gametos femeninos y masculinos fueron detectados entre marzo y octubre, alcanzando su tamaño máximo entre julio y agosto, coincidiendo con la temperatura máxima del agua de mar. La drástica reducción del número de gametos entre agosto y octubre indica que la liberación se produjo a finales del verano. Estos resultados difieren de los obtenidos en el Mar Adriático, dónde esta especie ha sido descrita como hermafrodita y la liberación de gametos ocurre a principios del verano. La sorprendente plasticidad de esta especie longeva y de lento crecimiento pone de manifiesto la necesidad de nuevos estudios sobre esta temática.

Palabras clave:
Arrecifes de coral, *Cladocora caespitosa*, Reproducción, Mar Mediterráneo

INTRODUCTION

Understanding reproductive biology is essential to comprehending the population dynamics of marine organisms (Fadlallah 1983). Hence, knowledge of coral reproduction is necessary for the management and preservation of coral reefs. For example, successful reproduction can allow the addition of new individuals to existing populations, the colonization of new areas, and the recovery of populations damaged by natural or human disturbances.

The majority of scleractinian species can be classified as either hermaphroditic or gonochoric, however more complex sexual patterns have also been described (Harrison and Wallace 1990; Baird et al. 2009b; Guest et al. 2012a). Likewise, there are 2 types of larval development or reproductive modes. Fertilization is either internal, i.e., the embryo develops within the polyp and is released as a motile planula larva (brooding), or external, with the embryo developing in the water column (broadcast spawning) (Harrison and Wallace 1990; Baird et al. 2009b). However, information on the reproductive biology of temperate scleractinian species is relatively scarce in comparison to tropical scleractinian corals (see Harrison 2011 for a review), particularly for the Mediterranean Sea (Goffredo and Zaccanti 2004; Goffredo et al. 2006; Goffredo et al. 2010).

Hermaphroditic broadcast spawners are the dominant group among tropical scleractinian corals (Harrisson and Wallace 1990; Harrison 2011; Kerr et al. 2011). In contrast, temperate scleractinians appear to display higher variability in their sexual condition and fertilization strategy, although the latter appears to be fairly consistent within the same family. Within the family Caryophyllidae, for example, the species *Caryophyllia inornata, C. smithi, Lophelia pertusa* and *Paracyathus stearnsii* are gonochoric, whereas *C. ambrosia, C. cornuformis* and *C. sequenzae* appear to be hermaphroditic (Fadlallah and Pearse 1982a; Waller et al. 2005; Waller and Tyler 2005; Goffredo et al. 2012). However, all these species, except for *C. inornata*, show the same fertilization mode (external, broadcast spawners), which appears to be more frequent among scleractinian corals (Fadlallah and Pearse 1982b; Waller et al. 2005; Waller and Tyler 2005; Goffredo et al. 2012). Similarly, in the family Dendrophylliidae, *Balanophylllia elegans* and *Leptopsammia pruvoti* are gonochoric, whereas *B. europaea* is described as a hermaphroditic species (Fadlallah and Pearse 1982a; Goffredo and Zaccanti 2004; Goffredo et al. 2006). All species of the Dendrophylliidae are brooders and show internal fertilization of gametes (Fadlallah and Pearse 1982a; Goffredo and Zaccanti 2004; Goffredo et al. 2006).

The scleractinian *Cladocora caespitosa* (Linnaeus, 1767) is and was the only reef-forming Mediterranean endemic zooxanthellate coral (Morri et al. 1994; Aguirre and Jiménez 1998). This coral is physiologically and morphologically similar to the typical tropical reef-building scleractininans, being zooxanthellate, colonial and capable of forming extensive bioconstructions (Zibrowius 1982). *C. caespitosa* occurs from shallow waters to depths of approximately 40 m (where the amount of light still allows photosynthesis by the symbiotic zooxanthellae) and in sites characterized by calm waters or exposed to strong currents (Zibrowius 1982; Kružić and Benković 2008; Kersting and Linares 2012). Currently, living banks of the coral *C. caespitosa* appear to be restricted to a few Mediterranean locations and are threatened by the escalating impacts affecting coastal areas such as global warming and the spread of invasive species (Kružić and Požar-Domac 2007; Kružić et al. 2008b; Kersting and Linares 2012). Furthermore, *C. caespitosa* populations have been strongly affected during the past decade by mass-mortality events related to positive sea surface temperature (SST) anomalies (Perez et al. 2000; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009; Kersting and Linares 2009).

To date, only 2 studies based on *in situ* observations and preliminary histological analyses have provided insights into the reproduction of this emblematic species. The spawning of this coral species was first observed by Schiller (1993) in the Bay of Piran (Northern Adriatic Sea), where eggs and sperm bundles were released by a few colonies 4 days prior to the full moon in June. More recently, Kružić et al. (2008a) observed the timing and mode of spawning of *C. caespitosa* in the bank of Veliko jezero (Mljet National Park, Croatia) and described the species as colonial hermaphroditic but with colonies releasing either sperm or eggs during each spawning episode 2 nights before the full moon in June 2005.

The aim of this study was to enlarge our knowledge of the reproductive biology of *C. caespitosa* in the Western Mediterranean. The bioconstructions of this emblematic species along the Mediterranean Sea are currently threatened by seawater temperature increases and other anthropogenic impacts, and a thorough understanding of the reproductive characteristics of the species is now imperative. We used histological techniques to study the sexual condition, as well as the reproductive cycle of this species in order to assess the timing of spawning in the Columbretes Islands Marine Reserve (Western Mediterranean, Spain). This information was compared with results previously reported for the Adriatic Sea. In addition, we examined the sexual condition of *C. caespitosa* in 5 Western Mediterranean locations to determine the general patterns of reproduction in this area.

MATERIALS AND METHODS

To determine the sexual condition of *Cladocora caespitosa* (gonochoric vs. hermaphroditic) at both polyp and colony levels, colonies of *C. caespitosa* were sampled by SCUBA divers in 5 Western Mediterranean locations: Columbretes Islands Marine Reserve (Spain), Eivissa (Spain), Medas Islands Marine Reserve (Spain), Cap de Creus Natural Park (Spain), and Natural Reserve of Scandola (Corsica) (Fig. 1). The number of sampled colonies per site was variable, depending on the abundance of the species at each sampling site (Table 1).

Population	Males	Females	Immature
Columbretes Is.	4	5	1
Eivissa	0	2	0
Medas Islands	2	6	2
Scandola	2	0	0
Cap de Creus	4	0	0

Table 1. Summary of the sexual condition of *Cladocora caespitosa* colonies sampled at 5 study sites in the Western Mediterranean. No colonies were hermaphrodite.

To investigate the reproductive cycle of *C. caespitosa*, 10 colonies were surveyed monthly from April 2008 to July 2009 in the Columbretes Islands Marine Reserve (Spain, NW Mediterranean, 39°53.825' N, 0°41.214' E) at a depth of 15 m. These colonies (20 - 50 cm in diameter) were individually marked in one of the areas with higher coral cover values in Illa Grossa Bay (Kersting and Linares 2012). Particular efforts were made to select healthy colonies with no signs of recent or past mortality. Seawater temperature was measured daily during the study period with Stowaway Tidbits (ONSET, Cape Cod, MA, USA) autonomous sensors installed at the same depth and location as the studied colonies.

Initial histological analyses were conducted to assess the sexual condition of *Cladocora caespitosa* in all sampled sites, as well as to assess the sex of each of the 10 marked colonies in the Columbretes Islands; 3 polyps per colony were sampled for this purpose. According to these results, 3 male and 3 female colonies were selected and 3 polyps for each were further investigated. By the end of the study, 33 polyps per colony had been analyzed. The collected samples were fixed in 4% formaldehyde in seawater, decalcified in a solution of HCl (37%), formaldehyde and water (1.3:0.8:7.9) during 24 hours, dehydrated through a graded alcohol series and finally embedded in paraffin. Cross sections of polyps (5 - 6 µm thick) were stained with haematoxylin-eosin and examined under a light microscope equipped with a micrometer. In female colonies, the total number of oocytes per polyp was counted and minimum and maximum diameters of oocytes (sectioned through the nucleolus) were measured. The number of oocytes per polyp was counted when less than 100; whenever more than 100 oocytes were observed, the polyp was classified in class > 100. Oocyte diameter were measured in a maximum of 30 oocytes per polyp. In male colonies, only the number of spermaries was recorded due to the impossibility of measuring their size accurately. Due to the low number of either oocytes or spermaries in some samples, we were unable to establish specific stages of maturation; however, as maturation and oocyte size are correlated (Schlesinger et al. 1998) we used the later to estimate oocyte development.

Pearson's product-moment correlation was computed to examine the relationship between oocyte size (diameter) and seawater temperature at 15 m depth using the software package STATISTICA 8.0.

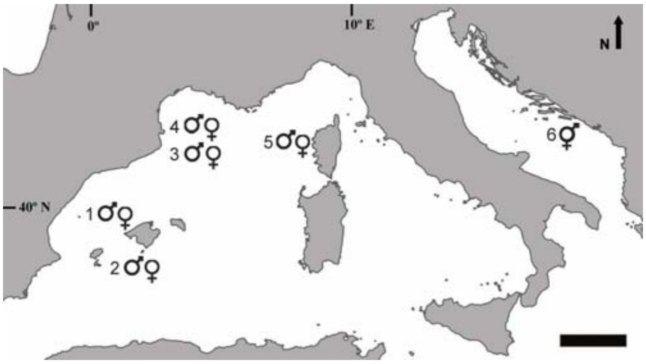

Figure 1. Map of the study sites in the Western Mediterranean and Adriatic Sea (sites 1-5 in this study and site 6 in Kružić et al. 2008a). 1 Columbretes Islands Marine Reserve (W Mediterranean, Spain), 2 Eivissa (W Mediterranean, Spain), 3 Medas Islands Marine Reserve (NW Mediterranean, Spain), 4 Natural Reserve of Scandola (NW Mediterranean, France), 5 Cap de Creus (NW Mediterranean, Spain) and 6 Mljet National Park (Adriatic Sea, Croatia). Scale bar: 200 km.

RESULTS AND DISCUSSION

Colonies of the scleractinian coral *Cladocora caespitosa* from the Western Mediterranean were determined to be gonochoric, since all polyps examined within the same colony exhibited the same sex. This species was described as hermaphroditic in the Adriatic Sea by Kružić et al. (2008a), i.e., polyps within a same colony had both male and female gonads. In fact, the preliminary histological analysis made by these authors showed that oocytes and spermaries developed on separate mesenteries within each polyp. Moreover, these authors found that the same colonies of *C. caespitosa* in the field released either female or male gametes, but not both simultaneously. In contrast, we found either oocytes or spermaries in a single polyp and colony and no signs of sex reversal, which are both typical signs of a gonochoric sexual condition (Fig. 2). Even though spawning was not directly observed in this study, the simultaneous maturation of gametes and the drastic decrease in the number of occytes and spermaries between August and October (Fig. 3) suggested that the release of sperm and eggs occurred at the same time in the Western Mediterranean *C. caespitosa.*

Although corals display great plasticity in their life history characteristics (Richmond and Hunter 1990), sexuality is generally consistent within most coral species and genera and within certain families (Harrison 2011). However, some examples of changes in sexual condition among populations have been reported in the literature. The scleractinian reef builder coral *Diploastrea heliopora*, was first classified as gonochoric on the Great Barrier Reef (Harrison 1985) but in Singapore was recorded to have colonies with hermaphroditic polyps, showing concurrent male and female gametes (Guest et al. 2012a). This species may exhibit alternate sexual function, with an overlap occurring when the end of one gametogenic cycle coincides with the beginning of the next cycle. In other species, unidirectional protandry has been related to colony size and age (e.g., *Stylophora pistillata,* Rinkevich and Loya 1979), and bidirectional sex change has also been described for corals from the family Fungiidae (Loya and Sakai 2008). Sexual mode variation has been also documented for *Protopalythoa* species in the Great Barrier Reef (Babcock and Ryland 1990) and for *Palythoa tuberculosa* in Japan (Yamazato et al. 1973). In the later, colonies were reported to be hermaphroditic while polyps within these colonies were gonochoric (Hirose et al. 2011). The variability in sexual condition of *C. caespitosa* appears to be one of the first records of this unusual plasticity in a temperate coral, along with *Astroides calycularis*, which was described as hermaphroditic in Algeria (Lacaze-Duthiers 1873) and as gonochoric in Southern Tyrrhenian Sea (Italy, Goffredo et al. 2010).

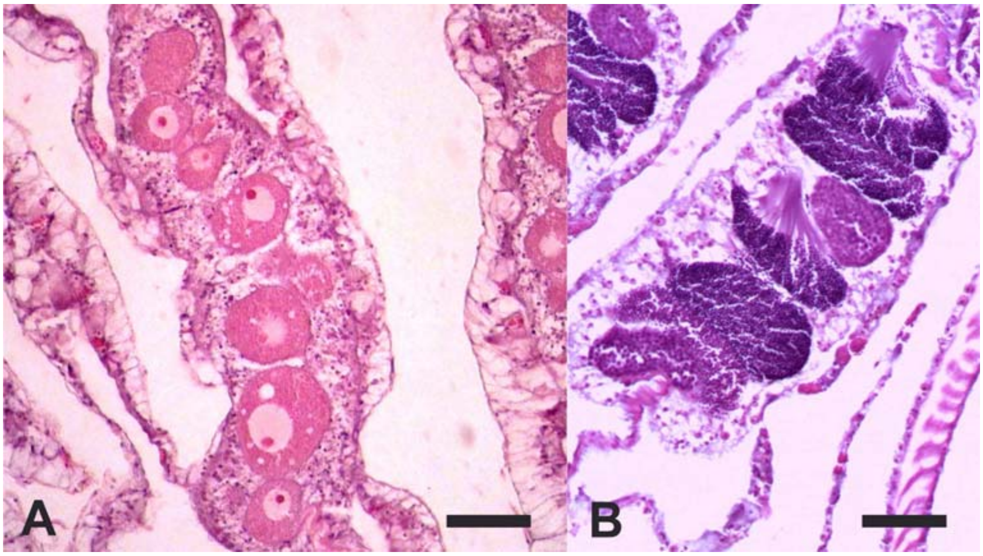

Figure 2. Gonads of *Cladocora caespitosa*. (A) Female septum packed with mature oocytes (July 2008) containing an oval nucleus and a spherical nucleolus. (B) Male with spermaries filled with spermatozoa (August 2008). Scale bars: 50 µm.

During the first year of study in the Columbretes Islands, oocytes and spermaries were detected in the mesenteries from April to October 2008 (Fig. 3) and reached their maximum development in August 2008, when oocyte mean diameter was 88,43 ± 22.53 µm (mean ± SD) and the number of oocytes and spermaries reached approximately 100 per polyp (Fig. 3). Gamete abundance showed a monthly increase during both study years, with a peak in July and August 2008 and a remarkable decrease in October 2008. The number of oocytes per polyp increased from an average of 10 ± 8.64 in April 2008 to approximately 100 in July 2008. Similarly, the number of spermaries increased from 7 ± 4.71 in April 2008 to approximately 100 in July 2008. During the second year of the study (2009), oocytes and spermaries were first detected in March and April, respectively. Although the monthly number of oocytes was very comparable between the 2 study years, the number of spermaries showed higher variability, especially in June (52 ± 30.4 in 2008 vs. 7 ± 4.7 in 2009). The maximal oocyte size found in this study also contrasted with previous findings reported for *C. caespitosa* in the Adriatic Sea (Kružić et al. 2008a). The mean diameter of spawned eggs described by these authors was 416 ± 73.12 µm, over four times greater than our measurements. Even though Kružić et al. (2008a) estimated oocyte sizes after spawning, the difference in size is extraordinary. To date, only small changes in oocyte sizes of scleractinian corals have been reported during the last month before spawning (Schlesinger et al. 1998).

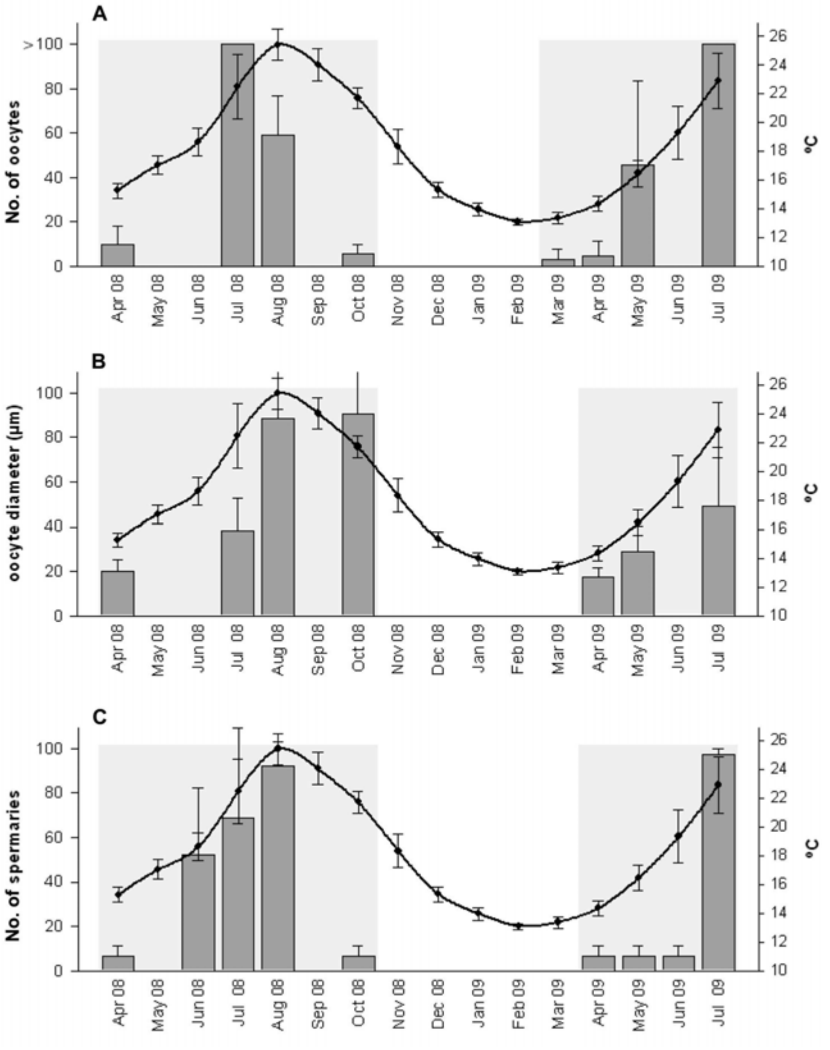

Figure 3. *Cladocora caespitosa***.** (A) Number of oocytes per polyp, (B) Oocyte diameter, (C) Number of spermaries per polyp and seawater temperature in the Columbretes Islands Marine Reserve. Oocyte and spermaries data are shown in bars (monthly average ± SD) and seawater temperature is shown as points connected by a smoothed line (monthly average ± SD). Grey background: reproductive season.

Oocyte development (in terms of size) were strongly correlated with seawater temperature ($r = 0.80$, $p < 0.05$, Fig. 3). The drastic decrease in number of gametes in October 2008 indicated that spawning occurred at the end of the summer in the Columbretes Islands, a pattern that differs from the spawning period described for the Adriatic Sea (early summer) (Schiller 1993; Kružić et al 2008a). Several studies have demonstrated that reproductive traits, including the spawning period, vary with latitude and geographic location (Rinkevich and Loya 1979; Kojis 1986; Richmond and Hunter 1990; Fan and Dai 1995; Baird et al. 2009b). Seasonal changes in seawater temperature are frequently cited as an important environmental factor controlling gametogenetic cycles or planulae release periods in scleractinian corals (see Richmond and Hunter 1990 and Harrison 2011 for reviews). Accordingly, we could hypothesize that differences in seawater temperature between the Western Mediterranean and the Adriatic Sea could result in a shift in the reproductive cycle of *C. caespitosa*. However, populations in both regions were located at similar latitudes and subjected to similar seasonal seawater temperature regimes (Kružić and Benković 2008 and present study). However, gamete spawning appeared to be related to contrasting periods of the seasonal SST regimes: increasing temperatures in the Adriatic versus decreasing temperatures in the Western Mediterranean. Thus, a temperature shift cannot explain the differences reported in gamete spawning, either in time (a > 2-mo lag occurs between spawning in the Adriatic and the Western Mediterranean Seas) or in the seasonal SST regime (decreasing vs. increasing SST).

Alternatively, the differences in the reproductive traits (i.e., oocyte size) observed for *Cladocora caespitosa* from the Western Mediterranean and the Adriatic could be due to genetic divergences between these geographic regions. Further analyses using nuclear DNA markers are needed to investigate whether these regional populations correspond to different lineages. In fact, the taxonomy and systematics of the entire order Scleractinia are being reviewed using several genetic markers and results to date have revealed several discrepancies between morphological observations and phylogenetic analyses (Pinzón and Lajeunesse 2011; Budd et al. 2012).

In contrast to the reproductive differences found between *C. caespitosa* populations in both Mediterranean regions investigated to date, our results revealed many reproductive similarities with the coral *Oculina patagonica* (cited as a Mediterranean alien species, Zibrowius 1974). *O. patagonica* has been described as gonochoric in both the Eastern and Western Mediterranean. In both regions, it reached its maximum gonadal development in August (oocyte mean diameter 100 µm), coinciding with the highest water temperatures (Fine et al. 2001). As our results suggested for *C. caespitosa*, spawning in *O. patagonica* was observed in September, when the temperature began to decrease (Fine et al. 2001). Consequently, our results indicated that the driving factor for gonad development in *C. caespitosa* is directly related to increasing seawater temperatures in summer, although other factors, such as changes in photoperiod, were not investigated and cannot be excluded. As observed by Glynn et al. (2012), coral sexual traits in several taxa demonstrate strong phylogenetic relationships. The similarities reported here between the reproductive cycles of *C. caespitosa* and *O. patagonica* support recent molecular phylogenies grouping both species within the same family (Oculinidae, Fukami et al. 2008).

Although *C. caespitosa* reefs were abundant in the past history of the Mediterranean Sea (Aguirre and Jiménez 1998), bioconstructions of this coral are currently very rare and should be considered endangered (Kružić and Benković 2008; Kersting and Linares 2012). A throughout knowledge of the sexual reproduction of *C. caespitosa* will allow the design of efficient protection and conservation plans for this emblematic species in the Mediterranean Sea. Further research on this topic is needed to better understand the unusual plasticity of this temperate coral and the effects on its ecology.

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Recruitment and mortality of the temperate coral *Cladocora caespitosa*: implications for the recovery of endangered populations

Photo: Juvenile colony of *Cladocora caespitosa*

CHAPTER III

Recruitment and mortality of the temperate coral *Cladocora caespitosa*: implications for the recovery of endangered populations

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Abstract

Long-lived species are characterized by low recruitment and mortality. In these species, longevity buffers low recruitment, but when catastrophic disturbances alter mortality, recruitment becomes fundamental for population recovery. In this study, we assessed basic biological traits, recruitment, post-settlement growth, and the mortality of juvenile corals and related these factors with the adult mortality of one of the most important populations of the Mediterranean reef-builder coral *Cladocora caespitosa* over a period of 6 years. Adult mortality and recruitment rates were low (~ 1 % and 0.30 recruits m^{-2} yr^{-1}, respectively), whereas the juvenile colony mortality was comparatively high (29 % in the smallest size-class, < 5 polyps). The low recruitment rates will hardly balance the recurrent climate-related mortalities that have affected this population. Conservation plans and the inclusion of this species in the protection lists are urgently needed given its slow dynamics and the escalating threats that it is subjected to.

Keywords
Recruitment, mortality, *Cladocora caespitosa*, long-lived species, Mediterranean Sea

Resumen

Las especies longevas se caracterizan por presentar tasas de reclutamiento y de mortalidad bajas. Este bajo reclutamiento se ve amortiguado por la longevidad de estas especies, pero cuando las tasas de mortalidad se incrementan notablemente, el reclutamiento juega un papel fundamental en la recuperación de las poblaciones. El objetivo de este trabajo ha sido estudiar durante 6 años el reclutamiento, crecimiento y mortalidad de corales juveniles y su relación con la mortalidad de adultos de una de las poblaciones más importantes del coral bioconstructor mediterráneo *Cladocora caespitosa*. Las tasas de mortalidad de adultos y reclutamiento registraron valores bajos (~1 % y 0,30 reclutas m^{-2} $año^{-1}$, respectivamente), mientras que los valores de mortalidad de las colonias juveniles fueron comparativamente altos (29 % en la clase de talla más pequeña, < 5 pólipos). Aunque las tasas de reclutamiento y mortalidad natural parecen estar compensadas, las bajas tasas de reclutamiento registradas podrán difícilmente compensar las altas tasas de mortalidad relacionadas con el aumento de la temperatura del agua que han afectado de manera recurrente a este coral. Estos resultados ponen de manifiesto la lenta dinámica de la especie y la necesidad de crear planes de conservación que puedan atenuar el impacto de las amenazas a las que está sujeta.

Palabras clave:
Reclutamiento, Mortalidad, *Cladocora caespitosa*, Especie longeva, Mar Mediterráneo

INTRODUCTION

Marine coastal habitats are being affected by multiple stressors such as overexploitation, habitat loss, invasive species, and climate change (Chapin et al. 2000; Halpern et al. 2008; Polunin 2008). Given these cumulative disturbances corals are declining worldwide at unprecedented rates (Hughes et al. 2003; Hoegh-Guldberg et al. 2007). Knowledge on population dynamics, especially demographic processes, is crucial to assess the viability of coral populations (Done 1992; Bellwood et al. 2004; Hughes et al. 2010). Recruitment and survival are essential for population recovery after disturbances (Connell et al. 1997; Hughes et al. 2000; Adjeroud et al. 2007; Coles and Brown 2007), and these traits become even more critical in long-lived, slow-growing species (Hughes and Tanner 2000). Long-lived species are characterized by low recruitment rates. Whereas in natural conditions, these low rates are buffered by their longevity and low adult mortality (Edmunds 2000; Hughes and Tanner 2000; Adjeroud et al. 2007; Coles and Brown 2007; Linares et al. 2007), under catastrophic impacts, these species are highly vulnerable due to their slow dynamics (Hughes and Tanner 2000; Linares et al. 2007).

The endemic scleractinian *Cladocora caespitosa*, the unique zooxanthellate reef-builder coral from the Mediterranean Sea, is a long-lived species whose bioconstructions have become very rare in the Mediterranean (Kružić and Benković 2008; Kersting and Linares 2012). One of the major threats to this coral at the present time is recurrent mass mortalities, which have seriously affected *C. caespitosa* in many Mediterranean sites (Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009; Kersting et al. 2013a). Whereas several population dynamics studies have been conducted on tropical corals (e.g., Edmunds 2000; Hughes and Tanner 2000; Smith et al. 2005; Guzner et al. 2007), temperate gorgonians (Linares et al. 2007, 2008), and solitary corals during the last decade (Goffredo et al. 2004), to our knowledge, no study has assessed the recruitment and natural mortality of temperate colonial scleractinian corals and specially of *C. caespitosa*.

The main goal of this study was to assess recruitment, post-settlement growth, and the mortality of juvenile corals and to relate these factors with the adult mortality of one of the most significant *C. caespitosa* populations in the Mediterranean Sea over a period of 6 years. Studying these traits will improve our knowledge of the life-history traits of this reef-building coral. This information is crucial to evaluate the resilience of the endangered reefs of this species.

MATERIALS AND METHODS

Adult mortality and recruitment rates were assessed from 2006 to 2012 and 2007 to 2012, respectively, in the *Cladocora caespitosa* population of Illa Grossa Bay (Columbretes Islands, NW Mediterranean, Kersting and Linares 2012).

Recruitment was quantified annually using 50 x 50 cm quadrats in permanent plots that were installed in subvertical and horizontal substrata (24 and 12 plots, respectively). In each quadrat, recruitment was considered as the number of new individuals who settled and survived until the time of observation, following the procedure applied in other coral recruitment surveys (e.g., Connell et al. 1997; Salinas-de-León et al. 2013). Observations were made approximately 5 months after *C. caespitosa* reproduces, coinciding with the lowest algal coverage (end of winter-beginning of spring March) thus maximizing the detection of new recruits. The earliest stage of recruitment observed during this study was a single polyp that had already undergone asexual reproduction through budding (Fig. 1).

Once recruits were detected, annual juvenile mortality rates and colony growth through budding (i.e., asexual buds are produced by reorganizing a small area on the exosarc; Rodolfo-Metalpa et al. 2008b) were obtained from the monitoring of the permanent plots. Juvenile colonies were assigned to three size-classes (< 5, 5 - 10, and > 10 polyps). The number of new fully developed buds in each colony was normalized to the total number of polyps in the colony in order to compare budding rates between size-classes (Fig. 2).

Figure 1. *Cladocora caespitosa* recruit (scale bar: 0.5 cm).

Figure 2. Juvenile colony in 2010 (left) and in 2011 (right) (scale bar: 0.5 cm).

In addition to the permanent quadrats, between 25 and 50 random quadrats were surveyed annually in order to obtain more recruitment data covering a larger area within the bay.

Natural mortality was estimated monitoring annually 250 adult colonies, which were individually identified along a permanent transect in the study area. Natural mortality differs from the climate-related mortalities that have been affecting this species over the last decade, because it is mostly due to the breakage of the colonies as a result of the combined action of boring organisms and hydrodynamics (Schiller 1993), whereas climate-related mortality is clearly noticeable in sizable necrosed areas that occur after the summer (Kersting et al. 2013b).

Generalized Linear Mixed Models (GLMM) with a Poisson distribution and a logit link function were used to test differences in recruitment in function of substrata (fixed factor) and time (random). GLMMs are used to model data that can cope with repeated measurements over time (Zuur et al. 2009).

One-way PERMANOVA, using Euclidian distances, was used to test both differences in the annual polyp budding rates and differences in mortality rates between juvenile colony size-classes.

The analyses were computed using lme4 package (Bates et al. 2013) implemented in R (R Development Core Team 2011) and Primer v6 with the PERMANOVA + add-on package (Anderson et al. 2008).

RESULTS AND DISCUSSION

The present study quantitatively revealed that the Mediterranean endemic reef-builder coral *Cladocora caespitosa* displayed both low mortality rates (1.06 ± 0.28 %, ± SE) and low recruitment (0.31 ± 0.09 recruits m^{-2} and 0.33 ± 0.13 recruits m^{-2} (± SE) in permanent plots and random quadrats, respectively). The results obtained corroborated the typical traits of a slow-growing species (Kersting and Linares 2012). Comparisons with recruitment rates reported for other scleractinian corals are difficult due to the different assumptions and methods (Harrison and Wallace 1990). However, whereas Coles and Brown (2007) reported similar annual recruitment rates for the long-lived tropical scleractinian *Porites lobata* (Lough and Barnes 1995), recruitment rates reported for other tropical corals were significantly higher (see Glassom et al. 2004 for comparisons).

Annual recruitment rates (obtained from permanent plots) were significantly different between horizontal and subvertical substrata ($Z = - 4.4$, $p < 0.0001$) and among years ($Z = - 0.4$, $p < 0.0001$). While in horizontal substrata, annual recruitment ranged from 0.33 ± 0.33 recruits m^{-2} to 2.33 ± 1.15 recruits m^{-2} (± SE), in subvertical substrata new recruits were only recorded in 2009 (0.17 ± 0.17 recruits m^{-2}, ± SE) (Fig. 3). The differences between both types of substrata may be explained by egg retention mechanisms described for this species (eggs released in a mucus coating), together with the possible negative buoyancy of the eggs (Kružić et al. 2008).

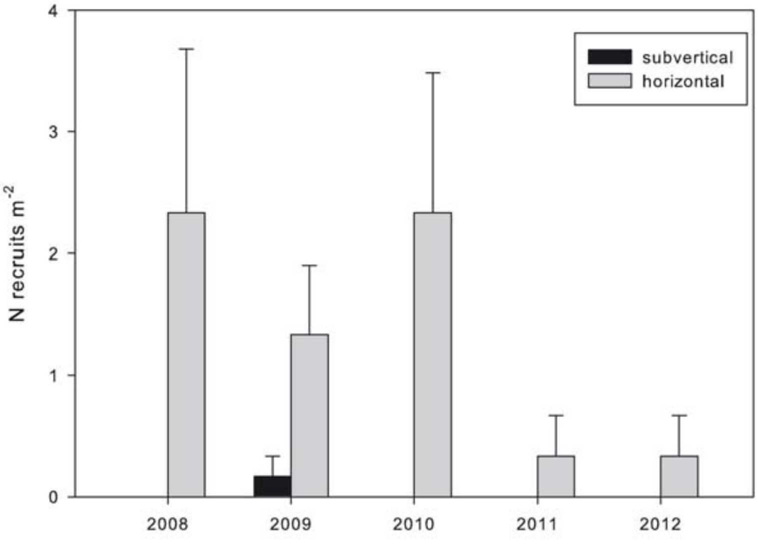

Figure 3. Annual recruitment in permanent plots in subvertical and horizontal substrata (mean ± SE).

Our findings are consistent with the overall paucity of sexual recruits in marine clonal organisms (Hughes and Jackson 1985; Garrabou and Harmelin 2002; Teixidó et al. 2011) and with the lack of conformity between adult abundance and recruitment in most coral reefs (Hughes et al. 1999). In long-lived species, longevity and a low natural mortality buffer the low recruitment rates (the storage effect, Warner and Chesson 1985), and low rates of recruitment may maintain high adult coral abundances, which are the result of the accumulation of recruit cohorts over many years (Edmunds 2000; Hughes and Tanner 2000; Adjeroud et al. 2007; Coles and Brown 2007). In this context, pulses of high recruitment may drastically influence adult abundance (Edmunds 2000). However, problems arise when catastrophic disturbances become more frequent and overcome the sporadic recruitment events (Hughes et al. 1999; Adjeroud et al. 2007). This could be the case with the *C. caespitosa* population in the Columbretes Islands, which has suffered recurrent climate-related mortalities between 2003 and 2012 (Kersting et al. 2013a).

The low recruitment rates recorded in *C. caespitosa* do not seem to be related to an extensive failure of gonad production, as *C. caespitosa* colonies seem to reproduce yearly (Kersting et al. 2013b), but rather to the low post-settlement survival. While, the natural mortality rates in adult *C. caespitosa* colonies were low (~ 1 %), juvenile colony mortality reached significant high values (29 % yr^{-1} for colonies < 5 polyps, Fig. 4) and no significant differences were found in the mortality rates among the three size-classes ($F_{2,\ 9} = 2.21$, $p = 0.269$). Although juvenile corals may show large spatiotemporal variability in mortality rates, which makes comparisons difficult even within the same species (Edmunds 2000), the mortality rates obtained for the smallest size-class of *C. caespitosa* colonies were in agreement with those reported for tropical scleractinian corals (e.g., *Siderastrea radians* [23 % yr^{-1}] or *Porites spp.* [27 % yr^{-1}]; Edmunds 2000). The early life stages in corals are defined by low survivorship, but survivorship increases appreciably with colony size (Vermeij and Sandin 2008). Juvenile *C. caespitosa* colonies grew in polyp number by asexual reproduction through budding. The smaller colonies grew significantly faster through this mechanism (1.14 buds $polyp^{-1}$ yr^{-1}), reaching higher budding rates than larger juvenile colonies (0.33 – 0.43 buds $polyp^{-1}$ yr^{-1}) ($F_{2,\ 34} = 4.44$, $p < 0.05$).

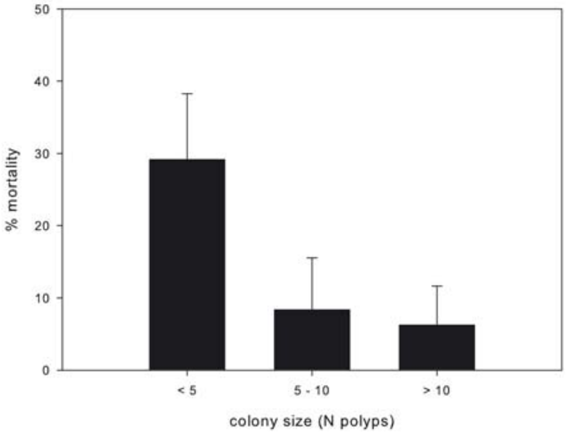

Figure 4. Variation in the annual mortality (mean ± SE) with colony size (N polyps).

Overall, the slow dynamics and reduced recovery potential highlight the vulnerability of the coral *Cladocora caespitosa* to the accelerating impacts affecting Mediterranean coastal waters. This species should be seriously considered for inclusion on the national and international lists of threatened species.

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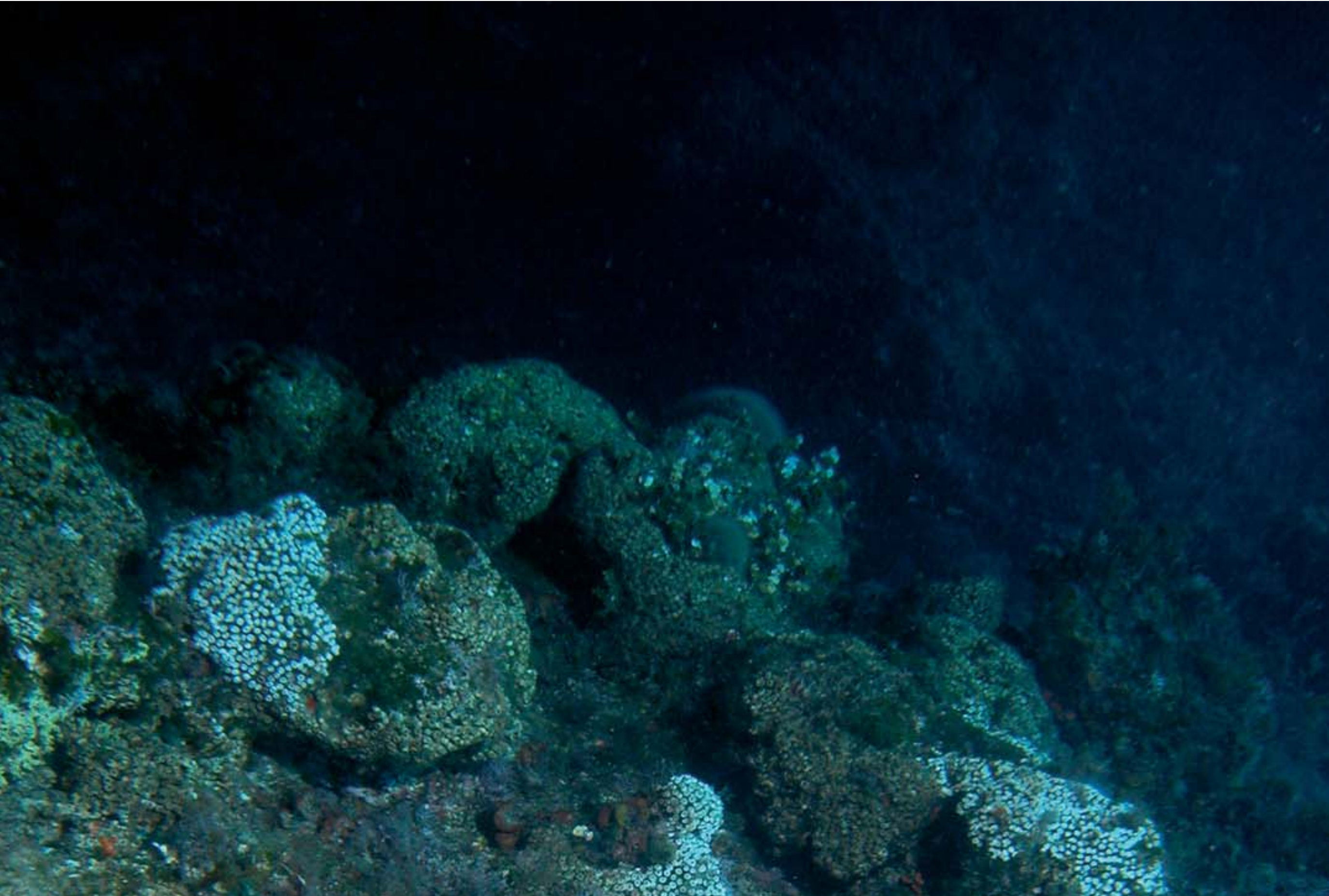

Long-term responses of the endemic reef-builder *Cladocora caespitosa* to Mediterranean warming

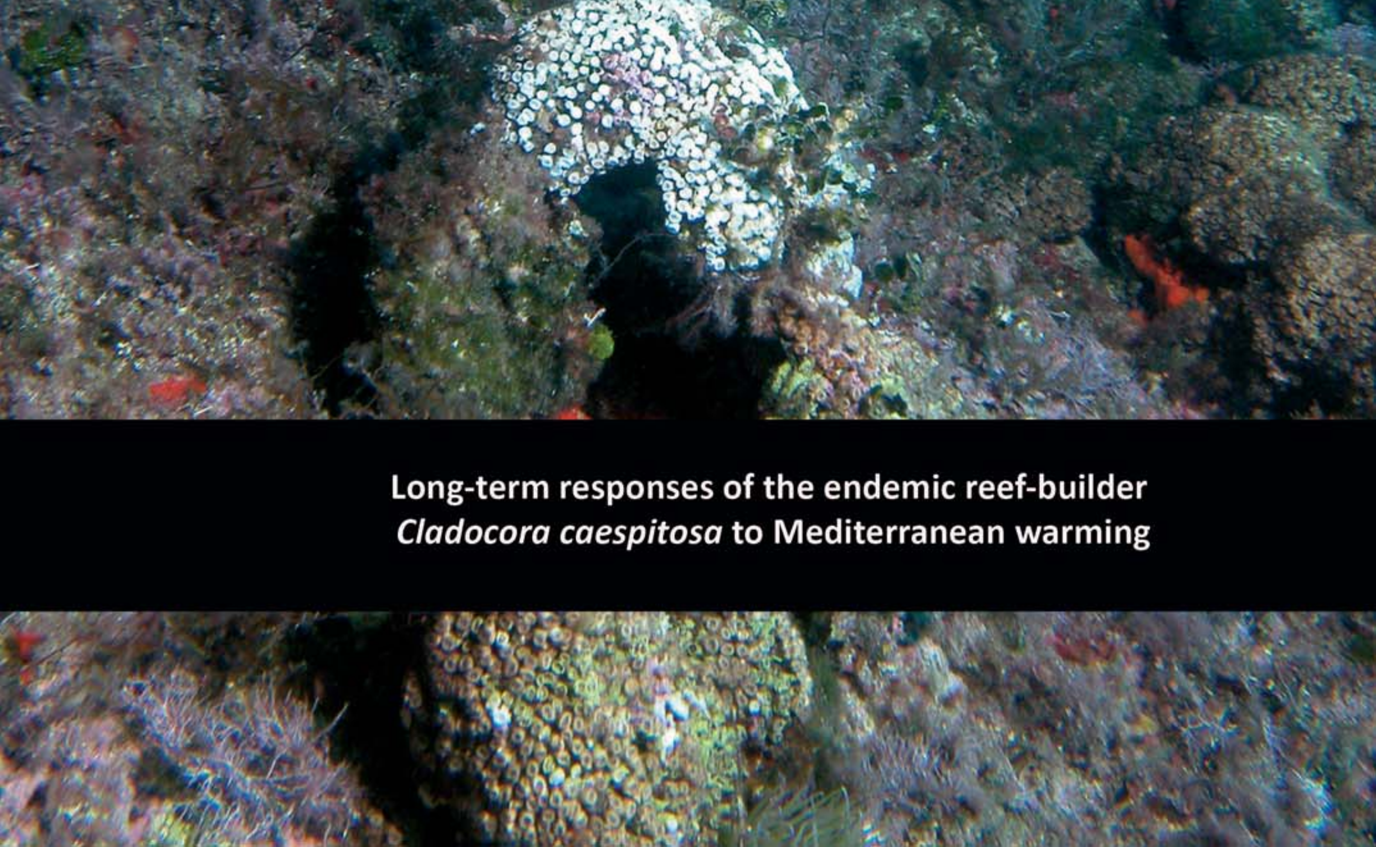

Photo: Necrosis affected *Cladocora caespitosa* colonies (2003)

CHAPTER IV

Long-term responses of the endemic reef-builder *Cladocora caespitosa* to Mediterranean warming

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Abstract

Recurrent climate-induced mass-mortalities have been recorded in the Mediterranean Sea over the past 15 years. *Cladocora caespitosa,* the sole zooxanthellate scleractinian reef-builder in the Mediterranean, is among the organisms affected by these episodes. Extensive bioconstructions of this endemic coral are very rare at the present time and are threatened by several stressors. In this study, we assessed the long-term response of this temperate coral to warming seawater in the Columbretes Islands (NW Mediterranean) and described, for the first time, the relationship between recurrent mortality events and local sea surface temperature (SST) regimes in the Mediterranean Sea. A water temperature series spanning more than 20 years showed a summer warming trend of 0.06 °C per year and an increased frequency of positive thermal anomalies. Mortality resulted from tissue necrosis without massive zooxanthellae loss and during the 11-year study, necrosis was recorded during 9 summers separated into 2 mortality periods (2003 - 2006 and 2008 - 2012). The highest necrosis rates were registered during the first mortality period, after the exceptionally hot summer of 2003. Although necrosis and temperature were significantly associated, the variability in necrosis rates during summers with similar thermal anomalies pointed to other acting factors. In this sense, our results showed that these differences were more closely related to the interannual temperature context and delayed thermal stress after extreme summers, rather than to acclimatisation and adaption processes.

Keywords
Climate-change, Coral, Mediterranean Sea, Mortality, SST

Resumen

Durante los últimos 15 años en el Mar Mediterráneo se han observado recurrentes mortalidades masivas de organismos bentónicos. El único coral escleractinio con zooxantelas y con capacidad bionconstructora en este mar, *Cladocora caespitosa*, se encuentra entre los organismos afectados por estos episodios. En el presente estudio se evalúa la respuesta a largo plazo de esta especie al calentamiento del agua de mar en las Islas Columbretes (Mediterráneo Noroccidental) y se describe, por primera vez, la relación entre las mortalidades recurrentes y el régimen térmico local en el Mediterráneo. La serie de datos de la temperatura del agua mar obtenida durante más de 20 años muestra una tendencia de calentamiento estival de 0,06 °C por año y un incremento en la frecuencia de las anomalías térmicas positivas. Durante los episodios de mortalidad la muerte de los pólipos se produjo por la necrosis del tejido sin que se observara una pérdida masiva de zooxantelas. Durante los 11 años de seguimiento de esta población se detectó necrosis durante 9 años (siempre a finales de verano), separados en 2 periodos de mortalidad (2003 - 2006 y 2008 - 2012). Las mayores tasas de mortalidad se registraron durante el primer periodo, tras el excepcionalmente caluroso verano de 2003. Aunque la tasa de necrosis y el régimen térmico presentaron una asociación significativa, la variabilidad en las tasas de necrosis durante los veranos con anomalías térmicas similares demuestran la implicación de otros factores en este proceso. En este sentido, los resultados obtenidos muestran que esta respuesta diferencial parece estar más relacionada con variabilidad interanual de las temperaturas y un posible efecto retardado del estrés térmico que con procesos de adaptación o aclimatación.

Palabras clave:
Cambio climático, Coral, Mar Mediterráneo, Mortalidad , Temperatura superficial del agua de mar

INTRODUCTION

Since the late 20th century, global warming has been enhanced by human activities (Oreskes 2005). In this ongoing climatic change, climatic models predict that the Mediterranean Sea will be among the regions that are most affected by the warming trend and the increase of extreme events (Déqué 2007; Diffenbaugh 2007). In fact, warming trends in the last decades are well documented for the Mediterranean Sea, in both deep and coastal waters (Bethoux et al. 1990; Romano and Lugrezi 2007; Vargas-Yanez et al. 2008; Coma et al. 2009).

In the Mediterranean Sea, the frequency of abnormally warm summers has increased, resulting in unprecedented mass-mortality events. Although some early mortalities were detected in the 1970s and 1980s (e.g., Harmelin and Marinopoulos 1994; Vacelet 1994), the first multispecies mass-mortality event was described in the NW Mediterranean in the summer of 1999 (Cerrano et al. 2000; Perez et al. 2000; Romano et al. 2000). In the summer of 2003, a new mass-mortality episode occurred in NW Mediterranean coastal waters, this time over a larger geographic area (Garrabou et al. 2009). Both events affected over 30 species of benthic invertebrates, mostly cnidarians, sponges and bryozoans (Garrabou et al. 2009; Lejeusne et al. 2010).

While the relationship of these mortalities to water temperature was unequivocal (Romano et al. 2000; Garrabou et al. 2009), different factors, such as energetic constraints due to prolonged summer stratification of the water column (Coma et al. 2009) and pathogens (Bally and Garrabou 2007), have been linked to the direct cause of death of the organisms. To date, several studies have examined the direct relationship between seawater temperature and the mortality patterns of affected species; however, these studies are basically field studies encompassing one or a few years of observations (Garrabou et al. 2009; Cebrian et al. 2011; Crisci et al. 2011) or laboratory experiments (Coma et al. 2009; Ferrier-Pagès et al. 2009). Hence, there is an important lack of long-term studies assessing the long-term responses of temperate species to ongoing warming.

Cladocora caespitosa, the sole zooxanthellate scleractinian reef-builder in the Mediterranean, is among the organisms affected by these mortalities (Perez et al. 2000; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009; Kersting and Linares 2009). Although it can be considered a conspicuous species, extensive bioconstructions of this endemic coral (i.e., banks, Peirano et al. 1998) are very rare at the present time and are threatened by global change-related disturbances, such as the above-mentioned mortalities as well as the presence of invasive species (Kružić and Benković 2008; Kersting and Linares 2012). Although an important effort has been made to study the thermal tolerance of this species in aquaria (Rodolfo-Metalpa et al. 2005, 2006a, 2006b), no study has assessed the long-term effects of warming-induced mortalities on natural *C. caespitosa* populations, especially on the endangered micro-reefs of this coral.

Here, we provide, for the first time, an analysis of the relationship between seawater warming and mortality in a *C. caespitosa* population over an 11-year period. We do so using data on the local water temperature regime for the period from 1991 to 2012 in the Columbretes Islands; this data set can also provide additional information on Mediterranean warming trends. The objectives of the present work are to study the existence of correlative evidence between the occurrence and intensity of the necrosis events and the local sea surface temperature (SST) regime and to compare the response of *C. caespitosa* throughout the recurrent mortality events in the Columbretes Islands to obtain information on the long-term effects of thermal anomalies on Mediterranean benthic species.

MATERIALS AND METHODS

Ethics Statement

This study was conducted according to the permitting requirements of the Columbretes Islands Marine Reserve Authority (Secretaría General de Pesca, MAGRAMA). The Secretaría General de Pesca specifically issued the required permission for the *Cladocora caespitosa* study in the Columbretes Islands Marine Reserve.

Study site

The Columbretes Islands emerge 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean). A marine reserve encircles the archipelago covering an area of 5,500 ha. Illa Grossa (39°53.825’N, 0°41.214’E), the largest of the islets in the Columbretes (14 ha), is a C-shaped, drowned, Quaternary volcanic caldera (Fig. 1). The studied *Cladocora caespitosa* population occurs in the central area of the bay formed by this islet (150,000 m^2, 5 - 30 m depth range); with the highest coral cover values in the NW and SE parts. The cumulative coral cover in the bay reaches 2,900 m^2 in a mixed bank-bed colony distribution (Kersting and Linares 2012).

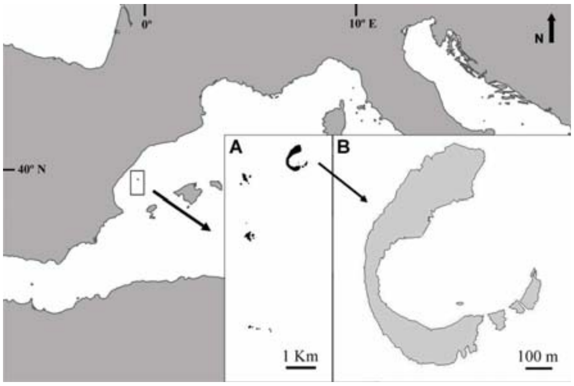

Figure 1. Map of the study site. A. The Columbretes Islands (NW Mediterranean, Spain). B. Illa Grossa Bay.

Cladocora caespitosa mortalities

The impact of tissue necrosis on the *Cladocora caespitosa* colonies was studied each year over the period 2002 - 2012. Mortalities were described and quantified by combining annual random transects and long-term monitoring of individually identified colonies. In the random transects, a total of 110 to 160 colonies were surveyed annually during the autumn (October – November). The long-term annual monitoring of identified colonies began in 2002 with 26 individually marked and mapped colonies; which were increased to 250 in 2006. The surveyed colonies occurred at a depth range of 5 to 20 m, and their maximum diameters ranged from 5 to 150 cm. Schemes and photographs of each colony were used in each survey to depict the areas affected by necrosis.

In each surveyed colony the following data were obtained: depth, percentage of the colony area affected by necrosis (in increments of 10 % and differentiating recent or old necrosis) and the size of the colony through its maximum axis. The percentage of necrosis was always related to the living area of the colony. Necrosed areas below 10 % were not considered to prevent confusion with other sources of natural mortality, such as those eventually induced by depredation by the gastropod *Babelomurex cariniferus* (D K Kersting, pers. obs.).

To detect delayed necrosis in the *C. caespitosa* colonies, additional surveys were undertaken four to five months after the first necrosis was detected.

Kolmogorov-Smirnov two-sample tests were used to determine whether there were significant differences in necrosis for the following comparisons: (i) along the depth gradient (5 - 15 m vs. 15 - 20 m; because vertical temperature profiles showed weak vertical gradients (< 1 °C) in the upper 15 m of depth during the warmest period), (ii) between the two main mortality periods (2003 - 2006 vs. 2008 - 2012) and (iii) during the second mortality period, between colonies that were previously unaffected (< 10 % necrosis) or affected (≥ 10% necrosis) during the first mortality period. This last test explored the existence of any degree of acclimatisation over time.

Kruskal-Wallis analysis was used to test for differences in necrosis depending on colony size (maximum diameter size classes: < 25 cm, 25-50 cm, > 50 cm).

Temperature measurements

The SST data have been recorded daily in the Columbretes Islands Marine Reserve since 1991 at depths of 1 m using a calibrated mercury-in-glass thermometer (Thies Clima, model 2.2141.00.64, Göttingen, Germany). The temperature was measured between 8:00 and 9:00 a.m. by the Marine Reserve wardens following the same protocol (bucket sampling in the first meter of water and direct measurement with the thermometer). Overall, 6,028 daily measurements of SST were collected, which covers 75 % of the 1991-2012 period, with a mean value of 274 data logs per year and a mean temporal cover of 87 % during the summer (June-September). However, with only 27 data logs during the summer, the year 2000 was not considered in the statistical analyses.

Uncertainty in SST from bucket measurement is on the order of a few tenths of a degree C (Kent and Kaplan 2006). Comparisons with hourly records recorded by autonomous data loggers (Water Temp pro v2, ONSET, Cape Cod, MA, USA; accuracy: 0.21 °C, resolution: 0.02 °C) at 1 m depth from June 2011 to October 2012 yielded very good results, indicating that these punctual measurements reflected the near surface thermal environment ($T_{1m} = 0.97\ SST + 0.64$, $r = 0.99$, $p < 0.001$, $N = 446$). Additional temperature profiles (0 - 20 m) were recorded monthly in the Illa Grossa Bay from 2004 to 2007 using an SBE 39 temperature and pressure sensor (Sea-Bird Electronics, Bellevue, WA, USA). Since 2007, the bay was equipped with Stowaway Tidbits (ONSET, Cape Cod, MA, USA; accuracy: 0.2 °C, resolution: 0.14 °C) autonomous sensors set at depths of 5, 10, 15 and 20 m (1 hour data-sampling frequency). These sensors were installed in the same area as the permanent *Cladocora caespitosa* transects.

Data obtained from the temperature profiles and the autonomous sensors were used to obtain information on the vertical gradients during the summer (June - September). Data from the autonomous sensor located at a depth of 15 m (average depth of the *C. caespitosa* population, Kersting et al. 2012) were compared to the SST data for the summers from 2007 to 2012 to validate the use of the latter longer temperature series for the posterior necrosis-temperature correlation analyses ($T_{15m} = 1.13$ SST - 4.74 , $r = 0.76$, $p < 0.001$, $N = 678$). Summer SST anomalies (i.e., the temperature obtained in the studied summer minus the average of the summers from the original data set [1991 - 2012]) were obtained for the studied summers (June - September, 2002 - 2012). Differences in summer SST anomalies among years were analysed using a one-way ANOVA and a Scheffé test for multiple comparison.

The persistence of high water temperatures during the studied summers was recorded as the number of days in which the SST exceeded certain temperature thresholds (from 24 to 28 °C).

Correlation between mortality and water temperature

Three mortality descriptors were selected to study the relationship between mortality events and SST anomalies: 1) The mean percentage of the coral's injured surface (hereafter, "necrosis"); 2) the percentage of colonies that were affected in their entirety by the necrosis (hereafter, "total mortality") and 3) the percentage of colonies that were affected by the necrosis to some extent (hereafter, "affected colonies").

Pearson's product-moment correlation was used to examine the relationship among the three descriptors (necrosis-affected colonies: $r = 0.97$, $p < 0.001$; necrosis-total mortality: $r = 0.81$, $p < 0.005$; affected colonies-total mortality: $r= 0.70$, $p < 0.05$; $N = 11$). Necrosis was chosen as the principal mortality descriptor because its use has been generalised in previous mortality studies (e.g., Perez et al. 2000; Garrabou et al. 2001; Linares et al. 2005; Rodolfo-Metalpa et al. 2005; Coma et al. 2006, 2009).

The SST descriptors used were as follows: 1) summer SST anomalies and 2) persistence of temperature thresholds (i.e., the number of days over temperature thresholds 24, 25, 26, 27 and 28 °C).

Multiple linear correlation analyses were performed to explore the relationship between the temperature and mortality descriptors. These analyses were performed for the whole studied period (2002 - 2012) and for the different mortality periods separately, in order to search for differences between them. The correlation analyses were also performed without the non-mortality years in order to study the role of the necrosis intensity in the correlation with the temperature descriptor.

RESULTS

Cladocora caespitosa mortalities: pattern of necrosis and inter-annual incidence

Old basal necrosis (i.e., accumulated necrosis prior to 2002) of approximately 3 % was registered during the first colony surveys in 2002 and 2003. The first mass-mortality event affecting *Cladocora caespitosa* was detected in September 2003. Recurrent mortalities were then detected at the end of the summers of 2004, 2005 and 2006. No mortality was detected in 2007. Although less virulent, necrosis events occurred again during every summer from 2008 to 2012.

The polyp mortality was always characterised by direct tissue necrosis without massive loss of zooxanthellae (i.e., the polyps never lost the brownish-green colour given by the zooxanthellae). Tissue necrosis began at the basal part of the polyps; in these first stages, the polyps often remained expanded. Necrosis gradually affected polyp structure until all tissue disappeared, leaving the bare skeleton (Fig. 2). When colonies were only partially affected by necrosis, the dead polyps were always adjacent to each other, and the colony necrosis had a patched appearance. The evaluation of the accumulated occurrence of the necrosis patches in each colony showed that necrosis occurred both in the upper part and lower sides of the colony in 89.5 % of all cases. The first signs of mortality were always detected during August and the beginning of September.

No delayed necrosis was ever detected, when the event was over, the necrosed areas of the colonies remained unchanged, and epibionts rapidly covered the damaged parts.

Recovery of these necrosed areas was never detected. However, in the last years of the survey (2010, 2011 and 2012), the recolonisation of dead colony areas was registered; this occurred through the recruitment of new *C. caespitosa* colonies on the old, dead polyps (Fig. 3). This colony-on-colony recruitment was recorded in 16.26 % of the colonies that had experienced partial or complete mortality (average necrosis 80.60 ± 20.3 % (± SD)). Through this process, the recolonised colonies gained between 10 and 30 % of new, living colony area.

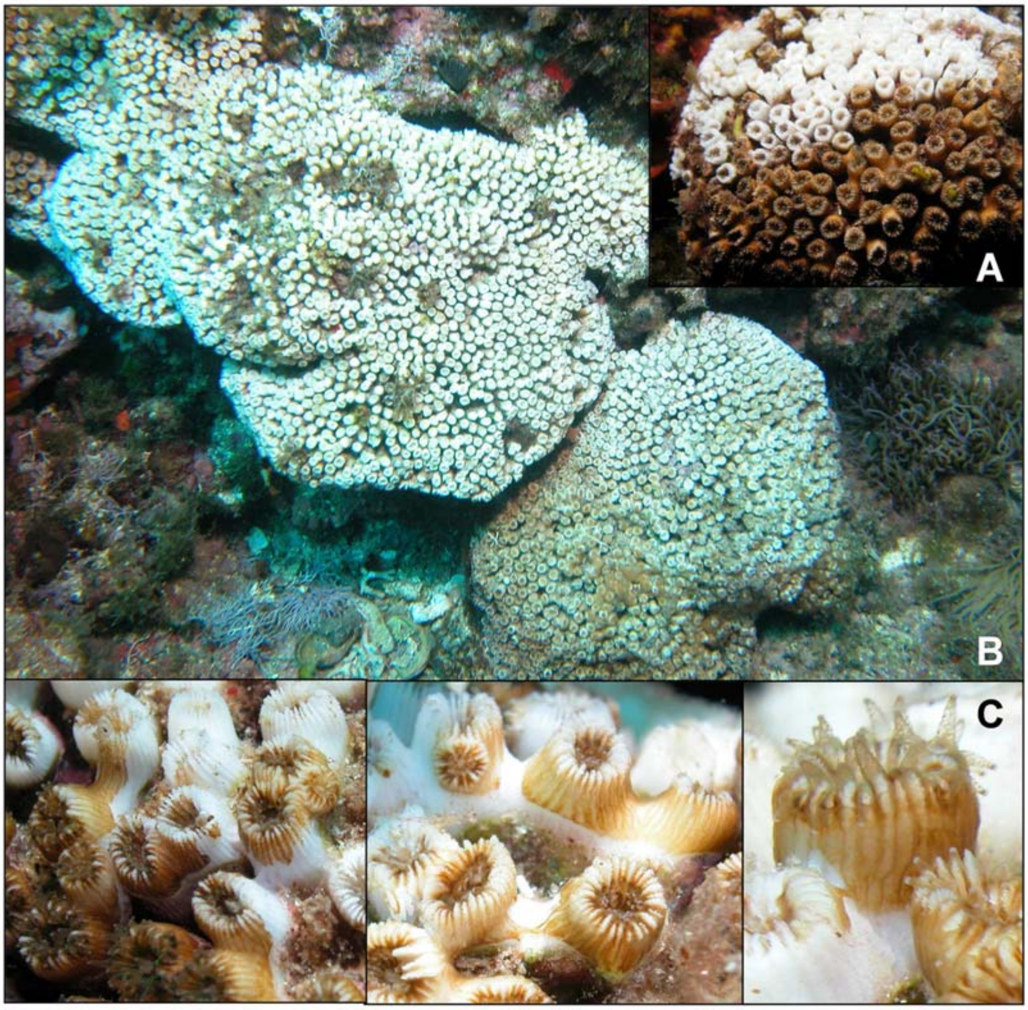

Figure 2. Mortality of *Cladocora caespitosa*. (A) *C. caespitosa* colony showing partial necrosis. (B) Totally affected colony. (C) The necrosis process in the polyps of *C. caespitosa*.

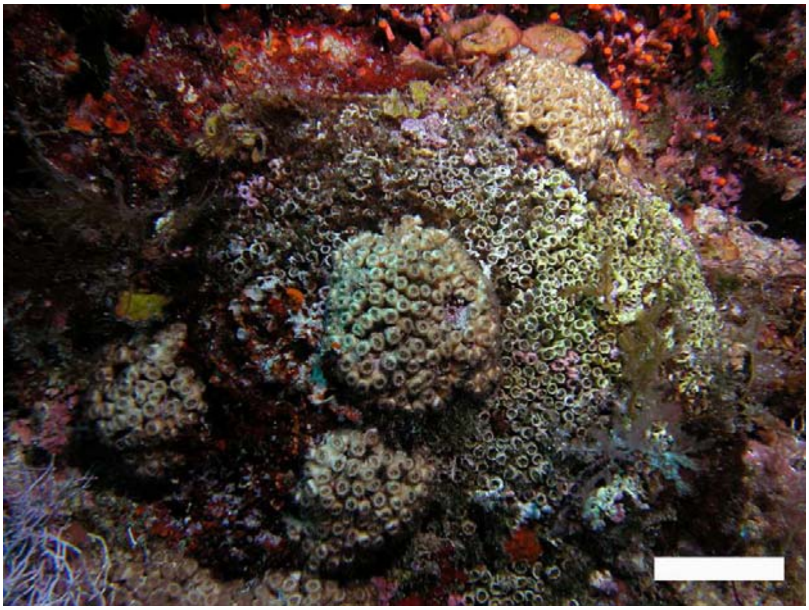

Figure 3. "Colony-on-colony" recruitment in a necrosis-affected *Cladocora caespitosa* colony. Scale bar: 5 cm.

Over the studied period, 80 % of the monitored colonies (N = 250) were affected to some extent (partially or totally) by multiple mortality events. Considering the information from the fixed and random colony transects, the total colony area that was affected by the accumulated, recurrent necrosis was estimated to range between 55 and 80 %.

The highest necrosis values were recorded during the 2003 event, during which 13.39 % of the surveyed colonies died completely and necrosis reached an average of 25 % (24.94 ± 37.82 %). Important mortalities occurred after the following summers (2004-2006), with necrosis values ranging between 12.91 ± 27.46 % and 19.62 ± 29.49 %. The recurrent mortality events that followed from 2008 to 2012 registered much smaller percentages of necrosis (ranging between 1.95 ± 10.78 % and 6.67 ± 18.11 %). See Figure 4 and Table 1. Generally, necrosis rates showed high variability between colonies, and affected and unaffected colonies were commonly found one beside each other.

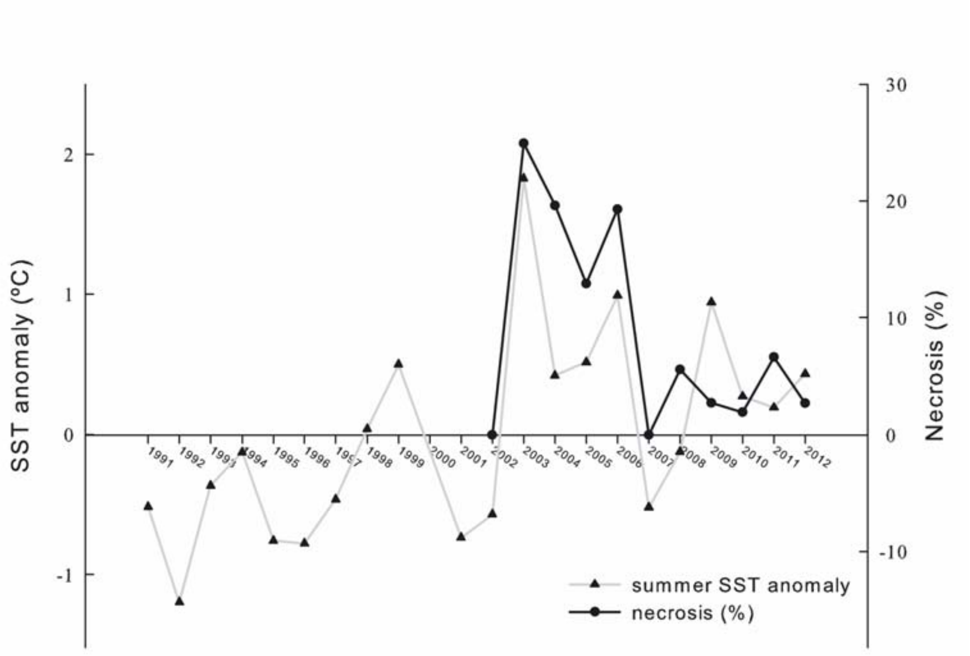

Figure 4. *Cladocora caespitosa* necrosis rates (2002-2012) and summer SST anomalies (1991-2012).

	Necrosis (% ± SD)	**Affected colonies** (%)	**Total mortality** (%)	**SST anomaly** (°C)	**24 °C**	**25 °C**	**26 °C**	**27 °C**	**28 °C**
2002	0	0	0	-0.57	66	22	4	0	0
2003	24.94 ± 37.82	46.43	13.39	1.83	98	82	61	44	25
2004	19.62 ± 29.49	53.64	3.31	0.42	79	51	36	6	0
2005	12.91 ± 27.46	26.36	5.43	0.52	85	64	33	0	0
2006	19.30 ± 31.02	38.46	2.43	0.99	89	72	43	19	13
2007	0	0	0	-0.52	63	27	8	0	0
2008	5.61 ± 18.50	12.34	0.43	-0.12	75	47	25	7	0
2009	2.76 ± 10.26	11.69	0.43	0.94	90	75	61	22	11
2010	1.95 ± 10.78	4.78	0.43	0.27	87	66	43	2	0
2011	6.67 ± 18.11	17.47	0.87	0.19	86	52	27	8	3
2012	2.73 ± 11.93	10.55	0	0.43	81	66	37	12	2

Table 1. Mortality and temperature descriptors. Note that necrosis is given in reference to the remaining living colony area.

Total mortality (100 % of necrosed surface) was mostly due to a single mortality event rather than to accumulated necrosis from multiple, recurrent events. In this sense, 26.7 % of the studied colonies experienced total mortality following a single event (half of these colonies died in 2003), while 6.9 % experienced total mortality as a result of repeated necrosis events.

Significant differences were found in necrosis over the entire study period among the selected depth ranges (Kolmogorov-Smirnov test, $p < 0.001$). In contrast, no significant differences were found between necrosis and colony size (Kruskal-Wallis test, $p = 0.415$).

The average percentage of necrosis was significantly higher during the first period than the second one: 19.07 ± 31.45 % between 2003 and 2006 vs. 3.96 ± 14.52 % between 2008 and 2012 (Fig. 5a, Kolmogorov-Smirnov test, $p < 0.01$). In contrast, similar necrosis rates were recorded during the second period from colonies that were unaffected or affected during the first period (4.59 ± 17.06 % vs. 3.57 ± 12.84 %, respectively) (Fig. 5b, Kolmogorov-Smirnov test, $p > 0.1$).

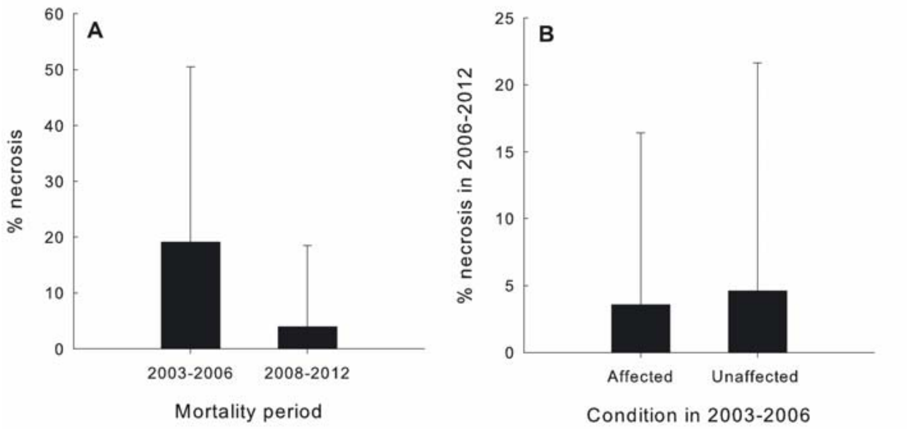

Figure 5. (A) Percentage of necrosis (mean ± SD) detected in each mortality period. (B) Percentage of necrosis (mean ± SD) detected in the second mortality period in colonies that were either affected or unaffected in the first mortality period.

Water temperature regime: annual cycle, warming trend and thermal anomalies

Annual cycles showed a minimum of ca. 12 °C in mid-February and a maximum between 24.9 and 29.6 °C in August (Fig. 6a). The seasonal warming typically had two phases: slow warming rates until mid-April, followed by steeper gradients through the end of June (0.19 vs. 0.87 °C per week). SST cooling was observed from the end of August to the end of year at a rate of 0.66 °C per week.

Over the period studied, SST exhibited a warming trend of 0.04 °C per year (r = 0.30, N = 227). Focusing only on summer SST (June to September), the warming trend was even stronger, reaching 0.06 °C per year (r = 0.55, N = 21) (Fig. 6b).

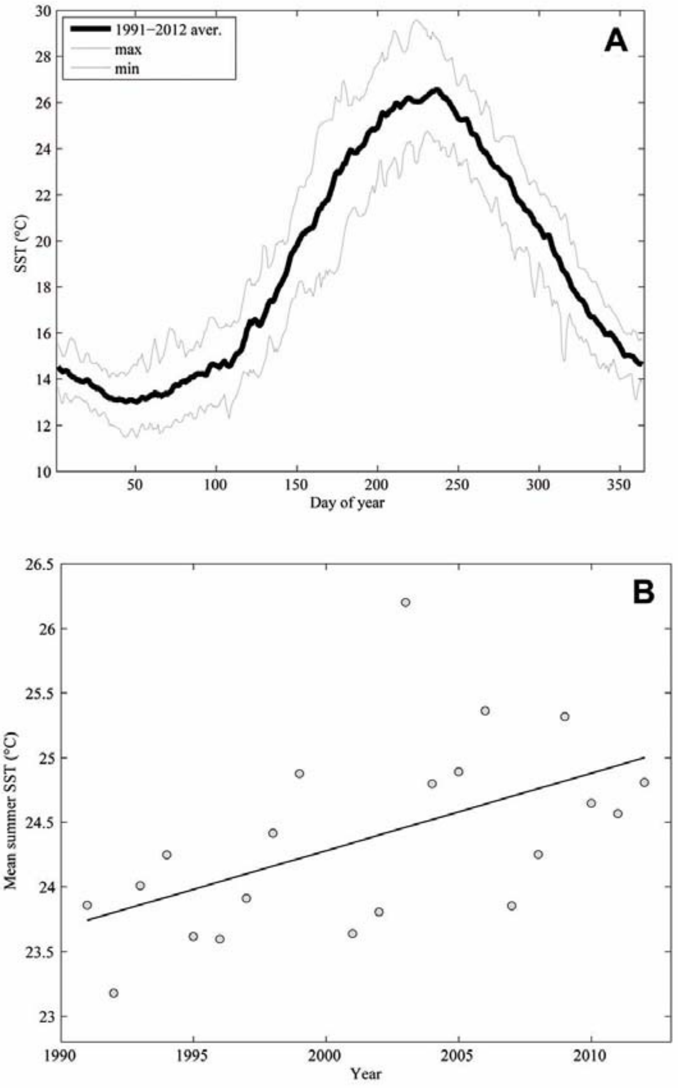

Figure 6. (A) SST mean annual cycle in the Columbretes Islands (1991-2012). (B) Mean summer SST (June-September, 1991-2012).

The frequency of positive thermal anomalies during the summer has increased markedly since 2003 (Fig. 4). In 1991-2002, all averaged summer thermal anomalies were negative, except in 1998 and 1999. Contrarily, in the second decade, positive anomalies were recorded during eight summers, occurring in two periods of four consecutive years and only interrupted by the 2007 and 2008 negative anomalies.

The summer SST anomalies varied significantly over time (one-way ANOVA, $F_{10, 1215} = 14.80$, $p < 0.001$). The maximum significant differences were found when comparing 2003 with all but the warmest summers (i.e., 2006 and 2009). The summers with marked negative thermal anomalies (2002 and 2007) were significantly different from the warmest ones (Table S1, Appendix I).

The summer of 2003 was the warmest of the 20-year-long SST data series, with an average positive anomaly of 1.83 °C. During this summer, SST maxima of over 29 °C were registered in the Illa Grossa Bay, and the average SST for the entire summer (June - September) was 26.20 ± 2.06 °C (Fig. 7). The following summers, i.e., 2004 and 2005, were characterised by moderate positive anomalies (0.42 °C and 0.52 °C, respectively). In the summer of 2006, high temperatures were reached again; temperature maxima were similar to those recorded in 2003, and an average anomaly of 0.99 °C was registered. A second cycle of positive thermal anomalies began in 2009 and lasted until 2012. During these years, the maximum positive anomaly was reached in 2009 (0.94 °C); positive anomalies were moderate in the summers of 2010, 2011 and 2012 (0.27 °C, 0.19 °C and 0.43 °C, respectively). See Figures 4 and 7.

Average vertical temperature profiles attested to weak vertical gradients (< 1 °C) in the upper 15 m of the water column during the warmest period (August) and in the upper 10 m over the entire summer (Fig. 8).

In the years with available data (2004 - 2012), water temperatures remained over 25 °C at depths of 15 m at least during August. The only year without mortality during this time span (2007) had 10 weeks over 24 °C, 3 weeks over 25 °C and 0.3 weeks over 26 °C at 15 m (Fig. 8b). In the years with mortality, water temperatures at depths of 15 m remained over 25 °C for between 5 and 10 weeks (Fig. 8c).

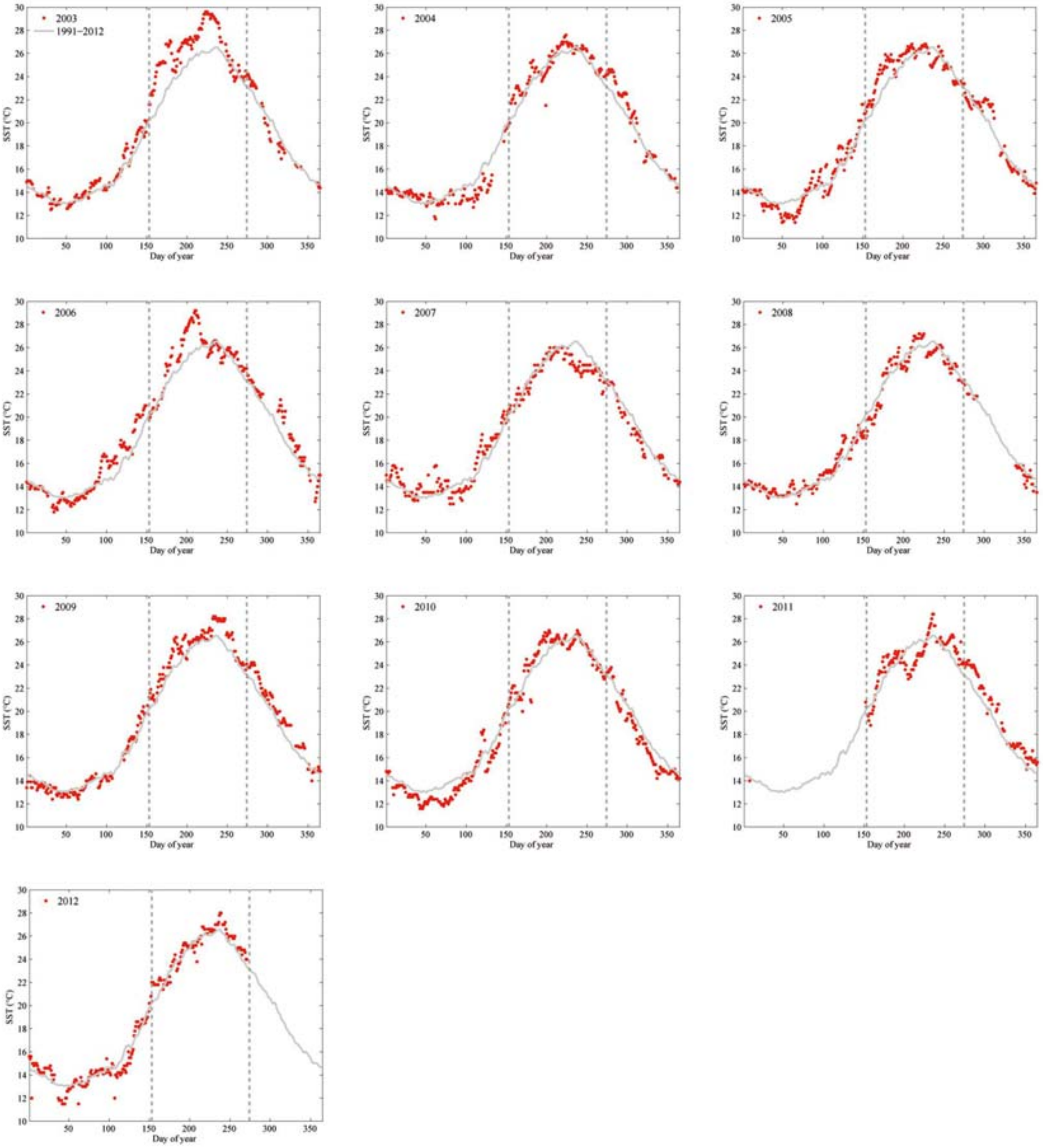

Figure 7. Annual thermal regime (2003-2012) and average SST for the data series 1991-2012. Dotted vertical lines delimit the summer period.

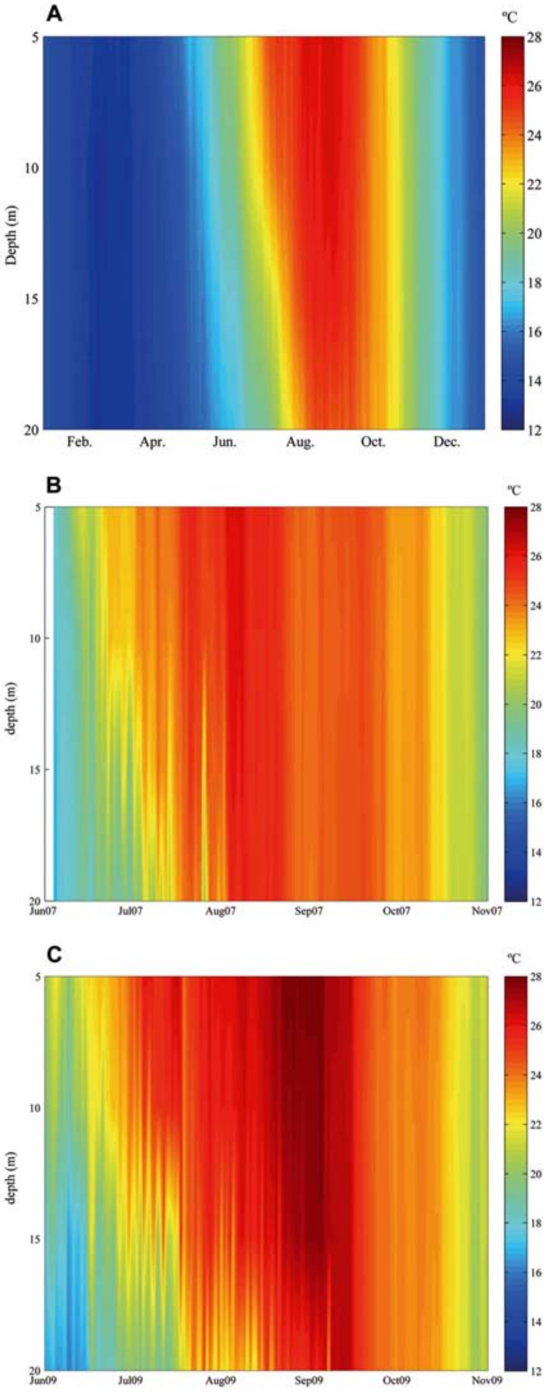

Figure 8. Thermal environment at a depth of 5 to 20 m. (A) The 2007-2012 annual average (B) Data from June to November in a summer with negative SST anomaly, 2007. (C) Data from June to November in a summer with highly positive SST anomaly, 2009.

Correlation between mortality and water temperature

Necrosis and SST anomalies showed a significant positive correlation over the entire studied period (2002 - 2012, $r = 0.75$, $p < 0.01$) (Table S2, Appendix I). Similarly, the other mortality descriptors also showed a positive relationship with SST anomalies (total mortality, $r = 0.75$, $p < 0.01$; affected colonies, $r = 0.70$, $p < 0.05$).

When performing the analyses with the two mortality periods separately, the relationship between mortality descriptors and SST anomalies was highly correlated during the first period (necrosis-SST anomalies, $r = 0.94$, $p < 0.01$) but lost significance during the second period. If the non-mortality years (2002 and 2007) were not considered, the correlation between these variables over the entire studied period lost significance (Table S2, Appendix I).

The correlation between necrosis and persistence of temperature thresholds over the whole studied period was significant only for the warmest limits (necrosis-27 °C, $r = 0.61$, $p < 0.05$; necrosis-28 °C, $r = 0.63$, $p < 0.05$), while during the first mortality period the correlation was significant for the colder thresholds (necrosis-24 °C, $r = 0.93$, $p < 0.01$; necrosis-25 °C, $r = 0.92$, $p < 0.01$; necrosis-26 °C, $r = 0.97$, $p < 0.01$). No correlation between necrosis and persistence of temperature thresholds was found when analyzing the second period separately (Table S2, Appendix I).

DISCUSSION

Historically, mass coral bleaching has been linked to episodes of thermal stress in tropical corals; this is an increasing concern around the world (see Hoegh-Guldberg 1999 for a review). Nonetheless, monitoring the mortalities in the temperate scleractinian reef-builder *Cladocora caespitosa* in the Columbretes Islands (NW Mediterranean Sea) over an 11-year period allowed describing, for the first time, the relationship between recurrent mortality events and local SST regimes in the Mediterranean Sea.

Patterns of mortality

The observed necrosis process in the Columbretes Islands was very similar to previous descriptions of *Cladocora caespitosa* necrosis in the Ligurian Sea (Rodolfo-Metalpa et al. 2005). In accordance with previous studies based on field and laboratory data, *C. caespitosa* polyps died due to progressive tissue necrosis with no signs of zooxanthellae loss (Rodolfo-Metalpa et al. 2000, 2005, 2006b).

The absence of bleaching is most likely related to the resistance to increases in temperature shown by the *Symbiodinium* (clade temperate-A, Visram et al. 2006) in symbiosis with *C. caespitosa* (Rodolfo-Metalpa et al. 2006a).

Tissue regeneration after mortality episodes was not detected in the Ligurian Sea (Rodolfo-Metalpa et al. 2005) or in the present study. This could be due to the phaceloid morphology of *C. caespitosa* colonies, built up by independent polyps, which makes the regeneration of adjacent damaged tissue by unaffected polyps difficult (Rodolfo-Metalpa et al. 2005). Conversely, the autonomy of the *C. caespitosa* polyps could also be responsible for the lack of delayed necrosis following mortality events as well as the lack of correlation between colony size and necrosis, as has been detected in temperate gorgonians (Garrabou et al. 2001, Linares et al. 2005, Coma et al. 2006). Unexpectedly, although tissue recovery was not observed, another indirect but non-trivial mechanism of colony recovery was detected during the last years of the study. *C. caespitosa* recruits settled on the newly available space on the dead colony parts.

Decreases in necrosis rates with depth have been described for species living at greater depths than *C. caespitosa*, e.g., the gorgonian *Paramuricea clavata* (Linares et al. 2005). Although the depth range of the studied *C. caespitosa* colonies places them above the thermocline depth during most of the summer, the relationship between necrosis and depth was consistent with the fact that the summer conditions begin sooner for shallower colonies because the thermocline typically reaches a depth of 15 m at the beginning of August. Therefore, *C. caespitosa* colonies living at shallower depths were more exposed to thermal stress and showed greater mortality rates. As a result, changes in the depth distribution of this population are expected in the future due to the disappearance of the shallower colonies.

Relationship between mortality and temperature

Mortalities were recorded in the context of regional warming and occurred concomitantly with a shift in the regime of positive thermal anomalies in the Columbretes Islands. In particular, the first mortality was triggered by exceptionally warm conditions accompanied by the persistence for several days of extreme (> 28 °C) temperatures.

However, it is worth mentioning that our results are not in concordance with those found in the laboratory. During different aquaria thermo-tolerance experiments with *Cladoocora caespitosa* polyps (collected in the Ligurian Sea), the first signs of necrosis were detected after 5-7 weeks at 24 °C, and all polyps that were exposed at 26 °C and 28 °C died after the treatments (Rodolfo-Metalpa et al.

2005, 2006b). Based on these experiments, the authors proposed that *C. caespitosa* is living close to its thermal limit during the summer period in the Ligurian Sea and a long-term increase at 24 °C or above could be lethal for it. In the Columbretes Islands, water temperatures at 15 m remained over 24 °C for 10 weeks during the summer of 2007, which recorded negative thermal anomaly. This time span was 3 to 5 weeks longer than that reported in the mentioned experiments and no necrosis was detected. Similarly, in the summer of 2009, the average extent of necrosis was approximately 3 %, and *C. caespitosa* colonies at 15 m were exposed to temperatures greater than 24 °C for 68 days and to temperatures greater than 26 °C for 34 days; this exposure was approximately three times longer than the exposure that caused necrosis in 100 % of the polyps in the aquaria experiment (Rodolfo-Metalpa et al. 2006b). The differences found between the mortalities in aquaria (Ligurian Sea) and *in situ* (Columbretes Islands) could be related to two major points: differences in the thermal acclimatisation of *C. caespitosa* between both sites, taking into account that the colonies are naturally subjected to different thermal regimes, and the fact that aquaria experiments can only partially simulate the natural environmental conditions.

Another striking result is that the response of *C. caespitosa* to summers with positive thermal anomalies changed between the two mortality periods and particularly in relation to temperature thresholds. The correlation between necrosis and the persistence of water temperature thresholds for the entire data series was only significantly positive when assessed using the 27 °C and 28 °C threshold. However, a significant positive correlation between necrosis and temperature thresholds of 24 °C, 25 °C and 26 °C was found when considering only the first mortality period, while no correlation was found for the second period.

During this 11-year study, mortality events occurred in two separated periods, i.e., 2003-2006 and 2008-2012. The average necrosis diverged significantly in these two periods (19 % vs. 4 %, respectively), and important differences in the average thermal anomaly were also found (1.00 °C and 0.39 °C, respectively). However, with the same positive thermal anomaly (approximately 1 °C), different years such as 2006 and 2009 registered contrasting necrosis (19 % vs. 3 %, respectively).

As our results prove, it is unequivocal that seawater temperature is one of the main factors that triggered *C. caespitosa* mortality events. Nevertheless, the differences found in necrosis between years with similar thermal anomalies show that other factors are also acting in this process.

Synergies with other factors

Water quality and ecosystem conservation has been ensured in the Columbretes Islands Marine Reserve since its creation in 1990. Furthermore, the location of the islands far from mainland (60 Km) guarantees low interaction with nearshore waters. Therefore, factors such as water quality or dysfunctions in trophic interactions derived from overfishing, that might be relevant in unprotected areas (Bruce et al. 2012), were excluded in the present study.

Although irradiance, especially photosynthetically active radiation (PAR), has been shown to be directly related to tropical coral bleaching (Brown et al. 1994; Dunne and Brown 2001; Dunne 2008), we disregarded it as a possible factor acting in the *Cladocora caespitosa* mortalities. Depending on the depth and water type, irradiance can be significantly attenuated (Brown et al. 1994; Dunne and Brown 1996). Bearing in mind the depth range of our studied *C. caespitosa* population we can assume an important reduction in irradiance. Furthermore, the zooxanthellae in symbiosis with *C. caespitosa* (*Symbiodinium* Clade A) are considered light-adapted (Rowan et al. 1997; Rodolfo-Metalpa et al. 2008a). Finally, a pattern in the necrosis scars related to the effects of irradiance, as reported in tropical corals (Brown et al. 1994), was not observed in *C. caespitosa*.

Disease outbreaks have affected an increasing range of marine organisms in different geographic regions worldwide (Harvell et al. 1999). In the Mediterranean Sea, thermally dependent pathogens have been considered co-responsible for mass-mortalities and coral bleaching (Bally and Garrabou 2007; Kushmaro et al. 1997; Toren et al. 1998). Although, as far as we know, no studies have dealt with this issue in *C. caespitosa*, the type of necrosis (lysis) suffered by this species could be related to a disease, such as that caused by *Vibrio coralliilityticus*, which synthesises a potent extracellular protease that lyses coral tissue (Santos et al. 2011). Although no analyses were conducted to detect opportunistic pathogens in the *C. caespitosa* mortalities, the possible role of pathogens or even polymicrobial consortiums as recently suggested in other tropical coral species (Garcia et al. 2013), should not be disregarded. Previous studies have demonstrated that the occurrence of *Vibrio* bacteria in the NW Mediterranean Sea is climate linked, greatly increasing under the influence of positive temperature anomalies as the observed ones in Columbretes Islands (Vezzulli et al. 2010).

In tropical corals, greater energy reserves or greater access to resources could compensate for decreased photosynthesis during bleaching events (Anthony et al. 2009; Grottoli et al. 2006). In the Mediterranean Sea, temperature-related mortalities have been associated with physiological stress due to energetic constraints (Coma et al. 2009). According to these data, Crisci et al. (2011)

considered physiological status to be a primary factor explaining differential mortality rates.

Cladocora caespitosa has the ability to upregulate heterotrophy and maintain symbiosis, even under suboptimal conditions (Hoogenboom et al. 2010). These authors detected maximum feeding effort when colonies were kept under high light with an irregular food source (typical Mediterranean summer conditions). Consequently, variation in the availability of food previous to and during warm summers could have an important effect in the energy budget of *C. caespitosa*. Furthermore, the impact of extreme summers (like 2003) on the energy budget of the polyps could be responsible for delayed effects in their physiological status.

Processes such as spawning that cause a reduction in tissue lipid content could also have an important effect on the severity of mortality (Anthony et al. 2009). Histological analyses showed that maximum gonadal development in *C. caespitosa* is reached in August (Kersting et al. 2013b) in coincidence with SST maxima, and spawning occurs at the end of the summer. Consequently, the interaction between sexual reproduction and necrosis could be reciprocal: necrosis could be enhanced due to increased energy investment in gonad development, and spawning could be affected by the mortality of the polyps.

With this in mind, we hypothesise that delayed physiological thermal stress could be the primary factor, acting together with temperature, that would explain the differences in necrosis during summers with similar thermal anomalies but with different interannual contexts. This sensitisation hypothesis has also been mentioned in regards to the mass-mortality of 1999 (Romano et al. 2000).

Searching for acclimatisation and adaption processes

The processes of acclimatisation (phenotypic response) and adaption (genotypic response) have been extensively studied and discussed in relation to thermal anomalies causing bleaching events in tropical corals (Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2002; Hughes et al. 2003; Baker et al. 2004; Berkelmans and van Oppen 2006; Baird et al. 2009a). While some authors extend hope for rapid evolution and adjustment (Baker et al. 2004; Baird et al. 2009a), others question the capacity of corals to adapt to rapid climate change (Hoegh-Guldberg 2002).

Through comparisons of bleaching events in tropical corals, several authors have found that corals were more resistant to temperature stress as the bleaching events repeated (Glynn et al. 2001; Thompson and van Woesik 2009; Guest et al. 2012b) and that the bleaching resistance shown by corals at sites dominated by high-frequency SST variability could be a consequence of rapid directional selection following an extreme event (Thompson and van Woesik 2009).

Although the SST series in the Columbretes Islands showed a dramatic increase in the frequency of positive thermal anomalies, as well as a positive warming trend, the differences in mortality detected between summers with similar thermal anomalies did not seem related directly to directional selection. *Cladocora caespitosa* colonies that survived the first mortality period were affected in the second period, although the thermal anomalies had lower positive values on average; therefore, survival was most likely not solely a result of differential survival of more tolerant genotypes.

In this sense, we found that necrosis in the second mortality period (2008 - 2012) showed no differences between colonies that were unaffected or affected during the first mortality period. Differences between these groups would have been expected if selection was acting on thermal tolerance.

Nevertheless, it is remarkable that approximately 20 % of the surveyed colonies remained unaffected over the entire study period and that a very low percentage experienced total mortality due to accumulated recurrent necrosis. These results may indicate the occurrence of more tolerant colonies or even parts of colonies; however, as discussed above, selection for thermal tolerant genotypes alone cannot explain the detected changes in necrosis. In conclusion, these mortalities do not relate to previous necrosis impacts on the same colonies; the occurrence of necrosis at the colony level seems more closely related, in general terms, to random processes involving the occurrence of pathogens or the energetic status of the polyps, as previously discussed.

The importance of context-dependent effects

The summer of 2003 was likely the warmest summer in Europe since 1500 (Luterbacher et al. 2004) and affected 25 rocky benthic macroinvertebrate species over several thousand kilometres of Mediterranean coastline (Garrabou et el. 2009). The mean SST anomaly registered in the summer of 2003 in the Columbretes Islands (1.83 °C) was 80 % warmer than the second positive SST anomaly recorded in the series (in 2006). During this summer, 25 % of the area covered by *Cladocora caespitosa* in the Columbretes Islands was necrosed.

As discussed above, the extreme conditions of 2003 could have been responsible for a delayed physiological stress in the colonies, influencing the mortalities registered in the following summers (2004 and 2005), which were quite important (approximately 20 % and 13 % of necrosis, respectively); however, the positive SST anomalies during these summers were relative low (0.42 °C and 0.52 °C, respectively).

The second mortality period (2008 - 2012) began after a year with negative SST anomalies and no necrosis (2007). This could have given *C. caespitosa* enough rest to withstand the mortality events of the next summers with much lower necrosis, in addition to the fact that no extreme conditions (such as those observed in 2003) were present during the second period. In this period, summers with similar or even higher SST anomalies than in the first period exhibited mortality events with less than 7 % necrosis. Although the first mortality event of the second period (2008) was registered after a summer with an average negative SST anomaly (- 0.12 °C), several weeks of strong positive anomalies were recorded during the middle of this summer.

However, what could have happened prior to 2002? The mortality of 2003 could be considered the first mass-mortality of *C. caespitosa* in the Columbretes Islands in the last two decades. Although necrosed colonies or sections of colonies were eventually covered by epibionts, they were perfectly noticeable over many years. Thus, a mass-mortality event prior to 2003 should have left a high percentage of detectable bare skeletons in the colonies, but the old necrosis detected was near 3 %. This is consistent with the thermal anomalies recorded in the available SST series during the first decade of record (1991 - 2002), which were much lower and less frequent than during the second decade. The summer of 1999 could have been the one in which some mortality would have been expected because the SST anomaly reached 0.50 °C; furthermore, this summer triggered a multispecies mass-mortality event in the NW Mediterranean (Cerrano et al. 2000; Perez et al. 2000; Garrabou et al 2001). That the summer of 1999 most likely did not cause high necrosis rates reinforces our hypothesis that some type of sensitisation or delayed stress occurred after the summer of 2003 because the summers of 2004 and 2005 had similar SST anomalies to those recorded for 1999 but triggered high necrosis.

Three important findings can be highlighted from the results obtained in this study. First, a significant positive correlation between mortality descriptors and SST anomalies was found over the entire studied period. Second, significant differences between the two mortality periods were found when correlation analyses were performed separately. Third, when removing the years without mortality (2002 and 2007) significance disappeared for the whole studied period. Two main conclusions can be drawn from these results. First, the significant, strong association between mortality descriptors and SST anomalies, when looking at the whole series, is more closely related to the concurrence of necrosis events and SST anomalies than to the specific intensity of these variables; as significance is lost when removing the years with no mortality and necrosis was generally detected in years with a positive SST anomaly, but summers with similar SST anomalies showed different responses in *C. caespitosa* necrosis. Second, the effects of the intensity of the SST anomalies on the necrosis rates seem to have been enhanced during the first period, which would be consistent with the delayed thermal stress hypothesis.

The complexity of the factors influencing these mortalities highlights the need for precise and continuous long-term monitoring of biotic and abiotic factors to move forward in our understanding of these events and their effects on the future viability of the benthic communities threatened by the increase in frequency and persistence of extreme events projected for the 21st century in the Mediterranean (Dequé 2007; Diffenbaugh et al. 2007). Recurrent extraordinary mortality episodes, such as the ones registered between 2003 and 2006, could likely be repeated and will threaten this species, which, due to its slow dynamics, will most likely not be able to cope with elevated mortality rates. Nevertheless, considering the less virulent mortalities registered in the second mortality period, the high coral cover in areas such as the Columbretes Islands (Kersting and Linares 2012), and the potential for colony-on-colony recruitment as an indirect mechanism of recovery, there can still be some hope for *C. caespitosa* banks in the Mediterranean Sea and particularly in the Columbretes Islands.

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Invasive macrophytes in a marine reserve: spread dynamics and interactions with the endemic scleractinian coral *Cladocora caespitosa*

Photo: Measuring invasive algal abundance around *Cladocora caespitosa* colonies

CHAPTER V

Invasive macrophytes in a marine reserve (Columbretes Islands, NW Mediterranean): spread dynamics and interactions with the endemic scleractinian coral *Cladocora caespitosa*

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Abstract

The invasive algae *Lophocladia lallemandii* and *Caulerpa racemosa* are becoming an important threat to benthic assemblages in the Mediterranean Sea. Both species were first detected in Illa Grossa Bay (Columbretes Islands Marine Reserve, NW Mediterranean) in 2006, and their invasion was monitored until 2012. *Lophocladia lallemandii* showed a rapid outburst, spreading around the entire bay in just 2 years and showing the highest abundances in the 5 - 10 m depth interval (82.07 ± 3.53 % (± SE) in 2011). *Caulerpa racemosa* showed a slower but steady spread and remained in deeper areas during the first years; however, drastic changes in the depth distribution, with algae invading toward shallower areas, were noted beginning in 2010 and reached abundances of 57.76 ± 1.07 % (± SE) in the 10 - 20 m interval in 2011. Illa Grossa Bay hosts one of the most important populations of the endemic coral *Cladocora caespitosa*. This study is the first to quantitatively assess interactions between the coral and invasive algae. Although both invasive species *L. lallemandii* and *C. racemosa* had overlapping distributions with *C. caespitosa,* we did not find any lethal or sublethal effects of either invasive algal species. On the other hand, *C. caespitosa* exhibited toxic activity, which could explain the low overgrowth of living colony parts by *C. racemosa*.

Keywords: Invasive species, *Lophocladia lallemandii*, *Caulerpa racemosa*, Coral, *Cladocora caespitosa*, Mediterranean Sea, Bioactivity

Resumen

Las algas invasoras *Lophocladia lallemandii* y *Caulerpa racemosa* son actualmente una importante amenaza para las comunidades bentónicas del Mar Mediterráneo. Ambas especies fueron detectadas en la Bahía de L'Illa Grossa (Reserva Marina de las Islas Columbretes, Mediterráneo Noroccidental) por primera vez en 2006 y su invasión hasta 2012 ha sido estudiada en el presente trabajo. *Lophocladia lallemandii* mostró una rápida expansión inicial, distribuyéndose por la totalidad de la bahía en tan solo 2 años, mostrando las mayores abundancias en el intervalo de profundidad de 5 - 10 m (82,07 ± 3,53 % (± SE) en 2011). En cambio, *C. racemosa* mostró una expansión más lenta pero continúa, permaneciendo en las zonas más profundas durante los primeros años. Sin embargo, en 2010 se inició un cambio en la distribución batimétrica de esta especie, invadiendo fondos más someros, registrándose abundancias de hasta 57.76 ± 1.07 % (± SE) en el intervalo de profundidad de 10 - 20 m en 2011. La Bahía de L'Illa Grossa alberga una de las poblaciones más importantes del coral endémico *Cladocora caespitosa.* Por tanto, el segundo objetivo del trabajo fue evaluar cuantitativamente, por primera vez, la interacción entre este coral y las dos especies de algas invasoras. Aunque ambas especies invasoras, *L. lallemandii* y *C. racemosa*, solaparon completamente su distribución con la de *C. caespitosa*, no se encontraron efectos letales ni subletales de las algas sobre el coral. Sin embargo, se detectó actividad tóxica por parte de *C. caespitosa*, lo que podría explicar el bajo recubrimiento de las partes vivas de sus colonias por *C. racemosa*.

Palabras clave:
Especies invasoras, *Lophocladia lallemandii*, *Caulerpa racemosa*, Coral, *Cladocora caespitosa*, Mar Mediterráneo, Bioactividad

INTRODUCTION

The invasion of coastal ecosystems by non-native species is widely recognized as a major threat to marine biodiversity (Molnar et al. 2008). Alien macrophytes have invaded local marine habitats around the world and they are considered to be especially worrying introduced marine species, as they may alter both ecosystem structure and function (Schaffelke et al. 2006; Williams and Smith 2007).

The Mediterranean Sea is a marine hot-spot for non-indigenous species, hosting almost 1,000 introduced species (Zenetos et al. 2010, 2012), of which 128 are macrophytes (Zenetos et al. 2012). The main vectors for these introductions are marine corridors (e.g., the Suez Canal), shipping and aquaculture (Zenetos et al. 2012). The non-indigenous macrophytes *Lophocladia lallemandii* (Montagne) F. Schmitz and *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman and Boudouresque are among the species that show invasive behavior (Boudouresque and Verlaque 2002a; Cebrian et al. 2011; Tomas et al. 2011), and they have rapidly spread across the Mediterranean Sea (Klein and Verlaque 2008; Zenetos et al. 2012).

The Indo-Pacific alga *Lophocladia lallemandii* was most likely introduced into the Mediterranean Sea via the Suez Canal (Verlaque 1994) and is currently distributed across most of the Mediterranean Sea. *Lophocladia lallemandii* can completely overgrow macroalgal assemblages both in infralittoral shallow and deep waters, as reported in the Balearic Islands (Patzner 1998; Ballesteros 2006; Cebrian and Ballesteros 2007, 2010) and the Tuscan Archipelago (Bedini et al. 2012). The impacts on indigenous species have been reported by few studies and they are mostly related to shading, enhanced sediment trapping, organic and nutrient enrichment and even oxygen reduction (Ballesteros et al. 2007; Cabanellas-Reboredo et al. 2010; Deudero et al. 2010).

Caulerpa racemosa var. *cylindracea* was first detected in the Mediterranean Sea in Lybia in 1990 (Nizamuddin 1991) and successfully spread through 12 countries and all of the major Mediterranean islands in less than two decades (see Klein and Verlaque 2008 for a review). Although the primary introduction of this species remains uncertain, the secondary dispersal is closely related to shipping, as recently established populations occur near harbors and fishing areas (Boudouresque and Verlaque 2002b; Verlaque et al. 2003; Ruitton et al. 2005). *Caulerpa racemosa* appears to be one of the most invasive species ever recorded in the Mediterranean Sea (Verlaque et al. 2003) because it grows from 1 to 70 m depth, on all types of soft and hard substrata, in different assemblages and under different environmental conditions (Verlaque et al. 2003; Klein and Verlaque 2008). This alga has had dramatic effects on native assemblages. The stolons of *C. racemosa* build up an intricate mesh that covers the entire available substratum, while the vertical growth of the thallus forms a multilayer structure that acts as a sediment trap that can negatively affect native algal assemblages (Piazzi et al. 2001, 2005, 2007) as well as some invertebrate species, such as sponges (Baldacconi and Corriero 2009; Žuljević et al. 2011) and gorgonians (Cebrian et al. 2012).

To date, most studies have assessed the direct effects of invasive algae on algal assemblages rather than on fauna. Moreover, most previous studies have been manipulative, showing opposing results for the effects of invasive algae on animal abundance and animal assemblages (Williams and Smith 2007; Wright and Gribben 2008; Baldacconi and Corriero 2009; Thomsen et al. 2009; Žuljević et al. 2011; Cebrian et al. 2012; Linares et al. 2012). Thus, further experimental and observational studies on the impacts of invasive macrophytes on native fauna are needed.

The aim of this study was threefold. First, we wanted to describe the spread of the invasion by the macrophytes *L. lallemandii* and *C. racemosa* in Illa Grossa Bay (Columbretes Islands Marine Reserve, NW Mediterranean Sea) from 2006 to 2012. The Illa Grossa Bay hosts one of the most important *Cladocora caespitosa* populations in the Mediterranean Sea, with a cumulative coral cover (i.e., the sum of the area covered by *C. caespitosa* colonies) of 2,900 m^2 (Kersting and Linares 2012). The scleractinian coral *C. caespitosa* (Linnaeus 1767) is the only Mediterranean endemic zooxanthellate coral with reef-forming capacity (Morri et al. 1994). Currently, living banks of this emblematic species appear to be restricted to a few Mediterranean locations and are threatened by the escalating impacts affecting coastal areas, such as global warming and the spread of invasive species (Kružić and Benković 2008; Kružić et al. 2008; Kersting and Linares 2012; Kersting et al. 2013a). We hypothesized that an increase of invasive algae in the bay could affect the coral *C. caespitosa*. Therefore, the second objective of the study was to assess the impact of both invasive algae on the species. Finally, the third aim of the study was to search for coral allelochemical defense mechanisms that could help to explain the observed patterns of algal overgrowth of coral colonies.

MATERIALS AND METHODS

Study site

The Columbretes Islands emerge 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean). A marine reserve encircles the archipelago covering an area of 5,500 ha. Illa Grossa (39°53.825’N, 0°41.214’E), the largest of the islets in the Columbretes (14 ha), is a C-shaped, drowned, Quaternary volcanic caldera (Fig. 1).

The bay formed by this islet has a total surface area of 150,000 m^2 and average and maximum depths of 15 m and 35 m, respectively. Rocky substrata and, to a lesser extent, biogenic coarse sand bottoms cover the bay. The coral *Cladocora caespitosa* is present throughout the bay; however, approximately 85 % of the cumulative colony area occurs in the center of the bay at the 10 – 20 m depth range (Kersting and Linares 2012). Monthly sea surface temperatures range from 13.16 ± 0.80 °C (February) to 26.19 ± 1.16 °C (August) (± SD), with minimum and maximum records of 12 °C and 29.6 °C, respectively (Kersting and Linares 2012; Kersting et al. 2013a).

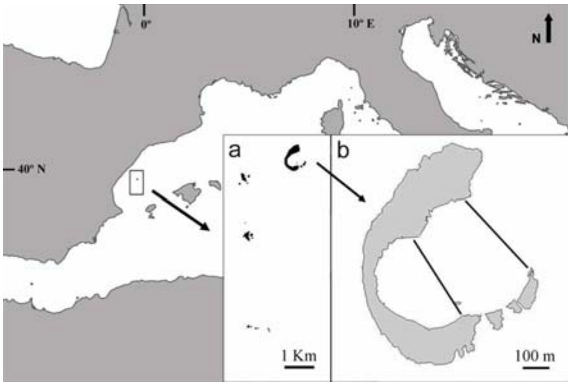

Figure 1. Study site. (a) The Columbretes Islands. (b) Illa Grossa Bay, including the location of transects.

Caulerpa racemosa and *Lophocladia lallemandii* invasion patterns

The evolution of the invasion of *Caulerpa racemosa* and *Lophocladia lallemandii* in Illa Grossa Bay was visually recorded by two divers during annual survey transects performed in August from 2006 to 2012. The two transects were located at the bay entrance and at the central part of the bay (Fig. 1) in order to follow both the progressive invasion of the bay (the invasive algae were first detected near to the entrance), as well as the potential invasion of the area with *Cladocora caespitosa* reefs. Transects covered a 0 to 33 m depth range, and the invasive algae abundance was quantitatively determined by means of 25 x 25 cm quadrats, divided into 25 subquadrats of 5 x 5 cm (Sala and Ballesteros 1997). The percentage of subquadrats in which a species appeared was recorded and used as the unit of abundance. Each transect was subdivided into 13 survey stations covering the depth range of the bay (exterior transect: 0 - 33 m depth and interior transect: 0 - 18 m depth). At each station, each of the two divers measured the invasive algae abundance in 15 randomly placed quadrats and abundances were averaged in four depth intervals (0 - 5 m, 5 - 10 m, 10 - 15 m, > 20 m). Substrata preferences were not tested since habitat in the bay is quite uniform, mainly rocky substrata with interspersed coarse sand pools.

Interactions between the invasive algae and *Cladocora caespitosa*

Additional surveys were performed to evaluate the cover of *Cladocora caespitosa* colonies by *Caulerpa racemosa* and *Lophocladia lallemandii*. For this purpose, 230 *C. caespitosa* colonies were individually tagged following a random positioned transect in the central part of the bay between 5 and 20 m depth, where coral cover reaches the highest values (Kersting and Linares 2012). Distance between colonies ranged from decimeters to meters. Colonies were surveyed annually from 2006 to 2012, and the presence of both algae on or near each colony was recorded. The growth of native algae, such as *Cystoseira sauvageauana*, *C. compressa* or *Codium bursa*, on *C. caespitosa* colonies was also studied. However, the information is not shown since the monitoring of the *C. caespitosa* colonies never revealed an increase of native algal abundance on them, being their cover very low during the study period.

Both algal species displayed contrasting growth patterns on the coral *Cladocora caespitosa*. While *L. lallemandii* grew mostly as an epiphyte on other native algal species that occur in interstices between coral polyps, increasing the whole canopy shading area (Fig. 2a), *C. racemosa* showed a rhizophytic and creeping growth directly over the coral (i.e., in direct contact with it) (Fig. 2b,c). For this reason, we also studied the percentage of coral colony that became directly overgrown by *C. racemosa*. Given that *C. caespitosa* has been suffering recurrent climate-induced necrosis since 2003, which have affected over 50 % of the coral cover in the bay (Kersting et al. 2013a), many colonies showed dead areas; when this was the case, the specific percentage of overgrowth by *C. racemosa* on dead or living colony parts was noted (the percentage always referred to living or dead areas). The percentages of necrosis and of *C. racemosa* overgrowth were visually estimated *in situ* using 10 % intervals following the methodology used in previous studies (Linares et al. 2005; Kersting et al. 2013a). Because our objective was to test for differences in the colonization on both colony surface types, to obtain information on possible defense mechanisms displayed by *C. caespitosa* polyps, the average percentage of *C. racemosa* cover (on dead or living areas) was calculated using only algal affected colonies. For this purpose, living and dead areas were monitored in 64 coral colonies (32 colonies for each type of area), from 2009 to 2012.

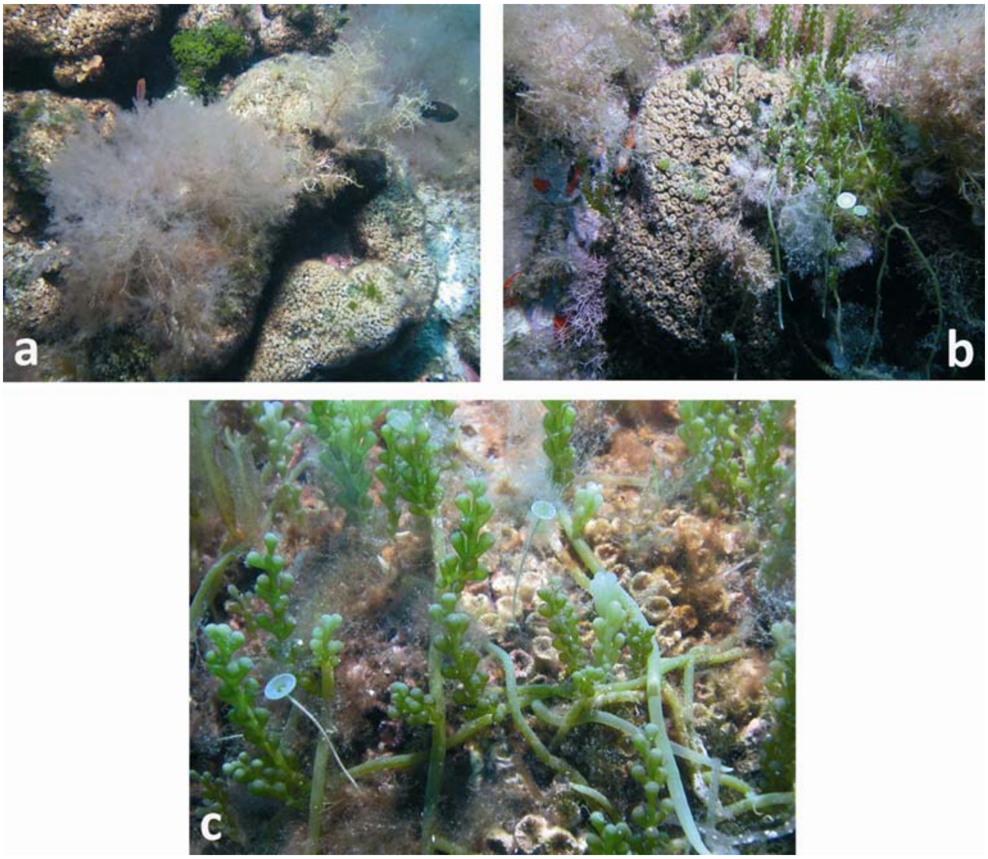

Fig. 2 Growth of *Lophocladia lallemandii* (a) and *Caulerpa racemosa* (b) on *Cladocora caespitosa* colonies. c. Detail of the rhizophytic growth of *C. racemosa* on dead coral polyps.

To detect the direct impacts of *C. racemosa* and *L. lallemandii* and to discriminate this impact from those caused by climate-induced necrosis events of *C. caespitosa*, we monitored necrosis annually in several coral colonies along the set transect, that were alternately unaffected or affected by *L. lallemandii* and *C. racemosa* (N = 20 for each group). This monitoring started when each of the invasive algae spread completely across Illa Grossa Bay, i.e., in 2008 for colonies affected by *L. lallemandii* and in 2010 for colonies affected by *C. racemosa*. Notice that here and hereafter the term affected refers to coral colonies being overgrown at some extent by the algae.

Repeated measures ANOVA was used to test for both, significant differences in the overgrowth of *C. racemosa* (cover) on dead or living areas of coral colonies, and significant differences in the percentage of necrosis of *C. caespitosa* colonies affected or unaffected by *L. lallemandii* and *C. racemosa*. Necrosis and *C. racemosa* cover data was arcsen (squareroot) transformed.

Because stress in corals is readily detectable as a change in the photosynthetic fitness of the zooxanthellae using non-invasive pulse amplitude modulation (PAM) fluorometry (Warner et al. 1996), photosynthetic efficiency ($\Delta F/F'_m$) was measured *in situ* in the field in 2011 using an underwater Pulse Amplitude Modulated fluorometer (Diving-PAM, Walz, Effeltrich, Germany). The 8 mm fiber was placed at a fixed distance from the coral tissue using a black jacket. After 5 - 10 seconds of darkness, the effective quantum yield was measured by exposing some polyps from 240 colonies (70 colonies per treatment, which were either affected by *C. racemosa*, affected by *L. lallemandii* or unaffected) to a 0.8 second period of saturating light, as performed by Rodolfo-Metalpa et al. (2009). After checking the data for normality and homoscedasticity, significant differences between the three treatments were tested using one-way ANOVA.

Standardized Microtox® bioassays (Microbics, Carlsbad, CA, USA) were used to quantify the bioactivity of *C. caespitosa*. In order to detect changes in toxicity as a reaction to *C. racemosa* cover, the test was performed in colonies with or without *C. racemosa* overgrowth. Microtox®-measured toxicity has been reported to accurately correlate with other ecotoxicological tests and with the concentration of the principal secondary metabolites (Botsford 2002; Martí et al. 2003). To assess bioactivity variation among colonies with or without *C. racemosa* overgrowth, 10 polyps from 10 colonies were sampled for each condition. The samples were freeze-dried, and the upper part of the corallite was pulverized in a mortar. Afterwards, approximately 40 mg per colony was extracted with dichloromethanol/methanol (1:1). Once the solvent was evaporated, the crude extract was weighed and resuspended through sonication in artificial seawater to a concentration of 6,000 µg/mL for the toxicity analyses.

The Microtox® bioassay measures the decrease in light emitted by the bioluminescent bacterium *Vibrio fischeri* when the bacteria are placed in contact with a likely toxic substance. In this case, the bacteria were placed in contact with the *C. caespitosa* crude extract for 5 minutes. In every assay, a control and four diluted concentrations with a dilution factor of two were run. A log-log regression between the concentrations of crude extract and output of light was generated. The EC50 value indicated in the regression equation is the concentration of crude extract that produces a 50 % decrease in light, which is assumed to represent the death of 50 % of the bioluminescent bacteria.

Differences in EC50 between colonies with and without *C. racemosa* overgrowth were analyzed using a t-test after checking the data for normality and homoscedasticity.

All analyses were performed using Statistica 8.0**.**

RESULTS

Caulerpa racemosa and *Lophocladia lallemandii* invasion patterns

Both invasive species were first detected in the Columbretes Islands in 2006 at the entrance of Illa Grossa Bay (Fig. 1). *Caulerpa racemosa* was first sighted directly below one of the mooring buoys installed in the bay, while with *Lophocladia lallemandii*, the original affected area was more dispersed.

Lophocladia lallemandii spread fast toward the bay reaching, in just one year (2007), 19.60 ± 4.53 % (mean ± SE) abundance at the 5 - 10 m depth interval. By the next year (2008), the distribution of *L. lallemandii* had extended through most of the bay, covering all depth intervals along the exterior transect and intermediate depths along the interior transect and reaching abundances of up to 74.70 ± 0.50 %. Following this fast, two-year outburst, *L. lallemandii* was observed at central depths (5 - 10 and 10 - 20 m) for the next three years (2009 - 2011) along both transects, with higher abundances at the 5 - 10 m interval. Abundance at this depth interval reached 82.07 ± 3.53 % along the exterior transect in 2011. In 2012, a similar pattern was maintained at the central depth intervals, but *L. lallemandii* also began colonizing the shallowest depth interval (0 - 5 m) along both transects; this depth range had been free of *L. lallemandii* since the explosive outburst of 2008 (Fig. 3).

We observed *L. lallemandii* in the bay during several occasional visits performed in the summer, autumn and early winter, but thalli disappeared completely during the remainder of the winter and spring. The alga exhibited epiphytic growth on the native macroalgae, building up a dense carpet when highly abundant.

Caulerpa racemosa, was first noticed at the entrance of the bay at 30 m depth in 2006 and spread primarily around this point during the next three years (2007 - 2009), with abundances ranging from 24.60 ± 2.65 to 29.37 ± 7.10 % (mean ± SE). Anecdotal evidence also suggested that *C. racemosa* was present at intermediate depths and inside the bay during this period. In 2010, the depth distribution of this alga changed drastically, reaching high abundances (approximately 40 %) at 10 - 20 m depths along both transects. The critical spread of *C. racemosa* throughout the bay and at all depth intervals began in 2011, with the alga reaching the 5 - 10 m interval along the interior transect. During this year, abundance at the deeper areas of the bay (entrance) recovered (49.67 ± 1.60 %), but the greatest abundance was observed along the interior transect at the 10 - 20 m interval (57.76 ± 1.07 %). In 2012, *C. racemosa* reached a homogeneous distribution throughout the bay and at all depths with intermediate abundances (12.40 ± 0.53 % - 30.83 ± 5.90 %) and was noticed, for the first time, at the 0 - 5 m depth interval (Fig. 4).

Unlike *L. lallemandii*, we observed *C. racemosa* throughout the year, i.e., with little seasonality, and forming tight, stolon, net-like structures in the highly developed patches.

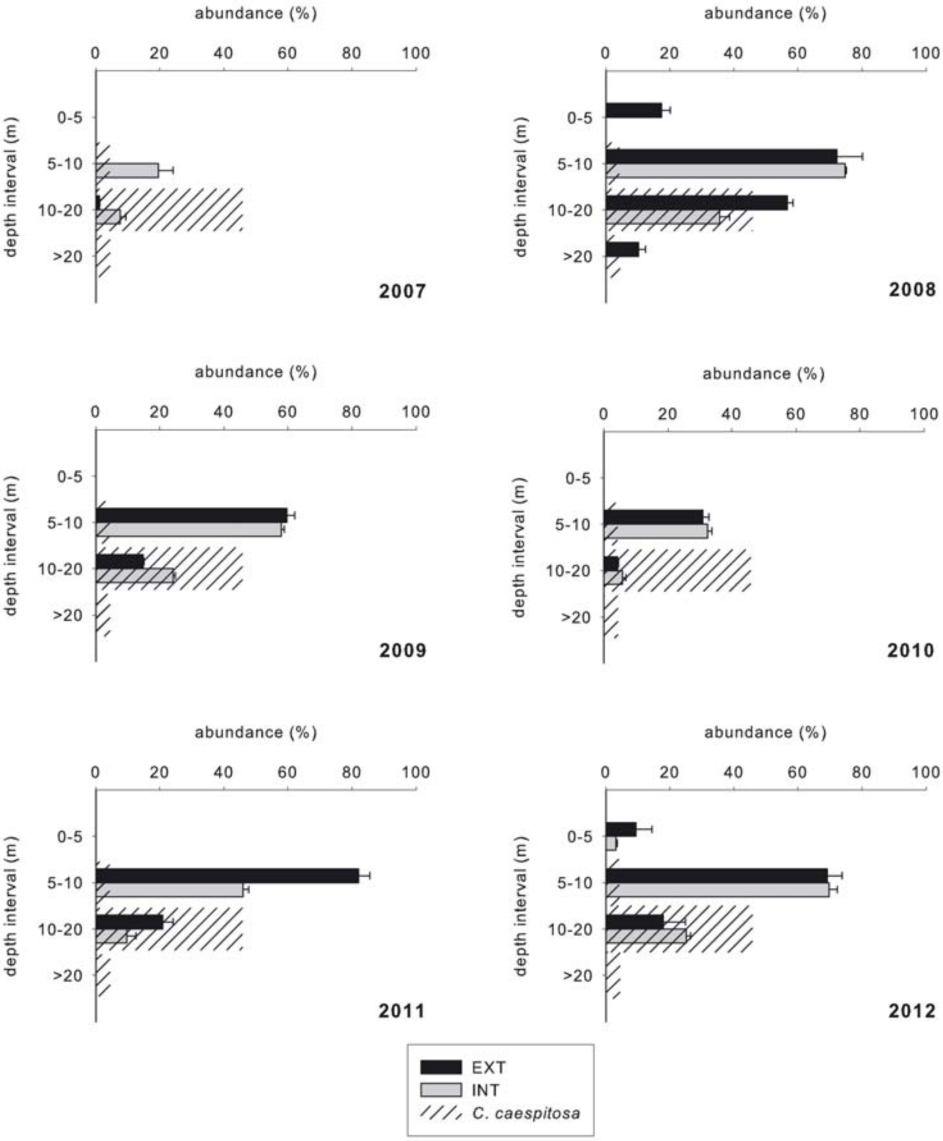

Figure 3. Annual abundances (mean ± SE) of *Lophocladia lallemandii* along both study transects (exterior and interior) at each depth interval. The depth distribution of *Cladocora caespitosa* [cumulative colony area (m^2), modified from Kersting and Linares (2012)] is overlaid on the graphs. Note that the invasion began in 2006 with low abundances.

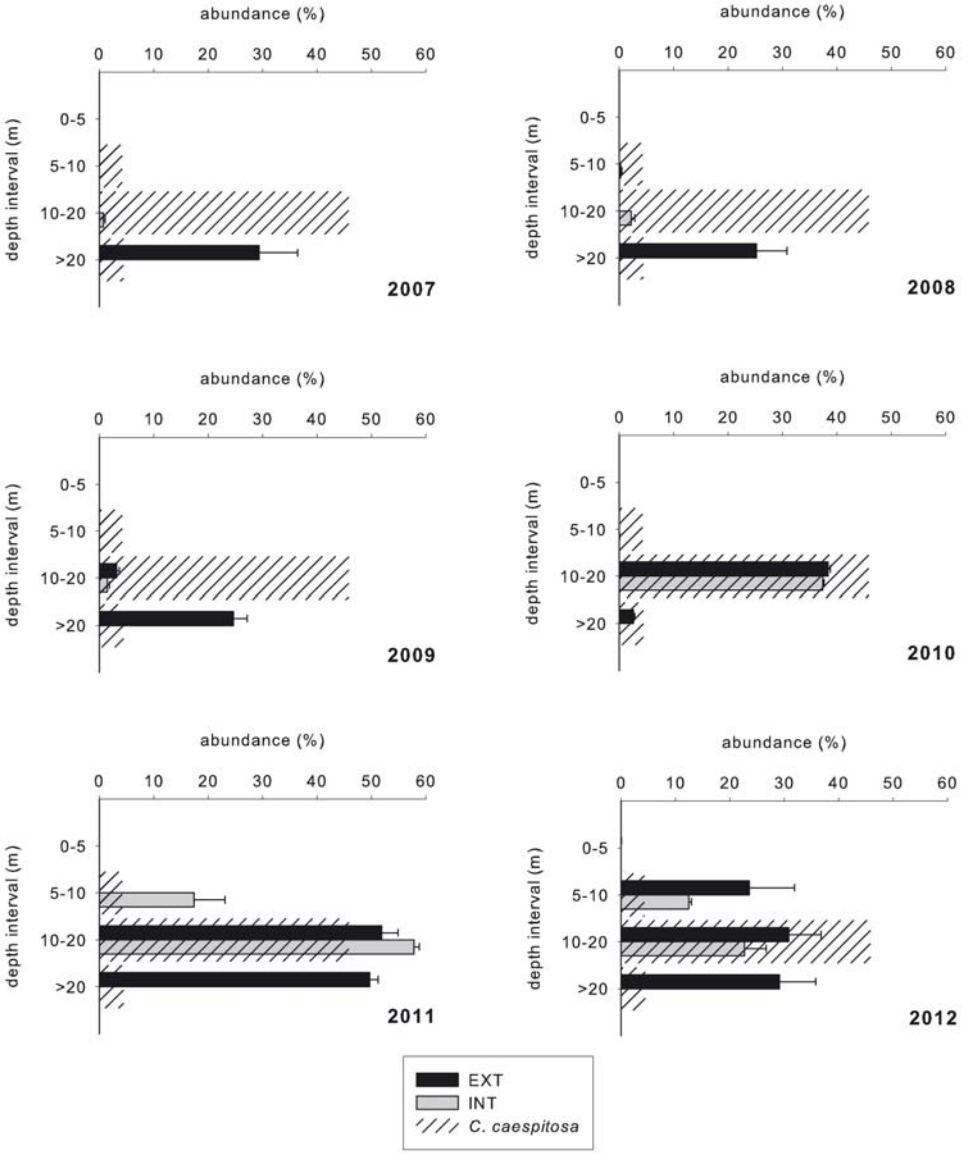

Figure 4. Annual abundances (mean ± SE) of *Caulerpa racemosa* along both study transects (exterior and interior) at each depth interval. The depth distribution of *Cladocora caespitosa* [cumulative colony area (m^2), modified from Kersting and Linares (2012)] is overlaid on the graphs. Note that the invasion began in 2006 with low abundances.

Interactions between invasive algae and *Cladocora caespitosa*

The spatial distribution of *Lophocladia lallemandii* and *Cladocora caespitosa* overlapped at the beginning of the invasion (2007) and in spite of some temporal variability, *L. lallemandii* persisted during summer and autumn with the same distribution. In contrast, *Caulerpa racemosa* occurred only in small and very disperse patches in the area with the highest concentration of *C. caespitosa* colonies between 2007 and 2009 but showed an overlapped distribution from 2010 throughout the remaining study period (Figs. 3 and 4).

Lophocladia lallemandii grew mostly as an epiphyte on the native algae that occur in the interstices of the *C. caespitosa* colonies, including *Cystoseira compressa* and *C. sauvageauana* and any other small algal fragment that may occur on the colonies. As a result, the whole canopy shaded area was increased. Increases in the native algae occurrence on the coral colonies were not detected during the study. In 2008, two years after detection of *Lophocladia lallemandii*, 69.7 % of the *C. caespitosa* colonies were affected by this alga, while 46.7 % to 72.9 % of colonies were affected in the following years (Fig. 5). The overgrowth of *C. caespitosa* colonies by *C. racemosa* showed a much slower but steadier pace; beginning in 2009, with 3.9 % of colonies affected and reaching values close to 30 % in 2011 and 2012 (Fig. 5).

There were no significant differences in the annual necrosis rate of *C. caespitosa* in colonies affected by *L. lallemandii* and *C. racemosa* compared with unaffected colonies (repeated measures ANOVA, $F_{2, 57} = 0.283$, $p = 0.754$) (Fig. 6).

The overgrowth of necrosed colony areas by *C. racemosa* was highly variable between years, with coverage values ranging from approximately 17 to 48 %. However, the colonization of living areas of colonies decreased linearly over time (Fig. 7). Significant differences were found between the overgrowth of *C. racemosa* on dead coral colony areas compared with living areas (repeated measures ANOVA, $F_{1, 62} = 18.294$, $p < 0.001$), among years (repeated measures ANOVA, $F_{3, 186} = 6.813$, $p < 0.001$) and within the interaction term (repeated measures ANOVA, $F_{3, 186} = 7.280$, $p < 0.001$).

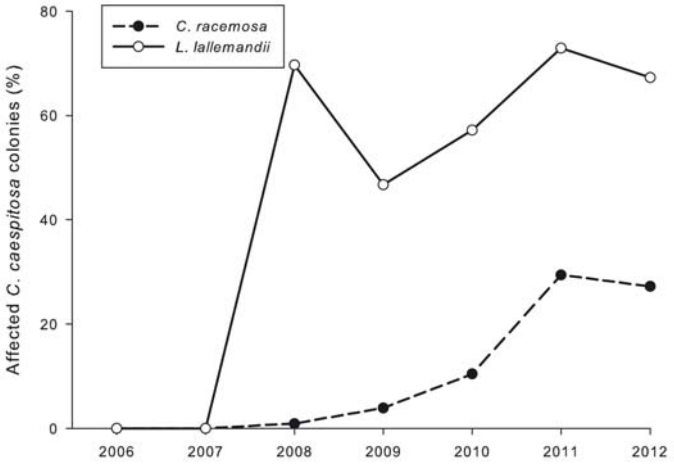

Figure 5. Annual percentage of *Cladocora caespitosa* colonies affected by *Lophocladia lallemandii* and *Caulerpa racemosa*.

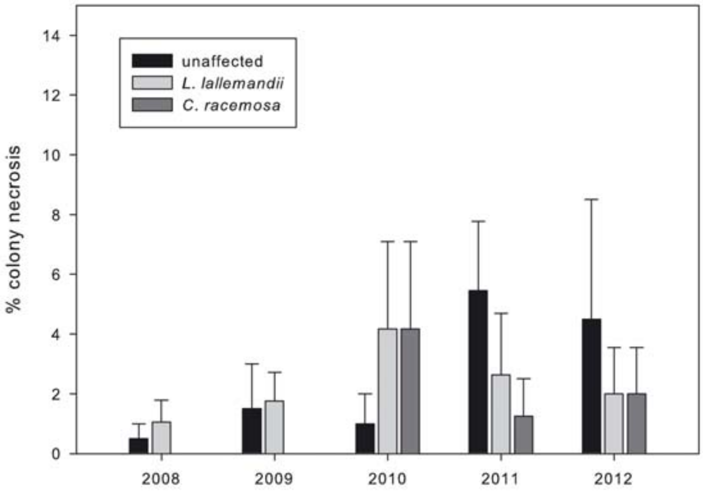

Figure 6. Annual mean necrosis (mean ± SE) in *Cladocora caespitosa* colonies affected and unaffected by *Lophocladia lallemandii* and *Caulerpa racemosa*.

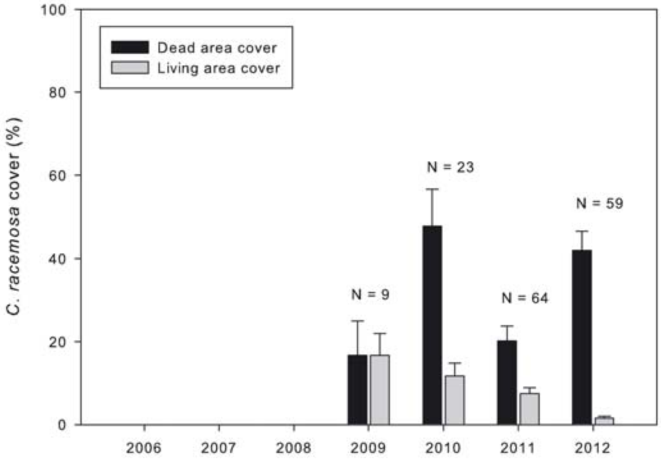

Figure 7. Annual percentage of necrosed and living coral colony areas (mean ± SE) covered by *Caulerpa racemosa* (N = number of *Cladocora caespitosa* colonies with *C. racemosa*).

Photosynthetic efficiency ($\Delta F/F'_m$) was similar for all colonies regardless of affected condition, i.e., unaffected, affected by L. *lallemandii* and affected by *C. racemosa* (one-way ANOVA, $F_{2,207} = 0.482$, $p = 0.618$, Fig. 8).

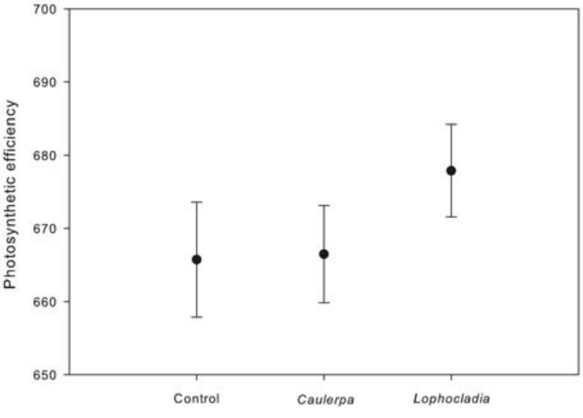

Figure 8. Mean effective photosynthetic efficiency ($\Delta F/F'_m$, mean ± SE) measured *in situ* on *Cladocora caespitosa* colonies affected and unaffected by *Caulerpa racemosa* and *Lophocladia lallemandii*.

The results of the Microtox® bioassay showed that the *C. caespitosa* colonies affected by *C. racemosa* had an EC50 of 391.70 ± 129.98 µg/mL (mean ± SE), while the unaffected colonies had an EC50 of 595.70 ± 138.28 µg/mL (mean ± SE). Although higher mean toxicity was reached by colonies affected by *C. racemosa,* no significant differences were found between the EC50s of these two groups (t-test, p = 0.303, Fig. 9).

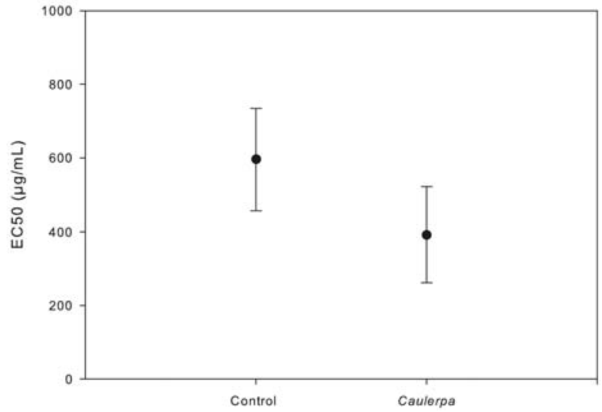

Figure 9. Toxicity (EC50, mean ± SE) of *Cladocora caespitosa* colonies affected or unaffected (control) by *Caulerpa racemosa*.

DISCUSSION

The invasive species *Lophocladia lallemandii* and *Caulerpa racemosa* successfully spread over Illa Grossa Bay from 2006 to 2012, albeit with different pace and variability, and algal distribution overlapped that of the endemic coral *Cladocora caespitosa*.

Spread dynamics

The spread of *Lophocladia lallemandii* throughout most of Illa Grossa Bay was very fast. By 2008, only two years after its detection at the entrance of the bay, *L. lallemandii* was observed at abundances of almost 75 % in both transects at the 5 - 10 m depth range and was present at all of the other depths surveyed. In contrast, *Caulerpa racemosa* showed a much slower expansion rate, which was also linked to changes in the colonization rate at different depths.

Illa Grossa Bay is frequented by tourist, diving and fishing boats, which always use the 10 mooring buoys installed therein. Both species were initially found close to these buoys – in the case of *C. racemosa* clearly below one of them – suggesting that shipping activities were the vector of introduction to the islands, as previously proposed by other authors (see Klein and Verlaque 2008 for a review). Most of the boats that moor in the bay come from the nearby continental coast (Castelló, approximately 60 km to the west) or the Balearic Islands (Eivissa lies approximately 120 km south-east). *Caulerpa racemosa* was reported in Castelló in 1999 (Aranda et al. 1999), and by 2003, more than 20 km along the coast of Castelló was widely affected by *C. racemosa* (unpublished data). *Lophocladia lallemandii* has never been reported from the coast of Castelló, and we suggest that this species may have come from the Balearic Islands where it is very abundant (Weitzmann et al. 2009).

The invasion pattern of *L. lallemandii*, characterized by a fast outburst, has been previously documented from other affected areas, such as in the MPA of "Freus d'Eivissa i Formentera" (Balearic Islands) (Cebrian and Ballesteros 2007). The high colonization capacity of *L. lallemandii* has been attributed to its high reproductive output (Cebrian and Ballesteros 2010). In addition to high spore production and dispersal, vegetative fragmentation may also contribute to the wide distribution of *L. lallemandii*, as it is easily broken and the algal filaments produce disc-like holdfasts that are able to attach to a wide variety of substrata (Ballesteros et al. 2007).

Caulerpa racemosa was restricted to deep waters during the first four years (2006 - 2009) but progressively spread to shallow waters in subsequent years. Strikingly, the abundance of the alga in deep waters was significantly reduced during 2010, when it spread to shallower depths. However, the high abundance in deep waters had recurred by 2011. Although exhibiting a 5-year time lag compared to *L. lallemandii*, *C. racemosa* occurred uniformly throughout the bay and at all depth ranges by the end of the study period. The occurrence of *C. racemosa* in deep waters, at the early stages of the colonization in Columbretes, is consistent with previous reports (Ruitton et al. 2005; Cebrian and Ballesteros 2009). Ruitton et al. (2005) also reported the colonization of shallower waters in the vicinity of densely colonized areas, which, as observed in the Columbretes, could be associated with the development of dense *C. racemosa* meadows in deep waters.

Both algal species displayed a marked interannual variability, both in abundance and depth range, as previously documented by Cebrian and Ballesteros (2009, 2010); the reason for this variability is unknown.

Interactions with *Cladocora caespitosa*

Although the concurrence of *Cladocora caespitosa* and *Caulerpa racemosa* has been previously reported (Kružić et al. 2008b), this is the first study to present a quantitative assessment of any interactions.

High algal cover was considered to be a limiting factor in the development of *C. caespitosa* (Peirano et al. 1998; Rodolfo-Metalpa et al. 1999), and the occurrence of shallow *C. caespitosa* beds has been attributed to factors inhibiting algal growth or persistence, such as water turbidity or the grazing activity of sea urchins (Herndl and Velimirov 1986; Morri et al. 2001). Nevertheless, Illa Grossa Bay contains one of the largest populations of *C. caespitosa* in the Mediterranean Sea, despite the extraordinarily high algal coverage that shapes infralittoral algal assemblages in the bay (Kersting and Linares 2012). As previously mentioned *Lophocladia lallemandii* primarily grows as an epiphyte on the native algae but never recruits directly over coral colonies. Therefore, the presence of this alga on the *C. caespitosa* colonies depends on the occurrence of other algae that grow in the interstices of the colonies (from erect to filamentous and calcareous algae). Thus, the main effect of *L. lallemandii* on the coral colonies is most likely related to shading as *L. lallemandii* clumps strongly reduce the irradiance underneath (Ballesteros et al. 2007, Deudero et al. 2010). Nevertheless, when highly abundant, *L. lallemandii* could produce other undesirable effects, such as reduced water removal, increased sediment trapping, nutrient enrichment and even oxygen depletion (Ballesteros et al. 2007). These effects, in keeping with the algal annual cycle, should be seasonal. In contrast, *C. racemosa* shows a very different type of growth; the stolons of this alga build up an intricate net that spreads tightly over the substratum and are present throughout the year. Therefore, *C. racemosa* could potentially overgrow the *C. caespitosa* colonies permanently, thereby causing starvation or asphyxia, as described in other invertebrates (Žuljević et al. 2011). Furthermore, the depth range at which *C. racemosa* became established (10 - 20 m) overlaps with the depth of maximum coral cover, while *L. lallemandii* showed higher abundances in shallower depths (5 - 10 m).

The unique *C. caespitosa* bioconstructions of Illa Grossa Bay (Kersting and Linares 2012) are severely threatened by the repeated occurrence of mortality related to thermal anomalies (Kersting et al. 2013a), and thus, the additional stress caused by *C. racemosa* overgrowth could have unknown consequences. Nevertheless, our results show that *C. racemosa* significantly avoids overgrowth of the living parts of coral colonies, although it does grow on the bare coral skeletons. Furthermore, the avoidance of living colony areas seems to have been enhanced as *C. racemosa* spread through the zones of greatest coral colony concentration.

Lethal effects related to the invasion of both algal species were not detected in *C. caespitosa* colonies. *Cladocora caespitosa* has been suffering severe mortalities in the recent past, and high levels of necrosis were linked to thermal anomalies that occurred before the algal invasion (Kersting et al. 2013a). This finding is supported by the monitoring of algal-affected and algal-unaffected coral colonies that showed no difference in mortality rates occurring after 2010. Moreover, the similar photosynthetic efficiency values between algal-affected and algal-unaffected coral colonies indicated that there is little impact to the symbiotic zooxanthellae.

In this study, the toxicity of *C. caespitosa* was measured for the first time; the coral has EC50 values that are comparable to those obtained for other bioactive sessile invertebrates (e.g., *Dysidea avara* and *Ircinia variabilis*, Martí et al. 2003 and *Oscarella balibaloi*, Ivanisevic et al. 2011). Two sesterpenoids have been described for *C. caespitosa* (Cladocoran A and B, Fontana et al. 1998), which could account for the toxicity values obtained. The terpenoids represent a large class of natural products produced by marine invertebrates, and several natural roles have been attributed to them, such as defense against predation (e.g., Becerro et al. 1998), antifouling (e.g., Hirota et al. 1996), and competition for space (e.g., De Caralt et al. 2013). The toxic activity displayed by *C. caespitosa* may be responsible for the low living colony overgrowth by *C. racemosa*, acting as an allelochemical defense mechanism preventing the direct effects of algal cover. Because of the high variability of the results and the potential importance for the survival of the species, the production of secondary metabolites and their function in controlling algal overgrowth in *C. caespitosa* should be studied further also considering other *C. caespitosa* reefs in the Mediterranean.

Continual spread of invasive algae to reefs have been documented in several tropical regions, as the case of Hawaii (Stimson et al. 2001; Smith et al. 2002) and nonindigenous algae are posing additional threats to coral-dominated habitats (i.e., branching coral overgrowth by the introduced *Caulerpa verticillata* in the Gulf of California, Pérez-Estrada et al. 2013). Although competition between algae and corals is widespread and is a well-known subject of study in coral reefs, their interaction varies considerably (McCook et al. 2001). While some studies have shown negative effects related to algal overgrowth or shading (Quan-Young and Espinoza-Avalos 2006; Vermeij et al. 2010), others reported corals to be competitively superior, providing evidence that some coral species may inhibit algal growth (De Ruyter van Steveninck et al. 1988; McCook 2001). Our results, together with the massive occurrence of *C. caespitosa* despite the high algal abundance in the bay, seem to be in concordance with the second group of studies. Nevertheless, despite we did not detect direct negative effects, we cannot disregard the possible occurrence of sublethal effects, for example, in other stages of the life cycle of *C. caespitosa*, such as recruitment and juvenile survival, as reported in other invertebrates (Arnold et al. 2010; Cebrian et al. 2012; Linares et al. 2012). In relation to this, it is important to note that *C. caespitosa* reproduction in the Columbretes Islands takes place at the end of the summer when both algal species are present (Kersting et al. 2013b).

Because the invasion by both algal species is quite recent in the Columbretes Islands, their evolution and the effects on native species should be investigated further to obtain valuable information to improve the current management plans for Mediterranean marine reserves.

Acknowledgements

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GENERAL DISCUSSION AND CONCLUSIONS

Photo: ***Muraena helena*** **under a** ***Cladocora caespitosa*** **colony**

CONSERVATION IN A GLOBALLY CHANGING ENVIRONMENT: A NEW AND DIFFICULT TASK

Marine reserves are widely recognized as an important tool for marine biodiversity conservation and fisheries management (Gell and Roberts 2003; Lubchenco et al. 2003; Micheli et al 2004). Their establishment is one of the few management tools available for local communities to combat the deleterious effect of global environmental impacts on marine ecosystems; even though it was not the initial objective of this conservation tool. Nevertheless, despite the common hope that marine reserves could play this role, empirical evidence of the effectiveness of local protection against global problems is lacking. In recent years, some studies have highlighted climatic impacts within marine reserves (Graham et al. 2008; Côté and Darling 2010; Mora and Sale 2011). Only a few studies have recently shown that coastal marine reserves enhance the resilience of organisms to climate change-related disturbances; as, for example, in exploited abalone populations by increasing their resilience to mass mortalities (Micheli et al 2012) or coral reefs where protection may increase their resilience to coral bleaching and other disturbances (Mumby et al 2013) where fishing is prohibited. As a result, active discussion is ongoing as to whether reserves can increase the resilience of marine populations and ecosystems (Graham et al. 2008; Côté and Darling 2010; Hughes et al.2010; Mora and Sale 2011; Micheli et al 2012).

Global change is rapidly altering Mediterranean marine habitats (Occhipinti-Ambrogi 2007; Lejeusne et al. 2010; Zenetos et al. 2012), primarily through warming and the invasion of new species. Mediterranean Marine Protected Areas (MPAs) provide no barrier to these disturbances, and thus impacts have been extensively observed in several Mediterranean MPAs (e.g., Linares et al. 2005; Garrabou et al 2009; Cebrian and Ballesteros 2009; Coll et al. 2010; De Caralt and Cebrian 2013). A clear example of this is the recent impact temperature increases have had on *Cladocora caespitosa* in the Columbretes Islands Marine Reserve. This MPA is well known for its conservation status (Templado and Calvo 2002) and the effectiveness of protection on the conservation of many commercial species living within the MPA has been well documented (Goñi et al. 2006, 2010; Stobart et al. 2009; Díaz et al. 2011). However, successful MPAs such as the Columbretes Islands are failing to protect fragile species and communities from global change-related disturbances; highlighting the need of new protection and management strategies. However, while MPAs may not offer protection from such disturbances, they still provide unique laboratories for the long-term monitoring of species and communities and are providing the needed knowledge to assess the magnitude of the problem and to improve conservation measures. This is because MPAs often contain the most undisturbed examples of species and communities available, as well as offering the opportunity to control for many anthropogenic influences.

This study shows the relative ineffectiveness of current conservation measures in protecting fragile species against the threat of global change. Disturbingly, only global-scale measures (e.g., lowering CO_2 emissions) are likely to be effective in tackling this problem. These measures depend on complex global politics and are not being implemented at the needed rate. While this indicates MPAs are powerless to solve the problem, it is hoped that filling the gaps in the knowledge available for this species will provide some useful information for the management of the species and possibly argument for a review of its conservation status. The outline of these results could be preventively used in the conservation of other species with similar characteristics.

CONTRIBUTION TO THE REQUIRED KNOWLEDGE FOR CONSERVATION

The ecology of tropical coral reefs has been deeply studied for decades (Goreau 1959; Connell et al. 1997, as examples of some of the first and long-term studies). Such scientific interest is consistent with the importance of coral reefs in tropical seas; due to their extent, ecological role and economic importance. In comparison, temperate corals do not form extensive reef systems, are much less abundant and have been subject to fewer studies. While reefs formed by zooxanthellate corals have a testimonial distribution in temperate seas such as the Mediterranean, the fossil register shows that such reefs used to dominate coastal ecosystems in the tropical and subtropical past (Esteban 1996; Aguirre and Jiménez 1998). This is the case of *Cladocora caespitosa*, the only example of an endemic zooxanthellate scleractinian reef-builder in the present Mediterranean Sea. Given these unique characteristics and the conditions threatening to impact on the species, studies of its basic ecological and biological traits are desirable. This PhD thesis aims to fill this gap in available knowledge at a temporal scale for which information is currently lacking. The aim is not only to understand the degradation of the *C. caespitosa* populations and reefs, but also to assess the impacts that are affecting benthic communities in a rapidly changing Mediterranean Sea. This knowledge will form the baseline for future conservation measures.

Cladocora caespitosa bioconstructions in the Illa Grossa Bay (Columbretes Islands): highlighting their ecological plasticity and relevance in the Mediterranean Sea

While *Cladocora caespitosa* formed extensive reefs in the Mediterranean after the Messinian event (Aguirre and Jiménez 1998; Esteban 1996), today, living banks of this coral are restricted to a few Mediterranean locations. Populations and/or bioconstructions, have been described (in most cases briefly) in barely 10 sites in the Mediterranean Sea (Fig. 8, Chapter I). Comparison of the *C. caespitosa* population in the Illa Grossa Bay with the other locations showed that the Illa Grossa population has exceptional cover and extension. The largest *C. caespitosa* bank known to date is located in the Mjlet National Park (Adriatic) (650 m^2 of covered area, Kružić and Benković 2008). While this bioconstruction forms a continuous reef, resulting from colony fusion and the inclusion of satellite colonies (Kružić and Benković 2008), the ones in the Columbretes form a mixed bank-bed distribution. Despite this difference in colony distribution, the accumulated area covered by *C. caespitosa* bioconstructions in the Illa Grossa Bay is estimated to be 2900 m^2, which is over four times the area reported in Mjlet.

Optimal conditions for the development of extensive *C. caespitosa* populations and bioconstructions seem to be related to the following physicochemical factors: hydrodynamic protection, turbidity (high concentration of nutrients), alluvial or karstic water input (which affects water temperature), and currents which allow water exchange. These factors are quite common in the fossil register of *C. caes*pitosa (Aguirre and Jiménez 1998; Peirano et al. 2004, 2009) and also show up in the best developed *C. caespitosa* reef currently in existence (Mljet National Park, Kružić and Benković 2008). However, some of these factors are lacking in the Illa Grossa Bay, Columbretes Islands. While relative protection exists, offered both from the islet and the submerged rock crests, currents and storms from the E and NE hit the bay in autumn and winter, which are a potential threat to shallow and unprotected big colonies or reefs. Therefore, a larger reef-like development in Columbretes is probably limited by the hydrodynamic conditions in the Illa Grossa Bay, which can carry enormous energy (Fig. 1). Water inputs from the continent assure nutrients and lower temperatures. Average water temperature of $\sim$ 17 °C has been reported as optimal for corallite growth in actual and fossil reefs and in most cases this temperature was enabled due to terrestrial water input (Peirano et al. 2009). The Columbretes Islands receive no water input from the continent (they are located 60 km off the nearest coast) and average annual SST is $\sim$ 19 °C (see Fig. 6 in Chapter IV for mean SST annual cycle). This temperature regime may be the cause of the lower growth rates obtained for *C. caespitosa* corallites in the Columbretes Islands, which are in the lower range of those reported in other Mediterranean sites (Table 1, Chatper I). Despite some of the “optimal conditions” being missing in the Illa Grossa Bay, the regime of hydrodynamic protection and water exchange has allowed the development of the extensive field of *C. caespitosa* colonies. This development has probably been assisted by other factors such as the ecological plasticity of this coral.

Figure 1. The Illa Grossa Bay during the strongest storm in the last decades (November 2001).

Although at least some of the main factors that seem to enhance the occurrence of *C. caespitosa* are present in the Illa Grossa Bay, there is also a high prevalence of macroalgae which have been considered a limiting factor in the development of this coral (e.g., Peirano et al. 1998; Rodolfo-Metalpa et al. 1999). Algal cover in the Illa Grossa Bay is high (Templado and Calvo 2002) and most of the coral colonies and banks occur within the infralitoral photophilic algal community. This reinforces the high level of ecological plasticity of this coral, which is capable of living in such contrasting environments.

Coral reefs typically occur in oligotrophic waters with low variability; however, Mediterranean waters are oligotrophic but highly variable (Zabala and Ballesteros 1989). This difference in variability has been suggested to be one of the main factors that hinders the development of coral reefs in this sea (Zabala and Ballesteros 1989). However, *C. caespitosa* seems to be adapted to this variability, being the exception to the rule (a zooxanthellate reef-builder coral in the Mediterranean Sea). This fact is probably related to the ecological plasticity shown by this coral; unusual for a long-lived species which are typically submitted to relatively stable environmental conditions. The capacity of this species of living in contrasting environments (in several Mediterranean locations, such as Medes Islands and Cap de Creus, the species is commonly found below 20 m depth in dim light conditions) and to withstand the mentioned Mediterranean variability (seasonality), is probably related to the ability of this species to regulate autotrophy and heterotrophy depending on the environmental light conditions, as suggested by Hoogenboom et al. (2010). Furthermore, recently Ferrier-Pagès et al. (2013) showed that this species is able to optimize autotrophy in high light conditions without suffering from photoinhibition. Altogether, this coral displays the main characteristics of the typical tropical reef-builders but is highly adapted to Mediterranean seasonality, being capable of living in both high and low light conditions.

One of the most surprising results of this thesis are the striking differences found in the sexual condition, gonadal cycle and oocyte size between the sampled sites in W Mediterranean and previous reports from the Adriatic Sea (Schiller 1993, Kružić et al. 2008b). Although this divergence could reflect as well the plastic nature of this species and corals are known to display great plasticity in their life-history characteristics (Richmond and Hunter 1990), such divergence in the reproductive traits in the same species is extremely rare. These contrasting results highlight the need for comparative analyses using nuclear DNA markers to determine possible genetic differences between *C. caespitosa* populations of the W Mediterranean and Adriatic. Anyway, we believe that given the nature of this species (long-lived and slow-growing), the results obtained in the Adriatic Sea should be taken in consideration and reviewed in a next future. On the other side, reproductive traits of *C. caespitosa* in the W Mediterranean were very similar to those reported for the coral *Oculina patagonica* (cited as a Mediterranean alien species, Zibrowius 1974). Not surprisingly, this similarity could be explained by recent molecular phylogenies that group both species within the same family (Oculinidae, Fukami et al. 2008).

The gonadal cycle and spawning period found in our study seems to be adapted to the natural conditions found in the field. For example, although algal abundance is high in the bay, most of the algal species have a marked seasonal cycle with their abundance and cover varying significantly between the beginning of the summer and the autumn, when most erect frondose algae disappear or reduce their cover. This provides coral larvae with easier access to settlement substrate in autumn than in summer and could be an important advantage for the coral in a place like the Illa Grossa Bay, where algal abundance is high.

Although several stages of the reproductive biology of *C. caespitosa* remain unknown, for example there is a gap in the knowledge of post-spawning processes (e.g., planula behaviour), Kružić et al. (2008b) reported that eggs of this coral were released in a mucus coating that binds them together, which surely facilitates the fertilization and posterior larval settlement near the parental colonies. Results on recruitment presented in Chapter III showed that recruitment rates were higher in horizontal substrata, which may be due to the negative buoyancy of eggs bundles and planulae. The highly contagious distribution of this species in the bay described in Chapter I supports this theory. Although post-settlement studies are needed to be certain, given the available information, it seems likely that the dispersion capability of this coral is very low. As an example, while the Illa Grossa Bay hosts one of the most important populations of this coral, in the rest of the Columbretes Islands, despite suitable substrata being available close by, this species is almost absent.

Assessing the resilience of *Cladocora caespitosa* in a changing world

This is the longest, continuous register of mortalities and seawater temperature trends in Mediterranean benthic communities to date and for the first time allowed the description of the relationship between recurrent mortality events and SST regimes. Over the last 15 years *C. caespitosa*, as well as other Mediterranean benthic organisms, have suffered recurrent climate-induced mortalities (Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009). In the Columbretes Islands, *C. caespitosa* suffered necrosis after 9 summers in the period from 2002 to 2012; during this period over 50 % of the coral cover in the bay was necrosed. The long SST series for the Columbretes Islands spanning over 20 years showed a four-fold increase in summer positive thermal anomalies when the periods from 1991 to 2001 and 2002 to 2012 were compared (Fig. 4, Chapter IV), with a summer warming trend of 0.06 °C year^{-1}.

The highest necrosis rates were reached during the first mortality period from 2003 (probably the warmest summer in Europe since 1500, Luterbacher et al. 2004) to 2006. Thus there was a situation where not only were summers abnormally hot, but they also increased in frequency. The results obtained in the present thesis showed that extremely hot summers , such as the one recorded in 2003, have a large influence on the levels of necrosis triggered in the ensuing summers with milder thermal anomalies. This was evident when comparing summers from the first and second mortality periods with similar thermal anomalies where the necrosis rates were higher in the first period which included the very hot summer of 2003. Despite some summers triggered higher necrosis than others, the randomness in the colony necrosis occurrence between mortality years pointed out that, during this period, neither adaption or directional selection processes were playing a main role in the mortality patterns. Probably, other processes like delayed physiological stress and/or temperature dependent diseases, which may affect colonies more erratically, were having a large influence during the mortality summers.

Climatic models predict that the Mediterranean Sea will be among the regions most affected by the warming trend and the increase of extreme events (Déqué 2007; Diffenbaugh et al. 2007; IPCC 2013, Fig. 2). Therefore, there is a high probability that extreme hot summers, similar to that in 2003, will become more common. Taking into account the results obtained in this study, repeated extreme summers could decimate the population of *C. caespitosa* in the Illa Grossa Bay and the rest of the Mediterranean, specially if those abnormally hot summers are followed by summers with, even if mild, positive thermal anomalies. While summers with mild positive anomalies, not accompanied by extremely hot ones, should be expected to trigger low necrosis rates.

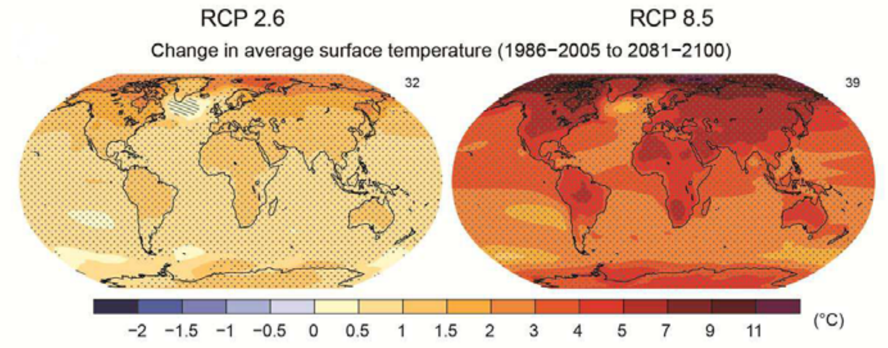

Figure 2. Change in average surface temperature (1986-2005 RCP 2.6 scenario to 2081-2100 RCP 8.5 scenario) (IPCC 2013).

Regarding to the impact of invasive species, the invasive algae *Lophocladia lallemandii* and *Caulerpa racemosa* were detected for the first time in the Columbretes Islands in the entrance of the Illa Grossa Bay in 2006. By 2012 both algal species had successfully spread over the bay, although with different pace and variability. During the studied period, the distribution of both invasive species overlapped with that of the coral *C. caespitosa*. During this time range, up to 70 % of the coral colonies were affected (i.e., overgrown to some extent) by *L. lallemandii* and 30 % by *C. racemosa*. While *L. lallemandii* grew as an epiphyte on native algae, *C. racemosa* showed a rhizophytic and creeping growth in direct contact with the coral. Interestingly, bioactivity was detected in *C. caespitosa*, with toxicity values close to those reported in bioactive benthic invertebrates. This would explain the invasion pattern of *C. racemosa* when encountering living coral surface. The bioactivity of the polyps could keep those living colonies out of the range of the algae invasion, thus avoiding direct negative effects such as asphyxia or enhanced sedimentation. Competition between algae and corals is widespread in tropical coral-reefs (McCook et al. 2001) and examples of both algal and coral success, in this case by inhibition of algal growth, have been reported (e.g., Vermeij et al. 2010; McCook 2001). The colony invasion pattern of *C.*

racemosa and the massive occurrence of *C. caespitosa* despite the high native algal cover in the bay, show that this coral may successfully compete in an algal dominated community.

Despite the fact that no lethal or sublethal effects of the invasive algae were detected on the *C. caespitosa* colonies, we can not disregard negative effects on other stages of the life cycle of *C. caespitosa*, such as recruitment and juvenile survival, as has been reported to occur in other invertebrates (Arnold et al. 2010; Cebrian et al. 2012; Linares et al. 2012). This may particularly be a risk for *C. caespitosa* as spawning occurs at the end of the summer - beginning of the autumn, coinciding with the period when the invasive algae cover a high percentage of the substrata.

Viability and conservation of the *Cladocora caespitosa* population at the Illa Grossa Bay

Cladocora caespitosa shows great ecological plasticity, mostly related to changing light conditions, being capable of living in different types of habitat and community at different depths. This plasticity is an advantage in a changing and diverse environment, as for example in shallow infralitoral communities, where significant changes in light intensity can occur at small temporal and space scales. However, while the characteristic communities that harbour *C. caespitosa* are normally subjected to significant changes in light conditions and hydrodynamics, climate change is causing extreme temperature conditions, especially in the summer, and to which this kind of organism may not be well adapted to endure. Therefore, although *Cladocora caespitosa* shows great ecological plasticity in relation to the environmental changes which occur in its natural habitat, it has proven to be extremely vulnerable to increases in seawater temperature.

While the effects of global warming have typically been studied for species living in much more stable environments (e.g., *Paramuricea clavata* or *Corallium rubrum*), results presented in this thesis show that species living in shallower and more dynamic environments are also being affected by these changes.

Twelve years of continuous monitoring showed that the steady warming of Mediterranean waters can be considered the most concerning actual threat, specifically to the *C. caespitosa* population of the Illa Grossa Bay, but also at a Mediterranean level (Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009). *Cladocora caespitosa* is a slow growing long-lived coral species, and thus is not likely to adapt fast to environmental change. Therefore, given that climate predictions (IPCC 2013) suggest that warming will continue to increase, and that *C. caespitosa* is vulnerable to temperature increases, the probability that *C.*

caespitosa populations will survive seems low. Furthermore, this main threat is accompanied by the community and environmental changes related to algal invasions. Climate-induced mortalities have so far reduced the coral cover in the bay to over a half, while lethal or sublethal effects of the invasive algae were not detected. However, the effects of invasive algae on recruitment and juvenile survival are unknown but may be considerable.

Recovery from coral mortalities relies mostly on recruitment (Connell et al. 1997; Hughes et al. 2000; Adjeroud et al. 2007; Coles and Brown 2007). For *C. caespitosa* there are two main obstacles that may hinder recovery through recruitment. Firstly, the high frequency of mortalities detected during the last decade probably exceed the recovery potential of the low recruitment rates; it has to be taken into account that while annual non-catastrophic mortality in this species is ~ 1 %, in years with catastrophic mortality this rate may suffer up to a 25-fold increase. Secondly, both seawater warming and invasive algae may have delayed and synergetic effects on reproduction, recruitment and juvenile survival. If this is the case, recovery of the coral cover through recruitment will be seriously compromised. Furthermore, Illa Grossa Bay population probably has a high reliance on self-recruitment given its geographic isolation and likely low capacity of dispersion of the species, and therefore recovery is not likely to be assisted by larvae from other populations. Nevertheless, *Cladocora caespitosa* was so abundant in the Illa Grossa Bay that even after the mass mortalities cover and colony density maintained high values, which does increase the likelihood of recovery. Furthermore, 20 % of the colonies in the Illa Grossa Bay were unaffected by the mortalities over the study period and may be more thermotolerant genotypes.

ESTABLISHING CONSERVATION MEASURES

Monitoring

The monitoring of both, *Cladocora caespitosa* and other similar species, and the ecological and environmental variables subjected to potential change (e.g., water temperature, biological invasions, pH), must continue. Our results showed the complexity of the factors influencing these mortalities, which highlights the need of regular, well designed long-term studies, to understanding the functioning of natural ecosystems and the responses of these long-lived species to rapid environmental changes. The main obstacle is that they require high dedication and related funding continuity, which are not always easy to find. Therefore, it is highly important that managers and politicians become aware of the need and value of long-term monitoring.

In the case of *C. caespitosa* the monitoring of populations and especially its rare reefs across the Mediterranean Sea will be essential to assess the status of the species and its temporal evolution. But, before setting up such a monitoring network, populations and reefs need to be identified and described.

Keeping controllable impacts at a minimum

Removing controllable impacts, such as direct pollution or damage by fishing gear, and keeping a good conservational status of the communities this coral lives in, will help to improve its conservation and resilience. In this sense even if, as previously discussed, MPAs are failing to protect these species from global change, they can significantly help to diminish other human induced impacts and thus improve resilience. However, even inside MPAs, permitted activities have to be critically reviewed to ensure the level of protection is sufficient and effective. For example, while the *C. caespitosa* populations in the Columbretes Islands are protected (Marine Reserve), they are located in the Illa Grossa Bay which is the area most frequented by tourists and divers. An annual average of ~ 1800 boats year^{-1} moor at the buoys installed in this bay and ~ 1900 dives are performed annually (Kersting 2002-2011). Although no direct impacts from the diving activities have been detected on the colonies, there is little doubt that boating activities were the vector for the introduction of the invasive algae that are now impacting the communities in the bay. Therefore, preventive protection measures aimed at keeping the impacts as low as possible should be implemented to prevent worsening the already endangered status of the species populations.

Endangered species status

The results of this thesis show that *C. caespitosa* and its bioconstructions are threatened. Large, centuries old, coral colonies have been described to be almost *immortal*, because once they have achieved a moderate size the probability of their being killed falls almost to cero (Hughes and Jackson 1985). This type of colony has survived extreme events over centuries; however, in the case of *Cladocora caespitosa* they are disappearing under the actual scenario of climate change in an unprecedented rate.

However, *C. caespitosa* is mostly not *Red Listed* at both the national and international level. With the updated knowledge on the ecology of *C. caespitosa*, we propose its inclusion in the protection lists, not only because of its fragile situation and ecological value, but also for the incalculable heritage value of the centenary bioconstructions. *C. caespitosa* is listed in the IUCN Red List of Threatened Species under the tag Data Deficient, but *it could fall into a* *threatened category if more information was known. Research on these aspects of this species' ecology is recommended* (IUCN, http://discover.iucnredlist.org/species/133142). With the gained information *C. caespitosa* could meet the IUCN's criteria to fall into the Endangered category. The IUCN considers: *A taxon is Endangered when the best available evidence indicates that it meets any of the criteria A to E for Endangered, and it is therefore considered to be facing a very high risk of extinction in the wild* (IUCN 2012).

Information on the health status of other *C. caespitosa* populations and reefs is mostly missing, here, as an example, the criteria will be applied to the studied population and/or the actual knowledge on the occurrence of *C. caespitosa* reefs throughout the Mediterranean. It is very important to bear in mind that under optimal ecological conditions *C. caespitosa* is a reef-forming species (Kružić and Benković 2008) and, therefore, reefs (banks) and fields built up by large colonies (beds) should be considered as a well preserved way of occurrence.

Cladocora caespitosa meets, at least, the following criteria:

A. 2. An observed, estimated, inferred or suspected population size reduction of ≥50% over the last 10 years or three generations, whichever is the longer, where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

A.4. An observed, estimated, inferred, projected or suspected population size reduction of ≥50% over any 10 year or three generation period, whichever is longer (up to a maximum of 100 years in the future), where the time period must include both the past and the future, AND where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

B criteria on geographic range would be probably met as well by *C. caespitosa* reefs. For example, *B.1.a. Severely fragmented or known to exist at no more than five locations*; *B.1.b. Continuing decline, observed, inferred or projected, in any of the following (extent of occurrence, area of occupancy, number of locations and subpopulations).*

Notice that compliance to just one of these criteria is enough to include this species in the IUCN Red List under the Endangered category.

For the same reasons, the species should be included in the different national protection lists as well, like in the Spanish *Catálogo Nacional de Especies Amenazadas*.

Global measures against the *new threats*

From a broader perspective, both geographically and ecologically, new measures are needed to stop the actual rate of ecological disassembly. Given the climate models predictions for the oncoming decades (IPCC 2013) and the acquired knowledge on its potential impacts in marine communities, catastrophic consequences are expected if no corrective measures are taken.

The most important global measure against climate change is the reduction of CO_2 and other greenhouse gas emissions to the atmosphere, as they contribute most to total radiative forcing since 1750 (IPCC 2013). However, the rate at which countries are reducing greenhouse gas emissions is far from enough to be effective and even if emissions were reduced to 0, a large fraction of anthropogenic climate change resulting from CO_2 emissions is already irreversible on a multi-century to millennial time scale (IPCC 2013).

New biological invasions could be significantly reduced by applying a more strict control to shipping activities, as they are considered one of the main vectors of introduction (Klein and Verlaque 2008); however, other vectors, like the artificial connection between seas (e.g., Suez Canal), are much more difficult to control. Most of the ongoing invasions will be difficult, if not impossible to stop, and it is highly unlikely that communities can be returned to their pre-invasion status. Therefore, the only practical measures are to; firstly stop new invasions; and secondly to monitor ongoing invasions to assess their evolution and impacts on the native communities and species.

The difficulty of implementing these global measures should not discourage the implementation of measures at a smaller scale, which need to be developed in parallel.

FUTURE RESEARCH

The study of the ecology of this endemic species has just started. During the development of the present PhD, many interesting and unresolved questions have arisen. Future research on the following topics is needed to further advance our knowledge of the ecology of *Cladocora caespitosa* and its application to its conservation.

Asexual reproduction of *Cladocora caespitosa*

Although there is some information on the growth of colonies through budding (Rodolfo-Metalpa et al. 2008; present PhD), knowledge of other mechanisms of asexual reproduction in this coral is lacking. While preliminary genetic studies suggest that asexual reproduction is not the main form of reproduction in *C. caespitosa* (Casado-Amenzua 2012), its relative contribution to the population dynamics of this species could change if disturbances limit the main form of reproduction of populations. One possible form of asexual reproduction may be polyp detachment which was observed *in situ* at the Columbretes MPA during the early stages of field work conducted for this PhD. Detachment could be an effective mechanism for broken living colony remains to recreate new colonies in suitable substrata.

Other factors acting in synergy with thermal stress: thermodependent pathogens and physiological status of the polyps

Climate-related mortalities of benthic organisms have been associated to both pathogens and physiological stress (e.g., Bally and Garrabou 2007; Coma et al. 2009). Although the relation between *Cladocora caespitosa* mortalities and SST anomalies in Columbretes was unequivocal, our results showed the occurrence of other factors acting in synergy with the thermal anomalies. Therefore, studies dealing with thermodependent pathogens and the physiology of this coral in relation to the mass-mortalities would complete our knowledge on the mechanisms involved in the mortalities.

Sublethal effects of thermal stress and invasive algae

As stated in this PhD, sublethal effects of thermal stress and invasive algae could affect different life-stages of *Cladocora caespitosa*, and thus their reproductive success and recruitment. Studies on the potential sublethal impacts on this species, particularly on the early life stages, are needed to fine-tune the present knowledge on its resilience to the drastic environmental changes this species is subjected to.

Characterization of other *Cladocora caespitosa* populations

As explained before, the Columbretes Islands were chosen as a field laboratory for this PhD because they harbour one of the most important and well developed populations of this coral. However, there is a general lack of descriptive studies and health status assessment in other *C. caespitosa* populations and bioconstructions. This information is crucial to have an overall picture of the conservation status of the species.

Genetic studies

By the time this PhD is presented, microsatellite markers have been already developed (Casado-Amenzua et al. 2011), preliminary genetic connectivity studies have been undertaken (Casado-Amenzua 2012) and more in depth studies on this last topic are submitted to scientific journals (P. Casado-Amenzua, pers. comm.). However, further genetic studies are needed to elucidate potential genetic differentiation of this species between the Adriatic and Mediterranean seas, given the striking divergences found in the reproductive biology of the species. In addition, small scale genetic studies, together with the study of the post-spawning behaviour of eggs and larvae, would be useful to increase our knowledge on the small scale connectivity and the processes that shape the population structure of this coral.

Cladocora caespitosa bioconstructions as a focus of biodiversity

The colonies of *Cladocora caespitosa* harbour many inhabitants that take advantage of the phaceloid structure of the coral as a refuge and food source. Some of these organisms live in the corallites walls (boring species), while others live in the interstices between corallites or even in the sediment that is trapped by the whole structure. While some information on this community appears in the early studies on this coral (Schiller 1993) a more detailed description is lacking. Increasing our knowledge on this issue will allow us to assess the ecological role of *C. caespitosa* in providing structure and biodiversity to the associated communities.

Comparisons between the actual and fossil *Cladocora caespitosa*

While *Cladocora caespitosa* dominated coastal communities in the subtropical Mediterranean, in the present time mass mortalities related to water warming are seriously affecting the species. A detailed comparison of actual and fossil *C. caespitosa* populations, the environmental conditions they endured in the past and present, as well as the plasticity or adaptability of this species to changing environments would help to solve the apparent contradiction in the thermal regime this species was able to tolerate in the past. Peirano et al. (2009) started some of this work, but many questions on this interesting topic are still unanswered.

Spawning and post-spawning processes

Spawning of *Cladocora caespitosa* has been solely observed in the Adriatic Sea (Schiller 1993; Kružić et al. 2008a). Given the detected divergences in the reproductive biology of the species, spawning events should also be described in Western Mediterranean populations. Furthermore, information on post-spawning processes, such as fertilization and planula behaviour, is completely lacking for this species.

CONCLUSIONS

Cladocora caespitosa bioconstructions in the Columbretes Islands Marine Reserve (Spain, NW Mediterranean): distribution, size structure and growth

- The Illa Grossa Bay hosts an exceptional *Cladocora caespitosa* population, formed by both banks and beds in a mixed manner, which shows a high degree of geographical isolation. These bioconstructions and the covered area (~ 2900 m^2) are, together with the ones described in Mjlet National Park (Adriatic Sea), the largest described to date.

- The distribution of the colonies in the bay is highly aggregated and associated to sea-floor morphology and, probably, to reproduction strategies. The semi-enclosed bay offers both hydrodynamic protection and high water exchange.

- *Cladocora caespitosa* colonies frequently occur amongst dense algal cover in bay.

- *Cladocora caespitosa* corallites showed growth rates of ~ 2.5 mm yr^{-1}.

Unexpected divergent patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*

- *Cladocora caespitosa* sexual reproduction showed striking divergences between the Western Mediterranean and Adriatic seas.

- *Cladocora caespitosa* has shown to be gonochoric in the Western Mediterranean Sea, while it was described as hermaphroditic in the Adriatic Sea.

- Gonadal cycle was associated with water temperature and maximum oocyte size (~ 100 µm) was reached in coincidence with SST maxima in August. Maximum oocyte size differed significantly between the Western Mediterranean and Adriatic seas.

- The drastic decrease in numbers of oocytes at the end of the summer - beginning of the autumn indicates that spawning occurs at this time of the year, while spawning in the Adriatic is reported to occur at the beginning of the summer.

- The reproductive characteristics of Western Mediterranean *Cladocora caespitosa* were similar to those described for *Oculina patagonica* (cited as a Mediterranean alien species). Both species have been recently grouped within the same family.

Recruitment and natural mortality of the temperate coral *Cladocora caespitosa*: implications for the recovery of endangered populations

- *Cladocora caespitosa* displays low recruitment and mortality rates (~ 0.30 recruits m^{-2} yr^{-1} and 1 %, respectively), and comparatively high juvenile colony mortality (29 % in the smallest size-class, < 5 polyps).

- Recruitment rates in horizontal substrata were significantly higher than in subvertical substrata. These differences could be related to the reproduction strategies of the species (i.e., egg retention mechanisms and possible negative buoyancy of the eggs).

- Juvenile colonies grew in polyp number through asexual reproduction, with significantly higher budding rates in the smallest size-class.

Long-term responses of the endemic reef-builder *Cladocora caespitosa* to Mediterranean warming

- The studied temperature series showed a summer warming trend of 0.06 °C yr^{-1} (~ 1.2 °C increase over the 20 yr series) and a significantly increased frequency of positive thermal anomalies.

- Mortality of *Cladocora caespitosa* was significantly related to positive thermal anomalies and resulted from tissue necrosis without massive zooxanthellae loss (i.e., no bleaching).

- *Cladocora caespitosa* suffered necrosis after 9 of the 11 studied summers, divided into two mortality periods (2003-2006 and 2008-2012). Over 50 % of the area covered by *Cladocora caespitosa* has suffered necrosis after these recurrent mortalities.

- The highest necrosis rates were recorded during the first mortality period, after the exceptionally hot summer of 2003 (~ 25 % necrosis).

- The differences in necrosis found after summers with similar thermal anomalies pointed out to the existence of other acting factors which could be more closely related to the interannual temperature context and delayed thermal stress after extreme summers, rather than to acclimatisation and adaption processes.

Invasive macrophytes in a marine reserve (Columbretes Islands, NW Mediterranean): spread dynamics and interactions with the endemic scleractinian coral *Cladocora caespitosa*

- The invasive algae *Lophocladia lallemandii* and *Caulerpa racemosa* successfully spread over Illa Grossa Bay from 2006 to 2012, albeit with different pace and variability.

- While *Lophocladia lallemandii* colonised the entire bay rapidly in just 2 years, *Caulerpa racemosa* showed a slower but steady spread.

- *Lophocladia lallemandii* overlapped its distribution in the bay with that of the coral *Cladocora caespitosa* as early as 2007, whereas *Caulerpa racemosa* did so by 2010.

- No lethal effects of the invasive algae were detected on the *Cladocora caespitosa* colonies. Nevertheless, the possible occurrence of sublethal effects (e.g., in recruitment or juvenile survival) can not be disregarded.

- *Cladocora caespitosa* showed toxic activity, which could explain the low overgrowth of living colony parts by *Caulerpa racemosa*.

General conclusion

The Illa Grossa Bay hosts a unique population of the Mediterranean endemic coral *Cladocora caespitosa*, a long-lived species capable of building large durable bioconstructions of invaluable ecological and heritage value. This species, and its rare bioconstructions, are dangerously threatened by global change-related disturbances and conservation plans are urgently needed. From a broad perspective, *Cladocora caespitosa* has revealed, as a case study, that Mediterranean benthic communities are seriously threatened by the drastic environmental changes, highlighting the need for general conservation measures and continuous long-term monitoring.

RESUMEN

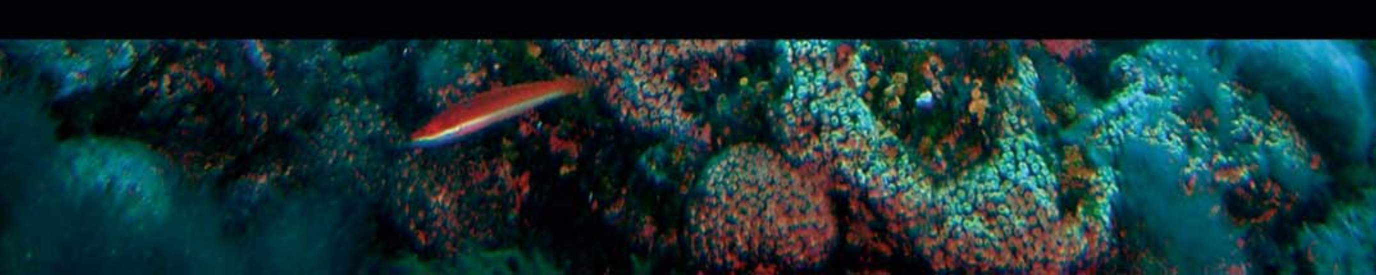

Photo: Monitoring *Cladocora caespitosa*

INTRODUCCIÓN

LA CONSERVACIÓN DE LAS COMUNIDADES BENTÓNICAS MARINAS

La mayoría de los ecosistemas marinos están en declive a causa de la sobreexplotación, contaminación, especies invasoras, enfermedades, fragmentación y pérdida de hábitat, y cambio climático (Jackson 2001; Harvell et al. 2002; Steneck and Carlton 2001; Hughes et al. 2003; Kappel 2005; Harley et al. 2006). Muchas especies están amenazadas por los rápidos cambios que están alterando la estructura de hábitats y comunidades. Sin embargo, las tan necesarias medidas de conservación son inexistentes en la mayoría de los casos, debido principalmente a la falta de información sobre las especies. Por lo tanto, es esencial conocer la biología y ecología de estas especies, así como los impactos a los que pueden estar sujetas. No obstante, la falta de información sobre estos aspectos es generalizada, incluso para especies clave y/o emblemáticas. Este conocimiento es clave para entender los procesos y mecanismos que intervienen en el declive de estas especies y por tanto para desarrollar medidas de conservación adecuadas.

Una de las herramientas de conservación más eficaces, especialmente a niveles regionales y locales, son las Áreas Marinas Protegidas (AMP). Las AMPs han mostrado su efectividad en la protección de las especies frente a amenazas como la sobreexplotación o la pérdida de hábitat (Lubchenko et al. 2003; Lester et al. 2009). Sin embargo, estas medidas pierden efectividad frente a las *nuevas amenazas*, de gran intensidad y que actúan en una escala geográfica mucho mayor; es decir: el cambio climático y la aparición de especies invasoras (Allison et al. 1998; Lubchenko et al. 2003). Por lo tanto, aunque estas medidas de conservación son herramientas muy potentes de cara a la protección frente a ciertos impactos, en este caso necesitan ser complementadas con otras medidas que generalmente no son fáciles de implementar debido a la escala global de los impactos que causan estas perturbaciones. Este es un difícil reto para la ciencia de la conservación en la actualidad, especialmente si las medidas necesarias para mitigar los impactos no son adoptadas a nivel global (por ejemplo, la reducción de emisiones de CO_2 a la atmósfera). Por otra parte, hay que destacar que las AMPs siguen teniendo un papel importante en el seguimiento de los efectos de estas *nuevas amenazas* sobre especies y comunidades, sirviendo como laboratorios privilegiados para su estudio.

Muchas especies marinas *anónimas* están fuera del campo de las acciones de estudio y conservación y su declive (si ocurre) pasará seguramente desapercibido; en este caso, las medidas de conservación, aunque en algún momento puedan llegar a desarrollarse, pueden llegar tarde. Sirva la especie objetivo de esta tesis, el coral endémico del Mediterráneo *Cladocora caespitosa*, como ejemplo y representante de todas aquellas especies sobre las que no se tiene información útil para su conservación; especialmente sobre procesos ecológicos clave y sus respuestas frente a los rápidos cambios ambientales que están ocurriendo a nivel global.

***Cladocora caespitosa*: el coral constructor de arrecifes endémico del Mediterráneo**

Cladocora caespitosa (Linnaeus 1767) es el único coral escleractinio colonial con zooxantelas simbiontes autóctono del Mediterráneo (Zibrowius 1980). Esta especie endémica está presente en un amplio rango de tipos de substrato, profundidad y condiciones de hidrodinamismo (Zibrowius 1980; Schiller 1993).

Peirano et al. (1998) propusieron los términos *bed* y *bank* (que se podrían traducir como lecho o campo de colonias y micro-arrecife o banco) para describir el tipo de distribución que presentan las colonias de esta especie, es decir: *bed*: gran número de colonias subesféricas independientes con diámetros comprendidos entre 10 y 30 cm y *bank*: grandes formaciones que alcanzan varios decímetros de altura y varios metros cuadrados en área.

Las colonias de este coral tienen una estructura faceloide, es decir, los coralitos tubulares se desarrollan verticalmente, presentando ejes de crecimiento subparalelos en sus ramas principales que mantienen la independencia entre coralitos. *Cladocora caespitosa* deposita en su esqueleto dos bandas anuales de distinta densidad: una banda de mayor densidad que se deposita durante el otoño e invierno y una banda menos densa que se deposita durante el verano (Peirano et al. 1999, 2005) (Fig. 1, Introducción General de la tesis). Estas bandas son fácilmente detectables mediante radiografías, método que ha mostrado ser efectivo para el estudio del crecimiento de los coralitos (Peirano et al. 1999; 2005, 2009; Kružić and Benković 2008; Kružić et al. 2012).

Cladocora caespitosa se ha adaptado a vivir en condiciones ambientales muy heterogéneas, desde comunidades fotófilas someras a comunidades circalitorales de mayor profundidad (Fig. 2, Introducción General de la tesis). Esta plasticidad podría estar relacionada con la habilidad de este coral para regular la alimentación heterotrófica en compensación de la autotrófica en condiciones de baja iluminación. La fisiología de la especie se caracteriza por la dominancia de la autotrofía y heterotrofía en verano e invierno, respectivamente (Ferrier-Pagès et al. 2011). Sin embargo, *C. caespitosa* tiene la capacidad de regular la heterotrofía y mantener la simbiosis con las zooxantelas, incluso en condiciones subóptimas (Hoogenboom et al. 2010). El papel de la heterotrofía es diferente en condiciones de alta y baja luminosidad (las condiciones de luz se ven modificadas tanto por cambios estacionales como ambientales). En condiciones de baja luminosidad la alimentación heterótrofa tiene un papel compensatorio en la adquisición de carbono (utilizado posteriormente para la calcificación). Mientras que en condiciones de alta luminosidad, el alimento es la fuente del nitrógeno y fósforo que permiten el crecimiento tisular y que permiten, a su vez, el crecimiento de la colonia a través de la reproducción asexual de los pólipos por gemación (Hoogenboom et al. 2010). Estos autores concluyen que las colonias de *C. caespitosa* con capaces de crecer en biomasa únicamente cuando tanto la luz (energía) como el alimento (nutrientes) están disponibles y que la alimentación juega un papel importante en el balance energético de esta especie.

Los coralitos de este coral, muestreados mediante ablación láser y analizados en búsqueda de ciertos elementos traza, han proporcionado un valioso archivo histórico de las condiciones climáticas en el Mediterráneo, obteniéndose registros de temperatura de resolución semanal en coralitos con edades comprendidas entre los 10 y 95 años (Silenzi et al. 2005; Montagna et al. 2006, 2007).

Este coral se encuentra entre el grupo de invertebrados bentónicos afectados por episodios de mortalidades masivas relacionadas con anomalías térmicas en el Mar Mediterráneo (Perez et al. 2000; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009; Kersting y Linares 2009). Experimentos de termo-tolerancia realizados en acuario han mostrado que un aumento de la temperatura del agua, por encima de la media y prolongado en el tiempo, puede provocar necrosis en los pólipos de este coral (Rodolfo-Metalpa et al. 2005, 2006b). No obstante, mientras que esta necrosis ha sido observada, tanto en acuario como *in situ*, nunca se ha detectado un “blanqueamiento” generalizado de los pólipos, que se produce en este tipo de corales por una pérdida masiva de las zooxantelas (Rodolfo-Metalpa et al. 2005, 2006b). De hecho, estudios previos han demostrado la resistencia de las zooxantelas simbiontes en *C. caespitosa* (descritas como *Symbiodinium* clade temperate-A; Visram et al. 2006) al estrés térmico (Rodolfo-Metalpa et al. 2006a).

Además de los impactos derivados del cambio climático, *C. caespitosa* está amenazada por otras perturbaciones como la presencia de algas invasoras (Kružić y Benković 2008; Kružić et al. 2008b) y la eutrofización (Kružić et al. 2007).

Las Islas Columbretes y la Bahía de L’Illa Grossa

La mayor parte del trabajo de esta tesis ha sido realizado en la Reserva Marina de las Islas Columbretes. Tal y como se describe en el Capítulo I, esta reserva marina alberga uno de los campos de colonias y arrecifes de *Cladocora caespitosa* más extensos del Mediterráneo, siendo un laboratorio privilegiado para el estudio de esta especie emblemática.

Las Islas Columbretes emergen a 30 millas náuticas de la costa de Castelló. Forman parte de un campo volcánico de 90 x 40 km que se localiza entre 80 y 90 m de profundidad. Estas islas y el campo volcánico asociado son uno de los raros ejemplos de vulcanismo Cuaternario en el Mediterráneo (Muñoz et al. 2005).

El archipiélago de las Islas Columbretes está compuesto por 4 grupos de islotes, que son de norte a sur: L'Illa Grossa, La Ferrera, La Foradada y El Carallot (Fig. 3, Introducción General de la tesis). L'Illa Grossa (Fig. 4, Introducción General de la tesis), de mayor tamaño, está formada por una caldera volcánica semisumergida, erosionada y abierta por su parte noreste, la dirección principal de los temporales de invierno (Aparicio y García 1995; Sánchez-Arcilla et al. 2008). La bahía formada por esta isla, tiene una superficie total de 150.000 m^2, una profundidad media de 15 m y fondos de substrato rocoso y arenas de origen biogénico.

Las aguas y fondos que rodean este archipiélago están protegidos desde 1990 por una reserva marina de 5500 hectáreas, gestionada por la Secretaría General de Pesca (MAGRAMA).

CONSERVACIÓN BASADA EN HECHOS. ¿POR QUÉ NECESITA ESTA ESPECIE PROTECCIÓN?

De comunidades arrecifales a micro-arrecifes relictos: declive histórico de una especie emblemática

Los ecosistemas arrecifales desaparecieron del Mediterráneo al final del periodo Messiniense (hace ~ 5 millones de años) en el Mioceno tardío (Esteban 1996). El arrecife fósil más antiguo de *Cladocora caespitosa* conocido hasta la fecha data del Plioceno tardío (hace ~ 3 millones de años), cuando este coral formaba verdaderos arrecifes monoespecíficos, tanto en el Mediterráneo Occidental como Oriental (Aguirre y Jiménez 1998; Dornbos y Wilson 1999). Estos arrecifes han sido considerados como la continuación hasta la actualidad de los ecosistemas arrecifales en el Mediterráneo, tras la extinción de los grandes arrecifes tropicales de este mar. Otros grandes arrecifes de este coral datan del Pleistoceno inferior (Bernasconi et al. 1997), del Pleistoceno medio y tardío y del Holoceno (ver revisión de Peirano et al. 1998). Por lo tanto, *C. caespitosa* fue mucho más abundante en las costas del Mediterráneo antiguo, en un clima más cálido y apareciendo mayoritariamente en ambientes con aportes aluviales, caracterizados por su turbidez (Peirano et al. 2004). Sin embargo, estudios recientes han mostrado que, aunque estos arrecifes fósiles de *C. caespitosa* eran muy abundantes en esta época de clima subtropical, los arrecifes se encontraban generalmente en ambientes más frescos, en lagunas costeras saladas pero con aportes de agua dulce de temperatura menor que el agua marina (Peirano et al. 2009). Estas condiciones serían comparables a las que se dan en el mayor arrecife de *C. caespitosa* descrito en la actualidad, en el Parque Nacional de Mjlet (Mar Adriático, Kružić and Benković 2008). El estudio realizado por Peirano et al. (2009) muestra que el arrecife fósil investigado creció bajo temperaturas ambientales que rondaban los 17 °C; esta temperatura es comparable a la que se registra en la actualidad en el Adriático Norte. De hecho, los mismos autores descubrieron que las mayores tasas de crecimiento de *C. caespitosa* en la actualidad se dan en condiciones de temperatura del agua de mar parecidas a las descritas para el arrecife fósil (17,5 °C – 18 °C).

En los ambientes costeros del Mediterráneo actual las grandes bioconstrucciones de *C. caespitosa* son excepcionales y en general, sus poblaciones están formadas por colonias pequeñas y dispersas. Aunque esta es la tónica general, aún quedan micro-arrecifes de este coral, como los existentes en el Parque Nacional de Mjlet (Mar Adriático, Kružić and Benković 2008) o en las Islas Columbretes (Mediterráneo Noroccidental, descritos en esta tesis) (Fig. 6, Introducción General de la tesis). Sin embargo, estos arrecifes se consideran como relictos o incluso como el “eslabón perdido” con los ecosistemas Mediterráneos del pasado (Kühlman et al. 1991).

La drástica reducción histórica en la distribución y abundancia de los arrecifes de *C. caespitosa* es evidente en la actualidad. Dada la evidencia del registro fósil y los procesos recientes que afectan a la especie, como las mortalidades masivas, el declive de los arrecifes de este coral ha actuado en dos escalas temporales diferentes: una escala en términos de tiempo geológico (orden de magnitud $\sim 10^6$ años) y otra basada en la historia reciente (siglos XX y XXI). Varios trabajos han mostrado claramente la preocupación sobre el declive en la historia reciente, asociado principalmente a causas humanas. Augier (1982) ya incluyó esta especie en una lista de especies amenazadas hace 3 décadas y Morri et al. (2000) afirman que este declive continúa, relacionándolo principalmente con el cambio climático.

La presencia masiva de arrecifes de *C. caespitosa* en el Mediterráneo subtropical parece contradecir que en la actualidad, en un mar más templado, este coral sufra mortalidades provocadas por un aumento en la temperatura del agua (Rodolfo-Metalpa et al. 2000; Garrabou et al. 2009; presente tesis). Sin embargo, como ya se ha comentado, los arrecifes fósiles de este coral parece que se localizaban en ambientes costeros con temperaturas menores que las de mar abierto y, de hecho, el aumento prolongado de la temperatura del agua ha sido atribuido como posible causa de la muerte de alguno de estos arrecifes (Peirano et al. 2009). Esto podría explicar, al menos en parte, la contradicción planteada; sin embargo, las causas históricas del declive de los arrecifes de *C. caespitosa* y la comparación entre la *C. caespitosa* actual y fósil es un tema apasionante que necesita ser estudiado en profundidad.

A pesar de las diferencias en las escalas temporales y en los posibles procesos involucrados en el declive de los arrecifes de *C. caespitosa* en el Mediterráneo, un hecho común relaciona todos estos aspectos: el desconocimiento sobre sus causas. Las poblaciones y su rango de expansión geográfica han sufrido una reducción tal que esta especie y sus arrecifes podrían cumplir los requisitos marcados por la IUCN (Unión Internacional para la Conservación de la Naturaleza) para ser clasificados como *En Peligro* (IUCN 2012).

Especie estructural y longeva

Cladocora caespitosa es una especie con un importante rol estructural o ingeniera, en el sentido descrito por Jones et al. (1994) que define estas especies como aquellas capaces de modificar la disponibilidad de recursos y refugio para otras especies. Las especies longevas y estructurales, como *C. caespitosa* juegan un papel importante en la biodiversidad y estructura de las comunidades bentónicas debido a su actividad trófica, biomasa y estructuras perennes que modifican el medio en el que habitan (Dayton et al. 1974; Hughes y Jackson 1985; Sebens 1991).

Estas especies se caracterizan por su lenta dinámica (es decir, bajas tasas de crecimiento y reclutamiento), que es amortiguada por su elevada longevidad y por tanto, por la baja tasa de mortalidad natural (Emunds 2000; Hughes y Tanner 2000; Adjeroud et al. 2007; Coles and Brown 2007; Linares et al. 2007). Estas características hace que estas especies sean muy vulnerables a impactos catastróficos (Hughes y Tanner 2000; Linares et al. 2007).

En el Mediterráneo las especies estructurales y longevas están bien representadas en el coralígeno, dónde la gorgonia roja *Paramuricea clavata* es uno de los ejemplos mejor estudiados junto con el coral rojo *Corallium rubrum* (Garrabou and Harmelin 2002; Linares et al. 2007). Sin embargo, la representación de especies animales longevas con un importante papel estructural en las comunidades mediterráneas infralitorales es mucho menor. *Cladocora caespitosa* es uno de los pocos ejemplos de este segundo grupo, ya que habita mayoritariamente en comunidades someras (Zibrowius 1980), aunque también se pueden encontrar en profundidades mayores, incluso en comunidades circalitorales (Morri et al. 1994) (Fig. 2, Introducción General de la tesis).

Valor patrimonial

El concepto de patrimonio ha sido tradicionalmente aplicado en la arquitectura y en la cultura en general, sin embargo empieza a ser utilizado en un ámbito cada vez mayor dentro de las ciencias sociales y, de manera más reciente, está presente en debates y acciones relacionadas con la naturaleza y la biodiversidad (Cormier Salem et al. 2002, 2005). Un patrimonio es un conjunto de elementos materiales o inmateriales a los que se atribuye un valor o valores específicos, además de derechos que se asocian a un determinado grupo social y que se heredan y transmiten de generación en generación (ver Michon et al. 2012, para ésta y otras definiciones).

Bajo este punto de vista, *Cladocora caespitosa* constituye un patrimonio natural de valor incalculable, en base a la antigua historia que une esta especie con los ecosistemas Mediterráneos del pasado, sus características como especie de gran longevidad y capaz de construir estructuras de gran tamaño que perduran en el tiempo, y su gran fragilidad bajo las condiciones actuales de cambio climático.

Falta de conocimiento aplicado a la conservación

Aunque hay alrededor de una decena de bioconstrucciones de *Cladocora caespitosa* descritas hasta la actualidad (Fig. 8, Capitulo I), solamente unas pocas poblaciones de este coral han sido estudiadas de forma más detallada (Schiller 1993; Peirano et al. 2001; Kružić y Benković 2008) y la falta de conocimiento sobre su ecología es generalizada.

Cladocora caespitosa fue incluida por primera vez en un listado de especies amenazadas por Augier (1982) y desde entonces ha sido incluida en algunas listas de protección (Tabla 1, Introducción General de la tesis), así como en varios listados de hábitat a nivel europeo, mediterráneo y español (Tabla 2, Introducción General de la tesis). Sin embargo, su inclusión en estas listas es testimonial y deficitaria, seguramente debido a la falta de conocimiento sobre sus características demográficas y amenazas. Lo que muestra la necesidad de aumentar el conocimiento sobre esta especie con el objetivo de mejorar su protección. Un claro ejemplo de esta falta de información sobre esta especie es el comentario que aparece en la *Lista Roja* de la UICN (http://www.iucnredlist.org) con respecto a este coral.

No hay información disponible sobre sus poblaciones y amenazas. Por lo tanto, esta especie está catalogada como Falta de Información. Sin embargo, esta especie podría entrar en alguna de las categorías amenazadas si se dispusiera de más información. Se recomienda el estudio de estos aspectos de la ecología de la especie. Esta evaluación debería ser re-evaluada en 10 años para poder incluir nueva información y poder determinar los efectos, continuados o incrementados, de las amenazas derivadas del cambio climático y la acidificación.

HACIA LA CONSERVACIÓN DE *CLADOCORA CAESPITOSA*. ¿QUÉ NECESITAMOS SABER?

La biología de la conservación ha nacido como un componente esencial de la respuesta social al desmantelamiento ecológico y sus principales objetivos y preocupaciones son aquellas especies, poblaciones, comunidades y ecosistemas que se ven amenazados por la alteración, fragmentación y destrucción del hábitat (Soulé y Kohm 1989). La principal tarea de esta disciplina es proveer herramientas y conocimiento que permita anticipar, prevenir, minimizar y/o reparar el daño ecológico. A la hora de evaluar la viabilidad de una población sometida a un impacto, este conocimiento debe centrarse en los procesos clave implicados en el mantenimiento y recuperación de dicha población.

Pese a que algunos aspectos de la biología de *Cladocora caespitosa* han sido estudiados, hay una gran necesidad de conocimiento aplicable su conservación. En este contexto, la información sobre la estructura poblacional, distribución, reproducción, reclutamiento, tasas de mortalidad natural y catastrófica, y los efectos a largo plazo de los impactos derivados del cambio global, es esencial para evaluar la viabilidad de la especie, sus poblaciones y para establecer medidas adecuadas de conservación.

CARACTERÍSTICAS BIOLÓGICAS Y ECOLÓGICAS

Los corales coloniales son organismos clonales de larga vida, cuyas formas de crecimiento incrementan la complejidad de su historia vital y dinámica poblacional si los comparamos con los organismos aclonales (solitarios) (Hughes y Jackson 1985; Hughes y Connell 1987). Los organismos coloniales están formados por módulos (por ejemplo, los pólipos en corales) y después del asentamiento larvario la colonia crece mayoritariamente mediante el incremento del número de módulos genéticamente idénticos (Jackson 1977). El crecimiento de la colonia es potencialmente indeterminado y en muchas especies puede ser inhibido por estrés físico, la disponibilidad local de alimento o por la mortalidad de los módulos. Este conjunto de factores puede producir diferencias de tamaño entre colonias pertenecientes a la misma cohorte (Hughes y Jackson 1980). Se estima que las grandes colonias de coral, como las que puede llegar a formar *Cladocora caespitosa* en la Bahía de L'Illa Grossa, puede alcanzar varios siglos de edad y resumen en su estructura una larga historia de crecimiento modular, mortalidades parciales, fusiones y fisiones (Hughes y Jackson 1985).

Distribución y estructura poblacional

La distribución, la estructura poblacional y el hábitat que ocupa una población, junto con su interacción con las condiciones medioambientales es la base necesaria para describir y entender los procesos involucrados en la ecología de una especie. Aunque en la actualidad sabemos que la población de *Cladocora caespitosa* de la Bahía de L'Illa Grossa constituye uno de los pocos ejemplos de micro-arrecifes de esta especie en el Mediterráneo, la información básica sobre su distribución, cobertura, estructura poblacional y tasas de crecimiento, era completamente inexistente. En el **Capítulo I** se han estudiado las características de la población de *C. caespitosa* de la Bahía de L'Illa Grossa. Esta información es necesaria como punto de inicio para el estudio de la especie y para incidir en la importancia de esta población a nivel Mediterráneo.

Reproducción

El conocimiento de la biología reproductora de los organismos marinos es esencial para comprender su dinámica poblacional (Fadlallah 1983). La reproducción asegura la incorporación de nuevos individuos a la población, la colonización de nuevas áreas y la recuperación de las poblaciones tras los impactos. Por lo que se trata de información crucial para establecer medidas de protección y conservación.

Pese a la importancia de este tema, poco se sabe sobre la mayoría de las características reproductoras de *Cladocora caespitosa.* La liberación de gametos ha sido observada *in situ* en el Mar Adriático (Schiller 1993; Kružić et al. 2008b) y Kružić et al. (2008b) describieron a esta especie como hermafrodita en este mismo mar. El objetivo del **Capítulo II** ha sido contribuir al conocimiento actual de la biología reproductora de la especie mediante el estudio de su condición sexual y ciclo reproductor mediante análisis histológicos. Los resultados obtenidos han sido comparados con los estudios realizados en el Mar Adriático.

Reclutamiento, mortalidad y recuperación

En corales la recuperación tras un impacto depende en gran medida del reclutamiento y la supervivencia (Connell et al. 1997; Hughes et al. 2000; Adjeroud et al. 2007; Coles y Brown 2007) y estos procesos son aún más importantes en el caso de especies de gran longevidad (Edmunds 2000; Hughes y Tanner 2000; Adjeroud et al. 2007; Coles y Brown 2007). Consecuentemente, el conocimiento de estas características generales y concretamente de la dinámica poblacional de la especie, es de gran importancia para evaluar la viabilidad de las poblaciones.

El **Capítulo III** se centra en el estudio de las principales características de la historia vital de esta población. Al ser *Cladocora caespitosa* una especie longeva y estructural, sus tasas de reclutamiento y mortalidad natural son de gran importancia para evaluar su resiliencia; sobretodo en el contexto actual de impactos derivados del cambio global, que son evaluados en las secciones siguientes.

IMPACTOS DERIVADOS DEL CAMBIO GLOBAL

Cambio global es un término utilizado para describir los efectos de la actividad humana sobre la tierra (National Research Council 2000), en el se incluyen, entre otros, el cambio climático y las invasiones biológicas (Vitousek et al. 1997; Occhipinti-Ambrogi 2007). El consenso científico sobre el cambio climático global antropogénico es un hecho (Oreskes 2004; Cook et al. 2013) y sus efectos se están registrando en una gran variedad de ecosistemas, desde bosques (por ejemplo, Dale et al. 2001; Allen et al. 2010) a arrecifes de coral (por ejemplo, Hoegh-Guldberg 1999; Hughes et al. 2003).

Los mares y océanos han sufrido durante la última década un aumento neto en su contenido calórico (Levitus et al. 2000; IPCC 2013). Como resultado de este calentamiento las comunidades bentónicas se han visto alteradas a nivel mundial a través de cambios en su composición y abundancias de especies, enfermedades y mortalidades masivas, entre otros cambios en procesos biológicos básicos como la reproducción (Harrison y Wallace 1990; Harvell et al. 1999; Parmesan y Yohe 2003; Bianchi 2007; Garrabou et al. 2009).

El Mar Mediterráneo es un punto caliente en lo que al cambio global se refiere (Occhipinti-Ambrogi 2007; Lejeusne et al. 2010; Zenetos et al. 2012). La frecuencia de veranos anormalmente cálidos ha aumentado significativamente en esta zona, provocando mortalidades masivas sin precedentes. Aunque las primeras mortalidades fueron detectadas en los años 70 y 80 del pasado siglo (por ejemplo, Harmelin and Marinopoulos 1994; Vacelet 1994), la primera mortalidad masiva que afectó a un gran número de especies fue descrita en el Mediterráneo Noroccidental en el verano de 1999 (Cerrano et al. 2000; Perez et al. 2000; Romano et al. 2000). Algunos años después, en el verano de 2003, un nuevo episodio de mortalidad masiva afectó a las costas de esta parte del Mediterráneo, aunque esta vez en un área geográfica mayor (Garrabou et al. 2009). Ambos eventos afectaron a más de 30 especies de invertebrados bentónicos, en su mayoría cnidarios, esponjas y briozoos (Perez et al. 2000; Lejeusne et al. 2010).

Por otra parte, existe en este mar un gran número de especies alóctonas que presentan una amplia distribución. Se estima que el mar Mediterráneo alberga casi 1.000 especies introducidas (Zenetos et al. 2010, 2012), de las cuales 128 son macrófitos (Zenetos et al. 2012). Los corredores marinos (por ejemplo, el Canal de Suez) y las actividades relacionadas con el tráfico marítimo y la acuicultura (Zenetos et al. 2012) son considerados los principales vectores de introducción de estas especies.

Las comunidades bentónicas del Mediterráneo están siendo sujetas a grandes cambios relacionados con el mencionado cambio global. Especies emblemáticas y clave, como la gorgonia roja *Paramuricea clavata*, el coral rojo *Corallium rubrum* o la especie objetivo de esta tesis *Cladocora caespitosa*, se están viendo seriamente amenazadas por estos impactos (Garrabou et al. 2001; Linares et al. 2005; Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009). Además, los hábitats y comunidades que albergan a estas especies, junto con otros de gran interés como las praderas de *Posidonia oceanica*, están siendo invadidos por algas introducidas. Los efectos negativos de estas invasiones sobre las especies autóctonas y comunidades han sido ampliamente reseñados (Piazzi et al. 2001; Ballesteros et al. 2007; Baldacconi y Corriero 2009; Cabanellas-Reboredo et al. 2010; Deudero et al. 2010; Žuljević et al. 2011; Cebrian et al. 2012; Linares et al. 2012). Sin embargo, teniendo en cuenta que muchas de estas invasiones son relativamente recientes y el amplio número de especies autóctonas afectadas, es necesario que se continúe investigando para poder evaluar correctamente los impactos derivados.

Respuestas de *Cladocora caespitosa* al calentamiento del Mediterráneo

Como ya se ha mencionado, las comunidades bentónicas mediterráneas se han visto afectadas de forma recurrente y durante más de una década, por mortalidades relacionadas con el cambio climático. Los trabajos que hasta la fecha han tratado este tema se pueden clasificar en dos tipologías: estudios de campo que incluyen series temporales relativamente cortas o experimentos de termotolerancia realizados en acuario. Sin embargo, hay una clara falta de estudios de series temporales largas, necesarios para evaluar la resiliencia de estas poblaciones y comunidades. En el **Capítulo IV** hemos evaluado la respuesta a largo plazo de *Cladocora caespitosa* al calentamiento del mar.

Especies algales invasoras y su interacción con *Cladocora caespitosa*

Continuando con los impactos derivados del cambio global, el **Capítulo V** se centra en el estudio de la dinámica de la invasión de dos especies de algas (*Lophocladia lallemandii* y *Caulerpa racemosa*) en la Bahía de L'Illa Grossa, sus posibles impactos sobre la población del coral *Cladocora caespitosa* y en detectar posibles mecanismos de defensa del coral frente a estas algas.

OBJETIVOS

El tema central de la presente tesis es el estudio de la ecología del único coral zooxantelado del Mediterráneo con capacidad para formar arrecifes, *Cladocora caespitosa*, con el objetivo de contribuir a su conservación. Como se menciona en la sección anterior, los objetivos específicos recogidos en la presente tesis cubren desde la caracterización de las bioconstrucciones de *C. caespitosa*, hasta el estudio de la historia vital de la especie y sus respuestas a una de las mayores amenazas que afectan a los ecosistemas mediterráneos costeros, el calentamiento y las invasiones biológicas.

Esta tesis ha sido estructurada en dos partes y sus objetivos específicos se numeran a continuación:

Parte I. Características biológicas y ecológicas de *Cladocora caespitosa*.

- Describir la población de *C. caespitosa* de la Bahía de L'Illa Grossa en términos de distribución espacial, estructura de tallas, morfología de las colonias y crecimiento.
- Aumentar el conocimiento sobre la biología reproductora de la especie a través del estudio de su condición sexual y ciclo reproductor.
- Estudiar las características básicas de su historia vital (tasas de mortalidad natural y reclutamiento), necesarias para evaluar la resiliencia de la especie.

Parte II. Respuestas a las perturbaciones derivadas del Cambio Global.

- Evaluar la respuesta a largo plazo de este coral al calentamiento.
- Estudiar la invasión de las algas alóctonas *Lophocladia lallemandii* y *Caulerpa racemosa* en la Bahía de L'Illa Grossa y su interacción con el coral *C. caespitosa*.

RESULTADOS PRINCIPALES

Capítulo I. Las bioconstrucciones de *Cladocora caespitosa* de la Reserva Marina de las Islas Columbretes: distribución, estructura de tallas y crecimiento

En este capítulo se ha realizado la primera descripción de la población y bioconstrucciones del coral endémico del Mediterráneo *Cladocora caespitosa*, mostrando la relevancia de esta población en el Mediterráneo. Según muestran los resultados de extensión y cobertura obtenidos ($\sim$ 2900 m^2 de superficie ocupada), es, junto con el arrecife del Parque Nacional de Mjlet (Croacia, Mar Adriático, Kružić and Benković 2008), la población más extensa y mejor desarrollada del Mediterráneo.

La población se sitúa en la bahía que conforma la isla L'Illa Grossa, una caldera volcánica semisumergida de origen cuaternario. La Bahía de L'Illa Grossa tiene aproximadamente 150.000 m^2 de área y una profundidad media de 15 m. El fondo de la bahía esta formado principalmente por substrato rocoso con zonas de arena biogénica intercaladas. Los restos de las sucesivas erupciones volcánicas han creado en la bahía crestas sumergidas y fondos de bloques (Aparicio y García 1995). La temperatura del agua del mar en la bahía oscila entre una media de $\sim$ 13 °C en febrero y $\sim$ 26 °C en agosto.

Las colonias de *C. caespitosa* se encuentran sobre los fondos rocosos de la bahía (bloques y crestas, en substrato tanto horizontal como subvertical), en profundidades comprendidas entre los 5 y los 27 m, aunque el 85 % de la superficie ocupada por el coral se localiza entre los 10 y 20 m de profundidad.

Aunque la cobertura media del coral en la bahía es de 1,9 %, en algunas áreas las colonias se concentran alcanzado coberturas de hasta el 80 % (en 5 m^2) y 5,5 colonias m^{-2}. La distribución de las colonias y arrecifes del coral en la bahía es significativamente agregada en una escala de 10 a 50 m (Correlograma de la I de Moran, $p < 0,001$). Las principales zonas de concentración de colonias de *C. caespitosa* se localizan en la zona norte y sur de la bahía, coincidiendo espacialmente con dos de las zonas de mayor relieve de fondo de la bahía (Fig. 6, Capítulo I). En estas zonas se obtuvieron datos de cobertura medios de hasta 7 % y se estima que en las zonas norte y sur de la bahía se concentran 240 m^2 y 910 m^2 de área ocupada por las colonias y arrecifes.

La distribución de tallas de la población muestra el dominio de las clases de talla intermedias, con tallas máximas y mínimas de $\sim$ 150 cm y $\sim$ 2 cm, respectivamente. En cuanto a la morfología de las colonias, el índice de esfericidad Is-Index (= altura diámetro^{-1}) muestra valores medios de $\sim$ 0,4.

El estudio de las tasas de crecimiento de los coralitos a través de radiografías y el marcado de las colonias con rojo de alizarina muestra valores anuales en torno a los 2,5 mm.

Capítulo II. Patrones divergentes en la reproducción sexual del coral escleractinio mediterráneo *Cladocora caespitosa*

El estudio de la reproducción sexual de esta especie en el Mediterráneo Occidental ha puesto de manifiesto importantes diferencias con las características reproductoras descritas en el Parque Nacional de Mjlet (Mar Adriático).

Mientras que en las 5 zonas muestreadas en el Mediterráneo Occidental (Cap de Creus, Islas Medas, Islas Columbretes y Eivissa) esta especie es gonocórica, en el Adriático había sido descrita como hermafrodita (Kružić et al. 2008). Por otra parte, los resultados obtenidos en el estudio histológico de las colonias de las Islas Columbretes han mostrado la presencia de gametos femeninos y masculinos en los pólipos entre marzo-abril y octubre. Los gametos femeninos alcanzaron el tamaño máximo en agosto (~ 100 µm), al igual que el número de de gametos tanto masculinos como femeninos (~ 100 por pólipo en ambos casos). El desarrollo de las gónadas aparece fuertemente ligado al aumento de la temperatura durante el verano (Fig. 3, Capitulo II) y la drástica reducción de las mismas en octubre indica que la liberación ocurre a finales de verano - principios de otoño. Tanto el tamaño máximo de los oocitos como la época de su máximo desarrollo y liberación de gametos difiere drásticamente de lo descrito por Kružić et al. (2008). Sin embargo, *Cladocora caespitosa* comparte características reproductoras (tamaño de oocitos y ciclo gonadal) con *Oculina patagonica* (descrita como especie introducida en el Mediterráneo, Zibrowius 1974).

Capítulo III. Reclutamiento y mortalidad del coral de aguas templadas *Cladocora caespitosa*: conclusiones sobre la recuperación de poblaciones en peligro

Las características de la historia vital de *Cladocora caespitosa*, reclutamiento, crecimiento y mortalidad de los juveniles, y mortalidad de adultos, han sido estudiadas en este capítulo. Los datos obtenidos durante 6 años de estudio muestran la lenta dinámica poblacional de esta especie con tasas anuales de reclutamiento y mortalidad de adultos bajas (~ 1 % y 0,30 reclutas m^{-2}, respectivamente), mientras que la mortalidad de juveniles es relativamente alta (29 % en la clase de talla menor, < 5 pólipos). Las colonias juveniles crecen por reproducción asexual (gemación), siendo este proceso más rápido en las colonias más pequeñas (1,14 gemas $pólipo^{-1}$ $año^{-1}$).

Se encontraron diferencias significativas entre las tasas de reclutamiento en substrato horizontal y subvertical, siendo el reclutamiento mayor en el horizontal. Estas diferencias podrían explicarse mediante los mecanismos de retención de huevos descritos para la especie y a la posible flotabilidad negativa de los huevos.

Los resultados obtenidos están en concordancia con la pausada dinámica que muestran los organismos clonales marinos y con la discordancia entre la abundancia de adultos y las bajas tasas de reclutamiento descrita en la mayoría de arrecifes tropicales. Una elevada longevidad y las bajas tasas de mortalidad amortiguan las bajas tasas de reclutamiento, por lo que este bajo reclutamiento es capaz de mantener altas abundancias de adultos. Sin embargo, en el caso de darse mortalidades catastróficas y recurrentes, como las sufridas por la población de *C. caespitosa* de Columbretes, las tasas de reclutamiento no son suficientes para hacer frente a la pérdida masiva de adultos. En general, la lenta dinámica de *C. caespitosa* y el bajo potencial de recuperación a través del reclutamiento hacen a esta especie más vulnerable frente a mortalidades catastróficas.

Capítulo IV. Repuestas a largo plazo del coral endémico y bioconstructor *Cladocora caespitosa* al calentamiento del Mediterráneo

Este capítulo se analizaron las series de datos de necrosis en *Cladocora caespitosa* y temperatura del agua del mar (11 y 21 años respectivamente) y los resultados obtenidos han permitido describir, por primera vez, la relación entre episodios recurrentes de mortalidad y el régimen térmico local del agua del mar.
Los episodios de mortalidad se han registrado en la población de *C. caespitosa* de L'Illa Grossa tras 11 veranos separados en dos periodos de mortalidad 2003-2006 y 2008-2012. La muerte de los pólipos se ha caracterizado en todos los episodios por una necrosis progresiva del tejido que ocurre a finales del verano. A nivel de colonia, la muerte de los pólipos se ha registrado o bien de forma parcial en manchas de pólipos adyacentes o en la totalidad de la colonia. Cabe destacar que durante estas afecciones masivas no se han registrado nunca procesos de expulsión masiva de zooxantelas o blanqueamiento. Durante el estudio no se registró ni la recuperación de las partes necrosadas, ni un aumento de las mismas una vez finalizados los eventos de mortalidad tras el verano.

El episodio de mortalidad registrado tras el verano de 2003, verano que ha sido descrito como probablemente el más cálido en Europa desde 1500 (Lutherbacher et al. 2004), provocó ~ 25 % de necrosis media en la población estudiada. Los 2 veranos siguientes, 2004 y 2005, presentaron anomalías térmicas positivas moderadas, sin embargo se registraron episodios de mortalidad importantes con valores medios de necrosis de ~ 19 % y ~ 13 %, respectivamente. El último verano de este primer periodo de mortalidad, 2006, registró anomalías térmicas positivas de magnitud importante que desencadenaron valores altos de necrosis media (~ 19 %). El verano de 2007, con anomalía térmica negativa y sin necrosis en el coral, separa los periodos de mortalidad estudiados. El siguiente periodo de mortalidad (2008 – 2012) se caracterizó por la relativamente baja magnitud de los valores de necrosis (entre 2 % y 6 % de necrosis media). Los veranos de este segundo periodo, a excepción del 2009, se caracterizaron por anomalías térmicas positivas pero de intensidad moderada. Sin embargo, la anomalía térmica positiva del 2009, es equiparable a la registrada previamente en 2006. Ambos periodos registraron valores de necrosis y anomalías térmicas muy diferentes (~ 19 % vs. 4 % y 1 °C vs. 0,4 °C). Se estima que la superficie necrosada que se ha acumulado tras estas mortalidades recurrentes oscila entre el 55 % y el 80 %.

En cuanto a la serie de temperatura superficial del agua de mar (SST) cabe destacar que, durante el periodo estudiado (1991 – 2012), se ha registrado una tasa de aumento anual de 0,04 °C y una tasa de aumento estival de 0,06 °C. La frecuencia de veranos con anomalía térmica positiva ha aumentado de forma importante a lo largo de este periodo de tiempo; si se comparan la década de 1990 con la del 2000, esta frecuencia se ha visto multiplicada por 4.

Durante la totalidad del periodo estudiado los valores de necrosis y anomalía térmica del agua mostraron una correlación positiva y significativa. Sin embargo, al analizar la correlación de estas variables en los dos periodos de mortalidad por separado se observa que la correlación se mantiene durante el primer periodo pero se pierde durante el segundo. Algo parecido ocurre si se analiza la correlación entre necrosis y el tiempo en que ha permanecido el agua por encima de cierto umbral de temperatura. En este caso, la correlación durante todo el periodo fue significativa solamente para los umbrales más cálidos (27 °C y 28 °C), mientras que durante el primer periodo la correlación fue significativa con umbrales más templados (24 °C y 26 °C). La correlación se pierde si se analiza únicamente el segundo periodo. Estos resultados muestran, por una parte, la clara influencia de veranos anormalmente cálidos en la necrosis registrada tras los veranos siguientes, aunque sus anomalías térmicas sean mucho más moderadas. Lo que explicaría que veranos con anomalías térmicas similares registren valores de necrosis tan dispares, por ejemplo, 2004 y 2012 que presentan anomalía térmica estival de ~ 0,40 °C mientras que presentan valores respectivos de necrosis de ~ 19 % y 3 %. Por otra parte, la similitud en los valores de necrosis registrados entre colonias que previamente habían sufrido o no mortalidad, descarta que procesos de adaptación o aclimatación puedan tener un papel importante en la variabilidad de la necrosis.

Capítulo V. Algas invasoras en la Reserva Marina de las Islas Columbretes: dinámica de la invasión e interacción con el coral endémico *Cladocora caespitosa*

Este capítulo se analiza la información obtenida durante 6 años de seguimiento de la invasión de las algas alóctonas *Lophocladia lallemandii* y *Caulerpa racemosa* en la Bahía de L'Illa Grossa y su interacción con la población del coral *Cladocora caespitosa* que se localiza en este enclave.

Ambas especies invasoras fueron detectadas por primera vez en la Bahía de L'Illa Grossa en 2006. A partir de ese momento el ritmo de invasión fue muy diferente entre ambas especies. Mientras que *L. lallemandii* tuvo una expansión muy rápida, pasando en un solo año a estar presente en toda la bahía, la invasión por parte de *C. racemosa* mostró una evolución más pausada aunque continua. Los datos de mayor abundancia para ambas especies se registraron en el rango batimétrico 5 – 10 m en el año 2011 para *L. lallemandii* (~ 82 %), en el rango 10 – 20 m en el mismo año para *C. racemosa* (~ 58 %).

Mientras que *L. lallemandii* solapó su distribución con la población del coral *C. caespitosa* al principio de su invasión en 2007, *C. racemosa* lo hizo a partir de 2010. Ambas especies algales tienen un crecimiento muy diferente sobre el substrato, mientras que *L. lallemandii* crece mayoritariamente epífita sobre algas autóctonas, los estolones de *C. racemosa* crecen directamente sobre el substrato. Es decir, *L. lallemandii* aparece sobre las colonias de *C. caespitosa* como epífita sobre cualquier fragmento de alga que pueda existir en los huecos entre los pólipos, mientras que el crecimiento de *C. racemosa* sobre las colonias es en contacto directo con su superficie.

No se detectaron efectos letales ni subletales en el coral *C. caespitosa* derivados de la presencia de ambas especies algales. Sin embargo, se detectó que *C. racemosa* crecía sobre las partes muertas de las colonias del coral, pero evitaba de forma significativa las partes vivas. Los resultados obtenidos mediante el bioensayo Microtox® mostraron que los pólipos de *C. caespitosa* presentan valores de toxicidad equiparables a los encontrados en otros organismos bentónicos bioactivos, lo que podría explicar el patrón de invasión de las colonias del coral por parte de *C. racemosa*.

No se puede descartar la presencia de efectos negativos en otras fases del ciclo vital de *C. caespitosa*, como por ejemplo durante los procesos de reclutamiento o en la supervivencia de los juveniles.

DISCUSIÓN Y CONCLUSIONES

Las reservas marinas están reconocidas como una de las principales herramientas para la conservación de la biodiversidad y la gestión pesquera (Gell y Roberts 2003; Lubchenco et al. 2003; Micheli et al 2004). Su creación es una de las pocas herramientas de gestión disponibles para combatir los efectos negativos del cambio global sobre los ecosistemas marinos, aunque éste no sea su objetivo original. Sin embargo, pese a que existe la esperanza de que las reservas marinas puedan ser útiles en esta tarea, no existen muchas evidencias empíricas. Durante los últimos años algunos estudios han informado sobre la incidencia de impactos derivados del cambio climático en reservas marinas (Graham et al. 2008; Côté y Darling 2010; Mora y Sale 2011), mientras que solamente unos pocos trabajos han mostrado que estas reservas pueden aumentar la resiliencia de los organismos frente a los impactos derivados del cambio climático (Micheli et al 2012; Mumby et al 2013). Por tanto no es sorprendente que exista actualmente un intenso debate sobre el hecho de si las reservas marinas pueden favorecer la resiliencia de las poblaciones y ecosistemas marinos (Graham et al. 2008; Côté y Darling 2010; Hughes et al. 2010; Mora y Sale 2011; Micheli et al 2012).

El cambio global está alterando los hábitats marinos mediterráneos (Occhipinti-Ambrogi 2007; Lejeusne et al. 2010; Zenetos et al. 2012) y los impactos derivados del calentamiento y la introducción de especies invasoras están afectando grandes áreas geográficas. Las áreas marinas protegidas (AMP) mediterráneas están siendo significativamente afectadas por estos impactos (por ejemplo, Linares et al. 2005; Garrabou et al 2009; Cebrian y Ballesteros 2009; Coll et al. 2010; De Caralt y Cebrian 2013) y están fracasando en la protección de las especies en este contexto. Un claro ejemplo de esta problemática es *Cladocora caespitosa* en la Reserva Marina de las Islas Columbretes. Esta AMP es ampliamente reconocida por su estado de conservación (Templado y Calvo 2002) y por su efectividad en la conservación de especies comerciales (Goñi et al. 2006, 2010; Stobart et al. 2009; Díaz et al. 2011). Sin embargo, incluso estas AMPs bien protegidas y gestionadas, no están cumpliendo su cometido a la hora de proteger especies y comunidades frente a las nuevas amenazas derivadas del cambio global; lo que pone de manifiesto la urgente necesidad de establecer nuevas medidas de protección y gestión. Aún así, hay que remarcar que estas áreas protegidas están sirviendo como laboratorios privilegiados para el seguimiento a largo plazo de estas especies y comunidades, aportando el conocimiento necesario para evaluar la amplitud del problema y para mejorar las medidas de conservación.

Los resultados de esta tesis muestran la baja efectividad de las medidas de conservación actuales a la hora de proteger a las especies más frágiles frente a estas nuevas amenazas; pero lo más alarmante es que no sabemos como afrontar este nuevo reto para la conservación más allá de las medidas globales (como por ejemplo la reducción de las emisiones de CO_2), que dependen del complejo panorama político internacional y que no se están aplicando con el ritmo necesario. No obstante, se necesita un amplio conocimiento sobre este problema para poder implementar cualquier tipo de acción. El principal objetivo de esta tesis ha sido contribuir información sobre una especie tipo, de manera que el esquema de los resultados obtenidos pueda servir para establecer medidas de conservación para ésta y otras especies de características similares.

ADQUIRIR CONOCIMIENTOS CON EL FIN DE PROMOVER LA CONSERVACIÓN

La ecología de los arrecifes de corales tropicales ha sido intensamente estudiada durante décadas (Goreau 1959; Connell et al. 1997, como ejemplos de algunos de los primeros estudios y de mayor duración). Este interés científico es coherente con la importancia de los arrecifes de coral en los mares tropicales, por su abundancia, importancia ecológica e incluso su importancia en términos económicos. En comparación, los corales de aguas templadas son mucho menos abundantes y estudiados. Mientras que los arrecifes formados por corales zooxantelados tienen una distribución testimonial en los mares templados como el Mediterráneo, el registro fósil indica que estos arrecifes dominaban los ecosistemas costeros durante las épocas tropicales y subtropicales (Esteban 1996; Aguirre y Jiménez 1998). Este es el caso del coral endémico *Cladocora caespitosa*, el único ejemplo de coral escleractinio, zooxantelado y bioconstructor en el Mediterráneo actual. Siendo una especie de características tan excepcionales y que, además, se está viendo seriamente amenazada por distintos impactos en la actualidad, sería de esperar cierto conocimiento sobre su ecología y biología básica. El objetivo de esta tesis se ha centrado en aportar este tipo de información, de gran necesidad en el contexto actual. No sólo para entender el proceso de degradación que están sufriendo las poblaciones y arrecifes de *C. caespitosa*, sino para poder evaluar los impactos que están afectando a las comunidades bentónicas en un Mediterráneo sometido a importantes y rápidos cambios. Esta información es la base para establecer las medidas de conservación de esta especie.

Las bioconstrucciones de *Cladocora caespitosa* en la Bahía de L'Illa Grossa (Islas Columbretes): plasticidad ecológica y relevancia en el Mar Mediterráneo

Mientras que *Cladocora caespitosa* formó arrecifes de gran extensión tras la Crisis del Messiniense (Aguirre y Jiménez, 1998; Esteban 1996), en la actualidad los arrecifes de este coral son poco frecuentes y se localizan en unas pocas zonas del Mediterráneo. Poblaciones y/o bioconstrucciones de este coral han sido descritas en poco más de 10 localidades en el Mediterráneo (Fig. 8, Capitulo I). En este contexto, la población de *C. caespitosa* de la Bahía de L'Illa Grossa posee características excepcionales en cuanto a cobertura y extensión. El arrecife de *C. caespitosa* de mayor tamaño descrito hasta la fecha se localiza en el Parque Nacional de Mjlet (650 m^2 de área, Croacia, Adriático, Kružić and Benković 2008). Mientras que en Mjlet *C. caespitosa* forma un arrecife continuo, resultado de la fusión e inclusión de colonias satélite (Kružić y Benković 2008), en Columbretes las colonias forman una distribución mixta de campos de colonias aisladas y pequeños arrecifes. Aunque la distribución de las colonias es diferente en ambos casos, el área estimada que ocupan las bioconstrucciones de este coral en la Bahía de L'Illa Grossa (2900 m^2) es cuatro veces mayor que la descrita en el arrecife de Mjlet.

Las condiciones óptimas para el desarrollo de grandes poblaciones y bioconstrucciones de este coral parecen estar relacionadas con los siguientes factores físico-químicos: protección frente al hidrodinamismo, turbidez (alta concentración de nutrientes), aportes de agua de origen kárstico o aluvial (con efectos sobre la temperatura del agua) y la presencia de corrientes que permiten un gran intercambio de agua. Estos factores son habituales en el registro fósil de *C. caespitosa* (Aguirre y Jiménez 1998; Peirano et al. 2004, 2009) y están presentes en el arrecife del Parque Nacional de Mjlet (Kružić y Benković 2008). Sin embargo, en la Bahía de L'Illa Grossa, algunos de estos factores están ausentes. Aunque la bahía y la morfología de su fondo ofrecen cierta protección frente al hidrodinamismo, el oleaje y corrientes de este y noreste generados durante las grandes tormentas de otoño e invierno, afectan de manera significativa al interior de la bahía. Por lo que, seguramente, el desarrollo de un arrecife de gran extensión en la bahía está limitado por las condiciones hidrodinámicas, que puntualmente pueden ser de gran energía (Fig. 1, Discusión General de la tesis). Como se ha comentado, los aportes de agua continental aseguran nutrientes y una menor temperatura del agua. La temperatura media óptima para el crecimiento de los coralitos de este coral ha sido establecida en $\sim$ 17 °C y tanto en el caso de los arrecifes fósiles como en los actuales, estas suaves temperaturas resultan generalmente del aporte de agua continental (Peirano et al. 2009). Las Islas Columbretes no reciben aporte directo de aguas continentales (están localizadas a 60 km de la costa continental más cercana) y la temperatura media del agua es de ~ 19 °C (ver Fig. 6 en el Capítulo IV para información sobre el ciclo anual de temperatura). De acuerdo con estos datos de temperatura, las tasas de crecimiento que se han obtenido para *C. caespitosa* en Columbretes se encuentran en la parte inferior del rango de tasas de crecimiento obtenidas en otras zonas del Mediterráneo (Tabla 1 del Capítulo I). Pese a que algunas de estas "condiciones óptimas" están ausentes en la Bahía de L'Illa Grossa, el equilibrio entre la protección general frente al hidrodinamismo y el intercambio de agua con mar abierto han permitido, seguramente junto con otros factores como la plasticidad ecológica de esta especie, el desarrollo de la extensa población de *C. caespitosa*.

Mientras que algunos de los factores que facilitan el desarrollo de *C. caespitosa* están presentes en la bahía, las macroalgas, consideradas como limitantes para el desarrollo de este coral, abundan en la zona (Peirano et al. 1998; Rodolfo-Metalpa et al. 1999). La cobertura algal en la Bahía de L'Illa Grossa es significativamente alta (Templado y Calvo 2002) y la mayoría de las colonias del coral se localizan en la comunidad infralitoral de algas fotófilas. Este hecho refuerza la gran plasticidad ecológica de este coral, que es capaz de vivir en ambientes muy distintos.

Los arrecifes de coral se encuentran típicamente en aguas oligotróficas que presentan muy baja variabilidad; mientras que las aguas del Mediterráneo, aunque son oligotróficas, muestran una gran variabilidad (Zabala y Ballesteros 1989). Esta diferencia ha sido sugerida como uno de los principales factores que dificulta el desarrollo de arrecifes de coral en el Mediterráneo (Zabala y Ballesteros 1989). No obstante, *C. caespitosa* parece haberse adaptado a esta variabilidad, mostrándose como la excepción a la regla (un coral zooxantelado y formador de arrecifes en el Mediterráneo). Este hecho está seguramente relacionado con la plasticidad ecológica que muestra este coral, inusual en una especie longeva, ya que este tipo de organismos suelen estar sometidos a condiciones ambientales relativamente estables. La capacidad de esta especie para vivir en ambientes muy distintos y para soportar la variabilidad Mediterránea (estacionalidad), está probablemente relacionada con su capacidad para regular la alimentación autótrofa y heterótrofa dependiendo de las condiciones ambienales de luz, como ha sido sugerido por Hoogenboom et al. (2010). Además, recientemente Ferrier-Pagès et al. (2013) han demostrado que este coral es capaz de optimizar la autotrofía en condiciones de alta iluminación sin sufrir fotoinhibición. En conjunto, este coral posee las características típicas de los corales formadores de arrifes pero a la vez está adaptado en gran medida a la estacionalidad del Mediterráneo, siendo capaz de vivir en condiciones extremas de iluminación.

Uno de los resultados más sorprendentes de esta tesis son las importantes diferencias que se han encontrado en la condición sexual, ciclo gonadal y tamaño de oocitos entre las zonas muestreadas del Mediterráneo Occidental y la información publicada sobre esta especie en el Mar Adriático (Schiller 1993; Kružić et al. 2008b). Aunque estas diferencias podrían reflejar la plasticidad de esta especie y se debe tener en cuenta que los corales muestran una gran plasticidad durante su ciclo vital (Richmond y Hunter 1990), una divergencia tan significativa en las características reproductoras de una misma especie es extremadamente inusual. Estos resultados tan dispares evidencian la necesidad de realizar análisis genéticos en las poblaciones de estas dos zonas del Mediterráneo, con el objetivo de detectar una posible divergencia genética entre estas poblaciones de *C. caespitosa.* De todos modos, tratándose de una especie de lento crecimiento y elevada longevidad, esta plasticidad es muy insólita y por tanto pensamos que la información aportada desde el Mar Adriático debería ser revisada en profundidad. Por otra parte, las características reproductoras de *C. caespitosa* en el Mediterráneo Occidental han mostrado ser similares a las descritas para el coral *Oculina patagonica* (descrita como especie introducida en el Mediterráneo, Zibrowius 1974). Estas especies han sido agrupadas recientemente en la misma familia (Oculinidae, Fukami et al. 2008) lo que podría explicar esta similitud. El ciclo gonadal y la época de liberación de gametos observada en las Islas Columbretes tiene unas consecuencias positivas para la viabilidad de esta especie. Aunque la abundancia algal es muy alta en la bahía, la mayoría de las especies tienen un ciclo estacional muy marcado y tanto la abundancia como la cobertura varían de forma significativa entre el principio del verano y el otoño, cuando la mayoría de las macroalgas desaparecen o reducen su cobertura. Por lo tanto, las larvas deberían tener mayor acceso al substrato en otoño que en verano. Esta podría ser una ventaja importante para este coral en localidades como la Bahía de L’Illa Grossa, donde la especie convive con especies muy competitivas como las diferentes especies de algas fotófilas.

Por otra parte, aún quedan fases del ciclo reproductor de esa especie que permanecen desconocidas. Por ejemplo, existe un desconocimiento absoluto de los procesos que ocurren tras la liberación de los gametos, como por ejemplo el comportamiento de la plánula. Sin embargo, Kružić et al. (2008b) describieron que los huevos de este coral eran liberados embebidos en un mucus que los mantiene unidos y que, seguramente, facilita su fertilización y el asentamiento de la larva en la cercanía de las colonias parentales. En el Capitulo III se muestra como las tasas de reclutamiento en substrato horizontal son mayores que en el vertical, lo que podría ser indicativo de la flotabilidad negativa de los cúmulos de huevos y de las plánulas. La distribución agregada del coral en la bahía, tal y como se describe en el Capítulo I, también apunta a este mecanismo. A falta de estudios específicos, con la información disponible se puede concluir que la capacidad de dispersión de la especie es muy baja. Como ejemplo, mientras la Bahía de L'Illa Grossa alberga una de las poblaciones más importantes del Mediterráneo, en el resto del archipiélago de las Columbretes y pese a la existencia de substrato adecuado, la especie está prácticamente ausente.

La resiliencia de *Cladocora caespitosa* en un mar cambiante

La serie de registro de necrosis y temperatura del agua de esta tesis es la más larga estudiada hasta la actualidad en las comunidades bentónicas mediterráneas. Este estudio ha permitido por primera vez la descripción de la relación entre los eventos de mortalidad recurrentes y los registros de temperatura. Durante los últimos 15 años *Cladocora caespitosa*, al igual que otros organismos bentónicos, ha sufrido mortalidades recurrentes relacionadas con el cambio climático (Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009). En las Islas Columbretes se han registrado eventos de necrosis en este coral tras 9 veranos durante el periodo comprendido entre 2002 y 2012. Durante este periodo más de la mitad de la cobertura del coral en la Bahía de L'Illa Grossa ha sufrido necrosis. La serie de temperatura del agua estudiada ha mostrado que las anomalías térmicas positivas han multiplicado su frecuencia por cuatro si se compara el periodo entre 1991 y 2001 con los 11 años siguientes (2002-2012) (Fig. 4, Capitulo IV), a la vez que una tendencia de calentamiento estival de 0,06 °C anuales.

Las tasas de necrosis más altas se registraron durante el primer periodo de mortalidad, desde 2003 (probablemente el verano más caluroso en Europa desde 1500, Luterbacher et al. 2004) hasta 2006. Por lo que no solo los veranos fueron anormalmente cálidos, sino que además aumentaron su frecuencia. Los resultados obtenidos muestran que veranos extremadamente calurosos, como el de 2003, tienen una gran influencia en los niveles de necrosis registrados en los veranos siguientes, aunque sus anomalías térmicas positivas fueran más moderadas. De hecho, veranos con anomalías térmicas similares pero pertenecientes a cada uno de los periodos de mortalidad registraron valores de necrosis muy diferentes, siendo mayores lo valores registrados en los veranos que pertenecen al primer periodo. La aleatoriedad en la ocurrencia de la necrosis de las colonias entre los distintos años de mortalidad apunta a que procesos relacionados con la adaptación o selección no han actuado de forma significativa en estos eventos. Sin embargo, otros procesos que actúan de manera más azarosa, como el estrés fisiológico retardado o infecciones de patógenos termodependientes, podría haber tenido una influencia mayor en las mortalidades.

Los modelos climáticos predicen que el Mar Mediterráneo se encontrará entre las regiones más afectadas por el calentamiento y el aumento de eventos extremos (Déqué 2007; Diffenbaugh et al. 2007; IPCC 2013, Fig. 2, Discusión General de la tesis). Por lo tanto, la probabilidad de que veranos extremos, como el de 2003, aumenten en frecuencia es muy alta. Si se tienen en cuenta los resultados obtenidos en este estudio, estos veranos extremos y recurrentes podrían diezmar la población de *C. caespitosa* de la Bahía de L'Illa Grossa y afectar negativamente a otras poblaciones de esta especie. Por otra parte, si los veranos muestran anomalías positivas pero moderadas y no vienen acompañados por eventos extremos, los valores de necrosis esperados serían menores.

En cuanto al impacto derivado de las especies invasoras, *Lophocladia lallemandii* y *Caulerpa racemosa* fueron detectadas por primera vez en Columbretes en 2006 en la entrada de la Bahía de L'Illa Grossa. En 2012 ambas especies habían invadido la totalidad de la bahía, aunque con ritmo y variabilidad diferente. Durante el periodo de estudio ambas especies solaparon su distribución con la del coral *C. caespitosa*. El 70 % de las colonias de este coral se vieron afectadas (es decir, invadidas de alguna manera) por *L. lallemandii* y un 30 % por *C. racemosa*. Mientras *L. lallemandii* crece epífita en las algas autóctonas, *C. racemosa* muestra un crecimiento rizofítico y reptante, en contacto directo con el coral. Cabe destacar que se ha detectado actividad tóxica en *C. caespitosa*, con valores comparables a los descritos para otros invertebrados bentónicos bioactivos, lo que explicaría el patrón de invasión de las colonias del coral mostrado por *C. racemosa*. Este mecanismo podría ayudar a mantener las partes vivas de las colonias fuera del alcance de la invasión algal, evitando efectos negativos directos como la asfixia o el aumento de la sedimentación. Los procesos de competencia entre algas y corales son frecuentes en los arrecifes de corales tropicales (McCook et al. 2001) y existen ejemplos de éxito tanto de algas como de corales; en este último caso por inhibición del crecimiento algal por parte de los corales (Vermeij et al. 2010; McCook 2001). El patrón de invasión de las colonias por parte de *C. racemosa* y la abundancia del coral *C. caespitosa* pese a la gran cobertura algal (algas autóctonas) en la bahía, muestran que este coral es capaz de competir exitosamente en una comunidad dominada por las algas.

Aunque no se detectaron efectos letales y subletales en *C. caespitosa* derivados de la invasión algal, no se puede descartar la ocurrencia de efectos negativos sobre otras fases del ciclo vital de este coral, como sobre el reclutamiento o la supervivencia de juveniles, tal y como se ha descrito en otros invertebrados bentónicos (Arnold et al. 2010; Cebrian et al. 2012; Linares et al. 2012). Se debe tener en cuenta que *C. caespitosa* se reproduce en otoño coincidiendo con la época en la que las dos especies invasoras ocupan grandes áreas de substrato en la bahía.

Viabilidad y conservación de la población de *Cladocora caespitosa* de la Bahía de L'Illa Grossa

Cladocora caespitosa muestra una importante plasticidad ecológica, principalmente en lo que se refiere a las condiciones de luz ambiental, siendo capaz de vivir en diferentes tipos de hábitat, comunidades y a distintas profundidades. Esta plasticidad es una ventaja en un medio cambiante y diverso, como en el que se establecen las comunidades infralitorales someras, dónde ocurren cambios importantes en la intensidad lumínica a escalas temporales y espaciales pequeñas. No obstante, mientras las comunidades que albergan a este coral están generalmente sujetas a cambios en las condiciones reinantes de luz e hidrodinamismo, el cambio climático está provocando condiciones de temperatura extremas, especialmente en verano, a las que este tipo de organismos no están adaptados. Por lo tanto, aunque *C. caespitosa* muestra una gran plasticidad ecológica en relación con ciertos cambios medioambientales frecuentes en su hábitat, ha mostrado ser extremadamente sensible y vulnerable al aumento de la temperatura del agua.

Mientras que los efectos del calentamiento global han sido típicamente estudiados en especies que viven en medios mucho más estables (por ejemplo, *Paramuricea clavata* o *Corallium rubrum*), los resultados presentados en esta tesis muestran que especies que habitan en medios más someros y dinámicos se están viendo afectadas de igual manera por estos cambios.

Doce años de muestreo continuo han mostrado que el aumento de la temperatura del Mar Mediterráneo puede considerarse como la amenaza más preocupante para la población de *C. caespitosa* de las Islas Columbretes en particular, pero también a nivel mediterráneo (Rodolfo-Metalpa et al. 2005; Garrabou et al. 2009). *Cladocora caespitosa* es una especie de dinámica lenta, por lo que no se espera que pueda adaptarse rápidamente a las nuevas condiciones. Por lo tanto, dadas las predicciones climáticas que auguran la continuidad del actual proceso de calentamiento (IPCC 2013) y la vulnerabilidad mostrada por *C. caespitosa*, la probabilidad de que las poblaciones de este coral puedan sobrevivir a estos cambios parece baja. Además, esta amenaza viene acompañada por los cambios ecológicos y ambientales provocados por la invasión de algas alóctonas. Las mortalidades relacionadas con el cambio climático han reducido a la mitad la cobertura de *C. caesptiosa* en Bahía de L'Illa Grossa y, aunque no se han detectado efectos letales y subletales derivados de la invasión algal, no se puede descartar que el reclutamiento y la supervivencia de los juveniles puedan verse afectados.

La recuperación de las poblaciones de coral sujetas a mortalidades depende altamente del reclutamiento (Connell et al. 1997; Hughes et al. 2000; Adjeroud et al. 2007; Coles y Brown 2007). Es muy probable que la recuperación de *C. caespitosa* a través del reclutamiento se vea obstaculizada por la alta frecuencia de las mortalidades sufridas, que seguramente sobrepasa la capacidad de recuperación derivada de las bajas tasas de reclutamiento y, además, tanto el calentamiento como las algas invasoras podrían tener efectos negativos sobre la reproducción, el reclutamiento y la supervivencia de los juveniles. Adicionalmente, la población de la Bahía de L'Illa Grossa tiene probablemente una alta dependencia del autoreclutamiento, dado su aislamiento geográfico y la seguramente baja capacidad de dispersión de la especie. No obstante, *C. caespitosa* era tan abundante en la bahía que incluso tras las mortalidades sufridas, la cobertura y densidad de colonias mantienen valores relativamente altos, lo que juega a favor de su recuperación. Por otra parte, el 20 % de las colonias de la bahía no se han visto afectadas por las mortalidades, por lo que podrían ser representantes de genotipos más resistentes al aumento de la temperatura.

MEDIDAS DE CONSERVACIÓN

Seguimiento

El seguimiento de *Cladocora caespitosa* (y especies similares) y de las variables ecológicas y ambientales sometidas a cambios (por ejemplo, temperatura, invasiones biológicas, pH) debe continuar. Los resultados obtenidos en esta tesis muestran la complejidad de los posibles factores que actúan en estas mortalidades, por lo que un seguimiento bien diseñado, regular y a largo plazo, es fundamental para entender el funcionamiento del ecosistema y las respuestas de estas especies longevas a los rápidos cambios que están sucediendo. El problema principal de este tipo de seguimientos es que necesitan un alto nivel de dedicación y una financiación regular que pueda asegurar su continuidad; factores que no siempre son fáciles de asegurar. Por lo tanto, es de gran importancia que los gestores y políticos responsables reconozcan la necesidad y el valor de los seguimientos a largo plazo.

En el caso de *C. caespitosa*, el seguimiento de sus poblaciones y de los raros arrecifes a lo largo y ancho del Mediterráneo es esencial para evaluar el estatus de la especie y su evolución temporal. Pero antes cabe identificar y describir estas poblaciones.

Minimizar los impactos controlables

La resiliencia de *Cladocora caespitosa* se puede ver favorecida si se minimizan aquellos impactos que son controlables a nivel de gestión local, como los impactos derivados de la contaminación directa o daños físicos provocados por anclas o artes de pesca, así como la conservación de las comunidades en las que habita. En este sentido, aunque las áreas marinas protegidas no pueden proteger a estas especies de los impactos derivados del cambio global, sí pueden ser de gran ayuda en la tarea de limitar los impactos derivados de la actividad humana y, por lo tanto, mejorar la resiliencia de estas especies. Por lo que, incluso en el interior de las áreas marinas protegidas, las actividades permitidas deben ser revisadas para asegurar el nivel de protección necesario. Por ejemplo, en el caso de las Islas Columbretes y *C. caespitosa*, pese a que la población del coral está protegida por la reserva marina, las colonias se encuentran en la zona más frecuentada de la reserva marina. Una media anual de ~ 1800 barcos amarran en las boyas de la bahía y ~ 1900 inmersiones se realizan anualmente en este enclave (Kersting 2002-2011). Y aunque no se han detectado impactos directos de estas actividades, como por ejemplo del buceo, es altamente probable que esta gran afluencia de embarcaciones haya sido el vector de introducción de las algas invasoras que están modificando las comunidades en la bahía. Por lo tanto, las medidas de protección deben dirigirse de forma preventiva a evitar cualquier tipo de impacto adicional que pueda empeorar la ya complicada situación de las especies afectadas.

Estatus de especie en peligro

Los resultados de esta tesis muestran que *Cladocora caespitosa* y sus bioconstrucciones se encuentran seriamente amenazadas. En general, las grandes colonias de coral, centenarias en muchos casos, han sido descritas como prácticamente *inmortales* porque una vez han llegado a alcanzar cierto tamaño la probabilidad de muerte se reduce casi a cero (Hughes y Jackson 1985). Este tipo de colonias han sobrevivido eventos extremos durante siglos y, sin embargo, en el caso de *C. caespitosa* están desapareciendo a un ritmo sin precedentes debido al escenario actual de cambio climático. No obstante, *C. caespitosa* está ausente en la mayoría de *Listas Rojas* tanto a nivel nacional como internacional. A partir de los conocimientos adquiridos sobre *C. caespitosa*, proponemos su inclusión en las listas de protección, no solo por su frágil situación actual y valor ecológico, sino también por el gran valor patrimonial de sus bioconstrucciones centenarias. Este coral aparece clasificado en la *Lista Roja de Especies Amenazadas* de la IUCN como *Falta de Información*, pero que podría ser incluida en alguna de las categorías de especies amenazadas si se conociera más información. Por tanto, desde la UICN se recomienda la investigación sobre la ecología de la especie

(IUCN, http://discover.iucnredlist.org/species/133142). Con el conocimiento adquirido es muy probable que *C. caespitosa* cumpla los criterios para ser clasificada como especie *En Peligro* (*Endangered*). La IUCN considera: *Un taxón está en peligro cuanto las evidencias disponibles indican que pueda cumplir cualquiera de los criterios (del A al E) de la categoría En Peligro* (IUCN 2012).

La información sobre el estado en el que se encuentran otras poblaciones de *C. caespitosa* es muy escasa, por lo que aquí los criterios de la UICN se aplicarán, a modo de ejemplo según el conocimiento actual sobre los arrecifes de esta especie en el Mediterráneo. Es importante tener en cuenta que se trata de una especie bioconstructora, que bajo condiciones óptimas forma campos de colonias y arrecifes de distinto tamaño (Kružić and Benković 2008), por lo que estas formaciones deberían considerarse como indicadores del buen desarrollo de la especie.

Cladocora caespitosa cumple al menos los criterios A.2. y A.4. relativos a la reducción del tamaño de las poblaciones (tomando como ejemplo la población de Columbretes) y los criterios B.1.a. y B.1.b. relativos al rango geográfico de la especie. Para más detalle sobre estos criterios: http://www.iucnredlist.org.

Cabe destacar que la especie entraría en esta categoría si cumple uno solo de estos criterios. Por las mismas razones esta especie debería ser incluida en las diferentes listas de protección a nivel nacional, como es el caso del *Catálogo Nacional de Especies Amenazadas*.

Medidas globales contra las nuevas amenazas

Desde un enfoque más amplio, tanto geográfica como ecológicamente, son necesarias nuevas medidas para hacer frente a la tasa actual de destrucción ecológica. Dadas las predicciones de los modelos climáticos para las próximas décadas (IPCC 2013) y el conocimiento actual sobre sus efectos sobre las comunidades marinas, cabe esperar que las consecuencias sean catastróficas si no se toman medidas correctoras.

La medida más importante e inmediata a este nivel es la reducción de las emisiones de CO_2 y otros gases de efecto invernadero, ya que son los principales responsables del calentamiento (IPCC 2013). No obstante, el ritmo de reducción que se está aplicando a estas emisiones está muy lejos de ser efectivo, teniendo en cuenta que incluso aunque las emisiones se redujeran a cero, una gran parte del cambio climático derivado de estos gases ya es irreversible a una escala de siglos a milenios (IPCC 2013).

En cuanto a nuevas invasiones biológicas, estas podrían verse significativamente reducidas aplicando medidas más estrictas por ejemplo a las actividades de transporte marítimo, ya que se consideran como uno de los principales vectores de introducción de especies (Klein y Verlaque 2008). Sin embargo, otros vectores de introducción como la conexión artificial entre mares, como por ejemplo el Canal de Suez, son mucho más difíciles de controlar. La mayoría de las invasiones actuales son muy difíciles o incluso imposibles de contener y es muy improbable que las comunidades autóctonas puedan recuperar el estado previo a la introducción de estas especies invasoras. Por lo tanto, las únicas medidas prácticas en este caso se pueden resumir en: primero evitar nuevas invasiones y segundo realizar un seguimiento periódico para poder evaluar la evolución de las invasiones existentes y los impactos derivados sobre las especies y comunidades autóctonas.

La dificultad que supone implementar estas medidas globales no debería desalentar la adopción de medidas de escala geográfica menor, que necesitan ser desarrolladas en paralelo.

FUTURAS LÍNEAS DE INVESTIGACIÓN

El estudio de la ecología de esta especie acaba de empezar. Durante la elaboración de esta tesis han surgido muchas preguntas interesantes que deberían ser abordadas en un futuro cercano. Investigar sobre los aspectos que vienen a continuación es clave para continuar avanzando en el conocimiento de la ecología de *Cladocora caespitosa* y en su conservación.

Reproducción asexual de *Cladocora caespitosa*

Aunque existe información sobre el crecimiento asexual de las colonias por gemación (Rodolfo-Metalpa et al. 2008; presente tesis), hay una falta de conocimiento total sobre otros posibles mecanismos de reproducción asexual en esta especie. Los estudios genéticos realizados sugieren que la reproducción asexual no es la estrategia reproductora predominante en esta especie (Casado-Amenzua 2012). Pero en el caso de que la reproducción sexual pueda verse afectada en poblaciones sometidas a impactos, los mecanismos de reproducción asexual podrían cobrar importancia. Uno de estos mecanismos ha sido observado *in situ* en la población de Columbretes, se trata de procesos de liberación de pólipos (los pólipos se liberan del esqueleto) y podría ser un mecanismo efectivo para regenerar nuevas colonias a partir de fragmentos rotos de colonias madre.

Factores que pueden actuar en sinergia con el estrés térmico: patógenos termodependientes y estado fisiológico de los pólipos

Las mortalidades relacionadas con el cambio climático han sido asociadas tanto a patógenos como a estrés fisiológico (Bally and Garrabou 2007, Coma et al. 2009). Aunque la relación entre las mortalidades de *Cladocora. caespitosa* y el calentamiento del agua en Columbretes es inequívoca, los resultados obtenidos muestran la probable sinergia de de otros factores con las anomalías térmicas. Por lo tanto, son necesarios estudios sobre su fisiología y la posible actuación de patógenos en esta especie. Esta información completaría el conocimiento sobre los mecanismos implicados en las mortalidades sufridas por este coral.

Efectos subletales del estrés térmico y las algas invasoras

Efectos subletales derivados del estrés térmico y de la invasión de algas alóctonas podrían afectar a diferentes fases vitales de *Cladocora caespitosa*, incluida su reproducción y el reclutamiento. Estudios sobre estos impactos potenciales, particularmente en las primeras fases del ciclo vital de la especie, son necesarios para conocer su resiliencia frente a los drásticos cambios ambientales que sufre en la actualidad.

Caracterización de otras poblaciones de *Cladocora caespitosa*

Las Islas Columbretes fueron elegidas como laboratorio de campo para la realización de esta tesis porque albergan una de las más importantes y mejor desarrolladas poblaciones de este coral. En cambio, hay una falta general de estudios descriptivos y de evaluación del estado de salud de otras poblaciones y bioconstrucciones de *Cladocora caespitosa*. Esta información es crucial para poder tener una visión general del estado de conservación de la especie.

Estudios genéticos

En el momento de finalizar esta tesis los microsatélites de *Cladocora caespitosa* ya han sido desarrollados (Casado-Amenzua et al. 2011), estudios preliminares sobre la conectividad genética entre las poblaciones de este coral han sido realizados (Casado-Amenzua 2012) y estudios en más profundidad sobre este último tema acaban de ser enviados a revistas científicas (P Casado-Amenzua, com. pers.).

A pesar de ello, aún quedan pendientes estudios genéticos con el fin elucidar la existencia de diferenciación genética entre las poblaciones del Adriático y del

Mediterráneo, dadas las importantes divergencias que se han encontrado en la biología reproductora de la especie. Además, estudios genéticos a pequeña escala espacial, junto con el estudio del comportamiento de las larvas de este coral, serían de gran utilidad para aumentar el conocimiento sobre la conectividad a pequeña escala de esta especie y sobre los procesos que modelan la estructura poblacional de este coral.

Las bioconstrucciones de *Cladocora caespitosa* como un foco de biodiversidad

Las colonias de *Cladocora caespitosa* albergan entre sus coralitos una gran cantidad de organismos que aprovechan la estructura de la colonia como refugio y fuente de alimento. Algunos de estos organismos viven en los coralitos (especies perforantes), mientras que otros viven en los intersticios entre los coralitos o incluso en el sedimento que queda atrapado en la estructura. Aunque Schiller (1993) describe brevemente esta comunidad, una descripción más exhaustiva sería de gran utilidad para poder evaluar el papel ecológico de este coral en su función de aporte de estructura y aumento de la biodiversidad de estas comunidades asociadas.

Comparaciones entre la *Cladocora caespitosa* actual y fósil

Mientras que *Cladocora caespitosa* dominó las comunidades costeras en el Mediterráneo subtropical, actualmente las mortalidades masivas relacionadas con el calentamiento del agua están afectando críticamente a esta especie. La comparación detallada entre las poblaciones actuales y fósiles de *C. caespitosa*, las condiciones ambientales del presente y pasado, así como la plasticidad y/o adaptabilidad de esta especie a cambios en su ambiente, ayudaría a resolver la aparente contradicción que supone la existencia masiva de esta especie en un ambiente subtropical mientras que en la actualidad muere a causa del calentamiento. Peirano et al. (2009) empezaron con este trabajo, pero muchas preguntas sobre este interesante tema quedan aún sin respuesta.

Procesos de liberación de gametos y post-liberación

La liberación de gametos en *Cladocora caespitosa* ha sido únicamente observada en el Mar Adriático (Schiller 1993; Kružić et al. 2008a). Dadas las divergencias detectadas en la biología reproductora de la especie, los eventos de liberación de gametos deberían ser descritos también en poblaciones del Mediterráneo Occidental. Además, no existe información sobre los procesos que ocurren tras la liberación de los gametos, es decir, la fertilización de los huevos y el comportamiento larvario.

CONCLUSIONES

Las bioconstrucciones de *Cladocora caespitosa* de la Reserva Marina de las Islas Columbretes: distribución, estructura de tallas y crecimiento

- La Bahía de L'Illa Grossa alberga una población excepcional de *Cladocora caespitosa*, formada por campos de colonias y arrecifes, y que muestra un alto grado de aislamiento geográfico. Estas bioconstrucciones y el área que ocupan (~ 2900 m^2) son, junto con las descritas en el Parque Nacional de Mjlet (Mar Adriático), las mayores descritas hasta la actualidad.

- La distribución de las colonias en la bahía es altamente agregada y asociada a la morfología de fondo y, seguramente, a las estrategias reproductoras de la especie. La Bahía de L'Illa Grossa ofrece cierta protección frente al hidrodinamismo reinante en la zona a la vez que favorece un importante intercambio de agua.

- Las colonias de *C. caespitosa* se encuentran en muchos casos entre la densa cobertura algal en la bahía.

- Las tasas de crecimiento de los coralitos de *C. caespitosa* oscilan alrededor de los 2,5 mm al año.

Patrones divergentes en la reproducción sexual del coral escleractinio mediterráneo *Cladocora caespitosa*

- La reproducción sexual de *Cladocora caespitosa* ha mostrado importantes y sorprendentes divergencias entre el Mediterráneo Occidental y el Adriático.

- *Cladocora caespitosa* es gonocórica en el Mediterráneo Occidental, mientras que está descrita como hermafrodita en el Mar Adriático.

- El ciclo gonadal está asociado a la temperatura del agua, los mayores tamaños de oocitos (~ 100 µm) se registraron en coincidencia con los máximos de temperatura en agosto. El tamaño máximo de los oocitos también difiere significativamente con el descrito en el Mar Adriático.

- La drástica reducción en el número de oocitos en otoño indica que la liberación de gametos ocurre durante esta época del año, mientras que en Adriático la liberación está descrita a principios del verano.

- Las características reproductoras de *C. caespitosa* en el Mediterráneo Occidental son muy similares a las descritas para *Oculina patagonica* (especie citada como alóctona). Ambas especies han sido agrupadas recientemente en la misma familia.

Reclutamiento y mortalidad del coral de aguas templadas *Cladocora caespitosa*: conclusiones sobre la recuperación de poblaciones en peligro

- *Cladocora caespitosa* muestra tasas de reclutamiento y mortalidad bajas (~ 0,30 reclutas m^{-2} $año^{-1}$ y 1 %, respectivamente) y tasas de mortalidad de los juveniles comparativamente altas (29 % en la clase de talla más pequeña, < 5 pólipos).

- Las tasas de reclutamiento en substrato horizontal son significativamente mayores que en substrato subvertical. Estas diferencias podrían estar relacionadas con las estrategias reproductoras de la especie (mecanismos de retención de huevos y su posible flotabilidad negativa).

- Las colonias juveniles crecen en número de pólipos a través de reproducción asexual, mostrando tasas de gemación significativamente mayores en la clase de talla menor.

Repuestas a largo plazo del coral endémico y bioconstructor *Cladocora caespitosa* al calentamiento del Mediterráneo

- La serie de temperatura estudiada ha mostrado una tasa de calentamiento anual de 0,06 °C (lo que supone un aumento de ~ 1,2 °C durante los 20 años de la serie) y un aumento significativo en la frecuencia de las anomalías térmicas positivas.

- La mortalidad de *Cladocora caespitosa* está significativamente relacionada con la ocurrencia de las anomalías térmicas positivas y es producida por procesos de necrosis del tejido de los pólipos, sin haberse observado una expulsión masiva de zooxantelas (es decir, no existe blanqueamiento).

- *Cladocora caespitosa* sufrió necrosis tras 9 de los 11 veranos estudiados, divididos en dos periodos de mortalidad (2003-2006 y 2008-2012). Más de la mitad del área cubierta por *C. caespitosa* ha sufrido necrosis tras estas mortalidades recurrentes.

- Las mayores tasas de necrosis se registraron en el primer periodo de mortalidad, tras el excepcionalmente caluroso verano de 2013 (~ 25 % necrosis).

- Las diferencias en necrosis registradas entre veranos con anomalías térmicas similares apuntan a la existencia de otros factores, que podrían estar más relacionados con el contexto interanual de temperatura y con procesos de estrés retardado tras veranos extremos, que con procesos de aclimatación o adaptación.

Macrófitos invasores en la Reserva Marina de las Islas Columbretes: dinámica de la invasión e interacción con el coral endémico *Cladocora caespitosa*

- Las algas invasoras *Lophocladia lallemandii* y *Caulerpa racemosa* invadieron exitosamente la Bahía de L'Illa Grossa desde 2006 hasta 2012, aunque mostrando diferencias en el ritmo y variabilidad.

- Mientras que *L. lallemandii* colonizó la bahía en solo 2 años, *C. racemosa* mostró un ritmo de expansión más lento aunque continuo.
- *Lophocladia lallemandii* solapó su distribución en la bahía con la del coral *Cladocora caespitosa* en 2007, mientras que *C. racemosa* lo hizo en 2010.

- No se detectaron efectos letales ni subletales de las algas invasoras en las colonias de *C. caespitosa*. Aunque no se puede descartar la ocurrencia de efectos subletales en ciertas fases vitales de la especie, como en los juveniles o en el reclutamiento.

- *Cladocora caespitosa* presenta actividad tóxica, lo que podría explicar la baja colonización de las partes vivas de las colonias por parte de *C. racemosa*.

Conclusión general

La Bahía de L'Illa Grossa alberga una población única del coral endémico del Mediterráneo *Cladocora caespitosa*, una especie longeva capaz de formar grandes y duraderas bioconstrucciones de valor ecológico y patrimonial incalculable. Esta especie y sus cada vez más escasas bioconstrucciones están amenazadas seriamente por los impactos derivados del cambio global, por lo que urge el establecimiento de planes de conservación. Desde un enfoque más amplio, *Cladocora caespitosa* ha mostrado, como especie ejemplo, la grave amenaza que suponen para las comunidades bentónicas mediterráneas los drásticos cambios ambientales que están ocurriendo; poniendo de manifiesto la necesidad de medidas de conservación de amplio espectro y el establecimiento de programas de seguimiento a largo plazo.

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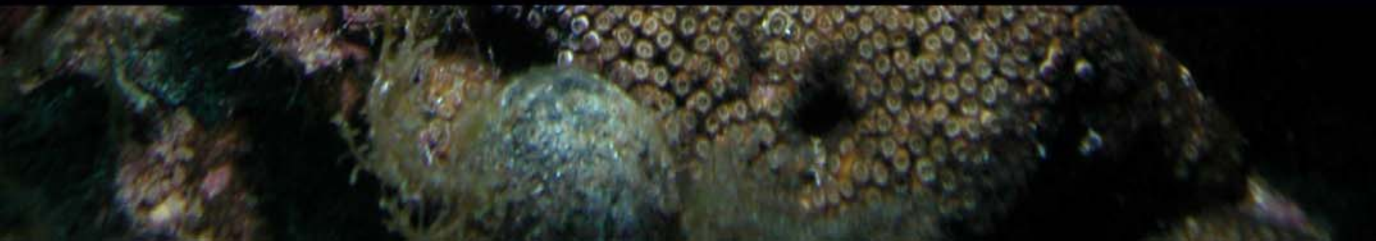

Photo: *Cladocora caespitosa* bank

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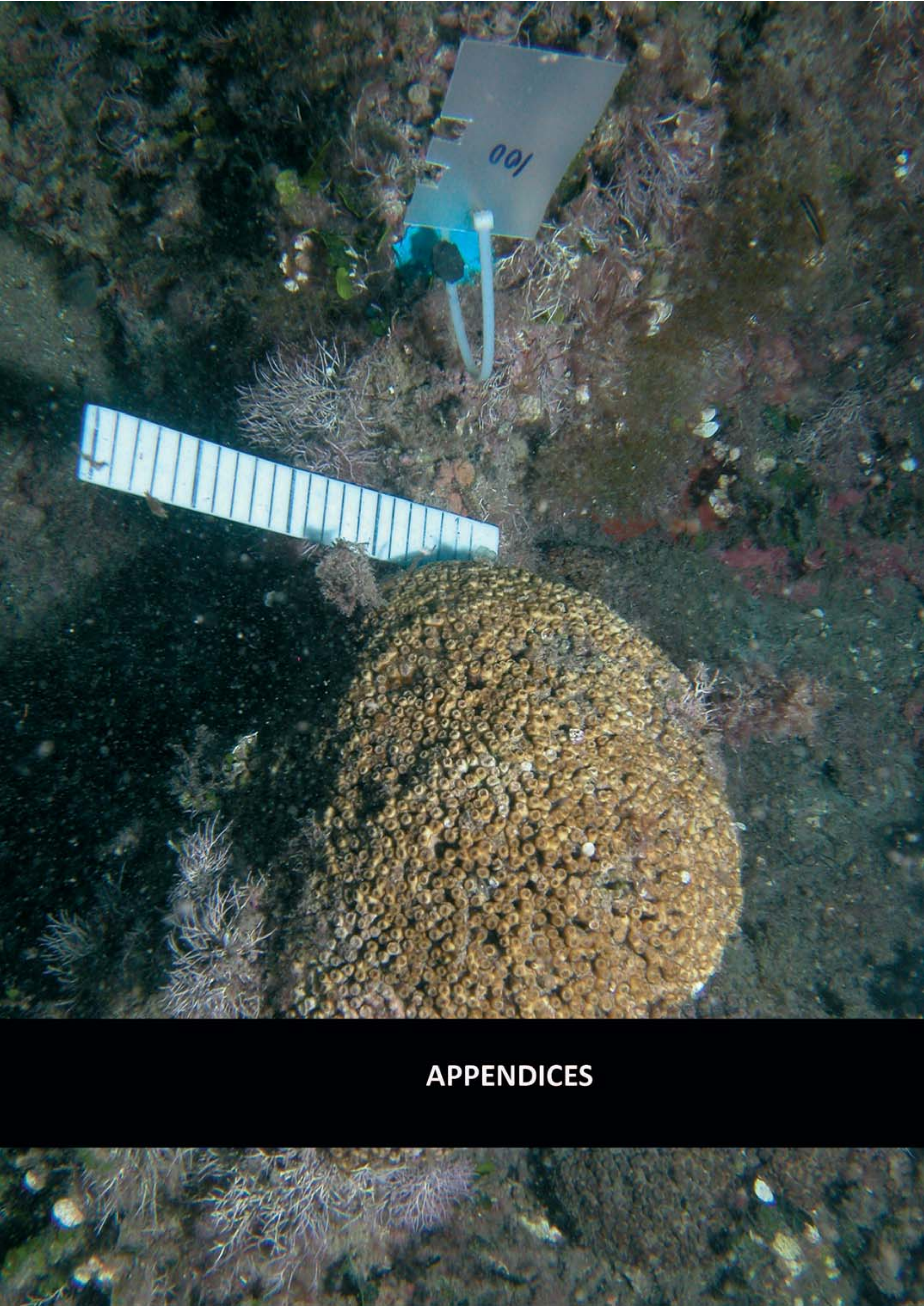

APPENDICES

Photo: Colony no. 100 in the monitoring transect

APPENDIX I
Supplementary tables

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
2002										
2003	**<0.001**									
2004	0.100	**<0.05**								
2005	**<0.05**	**<0.05**	1.000							
2006	**<0.001**	0.424	0.928	0.970						
2007	1.000	**<0.001**	0.177	0.087	**<0.001**					
2008	0.967	**<0.001**	0.923	0.826	0.063	0.988				
2009	**<0.001**	0.254	0.959	0.986	1.000	**<0.001**	0.081			
2010	0.304	**<0.001**	1.000	1.000	0.621	0.445	0.994	0.699		
2011	0.497	**<0.001**	1.000	0.998	0.441	0.646	0.999	0.517	1.000	
2012	0.077	**<0.05**	1.000	1.000	0.928	0.144	0.904	0.960	1.000	1.000

Table S1. Scheffé's contrast test obtained from a one-way ANOVA comparing summer SST anomalies among years.

	2002-2012 (N = 11)	**2002-2007 (N = 7)**	**2007-2012 (N = 6)**	**2002-2012* (N = 9)**
Necrosis - SST anomaly	**r = 0.746, p<0.01**	**r = 0.939, p<0.01**	r = 0.161, p = 0.761	r = 0.647, p = 0.060
Necrosis – 24 °C	r = 0.582, p = 0.061	**r = 0.928, p<0.01**	r = 0.404, p = 0.428	r = 0.399, p = 0.287
Necrosis – 25 °C	r = 0.536, p = 0.090	**r = 0.923, p<0.01**	r = 0.151, p = 0.775	r = 0.271, p = 0.480
Necrosis – 26 °C	r = 0.517, p = 0.104	**r = 0.974, p<0.01**	r = 0.059, p = 0.912	r = 0.257, p = 0.505
Necrosis – 27 °C	**r = 0.607, p<0.05**	r = 0.750, p = 0.086	r = 0.232, p = 0.658	r = 0.513, p = 0.158
Necrosis – 28 °C	**r = 0.629, p<0.05**	r = 0.695, p = 0.125	r = 0.063, p = 0.906	r = 0.581, p = 0.101

Table S2. Results of the multiple correlation tests between annual necrosis and temperature descriptors. Significant correlation is highlighted in bold.* Without 2002 and 2007.

APPENDIX II

Published papers

marine ecology

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ORIGINAL ARTICLE

Cladocora caespitosa bioconstructions in the Columbretes Islands Marine Reserve (Spain, NW Mediterranean): distribution, size structure and growth

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Keywords
Cladocora caespitosa; coral bioconstruction; growth rate; Mediterranean Sea; Scleractinia; spatial distribution.

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Abstract

Today, living banks of the coral *Cladocora caespitosa* appear to be restricted to a few Mediterranean locations and are threatened by the escalating impacts affecting coastal areas. In this study the exceptional occurrence of the Mediterranean coral *C. caespitosa* in the Columbretes Islands Marine Reserve (NW Mediterranean, Spain) is characterised in terms of spatial distribution, cover area, colony size and growth rates. The coral colonies form beds and banks in rocky bottoms within a semi-enclosed bay that offers both hydrodynamic protection and high water exchange. The spatial distribution of the *C. caespitosa* colonies, from 5 to 27 m depth, is highly aggregated, depending on sea-floor morphology and showing up to 80% of substrate coverage. The annual corallite growth rates obtained through the alizarin red staining method and x-ray image analysis are similar, and range between 2.55 ± 0.79 mm and 2.54 ± 0.81 mm, respectively. The exceptional nature of these bioconstructions is due to their cumulative cover area, which is comparable in size to the largest *C. caespitosa* bioconstructions described to date in Mljet National Park (Croatia, Adriatic Sea).

Introduction

The scleractinian *Cladocora caespitosa* (Linnaeus, 1767) is the only endemic zooxanthellate coral in the Mediterranean with reef-forming capacity at the present time (Morri *et al.* 1994) and in the past (*e.g.* Aguirre & Jiménez 1998). The oldest fossil reef known to date is after the Messinian Event (Late Pliocene, Aguirre & Jiménez 1998), following the extinction of ancient tropical reef ecosystems in the Mediterranean (*e.g.* Esteban 1996). Therefore, *C. caespitosa* banks may be considered the unique continuation of the reef ecosystems to the present day (Kühlman *et al.* 1991; Aguirre & Jiménez 1998).

This species occurs at a wide range of substratum, depth and hydrodynamic conditions (Zibrowius 1980; Schiller 1993). Schuhmacher & Zibrowius (1985) classified *C. caespitosa* as a constructional but ahermatypic coral, as it does not contribute significantly to the framework of reefs. In the last few years, the family to which the genus *Cladocora* belongs has been revised based on contrasting molecular investigations. The genus has been excluded from Faviidae and included first in Caryophylliidae (Romano & Cairns 2000) and afterwards in Oculinidae (Fukami *et al.* 2008).

The distribution of extant *Cladocora caespitosa* colonies has decreased compared with the fossil distribution (Laborel 1987). The causes of this historic reduction are not clear but they could be associated with environmental changes. Such decreases seem to be continuing today (Morri *et al.* 2001). This decline is being reinforced by recurrent mass mortality events that were recorded for *C. caespitosa* during the last decade (Perez *et al.* 2000; Rodolfo-Metalpa *et al.* 2005; Garrabou *et al.* 2009; Kersting & Linares 2009), probably caused by climate warming (Lejeusne 2010). Thus, global warming seems to be an important threat for this temperate coral, which was already included by Augier (1982) in a list of endangered marine species.

Although *Cladocora caespitosa* is a conspicuous species, large bioconstructions of this coral are very rare at the present time, and the common *C. caespitosa* populations are built up of small, disperse colonies. Some *C. caespitosa* bioconstructions have been described, but only in a few have the distribution patterns and main population characteristics been intensively studied (Schiller 1993; Peirano *et al.* 2001; Kružic & Požar-Domac 2003; Kružić & Benković 2008).

In this study, we describe the *Cladocora caespitosa* bioconstructions in the Illa Grossa Bay (Columbretes Islands, NW Mediterranean, Spain) in terms of spatial distribution, size structure and growth rates. The results are compared with published data from other areas providing new comparative information on biological and ecological features of different populations of this endangered coral species across the Mediterranean Sea.

Material and Methods

Study site

The Columbretes Islands emerge 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean) within a 90 × 40 km volcanic field at 80–90 m water depth (Muñoz *et al.* 2005). A marine reserve encircles the archipelago, covering an area of 5500 ha. Illa Grossa (39°53.825′ N, 0°41.214′ E), the largest of the islets in Columbretes (14 ha), is a C-shaped, drowned Quaternary volcanic caldera that is open to the NE in the main direction of winter storm waves (Fig. 1) (Aparicio & García 1995; Sánchez-Arcilla *et al.* 2008). The bay formed by this islet has a total surface of 150,000 m^2 and hosts the studied *Cladocora caespitosa* population.

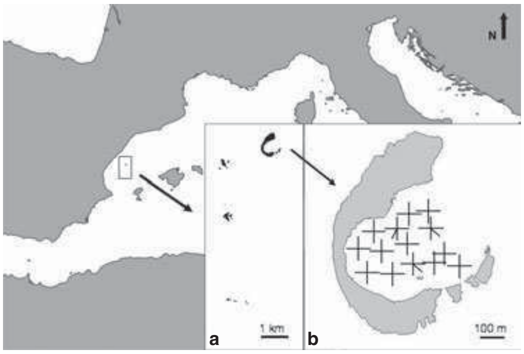

Fig. 1. (a) Location of Columbretes Islands (NW Mediterranean Sea, Spain). (b) Illa Grossa islet and the surveyed transects.

The sea bottom in the bay has an average depth of 15 m and is covered mainly by rocky substrata and biogenic sands in the central and deeper areas. A wide flat channel crosses the bay with the main storm direction (NE). In the bay, the rocky slopes of the islet sink abruptly, reaching at least 5 m depth in the shallowest areas. In the central part of the bay the sea floor becomes less steep, although eroded remnants of the successive volcanic eruptions, in the form of crests (rock formations over 5 m in height and 10 m in length) and blocks, are frequent in the NW and SE borders (Aparicio & García 1995). The infralittoral photophilic algal community covers the illuminated parts of the crests and blocks and is mainly dominated by dense facies of *Dictyopteris polypodioides* starting at 5 m depth (Templado & Calvo 2002).

Sea surface temperatures (SST) have been taken daily with a calibrated mercury-in-glass thermometer since 1991. The mean monthly SST in Illa Grossa Bay ranges from 13.16 ± 0.80 °C (February) to 26.19 ± 1.16 °C (August) (±SD) (average obtained from daily measures between 1991 and 2010; D.-K. Kersting & C. Linares, unpublished observations).

Spatial distribution

Due to the size of the bay and the ubiquity of the *Cladocora caespitosa* colonies, high resolution mapping techniques, such as used in Kružić & Benković (2008), had to be disregarded. Instead, an interpolation technique using transects was chosen and allowed to cover the whole bay. Therefore, Illa Grossa Bay was surveyed through radial transects starting from 14 homogeneously distributed points throughout the bay from 1 to 30 m depth (Fig. 1). There were at least four transects per point (North, East, South and West). Transects were 50 m long and 1 m wide (50 m^2) and each of them was subdivided into ten 5 m^2 areas to record: depth range, number of colonies and colony diameters (major axis D1 and minor axis D2 following Peirano *et al.* 2001). The colony diameters were measured to the nearest half centimeter with a 127-cm aluminium tree caliper. The *C. caespitosa* cover was obtained by approximating the colony base area to that of a circumference (D1 = D2) or an ellipse (D1 ≠ D2) depending on the shape of each colony.

The *C. caespitosa* cover in the bay was mapped through interpolation of the coral cover data obtained in transects with a gridding method (inverse distance to a power, SURFER version 9 software). In the mapping and estimations of coral cover, each of the 5-m^2 areas was considered as a single geographical location related to its coral cover data.

Spatial autocorrelation of coral cover at different distance classes was studied with spatial correlograms (Oden

& Sokal 1986) using Moran's I coefficient (Moran 1950). The autocorrelation coefficient Moran's I evaluates whether the spatial pattern observed is clustered, dispersed or random. The distance classes used for the correlogram were 10, 20, 30, 40, 50, 100 and 200 m and the distance matrix was obtained from the geographical position of each 5-m^2 transect subunit. Significant positive autocorrelation means that within a particular distance class, the coral cover value is more similar (clustered) than obtained randomly from any distance class.

The spatial analysis was undertaken using PASSAGE 2.0 software (Rosenberg & Anderson 2011; http://www.passagesoftware.net). Distance matrix used for the Moran's I correlogram was generated with GEOGRAPHIC DISTANCE MATRIX GENERATOR 1.2.3 software (http://biodiversityinformatics.amnh.org/open_source/gdmg). Relationships between the sea-floor morphology and coral cover were searched by overlaying the bathymetry of the bay (authorized by the Ministry of Environmental and Rural and Marine Affairs, Spanish General Secretariat for the Sea) and the coral cover map.

Size-frequency distribution and colony morphology

To choose a single size descriptor the correlation between diameter (D1, D2) and height (H) was studied in 115 colonies (Fig. 2). D1 showed a positive and significant relationship with D2 ($r^2 = 0.8946$; $P < 0.01$; $n = 115$) and with H ($r^2 = 0.8134$, $P < 0.01$; $n = 115$). Hence, D1 was selected as the colony size descriptor, given also its easy measurement. D1, measured on 1511 colonies in the bay, was used to obtain the size-frequency distribution of the population, which was analysed in terms of descriptive statistics using skewness (Sokal & Rohlf 1995).

As no quantitative data are available on the local current regime in the bay, the relationships between the hydrodynamics of the bay and the shape of *Cladocora caespitosa* colonies were investigated through the sphericity Is-index (maximum height/maximum diameter of a colony) (Riedl 1966; Kružić & Benković 2008; Fig. 2) and the correlation between D1 and the depth of colony occurrence.

Colony growth rates

The annual polyp growth rate was estimated using two methodologies: alizarin red staining technique and sclerochronology (x-ray image analysis of the corallites) (Fig. 3). The staining method was applied both in aquaria and *in situ* to colonies living between 14 and 16 m depth. The alizarin red concentration used in both cases was 10 mg·l^{-1}, and the staining lasted for 24 h (Lamberts 1978; Schiller 1993; Rodolfo-Metalpa *et al.* 1999). The colonies stained in aquaria were re-installed in the bay after the staining treatment. This transplantation was done using underwater putty to fix the base of the colony to the rock at the same location and depth where it had been previously collected. The *in situ* staining was undertaken by covering each colony with a semi-spherical transparent plastic structure well fitted to the ground to avoid significant losses in the alizarin red solution (as

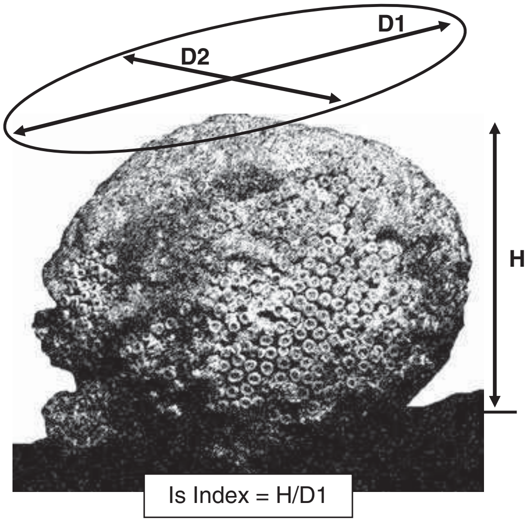

Fig. 2. Size descriptors used in the biometry of *Cladocora caespitosa*.

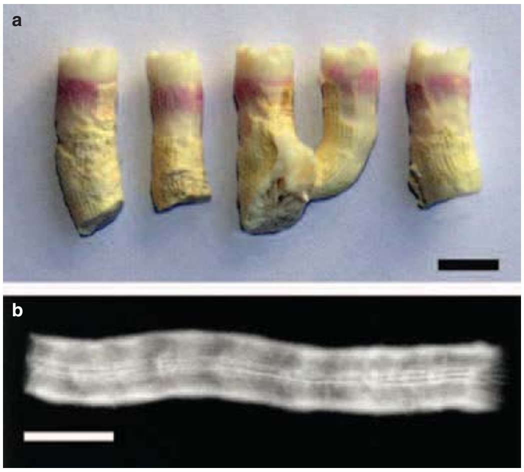

Fig. 3. (a) Alizarin red stain in *Cladocora caespitosa* corallites. (b) X-ray image of a *C. caespitosa* corallite with annual high and low density bands. Scale bars: 0.5 cm.

described in Lamberts 1978). This structure was large enough not to interfere with the colony, thus avoiding polyp retraction. Five colonies were stained in 2008 (four in aquaria and one *in situ*) and seven in 2009 (six in aquaria and one *in situ*). The corallites of each colony were collected 12 months after the staining. The corallites were cleared of organic material and tissue by submersion in H_2O_2 (30%) for 24 h and were then polished with an electric mini-borer with a diamond cutting wheel until the alizarin mark limit was clearly noticeable. A total of 540 corallites were used to measure the annual growth rate with this method. The measurement was performed with a caliper to the nearest 0.01 mm from the edge of the calyx to the upper limit of the staining (Schiller 1993; Rodolfo-Metalpa *et al.* 1999).

Evaluation of mean growth rates through corallite x-ray analyses was conducted on 13 colonies of *C. caespitosa*. Corallites were collected, cleaned in H_2O_2 (30%), x-radiographed with a medical unit and growth rates calculated with CORAL XDS software (http://www.nova.edu/ocean/ncri//projects/coralxds/index.html) (Peirano *et al.* 2005; Kružić & Benković 2008).

Given that the goal of the study was to provide mean growth rates of *C. caespitosa* colonies in Columbretes for comparison with similar published references, only the differences in polyp growth obtained from both methodologies (alizarin red and x-ray) were tested through a Kolmogorov–Smirnov two-sample test. The low number of colonies stained with alizarin *in situ* and in the aquaria prevented us from analysing statistically the differences between both techniques as well as differences relating to depth and sites. All statistical analyses were performed using STATISTICA 8 software.

Results

Spatial distribution

The depth distribution of *Cladocora caespitosa* colonies in the Illa Grossa Bay ranged from 5 to 27 m. The colonies occurred on rocky and small block (average diameter < 1 m) bottoms as well as on vertical, sub-vertical and horizontal substrata. The highest *C. caespitosa* cover was found between 10 and 20 m depth; about 85% of the cumulative *C. caespitosa* colony area was concentrated at this depth range (Fig. 4). Coral cover and depth showed no linear correlation ($r = -0.24$, $P < 0.01$).

Although *Cladocora caespitosa* colonies were present throughout the bay with an average cover of 1.9%, some areas displayed remarkably higher colony concentrations with contagious distributions. In these areas, the coral cover reached values up to 80% (in 5 m^2) and maximum colony densities of 5.5 colonies per m^2. Scattered banks of *C. caespitosa* (bioconstructions covering several square meters) were to be found within these colony beds.

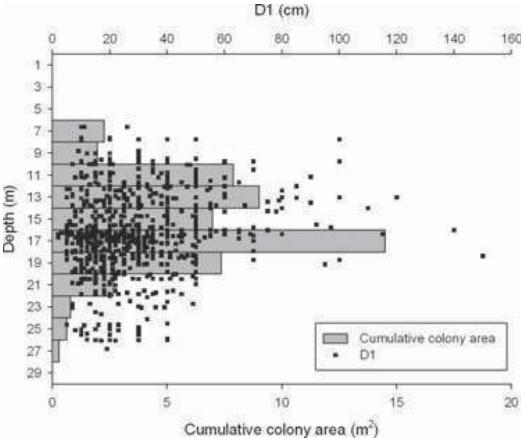

Fig. 4. Depth distribution of D1 and cumulative colony area per depth in the surveyed transects.

The Moran's I correlogram indicates a significant ($P < 0.01$) positive autocorrelation of the coral cover data, in agreement with contagious distribution at distance classes of 10–50 m (Fig. 5).

The main colony concentration zones were located at the NW and SE areas of the bay, where the steepest crests occur (Fig. 6). The mean cover obtained in these areas ranged from 2.7 to 7% in the NW and SE areas, respectively. The estimates of the overall surface covered by the colonies reached 240 m^2 in the NW area and 910 m^2 in the SE area. The cumulative cover area of *C. caespitosa* in the bay was estimated to be approximately 2900 m^2.

Size-frequency distribution and colony morphology

The size-frequency distribution of this population was unimodal and non-normal (K-S $d = 0.127$, $P < 0.01$, Fig. 7). The skewness of the distribution was significantly positive ($g1 = 1.667$; Sokal & Rohlf 1995), which indicates the prevalence of small classes in the population. The mean colony diameter was 31.48 ± 21.02 cm (±SD), and the maximum and minimum diameters recorded within transects were 150 and 2 cm, respectively. Regarding colony morphology, the average Is-index value obtained for this population was 0.55 ± 0.21 (±SD).

D1 and depth showed no correlation ($r = -0.19$, $P < 0.01$). All the size classes were represented in the middle depth range of distribution (10–20 m), whereas the largest colonies were absent in the upper and lower limits (Fig. 4).

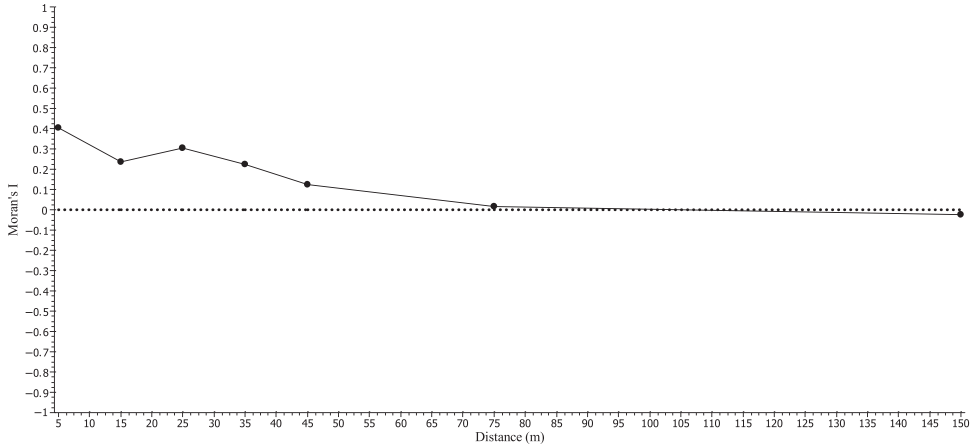

Fig. 5. Spatial correlogram (Moran's I) for *Cladocora caespitosa* cover in the Illa Grossa Bay. Solid dots show significance ($P < 0.01$) for the Moran's I coefficient in the distance classes.

Colony growth rates

In the *Cladocora caespitosa* corallites, two bands are deposited annually: a high-density band (HD) in the winter and a low-density band (LD) in the summer, as previously found by Peirano *et al.* (1999, 2005). However, the annual HD and LD bands were not always noticeable in radiographed corallites and, consequently, many samples had to be disregarded. The image analysis of the 30 corallites resulted in 95 annual HD and LD bands.

Based on the alizarin staining method, the mean annual growth rate obtained for 2008 and 2009 was 2.55 ± 0.79 mm (±SD). The individual minimum and maximum growths were 0.49 and 5.49 mm, respectively. The mean annual growth rate obtained by means of the x-ray images was 2.54 ± 0.81 mm (±SD), and the minimum and maximum growth rates were 1.41 and 5.19 mm, respectively. While alizarin staining method provided growth rates from a unique year, the x-ray method obtained growth data from different years. Nonetheless, growth rates obtained from both methodologies did not display significant differences (Kolmogorov–Smirnov two-sample test, $P = 0.1$).

Discussion

The characteristics of the *Cladocora caespitosa* population in the Columbretes Islands Marine Reserve were exceptional in the current framework of this species in the Mediterranean Sea. Despite the common occurrence of colonies of *C. caespitosa*, barely 10 living banks and beds of this species have been described in different locations at the Western and Eastern Mediterranean Sea to date, (Fig. 8). The mean colony diameter (D1) and coral cover values obtained in Columbretes were higher than those reported in other Mediterranean sites such as the Bay of Piran and La Spezia (Schiller 1993; Peirano *et al* 2001).

The overall surface covered by *Cladocora caespitosa* was comparable in size to the largest described *C. caespitosa* bank reef known in the Mediterranean, at Veliko jezero in the Mljet National Park (Croatia), as reported by Kružić & Benković (2008). However, the type of colony distribution in these two sites differed widely: there is a continuous reef in Veliko jezero and a combination of banks and separate colonies in Columbretes (Fig. 6). By applying the terminology proposed by Peirano *et al.* (1998), that is, bed (a great number of distinct subspherical colonies 10–30 cm in diameter) or bank (large formations reaching several decimeters in height and covering several square meters in surface area), the *C. caespitosa* population of the Columbretes Islands can be considered a combination of both types of colony distribution. A veritable reef development is almost certainly limited by the hydrodynamic conditions within the bay. There are frequent remains of broken colonies throughout the bay, the result of the combined action of boring organisms and hydrodynamics. In fact, some of the detritic deposits within the bay have an important fraction of corallite fragments.

The contagious distribution pattern of *Cladocora caespitosa* in the bay is probably related to at least two factors: reproductive strategies and sea-bottom morphology. Clumped distributions have been reported for benthic species with philopatric dispersion (*e.g.* Gori *et al.* 2011), including *C. caespitosa* (Peirano *et al.* 2001). In the case of *C. caespitosa* the only study dealing with reproduction

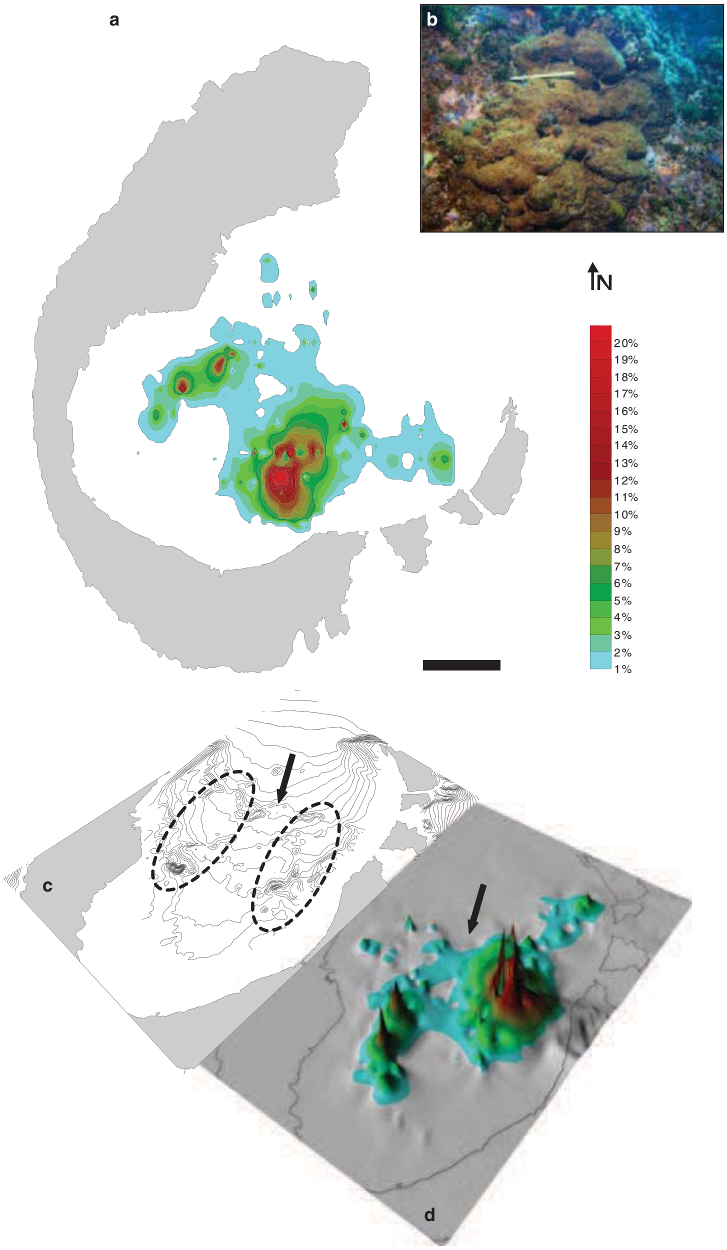

Fig. 6. *Cladocora caespitosa* bioconstructions in the Illa Grossa Bay. (a) Coral cover (%) map of the bay. Scale bar: 100 m. (b) Detail of a *C. caespitosa* bank in the study area (the scale is 30 cm). (c) Bathymetric map of the study bay, dotted ovals show the zones where rock crests are predominant and arrows show the NE–SW central channel in the bay. (d) 3D coral cover map of the area (note that the relief in this map reflects the coral cover values, not the sea-floor morphology).

(Kruzic *et al.* 2007) shows the occurrence of mechanisms that force the eggs to stay near the parental colonies (*e.g.* eggs covered in mucus coating). These mechanisms reduce the dispersion of eggs and consequently of larvae, which will finally develop new colonies near the parental ones. On the other hand, the distribution of *C. caespitosa* in the bay has been shown to be associated with sea-bottom morphology and hydrodynamic protection. The two areas in the bay displaying higher coral cover values occurred in sites with irregular bottom morphology,

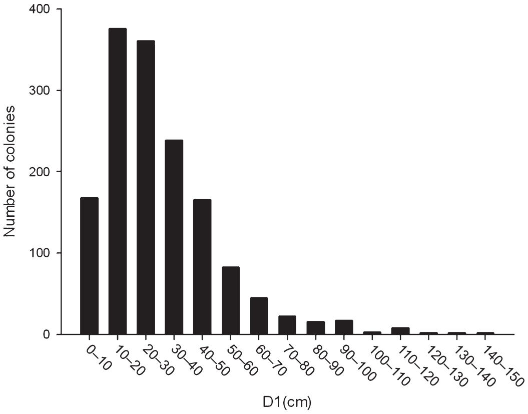

Fig. 7. Size frequency distribution of *Cladocora caespitosa* colonies in Illa Grossa (n = 1511).

Fig. 8. Main living *Cladocora caespitosa* bioconstructions described in the literature. 1. Columbretes Islands (Present work). 2. Port-Cros (Laborel & Laborel-Deguen 1978). 3. La Spezia region (Morri *et al.* 1994, 2000; Peirano *et al.* 2001, 2005; Rodolfo-Metalpa *et al.* 2005). 4. Bay of Piran (Schiller 1993). 5. Rovinj (Zibrowius 1980). 6. Prvić (Zibrowius 1980; Kružić & Benković 2008). 7. Pag (Kružić & Benković 2008). 8. Mljet (Kružic & Požar-Domac 2003; Kružić & Benković 2008). 9. Eubée, Gulf of Atalanta (Laborel 1961). 10. Tunisia (Zibrowius 1980).

where rock crests and blocks are common. These features and the relative location of these sites in the bay, at both sides of the central NE–SW channel (Fig. 6), ensure relative protection during the strong NE storms occurring in fall and winter (Sánchez-Arcilla *et al.* 2008).

No correlation between depth and the colony diameter (D1) or coral cover was found. Most of the Illa Grossa Bay *C. caespitosa* population is concentrated in the 10–20 m depth range. The low coral cover and the absence of the bigger colony sizes in the shallowest limit of the distribution (5–10 m) are probably related to a higher exposure to waves. But this absence is as also clearly noticeable in the deeper range (20–30 m); in this case the prevalence of low cover and smaller sizes could be related to sea-floor morphology and substrata, as detritic beds are more common in this depth range and rocks are rare.

The Is-index obtained for the Columbretes colonies showed a high degree of colony sphericity and is comparable to the minimum values obtained by Kružić & Benković (2008) in Mljet. These lower values were related by these authors to the influence of strong bottom sea currents. In spite of the protection given by the C-shaped Illa Grossa islet to waves and currents coming from the N, S and W, currents can be quite strong in the bay with E and NE winds. Hence, the combination of protection and elevated water exchange with the open sea seems to be a common factor in the development of large *C. caespitosa* bioconstructions; the reef described by Kružić & Benković (2008) in Mljet is a perfect example. Other similarities in the bioconstructions of Mljet and Columbretes were the depth range in which the colonies are found and the temperature regime.

High erect algal cover has been considered a limiting factor in *C. caespitosa* development (Peirano *et al.* 1998). The occurrence of shallow beds of this coral has been attributed to factors inhibiting algal growth, such as water turbidity or the grazing activity of sea urchins (Herndl & Velimirov 1986; Morri *et al.* 2001). It has even been hypothesised that the occurrence of *C. caespitosa* banks happens only below the compensation depth of photophilic algae (Rodolfo-Metalpa *et al.* 1999). The algal cover in Columbretes is significantly high (Templado & Calvo 2002), and in the Illa Grossa Bay, *C. caespitosa* often occurs within a high coverage of *Dictyopteris polypodioides* in the infralittoral photophilic algal community, although colonies in a sciaphilic habitat are also found. *Halimeda tuna*, *Cystoseira sauvageauana* and *Cystoseira compressa* frequently grow in the interstices between polyps of some of the colonies as well. Despite the high algal cover in the bay, only *Codium bursa* and *Codium coralloides* have been occasionally observed overgrowing *C. caespitosa* colonies. Therefore, contrary to the previous finding that large beds and banks are limited by high algal cover, an important *C. caespitosa* population has developed in Columbretes despite the dense photophilic algal community, reinforcing the high level of ecological plasticity of this coral, which is capable of living in such contrasting environments as photophilic communities (*e.g.* Columbretes) or circalittoral coralligenous assemblages, *e.g.* in Bonassola and Riomaggiore, Ligurian coast (Morri *et al.* 1994), or in Cap de Creus and Medes Islands, Catalan coast (D. Kersting & C. Linares, personal observation).

Alizarin staining technique and x-ray analysis showed no significant difference in the growth rates obtained. Kružić & Požar-Domac (2002) used both methodologies in parallel and with similar results. However, certain factors must be taken into account when considering

Table 1. *Cladocora caespitosa* annual growth rates obtained in this study and cited in the literature.

locality	annual growth (mm·year^{-1})	method	authors
Prvić (Adriatic)	3.2 ± 0.1	X-ray	Kružić & Benković (2008)
Pag (Adriatic)	3.1 ± 0.1	X-ray	Kružić & Benković (2008)
Mljet (Adriatic)	3.7 ± 1.3	X-ray	Kružić & Benković (2008)
Mljet (Adriatic)	4.7 ± 0.6/4.7 ± 0.6	Alizarin/X-ray	Kružić & Požar-Domac (2002)
Bay of Piran (Adriatic)	4.4 ± 0.6	Alizarin	Schiller (1993)
La Spezia (NW Med.)	3.01	X-ray	Peirano *et al.* (2005)
La Spezia (NW Med.)	1.3 ± 0.6–4.3 ± 1.4	X-ray	Peirano *et al.* (1999)
La Spezia (NW Med.)	4.8 ± 1.7	Alizarin	Rodolfo-Metalpa *et al.* (1999)
Mallorca (NW Med., Aquarium)	5	Direct measurement	Oliver Valls (1989)
N to S Adriatic	2.6 ± 0.2–4.1 ± 0.6	X-ray	Peirano *et al.* (2009)
Ligurian Sea	3.7 ± 0.5–3.3 ± 0.4	X-ray	Peirano *et al.* (2009)
S Italy	3.1 ± 0.3–3.2 ± 0.3	X-ray	Peirano *et al.* (2009)
Tunisia	2.3 ± 0.2	X-ray	Peirano *et al.* (2009)
Illa Grossa (NW Med.)	2.5 ± 0.8/2.5 ± 0.8	Alizarin/X-ray	Present work

these methodologies. As mentioned above, annual HD and LD banding are not always noticeable in radiographed corallites. The causes of this remain unknown and further research should investigate the factors which determine the pattern of seasonal calcium deposition. On the other hand, the staining was successful using both treatments, in aquaria and *in situ*. In this study, the *in situ* staining method was used to stain *C. caespitosa* corallites for the first time, and it was an effective and easily implemented method with minimal manipulation of the colonies. The only limitation was that the staining structure requires a tight fitting to the ground.

The annual growth rate obtained for *C. caespitosa* in Columbretes fits into the lower range of the results obtained by different authors using either alizarin staining or x-ray analysis on living colonies (Table 1). These rates demonstrate the slow growth of this species. Bearing in mind these growth rates, the mean age of the colonies in the bay of Columbretes Island could be roughly estimated at 50 years; almost 10% of the colonies may be over 100 years old, and some colonies in Columbretes could reach ages up to 300 years.

The large *C. caespitosa* bioconstructions in the Mediterranean have a high patrimonial value due to their rarity, their slow growth and the dynamics of this coral species. Moreover, their conservation is an important concern in the face of the increasing threats affecting these exceptional bioconstructions. Although the Columbretes Islands Marine Reserve protects *C. caespitosa* in the Illa Grossa Bay from direct human impacts, this population could be endangered by global change-related disturbances such as recurrent mortalities linked to positive thermal anomalies (Kersting & Linares 2009) or the presence of invasive algal species such as *Caulerpa racemosa* and *Lophocladia lallemandii*. Given that the Columbretes Islands are isolated at the edge of the continental shelf 60 km from the nearest coast, and the main current regime from north to south (Font *et al.* 1990) in this area, the connectivity of the Columbretes *C. caespitosa* population with the nearest populations at the coast west of the islands is probably very low. To evaluate the viability of these endangered Mediterranean bioconstructions, further scientific studies are needed on topics such as population dynamics and connectivity, especially in the present context of the impacts from global change.

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Unexpected patterns in the sexual reproduction of the Mediterranean scleractinian coral *Cladocora caespitosa*

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ABSTRACT: Knowledge of reproductive biology is essential to understanding population dynamics and ecological processes in corals. Sexual condition and the reproductive cycle of the Mediterranean endemic scleractinian *Cladocora caespitosa* was assessed through histological analyses. Our results showed that this species is gonochoric in the Western Mediterranean Sea. Oocytes and spermaries were detected annually from March to October, reaching their maximum size between July and August coincidentally with the highest seawater temperatures. A drastic decrease in gametes between August and October indicated that spawning occurred at the end of summer. These results differ from those obtained for the Adriatic Sea, where this species was described as hermaphroditic and spawning occurred at the beginning of summer. The unusual plasticity of this temperate coral and the endangered condition of *C. caespitosa* bioconstructions in the Mediterranean highlight the need for further research on this topic.

KEY WORDS: Coral reefs · *Cladocora caespitosa* · Reproduction · Mediterranean Sea

INTRODUCTION

Understanding reproductive biology is essential to comprehending the population dynamics of marine organisms (Fadlallah 1983). Hence, knowledge of coral reproduction is necessary for the management and preservation of coral reefs. For example, successful reproduction can allow for the addition of new individuals to existing populations, the colonization of new areas, and the recovery of populations damaged by natural or human disturbances.

The majority of scleractinian species can be classified as either hermaphroditic or gonochoric; however, more complex sexual patterns have also been described (Harrison & Wallace 1990, Baird et al. 2009, Guest et al. 2012). Likewise, there are 2 types of larval development or reproductive modes. Fertilization is either internal, i.e. the embryo develops within the polyp and is released as a motile planula larva (brooding), or external, with the embryo developing in the water column (broadcast spawning) (Harrison & Wallace 1990, Baird et al. 2009). However, information on the reproductive biology of temperate scleractinian species is relatively scarce in comparison to tropical scleractinian corals (see Harrison 2011 for a review), particularly for the Mediterranean Sea (Goffredo & Zaccanti 2004, Goffredo et al. 2006, Goffredo et al. 2010).

Hermaphroditic broadcast spawners are the dominant group among tropical scleractinian corals (Harrison & Wallace 1990, Harrison 2011, Kerr et al. 2011). In contrast, temperate scleractinians appear to display higher variability in their sexual condition and fertilization strategy, although the latter appears to be fairly consistent within the same family. Within the family Caryophylliidae, for example, the species *Caryophyllia inornata, C. smithi, Lophelia pertusa* and *Paracyathus stearnsii* are gonochoric, whereas *C. ambrosia,*

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C. cornuformis and *C. sequenzae* appear to be hermaphroditic (Fadlallah & Pearse 1982a, Waller et al. 2005, Waller & Tyler 2005, Goffredo et al. 2012). However, all these species, except for *C. inornata*, show the same fertilization mode (external; broadcast spawners) (Fadlallah & Pearse 1982b, Waller et al. 2005, Waller & Tyler 2005, Goffredo et al. 2012). Similarly, in the family Dendrophylliidae, *Balanophyllia elegans* and *Leptopsammia pruvoti* are gonochoric, whereas *B. europaea* is described as a hermaphroditic species (Fadlallah & Pearse 1982a, Goffredo & Zaccanti 2004, Goffredo et al. 2006). All species of the Dendrophylliidae are brooders and show internal fertilization of gametes (Fadlallah & Pearse 1982a, Goffredo & Zaccanti 2004, Goffredo et al. 2006).

The scleractinian *Cladocora caespitosa* (Linnaeus, 1767) is the only reef-forming Mediterranean endemic zooxanthellate coral (Morri et al. 1994, Aguirre & Jiménez 1998). This coral is physiologically and morphologically similar to the typical tropical reef-building scleractininans, being zooxanthellate, colonial and capable of forming extensive bioconstructions (Zibrowius 1982). *C. caespitosa* occurs from shallow waters to depths of approximately 40 m (where light still allows photosynthesis by the symbiotic zooxanthellae) and at sites characterized by calm waters or exposed to strong currents (Zibrowius 1982, Kružić & Benković 2008, Kersting & Linares 2012). Currently, living banks of the coral *C. caespitosa* appear to be restricted to a few Mediterranean locations and are threatened by the escalating impacts affecting coastal areas such as global warming and the spread of invasive species (Kružić & Požar-Domac 2007, Kružić et al. 2008b, Kersting & Linares 2012). Furthermore, *C. caespitosa* populations have been strongly affected during the past decade by mass-mortality events related to positive sea surface temperature (SST) anomalies (Perez et al. 2000, Rodolfo-Metalpa et al. 2005, Garrabou et al. 2009, Kersting & Linares 2009).

To date, only 2 studies based on *in situ* observations and preliminary histological analyses have provided insights into the reproduction of this emblematic species. The spawning of this coral species was first observed by Schiller (1993) in the Bay of Piran (Northern Adriatic Sea), where eggs and sperm bundles were released by a few colonies 4 d prior to the full moon in June. More recently, Kružić et al. (2008a) observed the timing and mode of spawning on banks of *C. caespitosa* in the saltwater lake Veliko jezero (Mljet National Park, Croatia) and described the species as colonial hermaphroditic but with colonies releasing either sperm or eggs during each spawning episode 2 nights before the full moon in June 2005.

The aim of this study was to increase our knowledge of the reproductive biology of *Cladocora caespitosa* in the Western Mediterranean. The bioconstructions of this emblematic species along the Mediterranean Sea are currently threatened by seawater temperature increases and other anthropogenic impacts, and a thorough understanding of the reproductive characteristics of the species is imperative. We used histological techniques to study the sexual condition, as well as the reproductive cycle of this species in order to assess the timing of spawning in the Columbretes Islands Marine Reserve (Western Mediterranean, Spain). This information was compared with results previously reported for the Adriatic Sea. In addition, we examined the sexual condition of *C. caespitosa* in 5 Western Mediterranean locations to determine the general patterns of reproduction in this area.

MATERIALS AND METHODS

To determine the sexual condition of *Cladocora caespitosa* (gonochoric vs. hermaphroditic) at both polyp and colony levels, colonies of *C. caespitosa* were sampled by SCUBA in 5 Western Mediterranean locations: Columbretes Islands Marine Reserve (Spain), Eivissa (Spain), Medas Island Marine Reserve (Spain), Cap de Creus Natural Park (Spain), and Natural Reserve of Scandola (Corsica) (Fig. 1). The number of sampled colonies per site was variable, depending on the abundance of the species at each sampling site (Table 1).

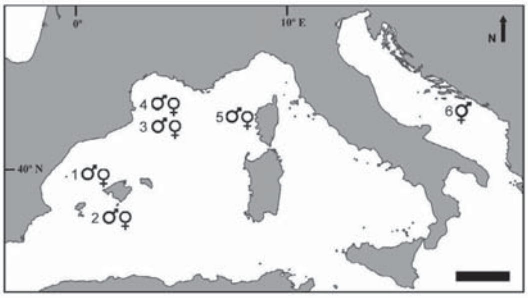

Fig. 1. Map of the study sites in the Western Mediterranean and Adriatic Sea (Sites 1–5 in this study and Site 6 in Kružić et al. 2008a). 1: Columbretes Islands Marine Reserve (W Mediterranean, Spain), 2: Eivissa (W Mediterranean, Spain), 3: Medas Island Marine Reserve (NW Mediterranean, Spain), 4: Natural Reserve of Scandola (NW Mediterranean, France), 5: Cap de Creus (NW Mediterranean, Spain) and 6: Mljet National Park (Adriatic Sea, Croatia). Scale bar = 200 km

To investigate the reproductive cycle of *Cladocora caespitosa*, 10 colonies were surveyed monthly from April 2008 to July 2009 in the Columbretes Islands Marine Reserve (Spain, NW Mediterranean, 39° 53.825′ N, 0° 41.214′ E) at a depth of 15 m. These colonies (20–50 cm in diameter) were individually marked in one of the areas with higher coral cover in Illa Grossa Bay (Kersting & Linares 2012). Particular efforts were made to select healthy colonies with no signs of recent or past mortality. Seawater temperature was measured daily during the study period with Stowaway Tidbits (ONSET, Cape Cod, MA, USA) autonomous sensors installed at the same depth and location as the studied colonies.

Initial histological analyses were conducted to assess the sexual condition of *Cladocora caespitosa* in all sampled sites, as well as to assess the sex of each of the 10 marked colonies in the Columbretes Islands; 3 polyps per colony were sampled for this purpose. According to these results, 3 male and 3 female colonies were selected and 3 polyps of each colony were further investigated. By the end of the study, 33 polyps per colony had been analyzed. The collected samples were fixed in 4 % formaldehyde in seawater, decalcified in a solution of HCl (37 %), formaldehyde and water (1.3:0.8:7.9) for 24 h, dehydrated through a graded alcohol series and finally embedded in paraffin. Cross sections of polyps (5–6 µm thick) were stained with haematoxylin-eosin and examined under a light microscope equipped with a micrometer. In female colonies, the total number of oocytes per polyp was counted and minimum and maximum diameters of oocytes (sectioned through the nucleolus) were measured. The number of oocytes per polyp was counted when less than 100; whenever more than 100 oocytes were observed, the polyp was classified in the class >100. Oocyte diameters were measured in a maximum of 30 oocytes per polyp. In male colonies, only the number of spermaries was recorded due to the impossibility of measuring their size accurately. Due to the low number of either oocytes or spermaries in some samples, we were unable to establish specific stages of maturation; however, as maturation and oocyte size are correlated (Shlesinger et al. 1998) we used the latter to estimate oocyte development.

Table 1. Summary of the sexual condition of *Cladocora caespitosa* colonies sampled at 5 study sites in the Western Mediterranean. No colonies were hermaphrodite

Population	Males	Females	Immature
Columbretes Is.	4	5	1
Eivissa	0	2	0
Medas Islands	2	6	2
Scandola	2	0	0
Cap de Creus	4	0	0

Pearson's product–moment correlation was computed to examine the relationship between oocyte size (diameter) and seawater temperature at 15 m depth using the software package STATISTICA 8.0.

RESULTS AND DISCUSSION

Colonies of the scleractinian coral *Cladocora caespitosa* from the Western Mediterranean were determined to be gonochoric, since all polyps examined within the same colony were of the same sex. This species was described as hermaphroditic in the Adriatic Sea by Kružić et al. (2008a), i.e. polyps within one colony had both male and female gonads. In fact, the preliminary histological analysis made by these authors showed that oocytes and spermaries developed on separate mesenteries within each polyp. Moreover, these authors found that a single colony of *C. caespitosa* in the field released either female or male gametes, but not both simultaneously. In contrast, we found either oocytes or spermaries in a single polyp and colony and no signs of sex reversal, which are both typical signs of a gonochoric sexual condition (Fig. 2). Even though spawning was not directly observed in this study, the simultaneous maturation of gametes and the drastic decrease in the number of occytes and spermaries between August and October (Fig. 3) suggested that the release of sperm and eggs occurred at or around the same time in the Western Mediterranean *C. caespitosa.*

Although corals display great plasticity in their life-history characteristics (Richmond & Hunter 1990), sexuality is generally consistent within most coral species and genera and within certain families (Harrison 2011). However, some examples of changes in sexual condition among populations have been reported in the literature. The scleractinian reef building coral *Diploastrea heliopora* was first classified as gonochoric on the Great Barrier Reef (Harrison 1985) but in Singapore was recorded to have colonies with hermaphroditic polyps, showing concurrent male and female gametes (Guest et al. 2012). This species may exhibit alternate sexual function, with an overlap occurring when the end of one gametogenic cycle coincides with the beginning of the next cycle. In other species, unidirectional protandry has been related to colony size and age (e.g. *Stylophora pistillata*; Rinkevich & Loya 1979), and bidirectional sex change has also been described for corals from the family Fungiidae (Loya & Sakai 2008). Sexual mode variation has been also documented for *Protopalythoa* species in the Great Barrier Reef (Babcock & Ryland 1990) and for *Palythoa tuberculosa* in Japan (Yamazato et al. 1973). In the latter study, colonies were reported to be hermaphroditic while polyps within these colonies were gonochoric (Hirose et al. 2011). The variability in sexual condition of *Cladocora caespitosa* appears to be one of the first records

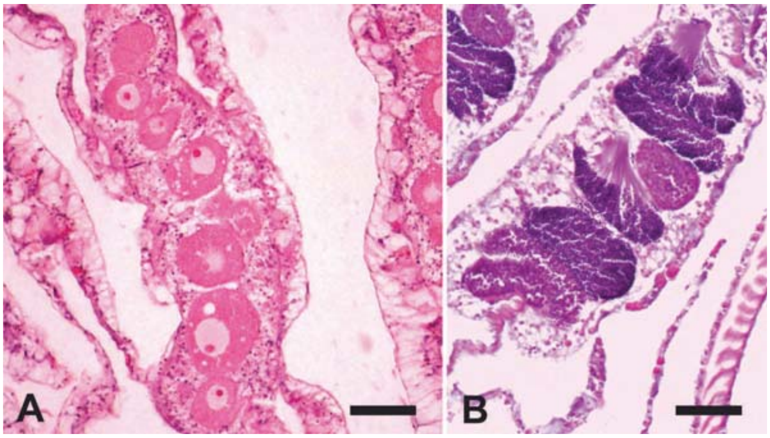

Fig. 2. Gonads of *Cladocora caespitosa*. (A) Female septum packed with mature oocytes (July 2008) containing an oval nucleus and a spherical nucleolus. (B) Male with spermaries filled with spermatozoa (August 2008). Scale bars = 50 µm

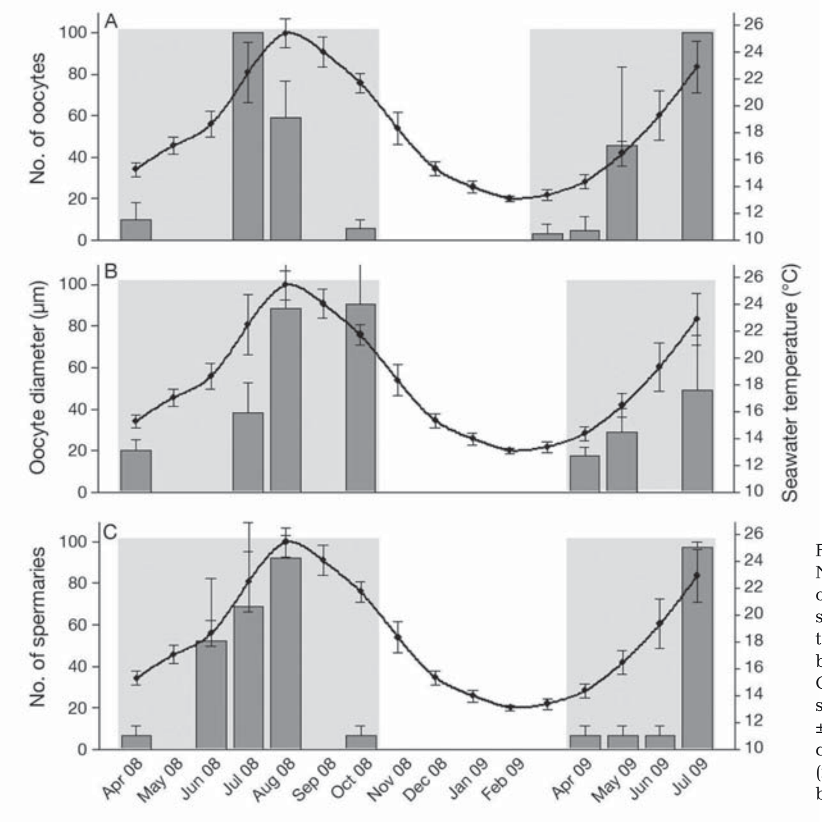

Fig. 3. *Cladocora caespitosa.* (A) Number of oocytes per polyp, (B) oocyte diameter, (C) number of spermaries per polyp and seawater temperature in the Columbretes Islands Marine Reserve. Oocyte and spermaries data are shown in bars (monthly average ± SD), and SST is shown as points connected by a smoothed line (monthly average ± SD). Grey background: reproductive season

of this unusual plasticity in a temperate coral, along with *Astroides calycularis*, which was described as hermaphroditic in Algeria (Lacaze-Duthiers 1873) and as gonochoric in the Southern Tyrrhenian Sea (Italy) (Goffredo et al. 2010).

During the first year of study in the Columbretes Islands, oocytes and spermaries were detected in the mesenteries from April to October 2008 (Fig. 3) and reached their maximum development in August 2008, when oocyte mean diameter was 88.43 ± 22.53 µm (mean ± SD) and the number of oocytes and spermaries reached approximately 100 per polyp (Fig. 3). Gamete abundance showed a monthly increase during both study years, with a peak in July and August 2008 and a remarkable decrease in October 2008. The number of oocytes per polyp increased from an average of 10 ± 8.64 in April 2008 to ~100 in July 2008. Similarly, the number of spermaries increased from 7 ± 4.71 in April 2008 to ~100 in July 2008. During the second year of the study (2009), oocytes and spermaries were first detected in March and April, respectively. Although the monthly number of oocytes was very comparable between the 2 study years, the number of spermaries showed higher variability, especially in June (52 ± 30.4 in 2008 vs. 7 ± 4.7 in 2009). The maximal oocyte size found in this study also contrasted with previous findings reported for *C. caespitosa* in the Adriatic Sea (Kružić et al. 2008a). The mean diameter of spawned eggs described by these authors was 416 ± 73.12 µm, over 4 times greater than our measurements. Even though Kružić et al. (2008a) estimated oocyte sizes after spawning, the difference in size is extraordinary. To date, only small changes in oocyte sizes of scleractinian corals have been reported during the last month before spawning (Shlesinger et al. 1998).

Oocyte development (in terms of size) were strongly correlated with seawater temperature ($r = 0.80$, $p < 0.05$, Fig. 3). The drastic decrease in number of gametes in October 2008 indicated that spawning occurred at the end of the summer in the Columbretes Islands, a pattern that differs from the spawning period described for the Adriatic Sea (early summer) (Schiller 1993, Kružić et al. 2008a). Several studies have demonstrated that reproductive traits, including the spawning period, vary with latitude and geographic location (Rinkevich & Loya 1979, Kojis 1986, Richmond & Hunter 1990, Fan & Dai 1995, Baird et al. 2009). Seasonal changes in seawater temperature are frequently cited as an important environmental factor controlling gametogenetic cycles or planulae release periods in scleractinian corals (see Richmond & Hunter 1990 and Harrison 2011 for reviews). Accordingly, we could hypothesize that differences in seawater temperature between the Western Mediterranean and the Adriatic Sea could result in a shift in the reproductive cycle of *Cladocora caespitosa*. However, populations in both regions are located at similar latitudes and subjected to similar seasonal seawater temperature regimes (Kružić & Benković 2008 and present study). However, gamete spawning appeared to be related to contrasting periods of seasonal SST regimes: increasing temperatures in the Adriatic versus decreasing temperatures in the Western Mediterranean. Thus, a temperature shift cannot explain the differences reported in gamete spawning, either in time (a >2-mo lag occurs between spawning in the Adriatic and the Western Mediterranean Seas) or in the seasonal SST regime (decreasing versus increasing SST).

Alternatively, the differences in the reproductive traits (i.e. oocyte size) observed for *Cladocora caespitosa* from the Western Mediterranean and the Adriatic could be due to genetic divergences between these geographic regions. Further analyses using nuclear DNA markers are needed to investigate whether these regional populations correspond to different lineages. In fact, the taxonomy and systematics of the entire order Scleractinia are being reviewed using several genetic markers and results to date have revealed several discrepancies between morphological observations and phylogenetic analyses (Pinzón & LaJeunesse 2011, Budd et al. 2012).

In contrast to the reproductive differences found between *Cladocora caespitosa* populations in both Mediterranean regions investigated to date, our results revealed many reproductive similarities with the coral *Oculina patagonica* (cited as a Mediterranean alien species; Zibrowius 1974). *O. patagonica* has been described as gonochoric in both the Eastern and Western Mediterranean. In both regions, it reached its maximum gonadal development in August (oocyte mean diameter 100 µm), coinciding with the highest water temperatures (Fine et al. 2001). As our results suggested for *C. caespitosa*, spawning in *O. patagonica* was observed in September, when the temperature began to decrease (Fine et al. 2001). Consequently, our results indicated that the driving factor for gonad development in *C. caespitosa* is directly related to increasing seawater temperatures in summer, although other factors, such as changes in photoperiod, were not investigated and cannot be excluded. As observed by Glynn et al. (2012), coral sexual traits in several taxa demonstrate strong phylogenetic relationships. The similarities reported here between the reproductive cycles of *C. caespitosa* and *O. patagonica* support recent molecular phylogenies grouping both species within the same family (Oculinidae; Fukami et al. 2008).

Although *Cladocora caespitosa* reefs were abundant in the past history of the Mediterranean Sea (Aguirre & Jiménez 1998), bioconstructions of this coral are currently very rare and should be considered endangered (Kružić & Benković 2008, Kersting & Linares 2012). A thorough knowledge of the sexual reproduction of *C. caespitosa* will allow the design of efficient protection and conservation plans for this emblematic species in the Mediterranean Sea. Further research on this topic is needed to better understand the unusual reproductive plasticity of this temperate coral and how its reproductive biology might affect its ecology.

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Long-Term Responses of the Endemic Reef-Builder *Cladocora caespitosa* to Mediterranean Warming

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Abstract

Recurrent climate-induced mass-mortalities have been recorded in the Mediterranean Sea over the past 15 years. *Cladocora caespitosa,* the sole zooxanthellate scleractinian reef-builder in the Mediterranean, is among the organisms affected by these episodes. Extensive bioconstructions of this endemic coral are very rare at the present time and are threatened by several stressors. In this study, we assessed the long-term response of this temperate coral to warming sea-water in the Columbretes Islands (NW Mediterranean) and described, for the first time, the relationship between recurrent mortality events and local sea surface temperature (SST) regimes in the Mediterranean Sea. A water temperature series spanning more than 20 years showed a summer warming trend of 0.06°C per year and an increased frequency of positive thermal anomalies. Mortality resulted from tissue necrosis without massive zooxanthellae loss and during the 11-year study, necrosis was recorded during nine summers separated into two mortality periods (2003–2006 and 2008–2012). The highest necrosis rates were registered during the first mortality period, after the exceptionally hot summer of 2003. Although necrosis and temperature were significantly associated, the variability in necrosis rates during summers with similar thermal anomalies pointed to other acting factors. In this sense, our results showed that these differences were more closely related to the interannual temperature context and delayed thermal stress after extreme summers, rather than to acclimatisation and adaption processes.

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Introduction

Since the late 20th century, global warming has been enhanced by human activities [1]. In this ongoing climatic change, climatic models predict that the Mediterranean Sea will be among the regions that are most affected by the warming trend and the increase of extreme events [2,3]. In fact, warming trends in the last decades are well documented for the Mediterranean Sea, in both deep and coastal waters [4–7].

In the Mediterranean Sea, the frequency of abnormally warm summers has increased, resulting in unprecedented mass-mortality events. Although some early mortalities were detected in the 1970s and 1980s (e.g., [8,9]), the first multispecies mass-mortality event was described in the NW Mediterranean in the summer of 1999 [10–12]. In the summer of 2003, a new mass-mortality episode occurred in NW Mediterranean coastal waters, this time over a larger geographic area [13]. Both events affected over 30 species of benthic invertebrates, mostly cnidarians, sponges and bryozoans [13,14].

While the relationship of these mortalities to water temperature was unequivocal [11,13], different factors, such as energetic constraints due to prolonged summer stratification of the water column [5] and pathogens [15], have been linked to the direct cause of death of the organisms. To date, several studies have examined the direct relationship between sea-water temperature and the mortality patterns of affected species; however, these studies are basically field studies encompassing one or a few years of observations [13,16,17] or laboratory experiments [5,18]. Hence, there is an important lack of long-term studies assessing the long-term responses of temperate species to ongoing warming.

Cladocora caespitosa, the sole zooxanthellate scleractinian reef-builder in the Mediterranean, is among the organisms affected by these mortalities [12,13,19,20]. Although it can be considered a conspicuous species, extensive bioconstructions of this endemic coral (i.e., banks; [21]) are very rare at the present time and are threatened by global change-related disturbances, such as the above-mentioned mortalities as well as the presence of invasive species [22,23]. Although an important effort has been made to study the thermal tolerance of this species in aquaria [20,24,25], no study has assessed the long-term effects of warming-induced mortalities on natural *C. caespitosa* populations, especially on the endangered micro-reefs of this coral.

Here, we provide, for the first time, an analysis of the relationship between seawater warming and mortality in a *C. caespitosa* population over an 11-year period. We do so using data on the local water temperature regime for the period from 1991 to 2012 in the Columbretes Islands; this data set can also provide additional information on Mediterranean warming trends. The objectives of the present work are to study the existence of correlative evidence between the occurrence and intensity of the necrosis events and the local sea surface temperature (SST) regime and to compare the response of *C. caespitosa* throughout the recurrent mortality events in the Columbretes Islands to obtain information on the long-term effects of thermal anomalies on Mediterranean benthic species.

Materials and Methods

Ethics Statement

This study was conducted according to the permitting requirements of the Columbretes Islands Marine Reserve Authority (Secretaría General de Pesca, MAGRAMA). The Secretaría General de Pesca specifically issued the required permission for the *C. caespitosa* study in the Columbretes Islands Marine Reserve.

Study site

The Columbretes Islands emerge 30 nautical miles off the coast of Castelló (Spain, NW Mediterranean). A marine reserve encircles the archipelago covering an area of 5,500 ha. Illa Grossa (39°53.825′N, 0°41.214′E), the largest of the islets in the Columbretes (14 ha), is a C-shaped, drowned, Quaternary volcanic caldera (Fig. 1). The studied *C. caespitosa* population occurs in the central area of the bay formed by this islet (150,000 m^2, 5–30 m depth range); with the highest coral cover values in the NW and SE parts. The cumulative coral cover in the bay reaches 2,900 m^2 in a mixed bank-bed colony distribution [22].

C. caespitosa mortalities

The impact of tissue necrosis on the *C. caespitosa* colonies was studied each year over the period 2002–2012. Mortalities were described and quantified by combining annual random transects and long-term monitoring of individually identified colonies. In the random transects, a total of 110 to 160 colonies were surveyed annually during the autumn (October – November). The long-term annual monitoring of identified colonies began in 2002 with 26 individually marked and mapped colonies; which were increased to 250 in 2006. The surveyed colonies occurred at a depth range of 5 to 20 m, and their maximum diameters ranged from 5 to 150 cm. Schemes and photographs of each colony were used in each survey to depict the areas affected by necrosis.

In each surveyed colony the following data were obtained: depth, percentage of the colony area affected by necrosis (in increments of 10% and differentiating recent or old necrosis) and the size of the colony through its maximum axis. The percentage of necrosis was always related to the living area of the colony. Necrosed areas below 10% were not considered to prevent confusion with other sources of natural mortality, such as those eventually induced by depredation by the gastropod *Babelomurex cariniferus* (Kersting DK, pers. obs.).

To detect delayed necrosis in the *C. caespitosa* colonies, additional surveys were undertaken four to five months after the first necrosis was detected.

Kolmogorov-Smirnov two-sample tests were used to determine whether there were significant differences in necrosis for the following comparisons: (i) along the depth gradient (5–15 m vs. 15–20 m; because vertical temperature profiles showed weak vertical gradients ($<1°C$) in the upper 15 m of depth during the warmest period), (ii) between the two main mortality periods (2003–2006 vs. 2008–2012) and (iii) during the second mortality period, between colonies that were previously unaffected ($<10\%$ necrosis) or affected ($\geq 10\%$ necrosis) during the first mortality period. This last test explored the existence of any degree of acclimatisation over time.

Kruskal-Wallis analysis was used to test for differences in necrosis depending on colony size (maximum diameter size classes: <25 cm, 25–50 cm, >50 cm).

Temperature measurements

The SST data have been recorded daily in the Columbretes Islands Marine Reserve since 1991 at depths of 1 m using a calibrated mercury-in-glass thermometer (Thies Clima, model 2.2141.00.64, Göttingen, Germany). The temperature was measured between 8:00 and 9:00 a.m. by the Marine Reserve wardens following the same protocol (bucket sampling in the first meter of water and direct measurement with the thermometer). Overall,

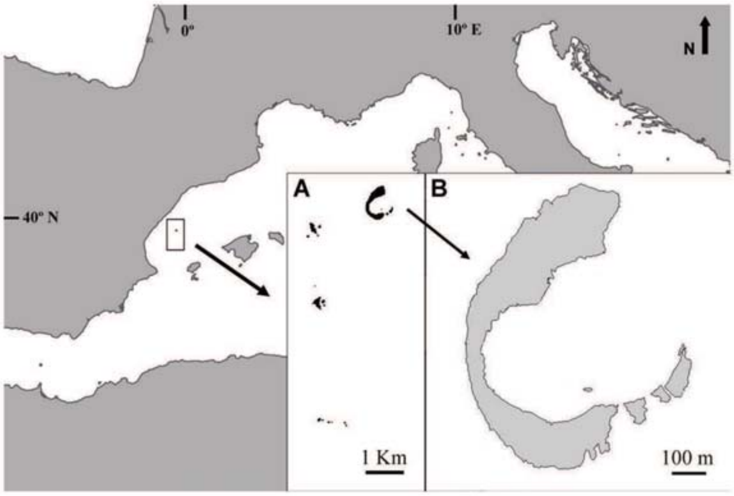

Figure 1. Map of the study site. A. The Columbretes Islands (NW Mediterranean, Spain). B. Illa Grossa Bay.
doi:10.1371/journal.pone.0070820.g001

6,028 daily measurements of SST were collected, which covers 75% of the 1991–2012 period, with a mean value of 274 data logs per year and a mean temporal cover of 87% during the summer (June-September). However, with only 27 data logs during the summer, the year 2000 was not considered in the statistical analyses.

Uncertainty in SST from bucket measurement is on the order of a few tenths of a degree C [26]. Comparisons with hourly records recorded by autonomous data loggers (Water Temp pro v2, ONSET, Cape Cod, MA, USA; accuracy: 0.21°C, resolution: 0.02°C) at 1 m depth from June 2011 to October 2012 yielded very good results, indicating that these punctual measurements reflected the near surface thermal environment ($T_{1m} = 0.97$ SST + 0.64, $r = 0.99$, $p < 0.001$, $N = 446$). Additional temperature profiles (0–20 m) were recorded monthly in the Illa Grossa Bay from 2004 to 2007 using an SBE 39 temperature and pressure sensor (Sea-Bird Electronics, Bellevue, WA, USA). Since 2007, the bay was equipped with Stowaway Tidbits (ONSET, Cape Cod, MA, USA; accuracy: 0.2°C, resolution: 0.14°C) autonomous sensors set at depths of 5, 10, 15 and 20 m (1 hour data-sampling frequency). These sensors were installed in the same area as the permanent *C. caespitosa* transects.

Data obtained from the temperature profiles and the autonomous sensors were used to obtain information on the vertical gradients during the summer (June-September). Data from the autonomous sensor located at a depth of 15 m (average depth of the *C. caespitosa* population; [22]) were compared to the SST data for the summers from 2007 to 2012 to validate the use of the latter longer temperature series for the posterior necrosis-temperature correlation analyses ($T_{15m} = 1.13$ SST – 4.74 , $r = 0.76$, $p < 0.001$, $N = 678$). Summer SST anomalies (i.e., the temperature obtained in the studied summer minus the average of the summers from the original data set (1991–2012)) were obtained for the studied summers (June-September, 2002–2012). Differences in summer SST anomalies among years were analysed using a one-way ANOVA and a Scheffé test for multiple comparison.

The persistence of high water temperatures during the studied summers was recorded as the number of days in which the SST exceeded certain temperature thresholds (from 24 to 28°C).

Correlation between mortality and water temperature

Three mortality descriptors were selected to study the relationship between mortality events and SST anomalies: 1) The mean percentage of the coral's injured surface (hereafter, "necrosis"); 2) the percentage of colonies that were affected in their entirety by the necrosis (hereafter, "total mortality") and 3) the percentage of colonies that were affected by the necrosis to some extent (hereafter, "affected colonies").

Pearson's product-moment correlation was used to examine the relationship among the three descriptors (necrosis-affected colonies: $r = 0.97$, $p < 0.001$; necrosis-total mortality: $r = 0.81$, $p < 0.005$; affected colonies-total mortality: $r = 0.70$, $p < 0.05$; $N = 11$). Necrosis was chosen as the principal mortality descriptor because its use has been generalised in previous mortality studies (e.g. [12,13,20,27–29]).

The SST descriptors used were as follows: 1) summer SST anomalies and 2) persistence of temperature thresholds (i.e., the number of days over temperature thresholds 24, 25, 26, 27 and 28°C).

Multiple linear correlation analyses were performed to explore the relationship between the temperature and mortality descriptors. These analyses were performed for the whole studied period (2002–2012) and for the different mortality periods separately, in order to search for differences between them. The correlation analyses were also performed without the non-mortality years in order to study the role of the necrosis intensity in the correlation with the temperature descriptor.

Results

C. caespitosa mortalities: pattern of necrosis and inter-annual incidence

Old basal necrosis (i.e., accumulated necrosis prior to 2002) of approximately 3% was registered during the first colony surveys in 2002 and 2003. The first mass-mortality event affecting *C. caespitosa* was detected in September 2003. Recurrent mortalities were then detected at the end of the summers of 2004, 2005 and 2006. No mortality was detected in 2007. Although less virulent, necrosis events occurred again during every summer from 2008 to 2012.

The polyp mortality was always characterised by direct tissue necrosis without massive loss of zooxanthellae (i.e., the polyps never lost the brownish-green colour given by the zooxanthellae). Tissue necrosis began at the basal part of the polyps; in these first stages, the polyps often remained expanded. Necrosis gradually affected polyp structure until all tissue disappeared, leaving the bare skeleton (Fig. 2). When colonies were only partially affected by necrosis, the dead polyps were always adjacent to each other, and the colony necrosis had a patched appearance. The evaluation of the accumulated occurrence of the necrosis patches in each colony showed that necrosis occurred both in the upper part and lower sides of the colony in 89.5% of all cases. The first signs of mortality were always detected during August and the beginning of September.

No delayed necrosis was ever detected, when the event was over, the necrosed areas of the colonies remained unchanged, and epibionts rapidly covered the damaged parts.

Recovery of these necrosed areas was never detected. However, in the last years of the survey (2010, 2011 and 2012), the recolonisation of dead colony areas was registered; this occurred through the recruitment of new *C. caespitosa* colonies on the old, dead polyps (Fig. 3). This colony-on-colony recruitment was recorded in 16.26% of the colonies that had experienced partial or complete mortality (average necrosis 80.60±20.3% (± SD)). Through this process, the recolonised colonies gained between 10 and 30% of new, living colony area.

Over the studied period, 80% of the monitored colonies ($N = 250$) were affected to some extent (partially or totally) by multiple mortality events. Considering the information from the fixed and random colony transects, the total colony area that was affected by the accumulated, recurrent necrosis was estimated to range between 55 and 80%.

The highest necrosis values were recorded during the 2003 event, during which 13.39% of the surveyed colonies died completely and necrosis reached an average of 25% (24.94±37.82%). Important mortalities occurred after the following summers (2004–2006), with necrosis values ranging between 12.91±27.46% and 19.62±29.49%. The recurrent mortality events that followed from 2008 to 2012 registered much smaller percentages of necrosis (ranging between 1.95±10.78% and 6.67±18.11%). See Figure 4 and Table 1. Generally, necrosis rates showed high variability between colonies, and affected and unaffected colonies were commonly found one beside each other.

Total mortality (100% of necrosed surface) was mostly due to a single mortality event rather than to accumulated necrosis from multiple, recurrent events. In this sense, 26.7% of the studied colonies experienced total mortality following a single event (half

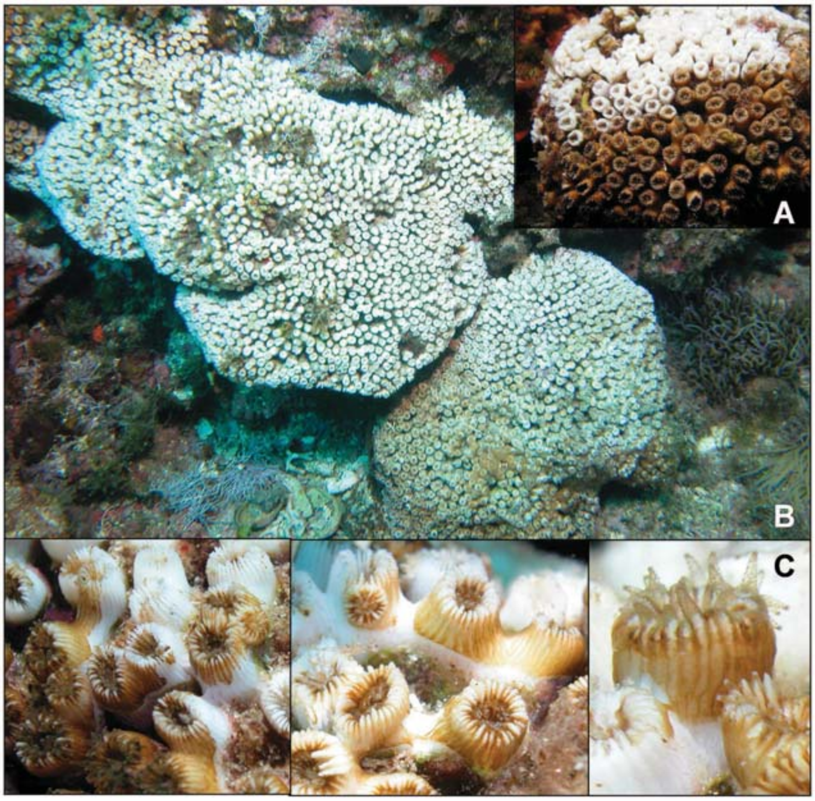

Figure 2. Mortality of *C. caespitosa*. A. *C. caespitosa* colony showing partial necrosis. B. Totally affected colony. C. The necrosis process in the polyps of *C. caespitosa*.
doi:10.1371/journal.pone.0070820.g002

of these colonies died in 2003), while 6.9% experienced total mortality as a result of repeated necrosis events.

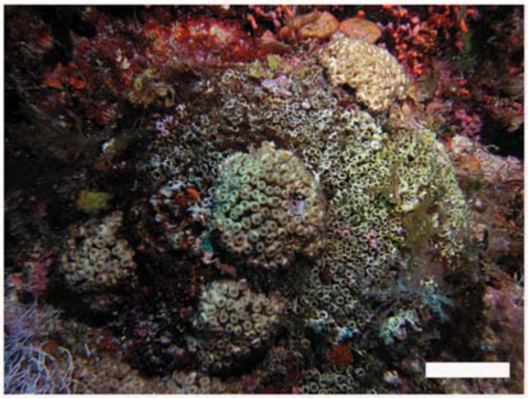

Figure 3. "Colony-on-colony" recruitment in a necrosis-affected *C. caespitosa* colony. Scale bar: 5 cm.
doi:10.1371/journal.pone.0070820.g003

Significant differences were found in necrosis over the entire study period among the selected depth ranges (Kolmogorov-Smirnov test, $p<0.001$). In contrast, no significant differences were found between necrosis and colony size (Kruskal-Wallis test, $p = 0.415$).

The average percentage of necrosis was significantly higher during the first period than the second one: 19.07±31.45% between 2003 and 2006 vs. 3.96±14.52% between 2008 and 2012 (Fig. 5a; Kolmogorov-Smirnov test, $p<0.01$). In contrast, similar necrosis rates were recorded during the second period from colonies that were unaffected or affected during the first period (4.59±17.06% vs. 3.57±12.84%, respectively) (Fig. 5b; Kolmogorov-Smirnov test, $p>0.1$).

Water temperature regime: annual cycle, warming trend and thermal anomalies

Annual cycles showed a minimum of ca. 12°C in mid-February and a maximum between 24.9 and 29.6°C in August (Fig. 6a). The seasonal warming typically had two phases: slow warming rates until mid-April, followed by steeper gradients through the end of June (0.19 vs. 0.87°C per week). SST cooling was observed from the end of August to the end of year at a rate of 0.66°C per week.

Over the period studied, SST exhibited a warming trend of 0.04°C per year ($r = 0.30$, $N = 227$). Focusing only on summer

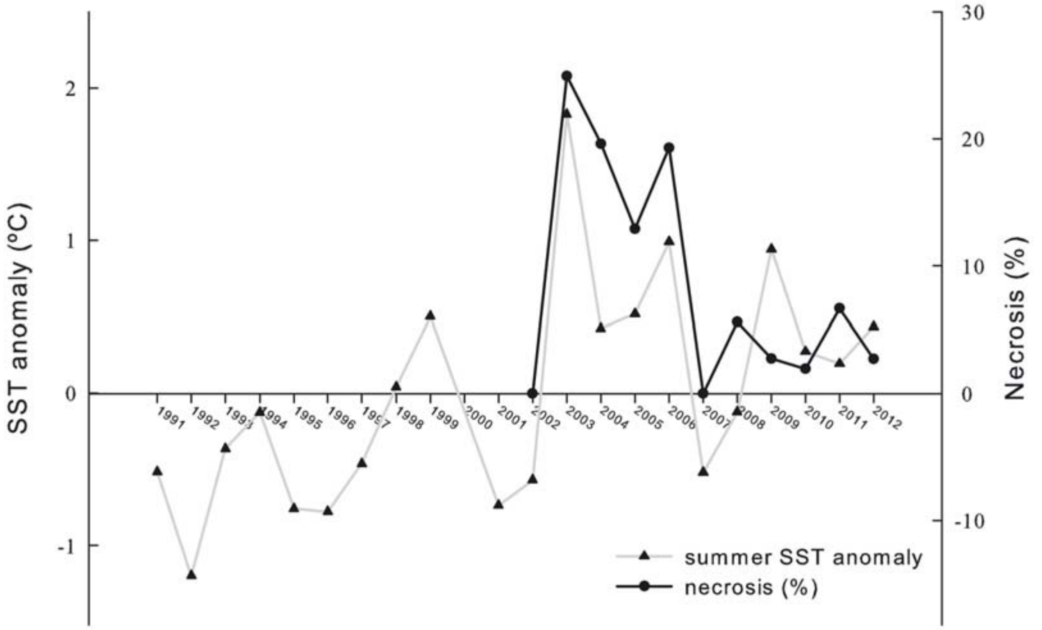

Figure 4. ***C. caespitosa*** **necrosis rates (2002–2012) and summer SST anomalies (1991–2012).**
doi:10.1371/journal.pone.0070820.g004

SST (June to September), the warming trend was even stronger, reaching 0.06°C per year ($r = 0.55$, $N = 21$) (Fig. 6b).

The frequency of positive thermal anomalies during the summer has increased markedly since 2003 (Fig. 4). In 1991–2002, all averaged summer thermal anomalies were negative, except in 1998 and 1999. Contrarily, in the second decade, positive anomalies were recorded during eight summers, occurring in two periods of four consecutive years and only interrupted by the 2007 and 2008 negative anomalies.

The summer SST anomalies varied significantly over time (one-way ANOVA, $F_{10,\ 1215} = 14.802$, $p<0.001$). The maximum significant differences were found when comparing 2003 with all but the warmest summers (i.e., 2006 and 2009). The summers with marked negative thermal anomalies (2002 and 2007) were significantly different from the warmest ones (Table S1).

The summer of 2003 was the warmest of the 20-year-long SST data series, with an average positive anomaly of 1.83°C. During this summer, SST maxima of over 29°C were registered in the Illa Grossa Bay, and the average SST for the entire summer (June-September) was 26.20±2.06°C (Fig. 7). The following summers, i.e., 2004 and 2005, were characterised by moderate positive anomalies (0.42°C and 0.52°C, respectively). In the summer of 2006, high temperatures were reached again; temperature maxima were similar to those recorded in 2003, and an average

Table 1. Mortality and temperature descriptors.

	Necrosis (% ± SD)	Affected colonies (%)	Total mortality (%)	SST anomaly (°C)	24°C	25°C	26°C	27°C	28°C
2002	0	0	0	−0.57	66	22	4	0	0
2003	24.94±37.82	46.43	13.39	1.83	98	82	61	44	25
2004	19.62±29.49	53.64	3.31	0.42	79	51	36	6	0
2005	12.91±27.46	26.36	5.43	0.52	85	64	33	0	0
2006	19.30±31.02	38.46	2.43	0.99	89	72	43	19	13
2007	0	0	0	−0.52	63	27	8	0	0
2008	5.61±18.50	12.34	0.43	−0.12	75	47	25	7	0
2009	2.76±10.26	11.69	0.43	0.94	90	75	61	22	11
2010	1.95±10.78	4.78	0.43	0.27	87	66	43	2	0
2011	6.67±18.11	17.47	0.87	0.19	86	52	27	8	3
2012	2.73±11.93	10.55	0	0.43	81	66	37	12	2

Note that necrosis is given in reference to the remaining living colony area.
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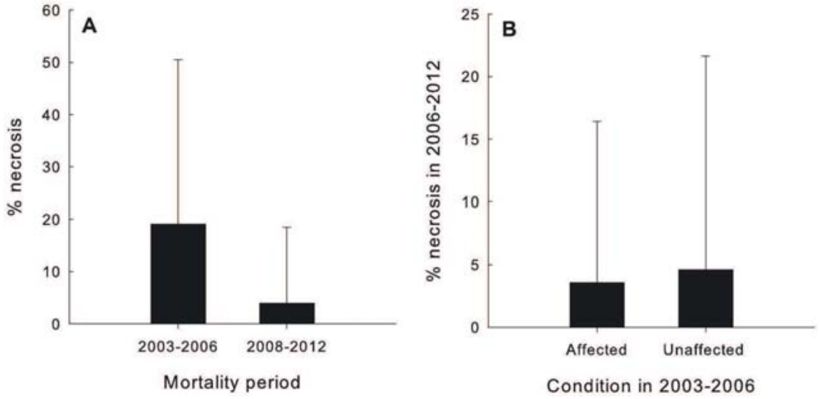

Figure 5. A. Percentage of necrosis (mean ± SD) detected in each mortality period. B. Percentage of necrosis (mean ± SD) detected in the second mortality period in colonies that were either affected or unaffected in the first mortality period.
doi:10.1371/journal.pone.0070820.g005

anomaly of 0.99°C was registered. A second cycle of positive thermal anomalies began in 2009 and lasted until 2012. During these years, the maximum positive anomaly was reached in 2009 (0.94°C); positive anomalies were moderate in the summers of 2010, 2011 and 2012 (0.27°C, 0.19°C and 0.43°C, respectively). See Figs 4 and 7.

Average vertical temperature profiles attested to weak vertical gradients (<1°C) in the upper 15 m of the water column during the warmest period (August) and in the upper 10 m over the entire summer (Fig. 8).

In the years with available data (2004–2012), water temperatures remained over 25°C at depths of 15 m at least during August. The only year without mortality during this time span (2007) had 10 weeks over 24°C, 3 weeks over 25°C and 0.3 weeks over 26°C at 15 m (Fig. 8b). In the years with mortality, water temperatures at depths of 15 m remained over 25°C for between 5 and 10 weeks (Fig. 8c).

Correlation between mortality and water temperature

Necrosis and SST anomalies showed a significant positive correlation over the entire studied period (2002–2012; $r = 0.75$, $p < 0.01$) (Table S2). Similarly, the other mortality descriptors also showed a positive relationship with SST anomalies (total mortality, $r = 0.75$, $p < 0.01$; affected colonies, $r = 0.70$, $p < 0.05$).

When performing the analyses with the two mortality periods separately, the relationship between mortality descriptors and SST anomalies was highly correlated during the first period (necrosis-SST anomalies, $r = 0.94$, $p < 0.01$) but lost significance during the second period. If the non-mortality years (2002 and 2007) were not considered, the correlation between these variables over the entire studied period lost significance (Table S2).

The correlation between necrosis and persistence of temperature thresholds over the whole studied period was significant only for the warmest limits (necrosis-27°C, $r = 0.61$, $p < 0.05$; necrosis-28°C, $r = 0.63$, $p < 0.05$), while during the first mortality period the correlation was significant for the colder thresholds (necrosis-24°C, $r = 0.93$, $p < 0.01$; necrosis-25°C, $r = 0.92$, $p < 0.01$; necrosis-26°C, $r = 0.97$, $p < 0.01$). No correlation between necrosis and persistence of temperature thresholds was found when analyzing the second period separately (Table S2).

Discussion

Historically, mass coral bleaching has been linked to episodes of thermal stress in tropical corals; this is an increasing concern around the world (see [30] for a review). Nonetheless, monitoring the mortalities in the temperate scleractinian reef-builder *C. caespitosa* in the Columbretes Islands (NW Mediterranean Sea) over an 11-year period allowed describing, for the first time, the relationship between recurrent mortality events and local SST regimes in the Mediterranean Sea.

Patterns of mortality

The observed necrosis process in the Columbretes Islands was very similar to previous descriptions of *C. caespitosa* necrosis in the Ligurian Sea [20]. In accordance with previous studies based on field and laboratory data, *C. caespitosa* polyps died due to progressive tissue necrosis with no signs of zooxanthellae loss [20,25,31]. The absence of bleaching is most likely related to the resistance to increases in temperature shown by the *Symbiodinium* (clade temperate-A, [32]) in symbiosis with *C. caespitosa* [24].

Tissue regeneration after mortality episodes was not detected in the Ligurian Sea [20] or in the present study. This could be due to the phaceloid morphology of *C. caespitosa* colonies, built up by independent polyps, which makes the regeneration of adjacent damaged tissue by unaffected polyps difficult [20]. Conversely, the autonomy of the *C. caespitosa* polyps could also be responsible for the lack of delayed necrosis following mortality events as well as the lack of correlation between colony size and necrosis, as has been detected in temperate gorgonians [27–29]. Unexpectedly, although tissue recovery was not observed, another indirect but non-trivial mechanism of colony recovery was detected during the last years of the study. *C. caespitosa* recruits settled on the newly available space on the dead colony parts.

Decreases in necrosis rates with depth have been described for species living at greater depths than *C. caespitosa*, e.g., the gorgonian *P. clavata* [29]. Although the depth range of the studied *C. caespitosa* colonies places them above the thermocline depth during most of the summer, the relationship between necrosis and depth was consistent with the fact that the summer conditions begin sooner for shallower colonies because the thermocline typically reaches a depth of 15 m at the beginning of August. Therefore, *C. caespitosa* colonies living at shallower depths were more exposed to thermal stress and showed

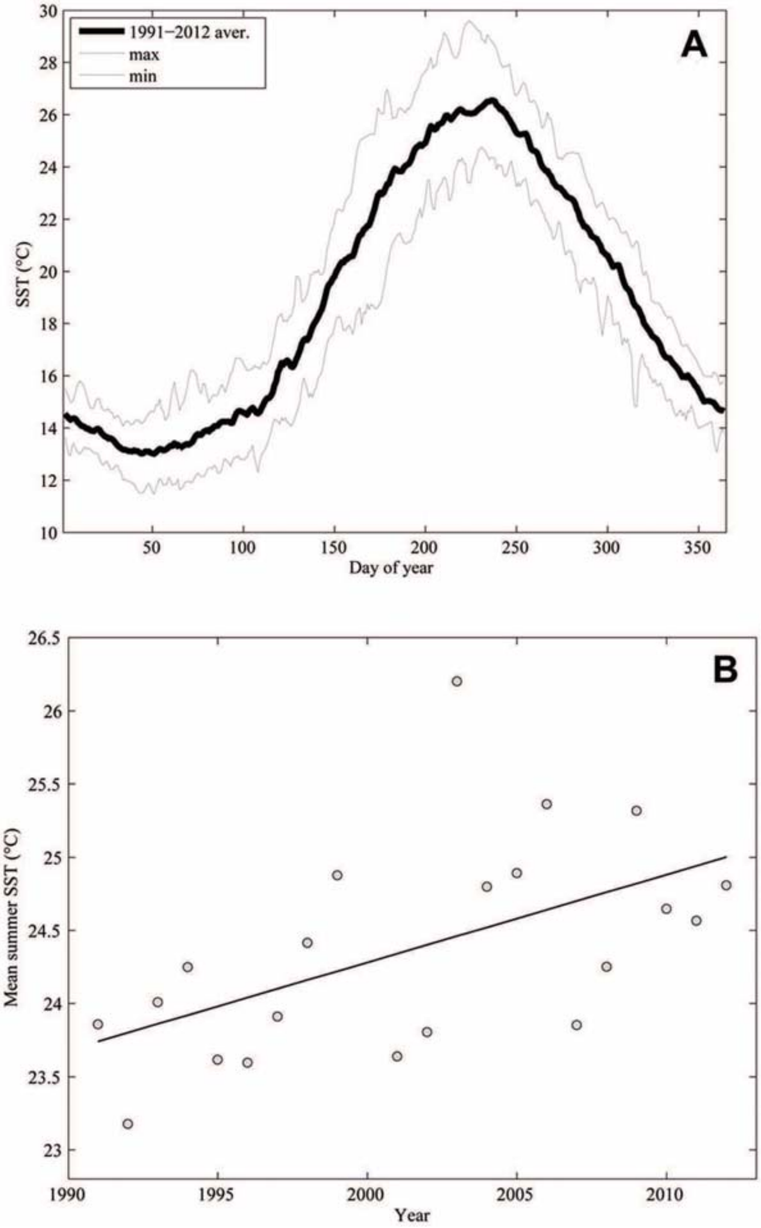

Figure 6. A. SST mean annual cycle in the Columbretes Islands (1991–2012). B. Mean summer SST (June-September, 1991–2012).
doi:10.1371/journal.pone.0070820.g006

greater mortality rates. As a result, changes in the depth distribution of this population are expected in the future due to the disappearance of the shallower colonies.

Relationship between mortality and temperature

Mortalities were recorded in the context of regional warming and occurred concomitantly with a shift in the regime of positive thermal anomalies in the Columbretes Islands. In particular, the first mortality was triggered by exceptionally warm conditions accompanied by the persistence for several days of extreme (>28°C) temperatures.

However, it is worth mentioning that our results are not in concordance with those found in the laboratory. During different aquaria thermo-tolerance experiments with *C. caespitosa* polyps (collected in the Ligurian Sea), the first signs of necrosis were

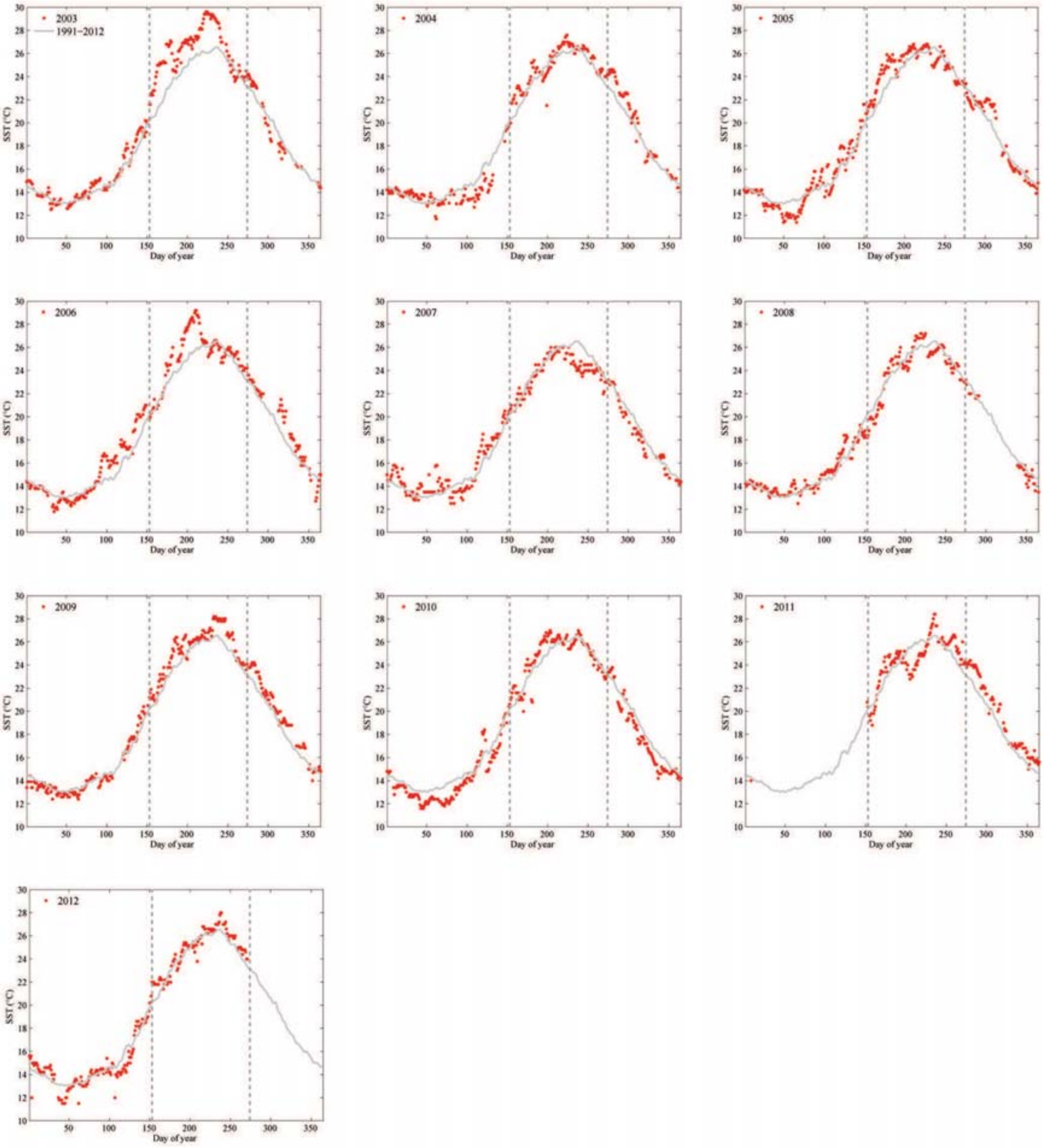

Figure 7. Annual thermal regime (2003–2012) and average SST for the data series 1991–2012. Dotted vertical lines delimit the summer period.
doi:10.1371/journal.pone.0070820.g007

detected after 5–7 weeks at 24°C, and all polyps that were exposed at 26°C and 28°C died after the treatments [20,25]. Based on these experiments, the authors proposed that *C. caespitosa* is living close to its thermal limit during the summer period in the Ligurian Sea and a long-term increase at 24°C or above could be lethal for it. In the Columbretes Islands, water temperatures at 15 m remained over 24°C for 10 weeks during the summer of 2007, which recorded negative thermal anomaly. This time span was 3 to 5 weeks longer than that reported in the mentioned experiments and no necrosis was detected. Similarly, in the summer of 2009, the average extent of necrosis was approximately 3%, and *C. caespitosa* colonies at 15 m were exposed to temperatures greater than 24°C for 68 days and to temperatures greater than 26°C for 34 days; this exposure was approximately three times longer than

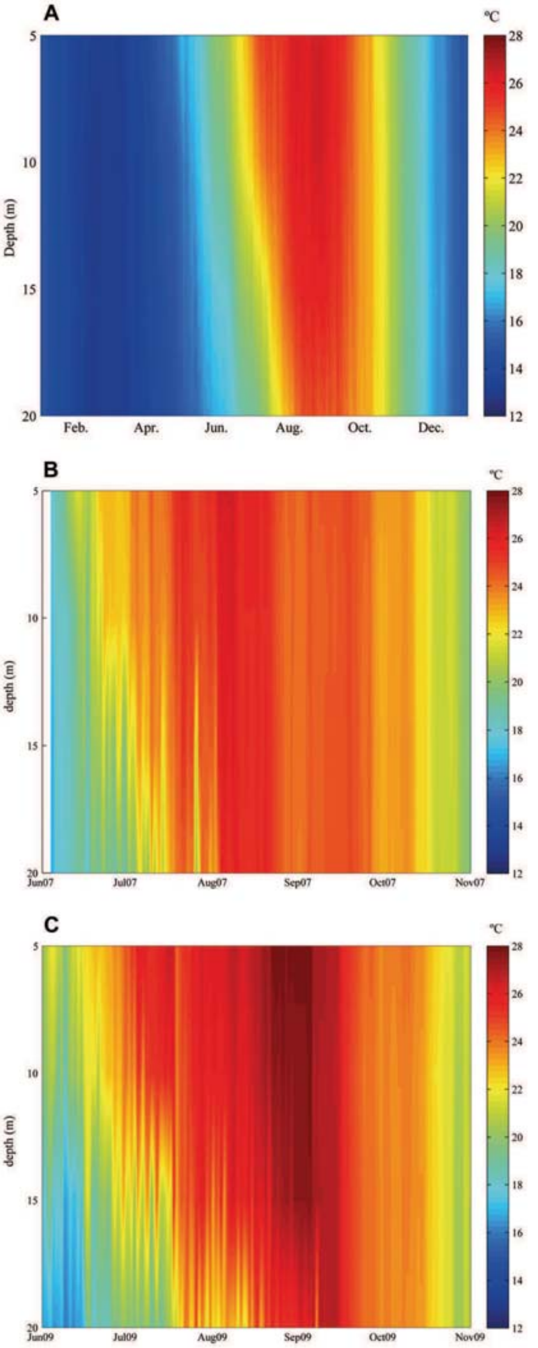

Figure 8. Thermal environment at a depth of 5 to 20 m. A. The 2007–2012 annual average B. Data from June to November in a summer with negative SST anomaly, 2007. C. Data from June to November in a summer with highly positive SST anomaly, 2009.
doi:10.1371/journal.pone.0070820.g008

the exposure that caused necrosis in 100% of the polyps in the aquaria experiment [25]. The differences found between the mortalities in aquaria (Ligurian Sea) and *in situ* (Columbretes Islands) could be related to two major points: differences in the thermal acclimatisation of *C. caespitosa* between both sites, taking into account that the colonies are naturally subjected to different thermal regimes, and the fact that aquaria experiments can only partially simulate the natural environmental conditions.

Another striking result is that the response of *C. caespitosa* to summers with positive thermal anomalies changed between the two mortality periods and particularly in relation to temperature thresholds. The correlation between necrosis and the persistence of water temperature thresholds for the entire data series was only significantly positive when assessed using the 27°C and 28°C threshold. However, a significant positive correlation between necrosis and temperature thresholds of 24°C, 25°C and 26°C was found when considering only the first mortality period, while no correlation was found for the second period.

During this 11-year study, mortality events occurred in two separated periods, i.e., 2003–2006 and 2008–2012. The average necrosis diverged significantly in these two periods (19% vs. 4%, respectively), and important differences in the average thermal anomaly were also found (1.00°C and 0.39°C, respectively). However, with the same positive thermal anomaly (approximately 1°C), different years such as 2006 and 2009 registered contrasting necrosis (19% vs. 3%, respectively).

As our results prove, it is unequivocal that sea water temperature is one of the main factors that triggered *C. caespitosa* mortality events. Nevertheless, the differences found in necrosis between years with similar thermal anomalies show that other factors are also acting in this process.

Synergies with other factors

Water quality and ecosystem conservation has been ensured in the Columbretes Islands Marine Reserve since its creation in 1990. Furthermore, the location of the islands far from mainland (60 Km) guarantees low interaction with nearshore waters. Therefore, factors such as water quality or dysfunctions in trophic interactions derived from overfishing, that might be relevant in unprotected areas [33], were excluded in the present study.

Although irradiance, especially photosynthetically active radiation (PAR), has been shown to be directly related to tropical coral bleaching [34–36], we disregarded it as a possible factor acting in the *C. caespitosa* mortalities. Depending on the depth and water type, irradiance can be significantly attenuated [34,37]. Bearing in mind the depth range of our studied *C. caespitosa* population we can assume an important reduction in irradiance. Furthermore, the zooxanthellae in symbiosis with *C. caespitosa* (*Symbiodinium* Clade A) are considered light-adapted [38,39]. Finally, a pattern in the necrosis scars related to the effects of irradiance, as reported in tropical corals [34], was not observed in *C. caespitosa*.

Disease outbreaks have affected an increasing range of marine organisms in different geographic regions worldwide [40]. In the Mediterranean Sea, thermally dependent pathogens have been considered co-responsible for mass-mortalities and coral bleaching [15,41,42]. Although, as far as we know, no studies have dealt with this issue in *C. caespitosa*, the type of necrosis (lysis) suffered by this species could be related to a disease, such as that caused by *V. coralliilityticus*, which synthesises a potent extracellular protease that lyses coral tissue [43]. Although no analyses were conducted to detect opportunistic pathogens in the *C. caespitosa* mortalities, the possible role of pathogens or even polymicrobial consortiums as recently suggested in other tropical coral species [44], should not be disregarded. Previous studies have demonstrated that the occurrence of *Vibrio* bacteria in the NW Mediterranean Sea is climate linked, greatly increasing under the inuence of positive temperature anomalies as the observed ones in Columbretes Islands [45].

In tropical corals, greater energy reserves or greater access to resources could compensate for decreased photosynthesis during bleaching events [46,47]. In the Mediterranean Sea, temperature-related mortalities have been associated with physiological stress due to energetic constraints [5]. According to these data, Crisci *et al.* [17] considered physiological status to be a primary factor explaining differential mortality rates.

C. caespitosa has the ability to upregulate heterotrophy and maintain symbiosis, even under suboptimal conditions [48]. These authors detected maximum feeding effort when colonies were kept under high light with an irregular food source (typical Mediterranean summer conditions). Consequently, variation in the availability of food previous to and during warm summers could have an important effect in the energy budget of *C. caespitosa*. Furthermore, the impact of extreme summers (like 2003) on the energy budget of the polyps could be responsible for delayed effects in their physiological status.

Processes such as spawning that cause a reduction in tissue lipid content could also have an important effect on the severity of mortality [46]. Histological analyses showed that maximum gonadal development in *C. caespitosa* is reached in August [49] in coincidence with SST maxima, and spawning occurs at the end of the summer. Consequently, the interaction between sexual reproduction and necrosis could be reciprocal: necrosis could be enhanced due to increased energy investment in gonad development, and spawning could be affected by the mortality of the polyps.

With this in mind, we hypothesise that delayed physiological thermal stress could be the primary factor, acting together with temperature, that would explain the differences in necrosis during summers with similar thermal anomalies but with different interannual contexts. This sensitisation hypothesis has also been mentioned in regards to the mass-mortality of 1999 [11].

Searching for acclimatisation and adaption processes

The processes of acclimatisation (phenotypic response) and adaption (genotypic response) have been extensively studied and discussed in relation to thermal anomalies causing bleaching events in tropical corals [30,50–54]. While some authors extend hope for rapid evolution and adjustment [50,51], others question the capacity of corals to adapt to rapid climate change [53].

Through comparisons of bleaching events in tropical corals, several authors have found that corals were more resistant to temperature stress as the bleaching events repeated [55–57] and that the bleaching resistance shown by corals at sites dominated by high-frequency SST variability could be a consequence of rapid directional selection following an extreme event [57].

Although the SST series in the Columbretes Islands showed a dramatic increase in the frequency of positive thermal anomalies, as well as a positive warming trend, the differences in mortality detected between summers with similar thermal anomalies did not seem related directly to directional selection. *C. caespitosa* colonies that survived the first mortality period were affected in the second period, although the thermal anomalies had lower positive values on average; therefore, survival was most likely not solely a result of differential survival of more tolerant genotypes.

In this sense, we found that necrosis in the second mortality period (2008–2012) showed no differences between colonies that were unaffected or affected during the first mortality period. Differences between these groups would have been expected if selection was acting on thermal tolerance.

Nevertheless, it is remarkable that approximately 20% of the surveyed colonies remained unaffected over the entire study period and that a very low percentage experienced total mortality due to accumulated recurrent necrosis. These results may indicate the occurrence of more tolerant colonies or even parts of colonies; however, as discussed above, selection for thermal tolerant genotypes alone cannot explain the detected changes in necrosis. In conclusion, these mortalities do not relate to previous necrosis impacts on the same colonies; the occurrence of necrosis at the colony level seems more closely related, in general terms, to random processes involving the occurrence of pathogens or the energetic status of the polyps, as previously discussed.

The importance of context-dependent effects

The summer of 2003 was likely the warmest summer in Europe since 1500 [58] and affected 25 rocky benthic macroinvertebrate species over several thousand kilometres of Mediterranean coastline [13]. The mean SST anomaly registered in the summer of 2003 in the Columbretes Islands (1.83°C) was 80% warmer than the second positive SST anomaly recorded in the series (in 2006). During this summer, 25% of the area covered by *C. caespitosa* in the Columbretes Islands was necrosed.

As discussed above, the extreme conditions of 2003 could have been responsible for a delayed physiological stress in the colonies, influencing the mortalities registered in the following summers (2004 and 2005), which were quite important (approximately 20% and 13% of necrosis, respectively); however, the positive SST anomalies during these summers were relative low (0.42 and 0.52°C, respectively).

The second mortality period (2008–2012) began after a year with negative SST anomalies and no necrosis (2007). This could have given *C. caespitosa* enough rest to withstand the mortality events of the next summers with much lower necrosis, in addition to the fact that no extreme conditions (such as those observed in 2003) were present during the second period. In this period, summers with similar or even higher SST anomalies than in the first period exhibited mortality events with less than 7% necrosis. Although the first mortality event of the second period (2008) was registered after a summer with an average negative SST anomaly (-0.12°C), several weeks of strong positive anomalies were recorded during the middle of this summer.

However, what could have happened prior to 2002? The mortality of 2003 could be considered the first mass-mortality of *C. caespitosa* in the Columbretes Islands in the last two decades. Although necrosed colonies or sections of colonies were eventually covered by epibionts, they were perfectly noticeable over many years. Thus, a mass-mortality event prior to 2003 should have left a high percentage of detectable bare skeletons in the colonies, but the old necrosis detected was near 3%. This is consistent with the thermal anomalies recorded in the available SST series during the first decade of record (1991–2002), which were much lower and less frequent than during the second decade. The summer of 1999 could have been the one in which some mortality would have been expected because the SST anomaly reached 0.50°C; furthermore, this summer triggered a multispecies mass-mortality event in the NW Mediterranean [10,12,28]. That the summer of 1999 most likely did not cause high necrosis rates reinforces our hypothesis that some type of sensitisation or delayed stress occurred after the summer of 2003 because the summers of 2004 and 2005 had similar SST anomalies to those recorded for 1999 but triggered high necrosis.

Three important findings can be highlighted from the results obtained in this study. First, a significant positive correlation between mortality descriptors and SST anomalies was found over the entire studied period. Second, significant differences between the two mortality periods were found when correlation analyses were performed separately. Third, when removing the years without mortality (2002 and 2007) significance disappeared for the whole studied period. Two main conclusions can be drawn from these results. First, the significant, strong association between mortality descriptors and SST anomalies, when looking at the whole series, is more closely related to the concurrence of necrosis events and SST anomalies than to the specific intensity of these variables; as significance is lost when removing the years with no mortality and necrosis was generally detected in years with a positive SST anomaly, but summers with similar SST anomalies showed different responses in *C. caespitosa* necrosis. Second, the effects of the intensity of the SST anomalies on the necrosis rates seem to have been enhanced during the first period, which would be consistent with the delayed thermal stress hypothesis.

The complexity of the factors influencing these mortalities highlights the need for precise and continuous long-term monitoring of biotic and abiotic factors to move forward in our understanding of these events and their effects on the future viability of the benthic communities threatened by the increase in frequency and persistence of extreme events projected for the 21st century in the Mediterranean [2,3]. Recurrent extraordinary mortality episodes, such as the ones registered between 2003 and 2006, could likely be repeated and will threaten this species, which, due to its slow dynamics, will most likely not be able to cope with elevated mortality rates. Nevertheless, considering the less virulent mortalities registered in the second mortality period, the high coral cover in areas such as the Columbretes Islands [22], and the potential for colony-on-colony recruitment as an indirect mechanism of recovery, there can still be some hope for *C. caespitosa* banks in the Mediterranean Sea and particularly in the Columbretes Islands.

Supporting Information

Table S1 Scheffé's contrast test obtained from a one-way ANOVA comparing summer SST anomalies among years.
(DOC)

Table S2 Results of the multiple correlation tests between annual necrosis and temperature descriptors. Significant correlation is highlighted in bold.* Without 2002 and 2007.
(DOC)

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Author Contributions

Conceived and designed the experiments: D-KK CL. Performed the experiments: D-KK. Analyzed the data: D-KK NB. Contributed reagents/materials/analysis tools: D-KK CL. Wrote the paper: D-KK NB CL.

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