



Conservation biology of the endangered orange coral

*Astroides calycularis*

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Tesis Doctoral 2015

A polyp of *Astroides calycularis* (modified image) from populations of Punta de la Mona (Almuñécar, Granada). Author: Terrón-Sigler A.



Laboratorio de Biología Marina  
Facultad de Biología  
UNIVERSIDAD DE SEVILLA

**Conservation biology of the endangered orange coral**  
*Astroides calycularis*

**Biología de la conservación del coral naranja amenazado**  
*Astroides calycularis*

Memoria presentada para optar al título de Doctor por la Universidad de Sevilla

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El director Dr. Free Espinosa Torre, profesor titular del Departamento de Zoología de la Universidad de Sevilla y el tutor Dr. Jesús Castillo Segura, profesor titular del Departamento de Biología Vegetal y Ecología de la Universidad de Sevilla,

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En Sevilla a 13 de octubre de 2015

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**Aquellos que afirman que no se puede hacer,  
no deben interrumpir a aquellos que lo estamos haciendo.**

(Regla romana)





**A Carmen Lacasa Madero**

**A David León Muez y Patricio Peñalver Duque**

**Y a todos aquellos que siguen creyendo en la Asociación Hombre y Territorio**



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Tras finalizar el informe de la primera parte del proyecto y los logros que habíamos conseguido con él, decidimos seguir realizando actuaciones con el



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## RESUMEN GENERAL

*Astroides calycularis* (Pallas 1766) es un coral escleractinio colonial azooxantelado con un característico color naranja. Es decir, es un coral que forma un exo-esqueleto de carbonato cálcico utilizado como protección de los pólipos de la colonia y que no presenta algas simbiotes en sus tejidos (zooxantelas).

La especie se distribuye principalmente en el mar Mediterráneo sur-occidental y, aunque existen citas de este coral en el mar Adriático y se adentre en el océano Atlántico hasta la ciudad de Rota (Cádiz, España) y Cabo Espartel (al norte del continente africano, en Marruecos), se considera que *A. calycularis* es endémico del Mediterráneo. Su estrecho rango de distribución se debe, entre otros aspectos, a los requerimientos ambientales de la especie, sobre todo en temperatura y aguas transparentes y bien oxigenadas. La especie habita fondos rocosos desde la superficie del mar hasta los 50 metros de profundidad, aunque principalmente se encuentra en los primeros 15 metros, tanto en sustratos verticales como horizontales y tanto en ambientes oscuros (grietas y entradas de cuevas) como bien iluminados. Localmente se pueden encontrar poblaciones muy abundantes de hasta un 95% de cobertura.

El coral naranja es una especie que se encuentra afectada por las actividades humanas desarrolladas en el litoral. Así, las obras litorales, la elevada sedimentación o las actividades recreativas desarrolladas en el mar, entre otros aspectos, impactan de forma negativa sobre sus poblaciones, mermándolas o degradándolas. Por todo ello, la especie se encuentra amenazada e incluida en catálogos regionales y nacionales (*Libro Rojo de Invertebrados Amenazados de Andalucía* y en el *Atlas y Libro Rojo de los Invertebrados Amenazados de España*) y en anexos internacionales como los convenios de Berna y Barcelona y la convención CITES (Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres). Además, recientemente la UICN (Unión Internacional para la Conservación de la Naturaleza) ha incluido al coral naranja en evaluación para su entrada en la Lista Roja.

En la costa andaluza, enclavada al norte del mar de Alborán (sur de la Península Ibérica), se encuentra el límite de distribución más occidental del coral naranja (el otro localizado en la costa marroquí). Con más de 800 kilómetros de costa, Andalucía se caracteriza por su turismo de sol y playa con un elevado desarrollo costero dirigido a cubrir las necesidades turísticas. Este hecho hace que las poblaciones presentes en este litoral se encuentren sometidas a diversos impactos. Por ello, el objetivo principal de la presente tesis es avanzar en el conocimiento de la biología y ecología de *Astroides calycularis* en el norte del mar de Alborán desde la perspectiva de la conservación. De esta forma, se han diseñado y desarrollado una serie de estudios y ensayos para que la información originada a partir de ellos sirva de utilidad a los gestores marinos

competentes en un futuro cercano. Siguiendo esta premisa, la presente tesis se estructura de la siguiente manera:

*Estudio sobre la estrategia reproductora del coral naranja.* Este apartado trata de probar las observaciones personales realizadas en campo sobre la estrategia reproductiva de la especie. Para ello se recolectaron colonias del medio a diferentes profundidades durante un ciclo anual y, mediante técnicas histológicas, se obtuvo la información necesaria sobre la estrategia reproductora de *Astroides calycularis* considerando dos factores: uno exógeno, la profundidad; y otro endógeno, el área de la colonia. Los resultados obtenidos son de gran relevancia a la hora de implementar estrategias de gestión dirigidas, por ejemplo, a la restauración o regeneración de poblaciones de *A. calycularis* mermadas o dañadas por cualquier tipo de impacto, ya que se debe tener en cuenta el factor exógeno de la profundidad.

*Astroides calycularis* como hábitat. La especie presenta un gran valor ecológico como tal, pero en este apartado se pretendía evaluar su valor añadido como posible micro-hábitat para otros macro-invertebrados marinos. De esta manera, se recolectaron colonias mensualmente a tres profundidades diferentes, se separaron los diferentes especímenes de macro-invertebrados asociados a las colonias, se identificaron y cuantificaron. Los resultados obtenidos ponen en valor esta especie como hábitat para muchas especies de invertebrados. Además, se ha levantado información sobre la importancia de la especie en las redes tróficas marinas de la zona objeto de estudio y las estrategias alimenticias de los principales macro-invertebrados asociadas a *A. calycularis*, así como el tipo de relación de los mismos con su hospedador.

*Amenazas.* Muchas son las amenazas a las que está sometida la especie a lo largo de toda su distribución mediterránea, pero la mayor parte de ellas no han sido cuantificadas ni caracterizadas. Es lo que ocurre con la actividad recreativa mediante buceo con escafandra autónoma. Por ello, uno de los objetivos específicos de este apartado es cuantificar y caracterizar el impacto que producen los buceadores autónomos en uno de los enclaves más importantes para el buceo recreativo de la costa andaluza (Almuñécar, Granada) y donde se encuentra una de las poblaciones más abundantes de coral naranja. Por otro lado, dentro de este mismo apartado, se ha registrado la distribución de las poblaciones de *Oculina patagonica*. Este coral zooxantelado, en un principio considerado como especie invasora por su gran capacidad de colonización, se está expandiendo y ha aumentado sus poblaciones en el Mediterráneo en las últimas décadas. De esta manera, la localización y censo de sus poblaciones en el litoral andaluz, aportará un punto de referencia para conocer si en el futuro la especie compete o excluye al coral naranja, sobre todo en aquellos ambientes donde esta especie es abundante (sustratos rocosos bien iluminados).

*Distribución geográfica de las poblaciones de Astroides calycularis del norte del mar de Alborán.* Es necesario obtener puntos de referencia para evaluar el estado de



las poblaciones de *A. calycularis*. Existen citas y estudios de seguimiento de algunas zonas a lo largo de su distribución, pero hasta ahora no existía una base de datos de la presencia y abundancia geo-referenciada de las poblaciones del coral naranja en el litoral andaluz. Por tanto, en este apartado se ha establecido por primera vez un punto de referencia o estado inicial de las poblaciones litorales de esta especie. Dicha información es de vital importancia para el seguimiento, evaluación y gestión de las poblaciones de *A. calycularis* más importantes del norte del mar de Alborán.

*Restauración.* Algunas de las poblaciones de *Astroides calycularis* se están viendo mermadas como consecuencia de los impactos antropogénicos originados en el litoral. Por ello, urge implementar metodologías o técnicas para la restauración y/o potenciar la recuperación de áreas donde la especie esté especialmente afectada por causas naturales o no naturales. Con este fin se han diseñado y puesto en marcha dos metodologías diferentes: una mediante un sistema de fijación traslocando y trasplantando colonias; y una segunda técnica mediante sustratos artificiales para la captación de larvas del medio natural. Las ventajas e inconvenientes de cada técnica han sido discutidos y analizados en el apartado correspondiente, estableciéndose una técnica fácil de desarrollar y eficaz para restaurar posibles poblaciones de coral naranja.

Finalmente, todos los resultados son discutidos en conjunto y se plantean nuevos horizontes sobre los estudios necesarios para la conservación de este emblemático coral en el litoral andaluz.



The background of the slide is a photograph of a rocky seabed covered with numerous bright orange sea anemones. The anemones are densely packed in some areas and more sparse in others, revealing the grey and brown rocks underneath. The lighting is somewhat dim, suggesting an underwater environment.

## 1. GENERAL INTRODUCTION

Abundant densities of *Astroides calycularis* from Marina del Este beach (Almuñécar, Granada). Author: Terrón-Sigler A.

## 1.1 GENERAL CHARACTERISTICS OF AHERMATIPIC SCLERACTINIAN CORALS

The phylum Cnidaria is a diverse group of relatively simple animals united by the ability to synthesise cnidocysts as a synapomorphy. These organelles are like capsules with eversible tubules (Weill 1934; Watson & Wood 1988), which function to catch prey, for defence and/or to make a structure. Of the three types of cnidae (nematocysts, ptychocysts and spirocysts), only nematocysts are found across the classes. These animals, generally marines, have been characterised by a radial symmetry. Nevertheless, analyses about the internal morphology demonstrate that the asymmetry of their corporal plan is common among classes (Gudo 2002) and present radial, bi-radial, tetra-radial, and bi-lateral patterns (Finnerty 2003). Species has oral and aboral differentiation, and the oral side are present one or more than one tentacles crown. These animals can be solitary or colonial, with medusa and/or polyp stage, and has both sexual and asexual reproduction.

Cnidarians comprise five classes: Hydrozoa; Scifozoa; Anthozoa; Cubozoa, and the recently designated Staurozoa (Marques & Collins 2004). Corals are located in the Anthozoa class and currently contain approximately 7,500 extant species, which comprise two reciprocally monophyletic lineages (Daly *et al.* 2007); Octocorallia corals with eight pinnate tentacles and septum; and Hexacorallia with a high number of tentacles and generally six or six multiple septums. All members are exclusively polypoid and may be colonial, clonal, or solitary, skeleton-less or with a mineral and/or proteinaceous skeleton.

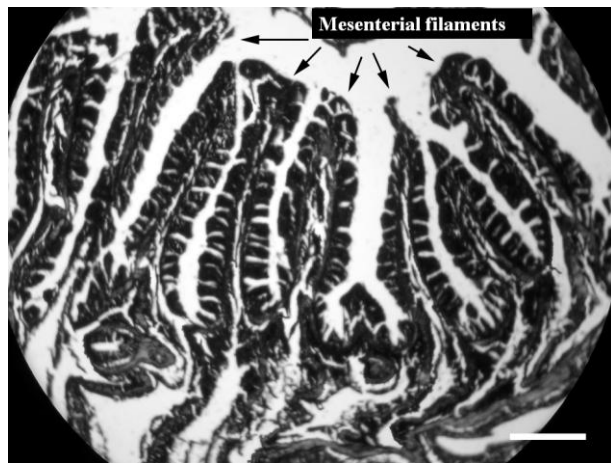
Hexacorallia currently contains about 4,300 extant species (Doumenc & van Praët 1987). As the name suggests, most hexacorallians have hexamerous symmetry, although eight- or ten-part symmetry are not uncommon. All members of Hexacorallia have spirocysts, a type of cnida with a single-walled capsule and a tubule composed of tiny entangling sub-threads (Mariscal *et al.* 1977). Hexacorallians currently contain six orders: Actiniaria (sea anemones), Antipatharia (black corals), Ceriantharia (tube anemones), Corallimorpharia (corallimorpharians), Zoanthidea (zoanthids) and Scleractinia (stony corals). The last can be solitaires or colonials with variable morphology, and whose principal character is that it presents a calcareous exoskeleton that is external to the soft tissues. Skeleton is secreted by epidermal cells at the base of polyps to form cup-like calyces subdivided by septa and into which the polyp can retract for protection. The Scleractinia are exclusively marine animals. Within the Anthozoa, such a skeleton is unique to the order. The approximately 1,300 described extant species (Cairns 1999) are divided ecologically into two main groups; reef builders and those which do not build reefs, the last is found in all regions of the oceans, including temperate and Polar Regions, and from relatively shallow waters to 6,000 m.

### 1.1.1 Anatomy

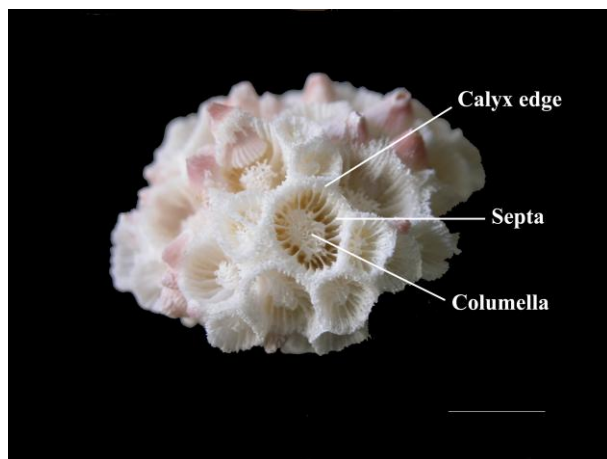
All Cnidarians are diblastic with two sheets embryological: ectoderm and endoderm; these are connected by connective tissue with relatively scarce cells, mesoglea (Chevalier 1987). Scleractinian polyps present a simple anatomy; inside there are folds (mesenteries) which give structural stability to the polyp, and in the free extreme of the mesenteries it can be found accumulation of mucus and digestive cells (mesenterial filaments) (Figure 1.1). The polyp wall (column or scapus) extends to the tentacles which are used both to catch food and for defence. The oral disc has the mouth (peristome) in the centre which extends inside, forming the pharynx (stomodeum) and contributes to the entrance and exit of the water and particles into the body cavity (gastrovascular cavity).

Scleractinian corals are different from other Anthozoans due to their ability to secrete a calcium carbonate exoskeleton (Vaughan & Wells 1943). Exoskeleton is secreted from the pedal disc, forming the calyx and sclerosepta (septa), from which the basic frame of the calcium skeleton originates. This is used as protection and defense. There are also species which make structures that protrude from the calyx, (pali and collumela). In the calyx wall, longitudinal elevations which run parallels to the septa (costa) may be differentiated (see Figure 1.2).

Polyps budding, forming new calyces contributes to the colony. In the colonies, the coenosteum is a skeletal matrix wherein polyps or individual calyces of a colony are embedded, and that cannot be assigned to any particular polyp. The polyps are connected by the cenosarco, a common tissue which is found over the calyx wall and the coenosteum.



**Figure 1.1:** Microscopic view of the *Astroides calycularis* mesenterial filaments. Scale bar = 100  $\mu$ m.



**Figure 1.2:** Exo-skeleton of *Astroides calycularis* which it can be diferenciate some structures. Scale bar = 1 cm.



### 1.1.2 Cnidom

Cnidae are the defining characteristic of phylum Cnidaria and are especially important in anthozoan taxonomy (Doumenc & Van Praët 1987; Fautin 1988; Fautin & Mariscal 1991). The cnidocysts are among the most structurally complex and enigmatic organelles in the animal kingdom (see Burnett *et al.* 1960; Mariscal 1974, 1984). They have diverse functions, such as capture of prey, defence, adhesion, construction of mucous tubes and locomotion (see Hand 1961; Mariscal 1974; Holstein & Tardent 1984; Fautin & Mariscal 1991; Kass-Simon & Scappaticci 2002). Shostak & Kolluri (1995) speculated about their possible origin in the symbiosis with protists. Referring to the diversity and distribution of cnidae of more than 800 species, these authors suggested that the present situation of cnidae was attained in at least two major evolutionary steps.

Three basic types of cnidae are usually distinguished: spirocysts, nematocysts and ptychocysts (Mariscal *et al.* 1977; Fautin & Mariscal 1991; Östman 2000). More recently, a fourth type of cnidae was proposed based on the study of agaricid scleractinians (Pires 1997), the agaricysts, but for a final acceptation more information on its variability and ultrastructure is needed.

The anthozoans differ from the hydrozoans, schyphozoans and cubozoans by possessing all three basic types of cnidae (see Mariscal 1984; Östman 2000). Nematocysts occur in all cnidarians, spirocysts only in hexacorals, and ptychocysts only in ceriantharians (see Mariscal 1984, Fautin & Mariscal 1991). The diversity of nematocysts is greatest within Hydrozoa, but Anthozoa is characterised by the greatest degree of cnidae diversity, as its members have two types of cnidae in addition to nematocysts (Watson & Wood 1988; Fautin & Mariscal 1991). Spirocysts have a tubule with threadlike mini-tubules that trap prey or other objects (Figure 1.3); these cnidae have been described as restricted to Hexacorallia (e.g. Mariscal 1974). Ptychocysts have an unarmed tubule that is pleated rather than helically folded; these cnidae are found only in ceriantharians and are used in the construction of their felt-like tube (Mariscal *et al.* 1977). The morphology, size and distribution of nematocysts are used to define groups, particularly within Actiniaria and Corallimorpharia, in which attributes of cnidae characterise families, genera or species (e.g. Carlgren 1940; 1945; Allcock *et al.* 1998).

Following Weill's (1934) nomenclature and Carlgren's (1940) amendments, nematocysts comprises:

1. **Haplonemes**, with a tubule lacking well-defined shaft; according with the spines and spine patterns it can be differentiate (Östman 2000): Athrics, without spines; Basitrichs (Figure 1.3), spines at the base of the tubule; and Holotrichs (Figure 1.3), tubule-spined throughout.

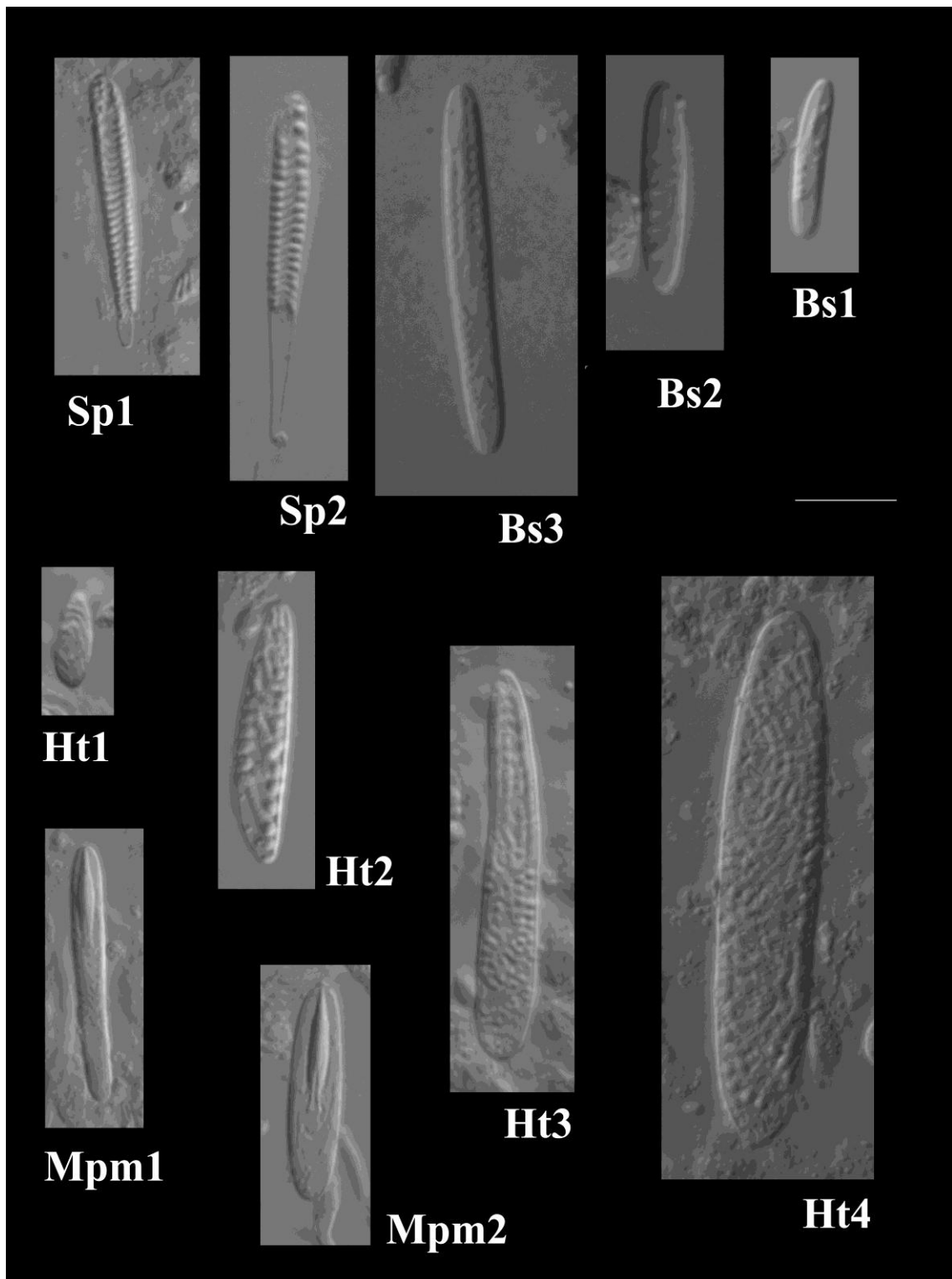
2. **Heteronemes**, nematocysts which have a tubule with a well-defined shaft. According with the shaft it can be differentiate (Östman 2000):

- *p*-mastigophore (Figure 1.3), V-shaped notch at base of unfired shaft. These can be microbasic, a shaft or prominent proximal armature less than one and a half times the capsule length; and macrobasic, a shaft more than four times longer than the capsule's long axis.
- *b*-mastigophore; no V-shaped notch at base of unfired, narrow shaft; discharged shaft or proximal tubule approximately the same diameter as the remaining tubule, proximal tubule with prominent armature with differences in size and shaft.

For each one, there are different sizes in length and width. Therefore, they must be represented and measured to be considered a taxonomic character.

The first information about the cnidocysts of *Astroides calycularis* was studied by Carlgren (1940). Nevertheless, recently the whole cnidom of *A. calycularis* of populations from South of Spain has been studied (Martínez-Baraldés *et al.* 2014). These authors have showed that cnidom is comprised of two types of cnidocysts: spirocysts (Sp) and nematocysts. Ten nematocysts categories were found; three basithrics (Bt); four holothrichs (Ht); and three microbasic *p*-mastigophore (Mpm). Tentacles comprised spirocysts, Bs3, Ht2 and Mpm3; while pharynx presented Bs2, Ht2 and Mpm1, and mesenterial filaments comprised only Ht1 and Mpm2. Significantly, scapus and columella obtained the same nematocyst composition: Bs1, Ht1, Ht4 and Mpm1 (see Martínez-Baraldés *et al.* 2014).





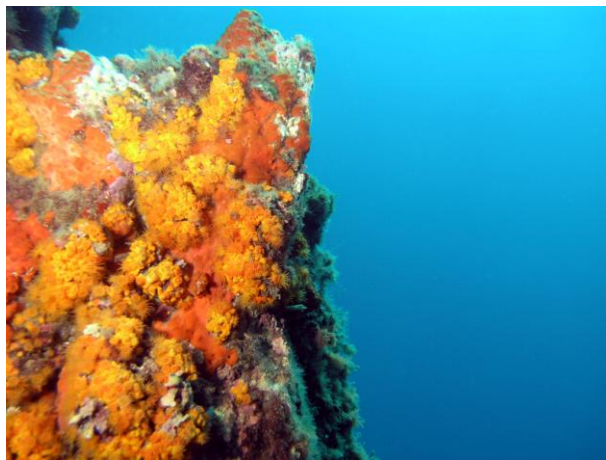
**Figure 1.3:** Some cnidocysts from scleractinian species. Sp = Spirocysts; Bs = Basitrichs; Ht = Holotrichs; Mpm = macrobasic *p*-mastigophore. Scale bar = 10  $\mu$ m.

## 1.2 ECOLOGY AND BIOLOGY OF *ASTROIDES CALYCULARIS*

*Astroides calycularis* is an azooxanthellate scleractinian colony coral with a carbonate calcium exoskeleton (Zibrowius 1980; 1983) that inhabits the rocky shore from the surface to 50-metres depth (Rossi 1971; Ocaña *et al.* 2000), but is typically found in the shallow infra-littoral (0 to 15-metre depth), on vertical walls or inside caves (Rossi 1971; Cinelli *et al.* 1977; Zibrowius 1978; Kružić *et al.* 2002) (Figure 1.4). It occupies both well-lit and dark habitats and appears to prefer a highly hydrodynamic environment (Cinelli *et al.* 1977; Zibrowius 1978; 1995; Kružić *et al.* 2002). The population density can be locally high, with colonies covering up to 90% of the sea bottom (Goffredo *et al.* 2011a). In places of high hydrodynamics, the species typically present forms with massive colonies and polygonal corallites. In sheltered or deeper places, colonies tend to have a bush-shaped morphology with almost circular corallites. The ecological importance of *A. calycularis* as a bio-builder and a producer of coral banks have been previously shown in a study based on North African populations (Ocaña *et al.* 2009).

This orange coral has a narrow distribution due to its temperature tolerance (Zibrowius 1995) and its need for clean and well-oxygenated waters (García-Gómez 2007; Casado-Amezúa 2012). It is affected by siltation caused by high coastal urbanisation (Ocaña *et al.* 2009) since it is a suspension feeder (Cebrián & Ballesteros 2004). Human activities on the littoral increase marine pollution and/or habitat destruction, and it have been shown that orange coral is being affected (Moreno *et al.* 2008). It has also been demonstrated that recreational activities like SCUBA diving have a negative effect on their populations, because colonies can be damaged or removed by fins, hands and other diving equipment parts (Moreno *et al.* 2008; Terrón-Sigler & León-Múez 2009-Section 5).

*Astroides calycularis* is considered a warm-water species with a tolerance for a narrow temperature range (Bianchi & Morri 1994). Based on fossil evidence, it was widely distributed throughout the Western Mediterranean Sea during certain periods of the Pleistocene (Zibrowius, 1995), but it disappeared from the Northern Mediterranean areas during colder periods. Thus, Zibrowius (1995) considers this coral as an indicator of Quaternary climate



**Figure 1.4:** Orange coral colonies from the bottom (11 meters depth) of the Natural Park of Acantilados Maro-Cerro Gordo.

oscillations. Some authors consider *A. calycularis* to be an ancient Tethyan species related to the genus *Tubastrea*, which is spreading through the Indo-Pacific region; this response is similar to that of other warm-water species (see Ocaña *et al.* 2007; Ocaña *et al.* 2009).

Harrison & Wallace (1990) reviewed the history of research on coral reproduction and noted that sexual reproduction in scleractinian had been studied for 200 years since Cavolini observed *Astroides calycularis* planulae in the Mediterranean region. This species has been characterised as gonochoric, both at the polyp and colony level, and as brooder (Goffredo *et al.*, 2010). Field observations performed by various authors have characterised the larvae as having negative buoyancy and a demersal behaviour, and thus crawling along the vertical rocky wall until finding a substrate to settle on (Lacaze-Duthiers 1893; Goffredo *et al.* 2010).

Recently, Casado-Amezúa (2012) designed thirteen microsatellite loci for *Astroides calycularis* that could be useful for studies on conservation genetic research on populations of this species and help improve the resolution of individual identification. Actually, this author showed a slight differentiation between the Italian samples and the samples from the westernmost Mediterranean, and within the westernmost Mediterranean samples, between those from Murcia (Southeast of Spain) and the rest of the samples (from the Alboran Sea coast along the southern Iberian Peninsula and northern Morocco).

Scarce information regarding the macrofauna associated with *Astroides calycularis* is known. Following Zibrowius (1980), the species is parasitized by *Boscia anglicum* (cirripid). López González (1993) noted that is parasitized by *Megatrema anglicum* (cirripid). Richter and Luque (2004) found *Epitonium dendrophylliae* (Gastropoda: Epitoniidae) feeding on *A. calycularis*. Nevertheless, this mollusc is a deep-sea species; therefore, this observation could be punctual.

Due to their ecology and biology features, *Astroides calycularis* is protected by national and international organisations as an endangered species (Berna and Barcelona Conventions and CITES). Recently, the International Union for Conservation of Nature (IUCN) has listed the orange coral as Least Concern in view of the low probability that the population is reducing rapidly enough to result in a higher threat category. However, given its relatively restricted distribution and the natural and human threats affecting the shallow water populations, it is recommended to be reassessed in five years.

### **1.2.1 Current distribution**

The family Dendrophylliidae is cosmopolitan and includes both solitary and colonial corals; 148 living species are described and divided into nineteen genera

(Cairns 1999). Seven species live in the Mediterranean Sea, and these are grouped into five genera; three of these (*Astroides*, *Cladopsammia*, and *Dendrophyllia*) are colonial (Minelli *et al.* 1995). The genus *Astroides* is made up of a single species, *A. calycularis* (Cairns 2001).

Currently, the range distribution of *Astroides calycularis* is restricted to the south-central part of the Western Mediterranean Sea. In particular, it is found in the following regions: the southeast Iberian Peninsula, from the Strait of Gibraltar to Cape Palos (Murcia); the northern coasts of Africa, from the Strait of Gibraltar to Cape Bon in Tunisia; around Sicily and nearby islands; and the Gulf of Naples in the Tyrrhenian Sea (Zibrowius 1980; 1995; Bianchi 2007; Goffredo *et al.* 2010). Moreover, it is also found in Malta. It is also present in Atlantic waters in the Espartel Cape (Morocco) and La Caleta (Cádiz; Spain), probably due to the currents dispersing larvae out of the Strait of Gibraltar (Ocaña *et al.* 2000, Casado-Amezúa 2012; Casado-Amezúa *et al.* 2012). Recently, *A. calycularis* has also been found in the Adriatic Sea, along the coast of Croatia (Grubelic *et al.* 2004) up to the Gulf of Venice (Casellato *et al.* 2007). The recent range expansion of this species into the Adriatic Sea has been influenced by the warming of seawater with the prevailing sea current system and the rocky coastal configuration (Grubelic *et al.* 2004). However, currently, this species is disappearing in some places because of destruction or loss of habitats caused by human activities, such as coastal development, pollution, diving, angling and illegal fishing of the endolithic date-mussel *Lithophaga lithophaga* (Templado *et al.* 2004; Moreno *et al.* 2008).

Inside the Iberian Peninsula, the highest densities can be found in the Andalusian shores (Alboran Sea). Andalusian rocky shores (South of Iberian Peninsula) host one of the highest densities of the endemic and endangered orange coral (*Astroides calycularis*).

### 1.2.2 Threats

As assessors of the last IUCN Red List Assessment of Mediterranean Anthozoa Workshop, several scientists (Terrón-Sigler A., Linares C., Goffredo S., García S. and Ocaña O.) accorded that some ecological constraints, such as its narrow range of thermal tolerance, make the species suitable as a climate change indicator (Zibrowius 1995). Moreover, orange coral could be considered as a good indicator of well-oxygenated waters (García-Gómez 2007, Casado-Amezúa 2012), being affected by coastal development which creates stressful conditions for the growth of the polyps and/or reduced recruitment and a decrease of the overall population density. The species is also affected by the siltation derived from a high coastal urbanisation (Ocaña *et al.* 2009), since it is a suspension-feeder, being more abundant in very shallow waters (Cebrián & Ballesteros 2004).

It is already demonstrated that recreational activities like SCUBA diving can have a negative effect on *Astroides calycularis* populations because colonies can be damaged or removed by the impact of fins, hands and others diving equipment parts (Moreno *et al.* 2007; Di Franco *et al.* 2009; Terrón-Sigler & León-Muez 2009-Section 5). Occasional collections by SCUBA divers also pose a threat (Zibrowius 1995; Ocaña *et al.* 2009). Moreover, populations are affected by recreational and professional fisheries (Terrón-Sigler & León-Muez 2009-Section 5). Finally, a new threat of lost or abandoned fishing gear (ghost fishing) is being observed because it affects this species (Figure 1.5).



**Figure 1.5:** Ghost fishing gear on *Astroides calycularis* colonies (Marina del Este Beach, Almuñecar, Granada, South of Spain).

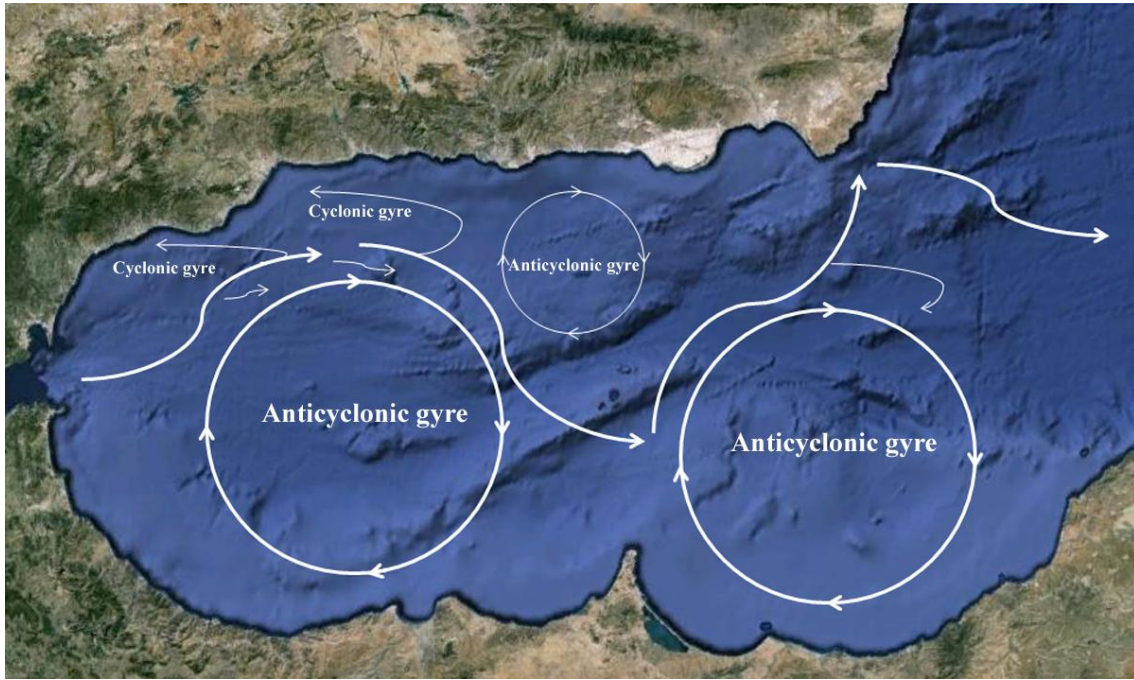
All these threats are linked to human activities; thus, a species framework should be addressed to mitigate these threats with specific management tools. Natural causes may produce mass mortality events, but also a rapid replacement of the populations was observed at least in deep waters (20-60 metres) (IUCN workshop Genoa, Italy, 2014).

Therefore, the last IUCN redlist assessment was that although the species has a restricted distribution, this is an abundant species in the South-Western Mediterranean basin. Colonial plasticity makes the species well adapted to a wide range of habitats, supporting competition for space with algae and sponges. The species is listed as Least Concern in view of the low probability that the population is reducing rapidly enough to result in a higher threat category. However, given its relatively restricted distribution and the natural and human threats affecting especially shallow-water populations, it is recommended to be reassessed in 5 years (IUCN workshop Genoa, Italy, 2014).

### 1.3 GENERAL CHARACTERISTICS OF THE ALBORÁN SEA

The Alborán Sea is where the Atlantic Ocean waters meet the Mediterranean Sea. This basin is located between the Strait of Gibraltar and a line that joins Cabo de Gata (South-eastern of Andalusia region-South of Spain) with Cape Fegalo (Orán-Algeria-North Africa) (Figure 1.6).





**Figure 1.6:** The Alborán Sea and the main surface streams.

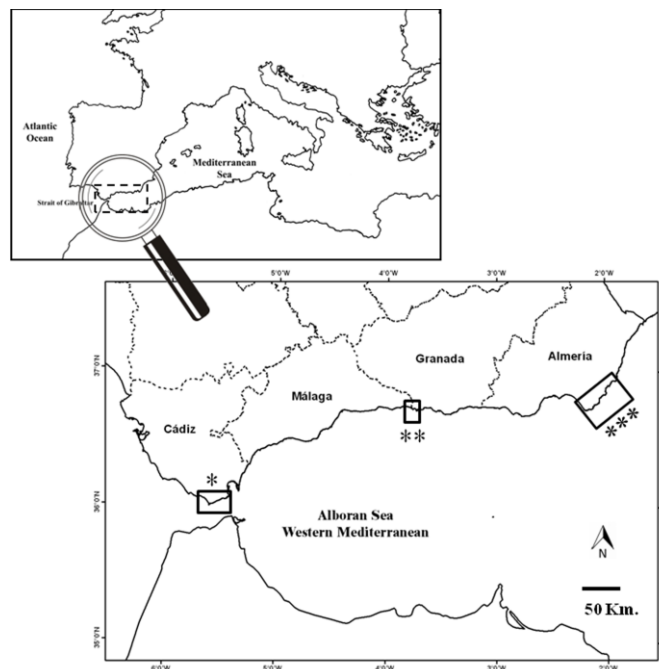
Mediterranean waters present a higher salinity than Atlantic Ocean waters due to the high evaporation and low water supply. By this situation, the Atlantic waters run superficially over Mediterranean waters in the Alborán Basin. Generally, the Alborán Basin is occupied by highly dynamic superficial waters originating from the Atlantic, which from one or two large anticyclonic gyres at the entrance to the Strait of Gibraltar, eventually covering the entire basin, and the several smaller cyclonic gyres near the coast. Initially, the Atlantic waters run superficially to the Northeast from the Strait of Gibraltar. Later, the waters of the front coastal line of Málaga and Granada (Andalusia region) run to the south making a big anticyclonic gyre (named “Alborán gyre”); this is very stable. Now, the Atlantic waters have two options: firstly, they may flow to the Alborán Island (South-eastern) and then back to the North-western region (origin of the cyclonic gyre); or secondly, waters from the Málaga-Granada coast may follow in the East direction and then turn to the South in the Algerian coast and follow to the West. This is the second anticyclonic gyre (Figure 1.6). There are areas where the waters mix and form an up-welling of deep waters, principally the “Alborán gyre” (Figure 1.6).

The submarine topography is singular; the western basin falls to around 1,500 metres and the eastern basin to over 2,000 m. of maximum depth. The continental shelf is narrow, with a depth between 100 and 150 metres (Templado *et al.* 2006).

This situation in the Alborán Sea may have led to the finding of Atlantic species, both from cold waters (European coasts) and warm waters (African coasts), together with Mediterranean species and several endemisms from this area.

### 1.3.1 Andalusian coast

Andalusian littoral covers about 812 km between the Atlantic and the Mediterranean coastal line. This special area harbours three specific Marine Protected Areas (MPAs) with different natural and geological characteristics: the Natural Park of the Strait of Gibraltar (Cádiz), the Natural Park of Acantilados Maro-Cerro Gordo (Málaga-Granada), and the Natural Park of the Cape of Gata-Níjar (Almería) (Figure 1.7).

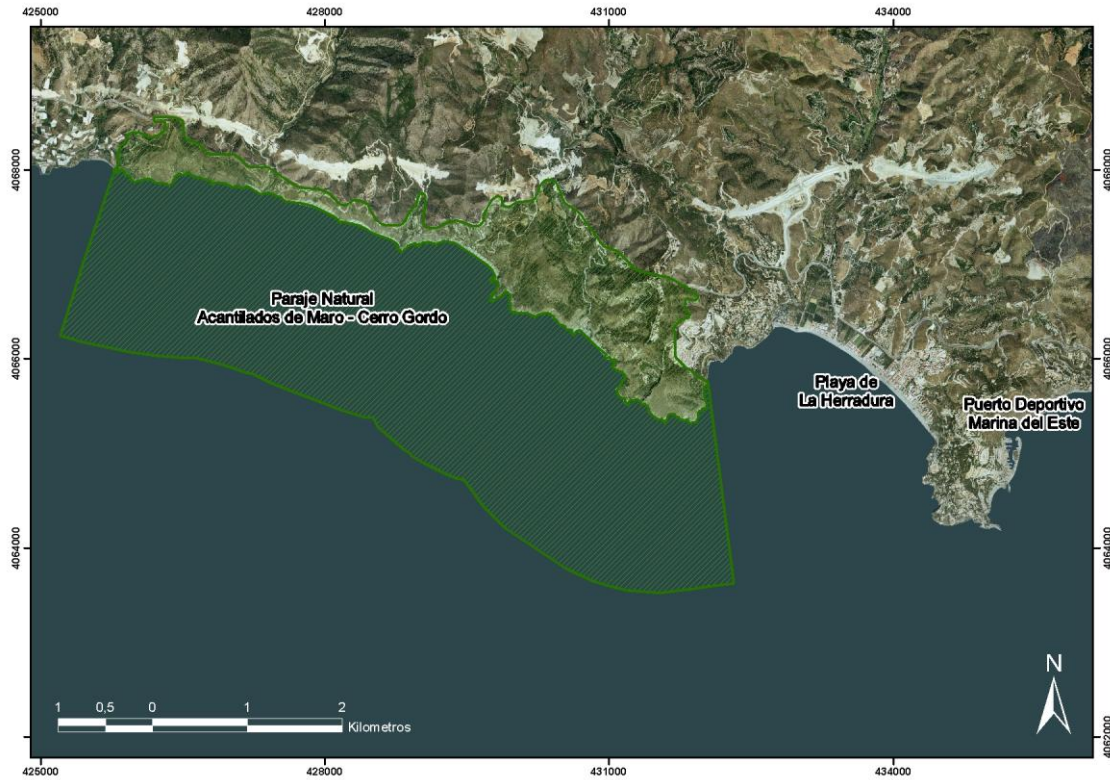


**Figure 1.7:** Andalusian coastal line (South of Spain). \* Natural Park of the Strait of Gibraltar; \*\* Natural Park of Acantilados Maro-Cerro Gordo; \*\*\* Natural Park of Cape of Gata.

The study area focuses on the coast of Granada and Málaga (South of Iberian Peninsula) in the Acantilados Maro-Cerro Gordo Natural Park and adjacent areas of the “Playa de La Herradura “and “Marina del Este”, near the Site of Community Interest of “Punta de la Mona”.

Both the coast of the province of Granada and Málaga are influenced by the proximity of the coastal mountains that border (Almijara and Tejada), preventing the

formation of wide beaches and leading to cliffs that are punctuated occasionally by small coves. The named “Andalusian Tropical Coast,” boasts a multitude of ways to enjoy quality marine tourism, including recreational diving, either scuba or snorkel, and spearfishing. These activities are regulated along the coast depending on the levels of demand.



**Figure 1.8:** General view where may observe Acantilados Maro-Cerro Gordo Natural Park, between Málaga and Granada littoral.

Granada’s coast has only a small marine protected area 12 kilometres long, shared with the province of Málaga; the Natural Park Acantilados Maro-Cerro Gordo. This area protects a submerged coastline reaching one mile from the littoral, covering an area of 1415 Ha. The Natural Park has 20.48% land surface area and 79.52% sea area (Figure 1.8). The special location of this natural area is in the Alboran Sea (bounded to the west by the Strait of Gibraltar and east by the Cabo de Gata), at the confluence of the masses of the Atlantic and the Mediterranean. The water and habitat diversity offers sandy, rocky and seagrass meadows. Funds determine a high level of biodiversity in the marine area of the Natural Park. Because of the many natural values, Acantilados Maro-Cerro Gordo Natural Park is within the Habitats Directive, as Site of Community Interest (SCI) Specially Protected Area of Mediterranean Importance (SPAMI), by agreement between Barcelona and the Birds Directive (D.79 / 409 EEC). Moreover, it is



one of the coastal areas with the highest population densities of orange coral which exist on the Andalusian coast and, Iberian Peninsula and, therefore, in the world.

In addition, the study area extends to the East and West sides of the beach of La Herradura, along the Acantilados Maro-Cerro Gordo Natural Park and the beach of Marina del Este (farthest from the MPA).

Punta de la Mona is characterised as a place of influence of upwelling of cold, nutrient waters and, because of the Site of Community Importance of cliffs and seabed of Punta de la Mona, reaches depths greater than 40-50 m in close proximity to the cliff points. These unusual conditions make a very narrow strip of submerged coast, having the highest concentration of threatened species of Andalusia. Indeed, 44 of marine species considered in the Red Book (Barea-Azcón *et al.* 2008) as endangered are in Punta de la Mona, which is a remarkable fact, given how small this space is. Additionally, in the area are species that normally only live at great depths in Mediterranean waters and in this unique enclave live in relatively shallow waters, observable by SCUBA diving, like the chandelier *Dendroplyllia ramea* (Linnaeus 1758) and the yellow coral *D. cornigera* (Lamarck 1816). Acantilados Maro-Cerro Gordo Natural Park (Málaga and Granada) also harbour high biodiversity funds, although they are somewhat lower than those observed at the Punta de la Mona, with the sea bottom being shallower.

Water sports and SCUBA diving has become overcrowded on this stretch of coastline, with repeated dives at certain points, some of them very fragile like caves. As a result of the massive amount of dives the viability of populations of species of marine invertebrates present in the area is being threatened. Most of them are very vulnerable to flutter or contact with the divers, due to their fragile calcareous skeletons.

### **1.3.2 Scleractinian corals from Alborán Sea**

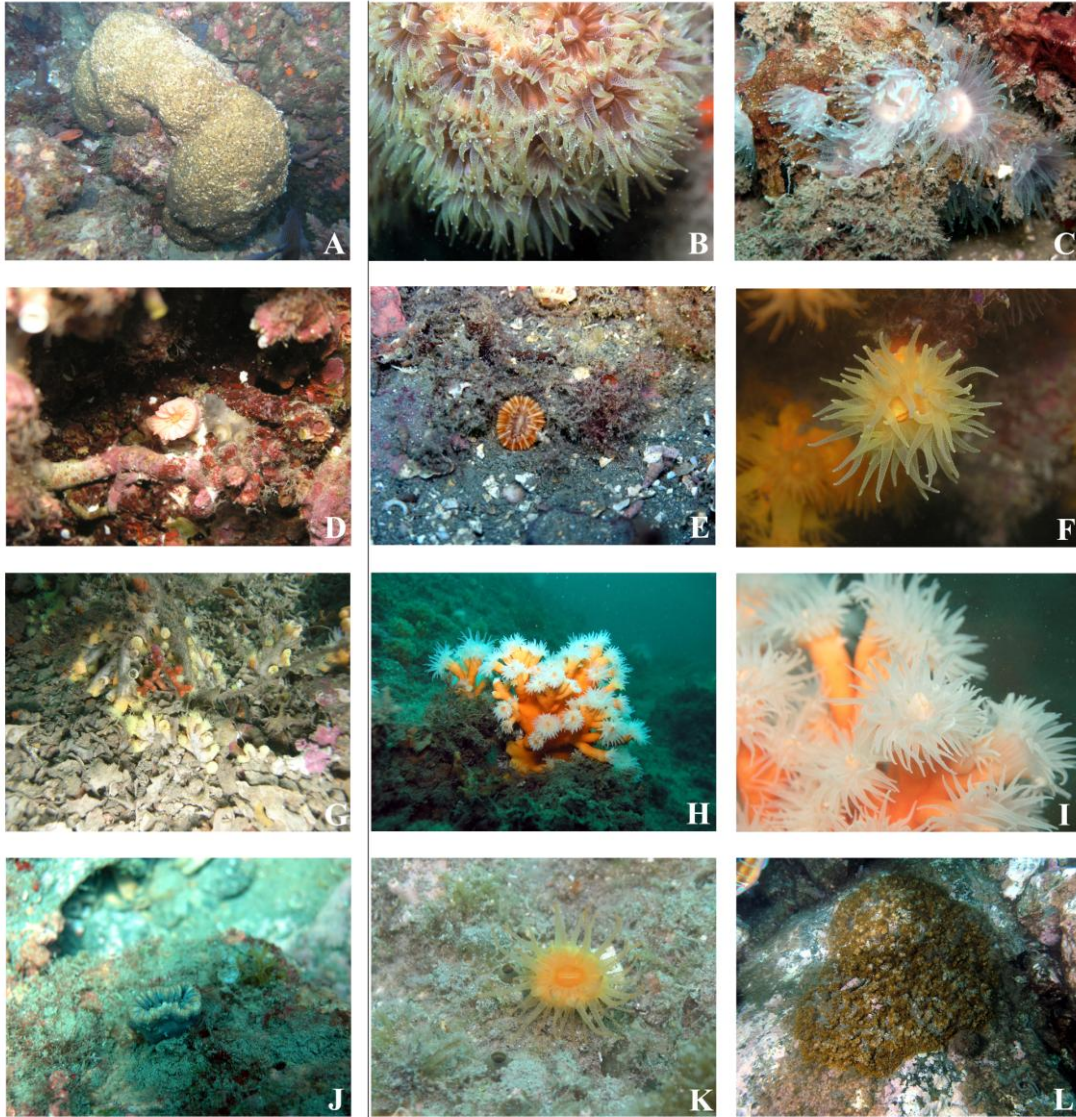
The Mediterranean harbours 25 genera and 33 species of Scleractinian corals, which is similar to the diversity found in the Late Miocene. Even the shallow water species currently found are very similar to the Pleistocene (Vertino *et al.* 2014). Nevertheless, the geographic distribution of some species have been highly reduced, such as *Astroides calycularis* that had a wide distribution on Pleistocene and nowadays the species has restricted their populations to coastal areas of the southwestern Mediterranean Basin (Zibrowius 1995). Moreover, *Cladocora caespitosa*, the only reef-builder scleractinian shallow waters present in the Mediterranean Sea, were much more abundant in warmer periods of the Pliocene and Pleistocene (Vertino *et al.* 2014). Zooxanthellate and azooxanthellate corals have been common of the benthic fauna of the Mediterranean basin in the Mesozoic and Cenozoic, but have been particularly sensitive to environmental modifications.

In the North of the Alborán Sea, the order Scleractinian is represented by thirteen genera and sixteen species (Ocaña *et al.* 2000) (Figure 1.9). The family Faviidae hosts only one species, *Cladocora caespitosa*, this is the only zooxantellate Mediterranean coral reef builder (e.g. Zibrowius 1980). In our study area, the species was described as an abundant coral (Ocaña *et al.* 2000). Nevertheless, following our field observations and according to Moreno *et al.* (2008) the species would be described as uncommon on Andalusian coasts. *Phyllangia mouchezii* and *Polycyathus muellererae* belong to the family Rhizangiidae and are very abundant from five metres depth. *P. mouchezii* can be found in their white variety. Family Caryophylliidae is represented by three species, two congeneric, *Caryophyllia inornata* and *C. smithii*, both abundant, and *Paracyathus pulchellus*. *Madrepora oculata* (Oculinidae) is a branched cold water coral present between 150-300 metres depth, is a scarce species in the North Alborán Sea (Ocaña *et al.* 2000) and outside of our studied area (as the abundant *Lophelia pertusa*: Desmophyllidae). Nevertheless, other species inside family Oculinidae (*Oculina patagonica*) was not reported in Punta de la Mona (Granada coast), being censused for the first time in the present study. The species inhabit horizontal rocky shore well illuminated (zooxantellate species) from one and a half metres depth (e.g. Terrón-Sigler *et al.* 2015-Section 6). *Pourtalosmilla anthophyllites* (Parasmiliidae) is an uncommon azoxantellate coral that inhabits the ceiling cave from seven metres depth. Finally, the most representative coral family is Dendrophylliidae, which harbor two *Dendrophyllia* genera, *D. ramea* and *D. cornigera*. The first species was described as uncommon or a little abundant in the Punta de la Mona area and appear from 30 metres (Cebrián & Ballesteros 2004). Nevertheless, we have found abundant populations that start to appear from 16 metres depth (isolated individuals). The second species (*D. cornigera*) had not been reported in this area above 40-45 metres depth (Moreno *et al.* 2008), but we have found the species at 30-35 metres depths with lower densities than *D. ramea*, and can be also found between 18-20 metres depths (Terrón-Sigler pers. obs.). Moreover, this family present two more congeneric species, *Balanophyllia europaea* and *B. regia*, both abundant solitary corals present in shallow water. *Leptopsammia pruvoti* is a very common species from the infralittoral and inhabits cave and dark areas from nine metres depth. The last species included in the present family is the orange coral *Astroides calycularis*, the scleractinian coral which is more abundant in the shallow waters from North of Alborán Sea, and objective of the present work.

### 1.3.3 Level of endangerment

All scleractinian corals are listed on the Appendix II of the Convention on International Trade in Endangered Species of wild fauna and flora (CITES). It is demonstrated that populations of *Cladocora caespitosa* have a rapid decline in shallow water, and is only included in the Annex 1 of Habitats Directive (Directive 92/43/CEE)

under Habitat 1170 “Reefs”. However, this species is under protection by national and regional laws, EU Directives and international conventions. In Andalusia *C. caespitosa* is listed as endangered in the regional red list from Andalusian region., as well as *Pourtalosmilla anthophyllites*, *Madrepora oculata* and *Lophelia pertusa*.



**Figure 1.9:** Some of the Scleractinian corals presents in the Granada coast. A: *Cladocora caespitosa*; B: *Phyllangia mouchezii*; C: *Phyllangia mouchezii* (white variety); D: *Caryophyllia smithii*; E: *Caryophyllia inornata*; F: *Leptopsammia pruvoti*; G: *Dendrophyllia cornigera*; H-I: *Dendrophyllia ramea*; J: *Balanophyllia europaea*; K: *Balanophyllia regia*; L: *Oculina patagonica*.

Additionally, *Madrepora oculata* and *Lophelia pertusa* is included in Annex I to the Habitats Directive as natural habitat types of community interest whose conservation requires the designation of special areas of conservation. The species are explicitly mentioned in the interpretation manual of EU habitats. Important conservation

actions for these species are to include it in national and regional legislation as protected species.

*Polycyathus muellerae*, *Caryophyllia inornata*, *Dendrophyllia ramea* and *D. cornigera* is also included in Annex 1 of the Habitats Directive (92/43/CEE) as priority habitats for conservation, Habitat 1170 “Reefs”. Moreover, *Phyllangia mouchezii*, *Dendrophyllia ramea* and *D. cornigera* are included in the regional red list from Andalusian region listed as vulnerable.

Finally, *Oculina patagonica* has been considered as alien species and for this reason the species was not assessed in the last IUCN anthozoan workgroup. Nevertheless, recently Leydet & Hellberg (2015) has shown that *O. patagonica* is not a recently introduced coral species in the Mediterranean. Therefore, the species must be assessed in the future. Regarding to the level of protection of *Astroides calycularis*, is presently protected by national and international organisations as an endangered species (i.e. the Bern and Barcelona Conventions and the Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES]).



## 2. OBJECTIVES



A polyp extending of the colony (Piedra del Hombre, La Herradura, Granada). Author:  
Terrón-Sigler A.

The main objective of this thesis was to advance in the knowledge of the biology and ecology of *Astroides calycularis* in the North of Alborán Sea from conservational perspective. The specific objectives could be useful for public managers to have knowledge of the current state of the populations, potential threats, as well as potential protocols for restoration purposes.

## 2.1 Specific objectives:

1. Study the influence of environmental factors on the reproductive cycle of *Astroides calycularis*.
2. Analyse the role of *Astroides calycularis* as habitat for macrofaunal groups.
3. To establish a reference point of the current state of the orange coral populations in the North of Alborán Sea.
4. To study the threats those are nowadays affecting on the *Astroides calycularis* populations.
5. To develop effective techniques of colonies transplantation in order to establish future protocols that could be used as a management tool in degraded areas, improving the conservation status of this endangered species.





The background image shows a close-up of a coral reef. A large, flat, brownish-tan coral plate is the central focus, with a small, bright yellow, elongated object resting on its surface. Surrounding this central plate are various other coral species, including some with reddish and orange hues. The overall scene is a detailed view of a diverse and colorful marine ecosystem.

3. INFLUENCING FACTORS ON THE  
REPRODUCTIVE BIOLOGY OF THE  
ENDANGERED CORAL *ASTROIDES CALYCULARIS*

An *Astroides calycularis* planula creeping on *Chondrosia reniformis* (Marina del Este beach, Almuñécar, Granada). Author: Terrón-Sigler A.

## Abstract

There are gaps on the biology of the *Astroides calycularis*, although the reproductive cycle has already been well studied. Nevertheless, there is little information about the exogenous and endogenous factors and those which may interact on the corals reproductive strategies. From 50 *A. calycularis* colonies, 18 colonies were collected at 0 metres; 15 colonies at 5 metres and 17 colonies at 10 metres. Sixteen of them were indeterminate, twenty were male, and fourteen female colonies. We did not observe sexual dimorphism in the colonies. Chi-square test showed significant differences between sex of the colony and depths but one-way ANOVA analysis did not show significant differences between sex and colony area. The results here presented highlight the importance of considering the depth as a variable within a population. These results must be taken in account for future management plans of the species.

## Resumen

Aunque el ciclo reproductivo del coral naranja (*Astroides calycularis*) se encuentra bien descrito, todavía faltan por estudiar muchos parámetros sobre la biología reproductiva de este coral amenazado. Factores exógenos y endógenos a la propia especie pueden interactuar en la estrategia reproductiva de *A. calycularis*. De esta forma, para estudiar la profundidad como un factor exógeno que puede afectar a la biología de la especie y la biometría de las colonias como factor endógeno, se han analizado 50 colonias de coral naranja: 18 de ellas recolectadas a 0 metros, 15 colonias a 5 metros y otras 17 a una profundidad de 10 metros. Los análisis histológicos aportaron información sobre el sexo de las colonias y se observó que 16 de ellas fueron colonias indeterminadas sexualmente, 20 colonias fueron machos y 14 hembras. No se observó dimorfismo sexual en las colonias. El test de Chi-cuadrado mostró diferencias significativas entre el sexo de las colonias y la profundidad. Sin embargo, el ANOVA de una vía no mostró diferencias entre el sexo de las colonias y el área de la misma. Los resultados obtenidos en el presente estudio aportan una información crucial en la importancia de la profundidad como factor exógeno en las poblaciones de *A. calycularis* del sur de la Península Ibérica. Así, estos resultados se deben tener en cuenta en futuros planes de gestión de la especie, por ejemplo, a la hora de aplicar medidas de restauración de poblaciones.



### 3.1 INTRODUCTION

*Astroides calycularis* is an endemic and endangered azooxanthellate scleractinian colony coral from Mediterranean Sea. This orange coral with a carbonate calcium exoskeleton inhabits the rocky shore from the surface to 50 m depth (Rossi 1971; Zibrowius 1980; Ocaña *et al.* 2000), but is typically found in the shallow infralittoral zone (Terrón-Sigler *et al.* 2015- Section 6), on vertical walls, or inside caves (Rossi 1971; Zibrowius 1978; Kružić *et al.* 2002). It occupies both light and dark habitats and appears to prefer a highly hydrodynamic environment (Zibrowius 1978; 1995; Kružić *et al.* 2002). The population density can be locally high, with colonies covering up to 90% of the sea bottom (Goffredo *et al.* 2011a; Terrón-Sigler *et al.* 2015- Section 6). It has a limited geographical distribution in the westernmost locations in Mediterranean Sea (Zibrowius 1995; Bianchi 2007). Recently, *A. calycularis* have been recognized as an important habitat for other species, which can vary depending on location and season (Terrón-Sigler *et al.* 2014a- Section 4).

Although there are gaps on the biology of the species, the reproductive cycle have already been well studied (Goffredo *et al.* 2010; 2011b; Casado-Amezua *et al.* 2013). The species is a gonochoric coral whose females brood their larvae (Goffredo *et al.* 2010). The morphologic aspects of spermatogenesis, ovogenesis, embryogenesis and larval development, as well as the quantitative data on the annual sexual reproduction cycle have been studied in Italian waters (Goffredo *et al.* 2010; 2011b). Moreover, Casado-Amezúa (2012) expanded the current knowledge on the sexual reproduction of *Astroides calycularis*, by studying the fertilization period of the coral between two distant localities (South of Spain and Italy) with different surface sea temperature (SST) during the late spring-summer. Thus, exogenous factors can interact with endogenous biorhythms to cause local shifts in reproductive strategies (Giese & Pearse 1974). Indeed, SST is one of the main parameters controlling the metabolic rates of marine organisms (e.g. Allen *et al.* 2006). Therefore, many marine invertebrates begin to reproduce when a certain temperature level is reached after a period of either increasing or decreasing temperature, or in response to sudden temperature changes (Kinne 1970; Bates 2005) often confining their reproductive period to relatively narrow thermal ranges (Riesgo & Maldonado 2008). Traditionally, SST has been the external factor more studied in scleractinian corals and SST has been considered as the most important seasonal environmental factor that affects reproductive timing of anthozoans, such that spawning time has been shown to correspond to the warming of seawater and the time when seawater reaches its annual maximum (Harrison & Wallace 1990; Baird *et al.* 2009). SST may change across a species distribution range, related to geographical factors such as latitudinal gradients (Kain 1989), and the appearance of oceanographic phenomena such as upwelling (Sarahn *et al.* 2000). Nevertheless, local SST variances

may be important in these aspects too. But, there is little information about these changes.

Moreover, there are other exogenous factors that may affect the reproductive strategy in scleractinian corals. For example, corals that dwell in shallow waters are susceptible to high irradiation, periods of emersion and sedimentation, and may have evolved a variety of adaptive responses to maximize their success in these environments (Kramarsky-Winter & Loya 1998). Indeed, some authors have hypothesized that, in response to environmental unpredictability and stress, organisms evolved r-strategy characteristics (Giesel 1976; Loya 1976; Stimson 1978). However, external factor such as nutrient availability, sediments, and/or depth although less studied, have also been regarded as important features for coral fecundity (Séré *et al.* 2010), and in those cases, has only been studied in tropical seas (e.g. Acosta *et al.* 2001), with less attention in temperate seas (Fine *et al.* 2001; Gori *et al.* 2007; Torrents & Garrabou 2011). Nevertheless, in the marine management the reproductive strategies of the species are essential to understand the resistance and resilience of populations following natural or anthropogenic impacts (Connell & Keough 1985; Torrents & Garrabou 2011).

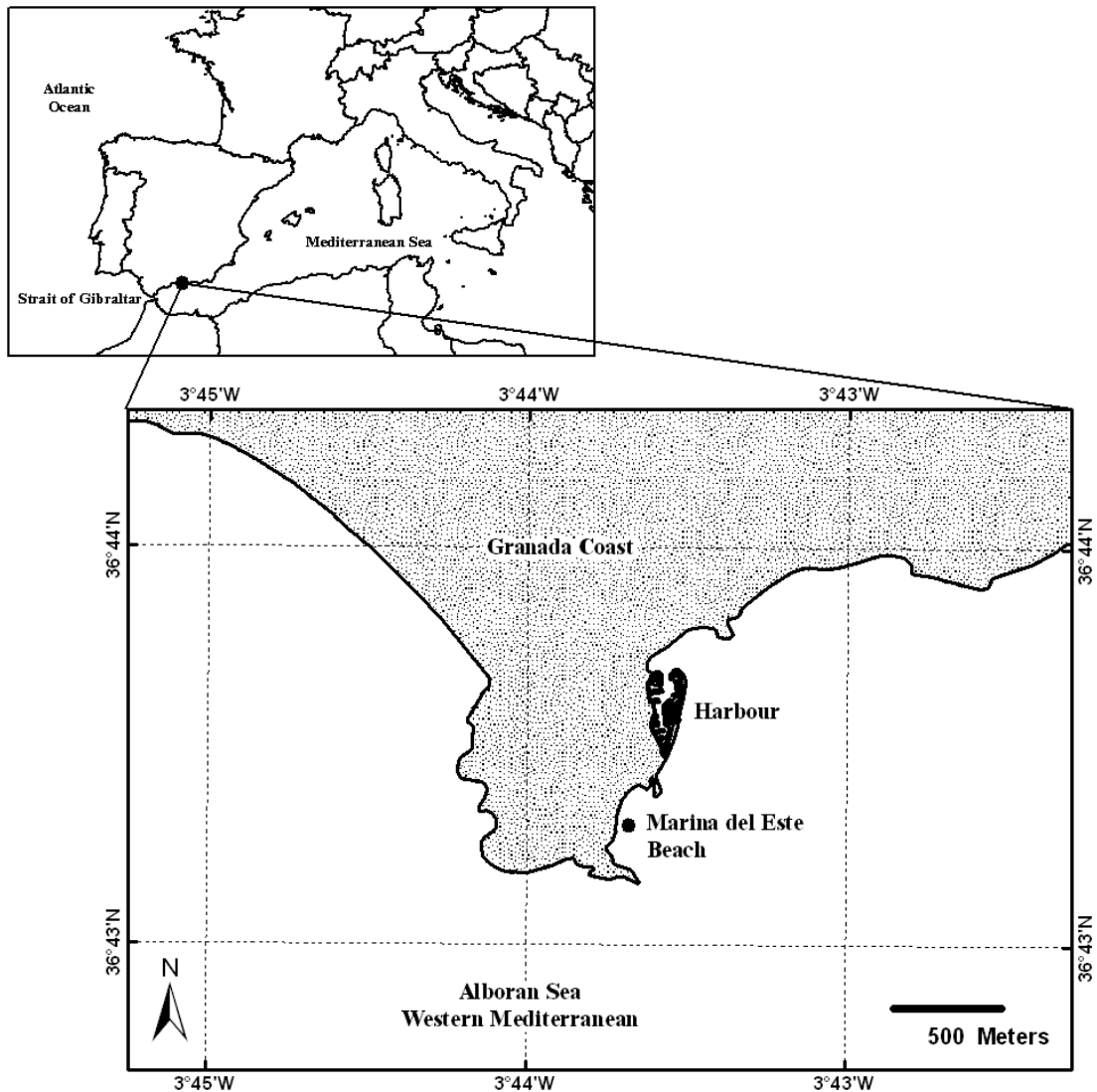
On the other hand, endogenous factors such as polyps and/or colony size have been showed key factor in some species to gonads maturity (e.g. Harvell & Grosberg 1988). But no interaction between exogenous and endogenous factors has been studied on this topic.

The aim of the present study was to test *in situ* personal observations done during a period of more than eight years, where the spawning of the *Astroides calycularis* larvae occur in shallow waters which are never seen in the deeper colonies on the South of Spain coasts. Therefore, this study tested whether depth (as exogenous factor) and/or colony size (as endogenous factor) could be keys in the reproductive strategy of *A. calycularis* populations from North of Alborán Sea.

### 3.2 MATERIAL AND METHODS

The study was conducted in Marina del Este beach (Granada coast; Andalusia; Spain) from August 2010 to July 2011 (Figure 3.1). On June 2011, several observations were made on releasing of the planula larvae of *Astroides calycularis*. Underwater photographs were taken with an Olympus C-8080 camera, and the sea surface temperature (SST) was registred with data loggers (Hobo pedant). A total of 50 colonies were collected by SCUBA diving at three depths (0, 5 and 10 m). We selected these depths range because on the Andalusian coast the higher populations of this species are between 0 and 15 metres depth (Terrón-Sigler *et al.* 2015-Section 6). Moreover, we chose a site with the same *A. calycularis* density coverage (between 50-75% cover) at the three depths.





**Figure 3.1:** Study site showing Marina del Este beach (Andalusia, Spain), where the colonies of *Astroides calycularis* were collected.

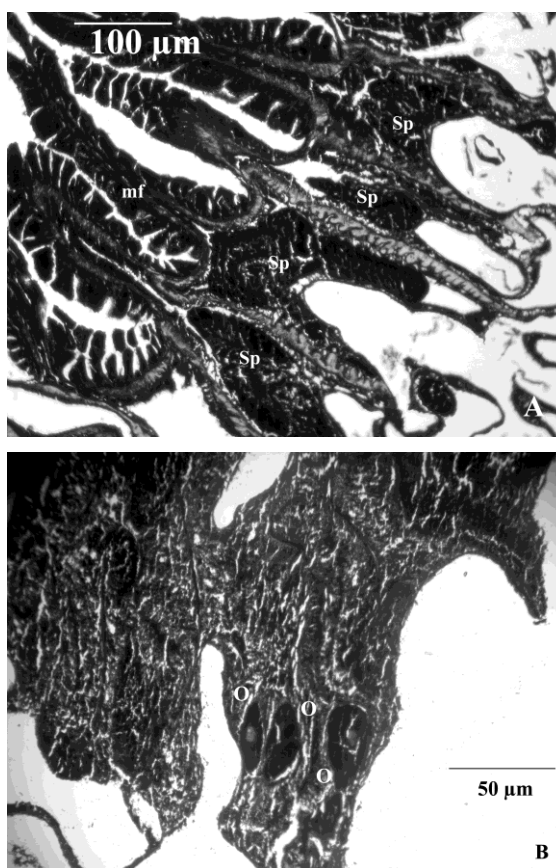
From 50 *Astroides calycularis* colonies, 18 colonies were collected at 0 m; 15 colonies at 5 m and 17 colonies at 10 m. The samples were fixed in 4% formalin and subsequently placed in 70% ethanol in the laboratory. Colony length ( $L_c$ , major axis of the colony) and colony width ( $W_c$ , minor axis of the colony) were measured, and colony area ( $A_c$ ) was calculated using the formula for an ellipse ( $A_c = \pi(L_c * W_c)/4$ ), according to Goffredo *et al.* (2011a). We selected the colony area as the main biometric parameter for the colonies, because it is a more accurate and representative measure of colony size than colony length (Vermeij & Bak 2002; Goffredo *et al.* 2011a; Terrón-Sigler *et al.* 2014a-Section 4).

After biometric measurements, the colonies were decalcified using a 10% solution of formic acid, and dehydration in a graded alcohol series from 80% to 100%. Tissues were embedded in paraffin and serial transverse sections were cut at 7  $\mu\text{m}$ . Then these were stained with Mayer's haematoxylin and eosin. Histological observations were made under a light microscope and, at least, three polyps of each colony were analysed to test the sex of polyps.

Chi-Square was used to test whether there were differences among sex of colonies regarding with the depth. Moreover, a one way analysis of variance (ANOVA) was used to test whether the colony area of *Astroides calycularis* was similar regarding with sex of colony. Prior to ANOVA, the heterogeneity of variance was tested via a Levene test. Analyses were conducted with SPSS© 15.0.

### 3.3 RESULTS

From fifty colonies analysed, sixteen of them were indeterminate, twenty were male, and fourteen were female colonies. Oocytes were oval and located in the mesenteries as well as spermaries (Figure 3.3). All colonies examined had male or female polyps we did not observe colonies with both sexes. Regards to depth, more than 85% of females colonies found were present at 0 metres, while the rest (14.29%) were located at 5 metres depth. No female colonies were found on 10 metres depth. From male colonies, 60 % were present at 10 metres depth, and the 35% at 5 metres, encompassing the 95% of the males between 5 and 10 metres depth. Only 5 % males were present at 0 metres depth. Indeterminate colonies did not show differences with dept (Figure 3.4 and Table 3.1). Additionally, sex of colonies showed significant differences regarding with depth (Chi-square test,  $\chi^2=9.1$ ;  $df=2$ ,  $p<0.05$  to males and  $\chi^2=17.58$ ;  $df=2$ ,  $p<0.001$  to females).



**Figure 3.3:** A: Spermaries and; B: oocytes from the mesentery filament of *Astroides calycularis* (mf: mesentery filament; sp: spermaries; o: oocytes).



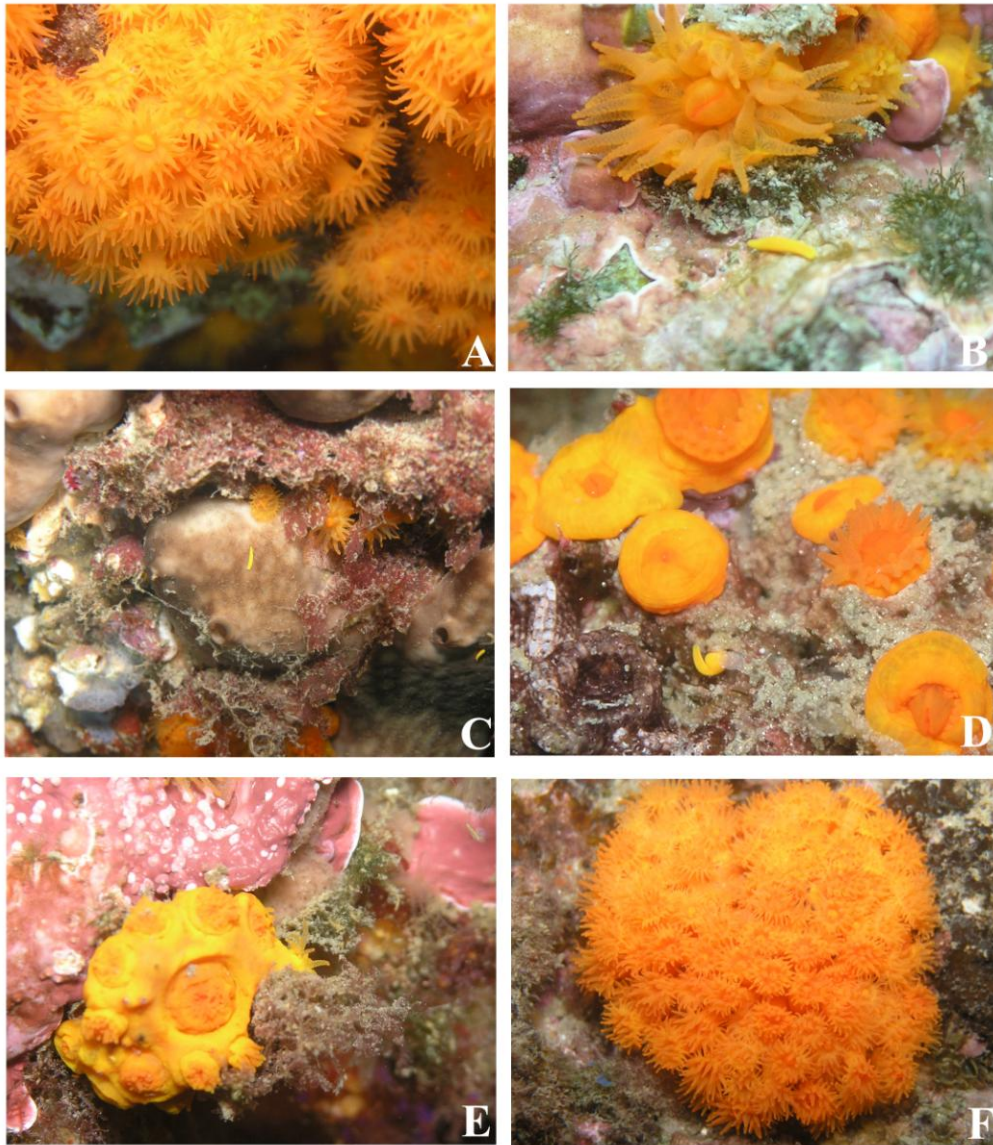
The releasing process in the study area start with embryos in coelenteric cavity, that migrate to top of tentacles, and coinciding with the full moon during June-July and when the SST reach 18-23°C (in Granada coast) the planulation release occur. The larvae creep on the substrate to be fixed (twisting) and form the first polyp that originates the colony by budding (Figure 3.2).

**Table 3.1.** Colonies sampled during the present study (*Lc*, major axis of colony; *Wc*, minor axis of colony; *Ac*, colony area; F female; M, male; I, Indeterminate).

Date	Depth	Lc (cm)	Wc (cm)	Ac (cm <sup>2</sup> )	Sex	Oogenesis*
August 2010	0	3	2.6	6.13	F	
	5	3.5	2.5	6.87	I	
	5	3	2.8	6.60	I	
	10	2.6	1.9	3.88	I	
September 2010	0	2.4	1.7	3.20	I	
	10	2.7	2.2	4.67	I	
October 2010	0	4.3	2.6	8.78	F	
	10	4.1	2.2	7.08	M	
January 2011	0	4	3.4	10.68	I	
	5	2.8	2.7	5.94	M	
	5	3.2	3	7.54	F	
	5	2.2	2.1	3.63	F	
February 2011	0	5.1	3.4	13.62	I	
	0	3.2	2.5	6.28	M	
	5	3.7	3.3	9.59	I	
	5	4	3.5	11.00	M	
	5	2.5	2.2	4.32	M	
	10	2.5	2.2	4.32	M	
March 2011	10	3	1.9	4.48	I	
	0	3.4	3	8.01	F	Early stage
	0	4.1	3	9.66	F	
	5	2.7	2.5	5.30	I	
	10	3.4	2.9	7.74	M	
April 2011	10	4.3	2.5	8.44	M	
	0	3.5	3.1	8.52	F	
	0	4.5	4	14.14	F	Intermediate Stage
	0	4.7	3.3	12.18	F	
	5	2.5	2	3.93	I	
	5	3.4	2.7	7.21	M	
	10	3.4	2.8	7.48	M	
May 2011	10	2.3	1.5	2.71	I	
	0	3	2.8	6.60	F	
	5	2.9	1.6	3.64	M	Intermediate Stage
	10	1.4	1	1.10	I	
	10	3.6	3	8.48	M	
June 2011	10	4.1	3.9	12.56	M	
	0	2.9	3.6	8.20	F	Late Stage
	0	3.5	2.6	7.15	I	
	0	3.2	2.6	6.53	F	
	0	3.8	3.3	9.85	F	
	5	6.4	5	25.13	M	
	5	4	3.1	9.74	I	
	10	2.8	2.1	4.62	M	
	10	3.3	3	7.78	M	
	10	3	2.2	5.18	M	
July 2011	10	6.1	5.2	24.91	M	
	0	5.2	4.1	16.74	I	
	0	3.8	3.6	10.74	F	
	5	5.2	4.5	18.38	M	
	10	1.5	1.2	1.41	M	

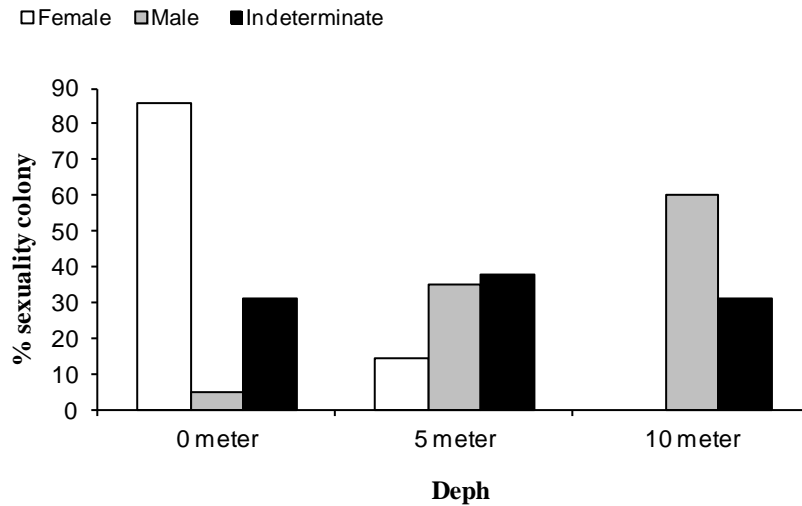
\* Following the oogenesis stage definition by Goffredo *et al.* (2010).

The males colony area ( $A_c$ ) studied ranged between 25.3-1.41 cm<sup>2</sup> with a mean of 9.1±6.53 cm<sup>2</sup>. Nevertheless, female colonies ranged between 14.14-3.63 cm<sup>2</sup> with a mean of 8.61±2.65 cm<sup>2</sup>. Finally, indeterminate colonies ranged between 16.74-1.13 cm<sup>2</sup> with a mean of 6.89±2.65 cm<sup>2</sup>. The one way-ANOVA analysis did not show significant differences between sex and colony area (Table 3.2).



**Figure 3.2:** Scheme of the steps of planula larvae release and development of the colony. A: Planula in the top of tentacles (it is can be differentiate because the top of several tentacles are more yellow). B: Larva creeping near a orange coral polyp. C: Larvae creeping look for an ideal substratum. D: Larva twisting to fix at substratum. E: Incipient colony with a bigger polyp in the middle and smaller around originates by asexual reproduction. F: Colony formed.

Early stage of oogenesis was found in March, intermediate stage between April and May, and late stage in June (Table 3.1). The oocytes increased from March to May.



**Figure 3.4:** Percentage of sexes colonies found in the three depths studied.

**Table 3.2:** Results of the one-way ANOVA for colony area and sex of colonies. Where df = degree of freedom, SS = sum square, MS = mean square, F = factor and P = p-value

Source	SS	DF	MS	F	P
<b>Colony area</b>	0.012	1	0.12	0.018	0.893
<b>Residual</b>	20.83	32	0.651		
<b>Total</b>	20.842	33			

Levene's Test = 0,071. Transformation: Square root

### 3.4 DISCUSSION

The present study shows for the first time that depth (exogenous factor) is an important variable that could be influencing the reproductive biology of the species. Additionally, it is shown that size colony has no correlation with the sex of the colony.

Most of reproductive strategies of scleractinian are hermaphroditic and release male and female gametes for external fertilization (e.g. Shlesinger *et al.* 1998). Gonochorism occurs in about 25% of the studied species (Fadlallah 1983; Harrison & Wallace 1990; Richmond 1997). Within the Scleractinia, the sexual condition tends to remain constant at the family level (Harrison 1985). Nevertheless, from seven species of Dendrophylliidae living in the Mediterranean, subdivided into five genera, *Leptopsammia pruvoti* is a gonochoric and brooding coral (Goffredo *et al.* 2005), while

*Balanophyllia* genus present hermaphroditic and brooding species such as *B. europaea* (Goffredo *et al.* 2002), whereas others such as *B. elegans* are gonochoric and brooding species (Fadlallah & Pearse 1982; Beauchamp 1993). On the other hand, *Astroides calycularis* was described as hermaphroditic species (Lacaze-Duthiers 1893), but Goffredo *et al.* (2010) demonstrated that the species was gonochoric, both polyps and colony. Here, it has shown the same results at colony level, already demonstrated by Casado-Amezúa *et al.* (2013) within the same geographical area. Sex ratio for active colonies from eastern Mediterranean populations was 1:1 for a population of the same depth (7 metres depth) (Goffredo *et al.* 2010), while the results from the present study suggest that the populations from the North Alborán Sea have variance in depth. On the other hand, the species has shown differences in the timing of fertilization and planulation period between eastern and western populations, surely due to differences in temperature of each site (Casado-Amezúa *et al.* 2013). These differences are already observed in coral species (e.g. Wilson & Harrison 2003; Gori *et al.* 2007; Kružić *et al.* 2008). In brooding scleractinians, reproductive cycles are extended for several months coinciding with environmental seasonality change (Fadlallah 1983; Richmond & Hunter 1990). Therefore, exogenous factors can strongly influence the reproductive cycles of coral species. Indeed, surface sea temperature has been demonstrated as an exogenous factor that has effects on gorgonian and scleractinian reproductive biology (e.g. Linares *et al.* 2008a; Airi *et al.* 2014). However, this is not the only factor that can act on the shift of coral biology reproductive cycles; photoperiod, current speed, the quantity and quality of available food, and nocturnal illumination occur as important factors indeed (Willis *et al.* 1985; Hunter 1989; Richmond & Hunter 1990; Gili & Coma 1998; Gardner 2000). In coastal areas, these factors may change considerably with depth, even among close locations (Rossi *et al.* 2003). In the Mediterranean Sea, the gorgonian species *Eunicella singularis* showed differences on gametes release strategy among depths, the release of larvae from the female colonies was more prolonged in the shallow than in deeper populations (Gori *et al.* 2012). This pattern was already observed in tropical corals (Vize 2006). Therefore, depth must be taken into account as a potential factor on the reproductive strategy of corals. Indeed, *A. calycularis* has showed clear segregation of males and females colonies depending on depth.

Differences in reproductive strategies have been found regarding with colony size of tropical corals, where small individuals were predominantly males, while very large were all females (Kramarsky-Winter and Loya 1998). Two congeneric stony corals, *Agaricia agaricites* and *A. humilis*, had significant differences in the size at which colonies started to produce planulae (Van Moorsel 1983). The morphology of the *Astroides calycularis* colonies may vary among localities (Casado-Amezúa *et al.* 2013) and depth (Terrón-Sigler *et al.* 2014a-Section 4). Moreover, some aspects of the reproductive biology can depend on the colony area (Casado-Amezúa 2012).

Nevertheless, neither Goffredo *et al* (2011b) nor the present study have found significant differences in mean colony size between males and females.

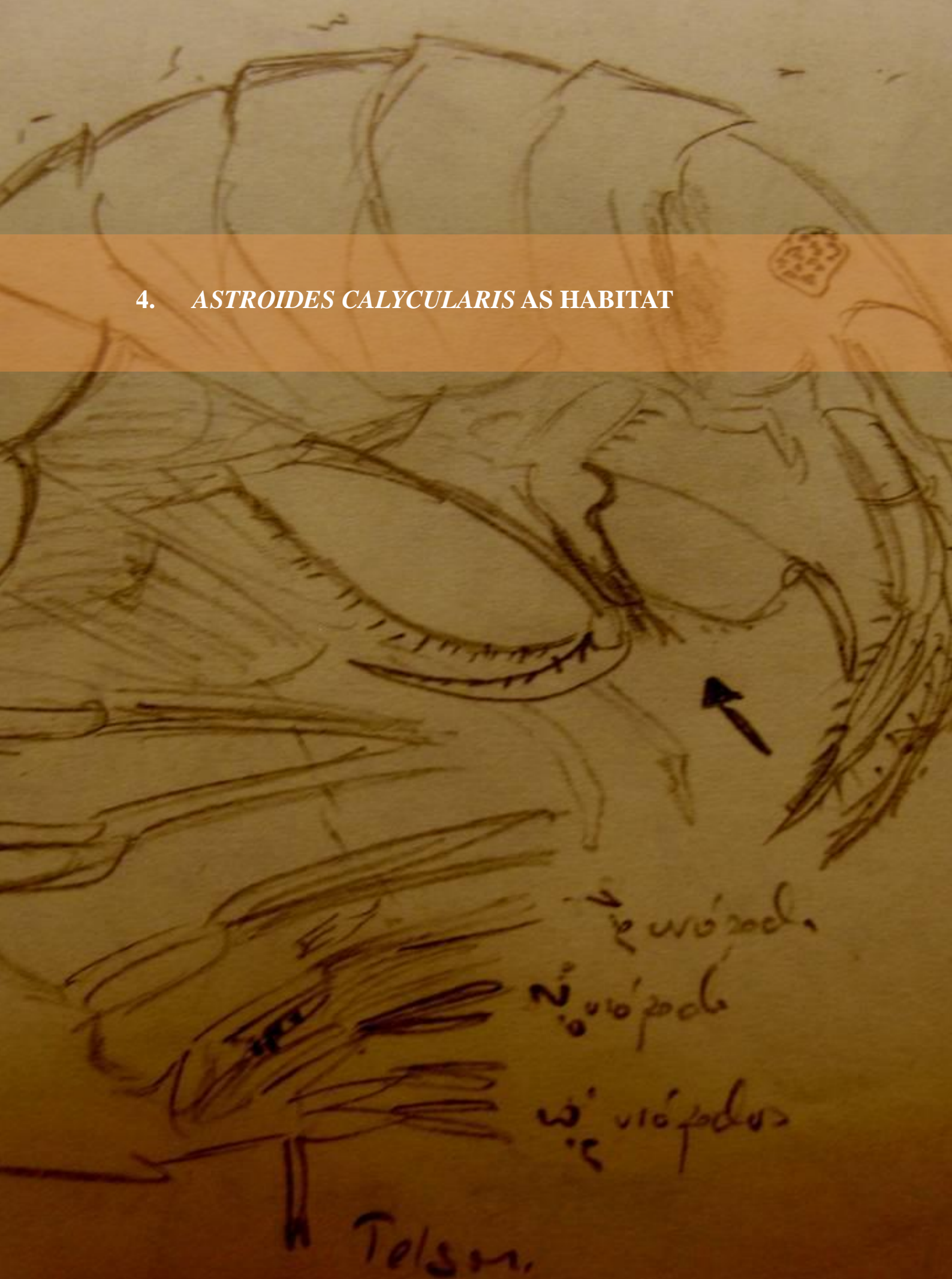
Gametogenesis may have differences among geographic areas, thus gametes of *Astroides calycularis* increased from March to May in the North of the Alborán Sea, while in the eastern Mediterranean area from November to March (Goffredo *et al.* 2010). According to energy devoted to male gametogenesis, this was higher than female gametogenesis, considering the species as an intermediate reproductive strategy on the r-K continuum (Goffredo *et al.* 2011b). Others Dendrophyllids species showed different ecology strategies, *Leptopsammia pruvoti* and *Balanophyllia elegans* presents an ‘r’-reproductive strategy, while *Balanophyllia europaea* is placed half-way along the r-K continuum (Goffredo *et al.* 2006).

It is crucial to know the reproductive strategy for a better understanding of the population dynamics of the species, and then to implement the appropriate tools to reach a sustainable management of their populations, especially when it is an endangered or endemic species. *Astroides calycularis* may have the sex of the colonies segregated in depth, which could be explained by quality food requirement (e.g. Gori *et al.* 2012); hydrodynamism, facilitating the fecundity (e.g. Kramarsky-Winter & Loya 1998); and/or temperature variance (e.g. Van Moorsel 1983), in shallow waters the increase of temperature occurs earlier than in deeper ones. Nevertheless, those populations that are near of the coastline are affected by human impacts (Terrón-Sigler *et al.* 2015-Section 6), and reproduction strategy is essential to understanding the resistance and resilience of populations under anthropogenic disturbances (Connell & Keough 1985). The orange coral are impacted by several anthropogenic activities (Ocaña *et al.* 2009; Moreno *et al.* 2008), and tools for the restoration of populations against future impacts are emerging (Ocaña *et al.* 2009; Terrón-Sigler *et al.* 2014b-Section 5). Therefore, the results here show and highlight the importance of considering the depth as variable within the same population in order to achieve a better understanding of the ecology of *A. calycularis*. Finally, our results may be useful for future management plans for the species, as well as improve management tools. Thus, depth factor must be taken into account in restoration projects (i.e. because of coastal impacts); or population regenerations (i.e. local environmental degradation). Further research is needed to test whether changes on the temperature may vary colony sexuality, and whether this aspect will have implications on the species in the context of global change.





4. *ASTROIDES CALYCCULARIS* AS HABITAT




1<sup>o</sup> ústí  
2<sup>o</sup> ústí  
3<sup>o</sup> ústí

Telson

Drawing of a gammarid done by Terrón-Sigler A.





4.1 Spatio-temporal macrofaunal assemblages associated with the endangered orange coral *Astroides calycularis* (Scleractinia: Dendrophylliidae)

*Caprella penantis* associated with colonies of *Astroides calycularis*. Author: Terrón-Sigler A.

## Abstract

The orange coral (*Astroides calycularis*) is internationally protected due to its narrow distribution, its sensitivity to environmental changes and human-induced activities developed on the coast. Spatio-temporal macrofauna assemblages associated with *A. calycularis* were studied in the South of the Iberian Peninsula. A total of 878 invertebrate specimens were collected, comprising 81 species. At the three depths studied (0, 5 and 10 metres), crustacean species were dominant in abundance, followed by annelids and mollusks. October and May showed higher macrofaunal densities, whereas August and September showed lower densities. *Janira maculosa* (isopod), *Lembos* spp. (gammarid) and *Sthenothoe cavimana* (gammarid) were present in almost all depths and months, and were the most abundant species. Monthly, ANOVA analyses reflected significant differences in species richness and Shannon-Wiener diversity, but did not show by differences among depth ranges. There was no interaction between time and depth factors. PERMANOVA analyses showed significant differences in the macrofauna associated either in time and depth, and no interaction was observed between both factors. The results could indicate the ecological relevance of this Mediterranean scleractinian coral as a habitat for many macrofaunal groups. Furthermore, the conservation of this endangered species contributes to the preservation of high values of marine biodiversity.

## Resumen

*Astroides calycularis* se encuentra protegido internacionalmente debido a su estrecho rango de distribución, su sensibilidad a cambios ambientales y a los impactos originados en la costa como consecuencia de las actividades humanas. Sin embargo, este coral bioconstructor puede aportar sustrato donde habitan otras especies. En el presente trabajo, se han estudiado por primera vez los cambios espacio-temporales de la comunidad de macrofauna invertebrada asociada a las colonias del coral naranja del sur de la Península Ibérica. Se encontraron un total de 878 especímenes de invertebrados que pertenecieron a 81 especies diferentes. En las tres profundidades estudiadas (0, 5 y 10 metros), los crustáceos fueron las especies dominantes en abundancia, seguidas por los grupos anélidos y moluscos. En los meses de octubre y mayo se obtuvieron las densidades de macrofauna más elevadas, mientras que en agosto y septiembre se encontraron las menores densidades. El isópodo *Janira maculosa* y los gammáridos *Lembos* spp. y *Sthenothoe cavimana* fueron las especies más abundantes y estuvieron asociadas al coral durante todos los meses objeto de estudio en casi todas las profundidades. En el estudio mensual, los análisis ANOVA mostraron diferencias significativas en riqueza de especie e índice de diversidad (Shannon-Wiener), pero no

mostraron diferencias entre las profundidades estudiadas. Además, no hubo interacción entre los factores tiempo (meses estudiados) y profundidad. Los análisis PERMANOVA mostraron diferencias significativas en la comunidad de macrofauna asociada, tanto entre los meses como entre las profundidades, pero tampoco hubo interacción entre ambos factores. Los resultados del presente estudio indican la importancia ecológica de *A. calycularis* como hábitat para muchos grupos de macro-invertebrados. Conservando esta especie amenazada se contribuirá a preservar muchas otras especies que se encuentran asociadas a ésta.

#### 4.1.1 INTRODUCTION

Mediterranean Sea comprises less than 1% of the World Ocean (Defant 1961; Bianchi 2007), but harbour between 4% and 18% of the world marine species depending to the group considered (Fredj *et al.* 1992; Bianchi & Morri 2000). Coll *et al.* (2010) listed in 17,000 marine species the Mediterranean biodiversity though could be much higher. This marine biota includes a high endemics species number (Roberts 1978; Giaccone 1999; Airoidi & Beck 2007), with more than one quarter of the whole Mediterranean species (Tortonese 1985; Fredj *et al.* 1992; Giaccone 1999). In this temperate sea, ten biogeographic sectors are described (Bianchi & Morri 2000) and one of them, Alboran Sea (Western Mediterranean), is a hot spot of biodiversity due to its ecological importance by the influx of Atlantic species and physicochemical conditions (Coll *et al.* 2010).

Taking into account the biodiversity on hard substratum, there is little information compared with those conducted on soft substrata (Chintiroglou *et al.* 2005). Nevertheless, studies addresses to show differences in distribution patterns on hard substratum have showed that the distribution and abundance of organisms differ among depths (Balata *et al.* 2006), and the structure of benthic assemblages may change seasonally (Coma *et al.* 2000; Piazzini *et al.* 2004; Balata *et al.* 2005). In this topic, bio-constructors species play an important role to increase the habitat complex and biodiversity (e.g. Bianchi & Morri 1996; Porras *et al.* 1996; Cocito 2001). In the Mediterranean Sea, *Cladocora caespitosa* (a shallow water zooxanthellate scleractinian coral), *Lophelia pertusa* and *Madrepora oculata* (a deep water azooxanthellate scleractinian corals) are recognized as the main bioconstructors (see Kružić 2014). The associated fauna with these species has been the subject of some studies (Koukouras *et al.* 1998; Antoniadou & Chintiroglou 2010; D'Onghia *et al.* 2010; Mastrototaro *et al.* 2010; Calcinai *et al.* 2013; Kružić *et al.* 2013). Nevertheless, none has assessed the spatio-temporal variability of the macrofaunal assemblages associated with temperate corals.

*Astroides calycularis* is protected by National and International organisations as an endangered species (Berna and Barcelona Conventions and CITES). Nevertheless, its relevance as a habitat for macrofaunal community has never been studied. Therefore, the aim of this study was to show the role of *A. calycularis* as a possible habitat for macroinvertebrate fauna.

#### 4.1.2 MATERIAL AND METHODS

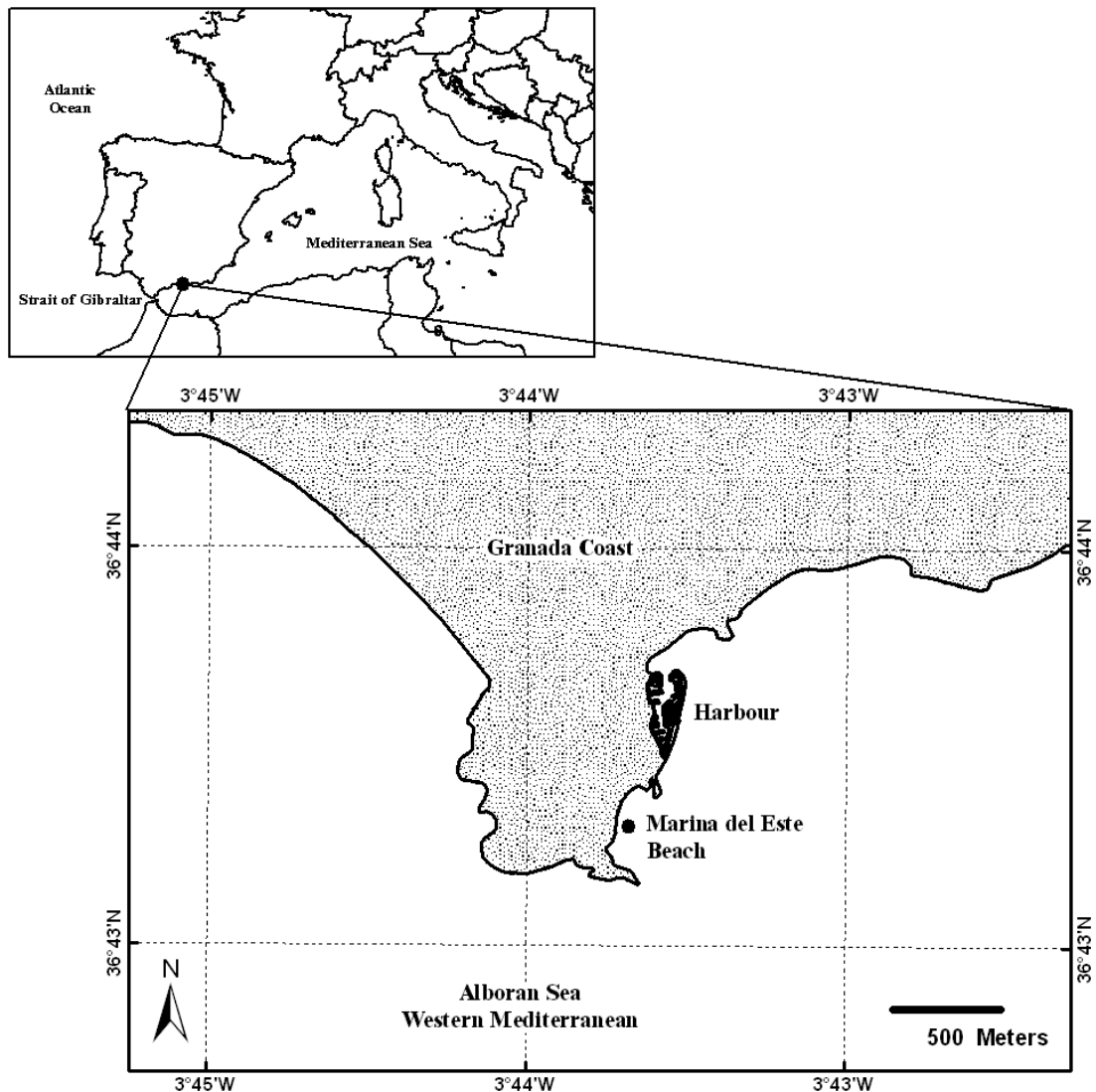
This study was conducted in Marina del Este beach (Granada coast; Andalusia; Spain) from August 2010 to July 2011 (Figure 4.1). The colonies were collected monthly by SCUBA diving at three depths (0, 5 and 10 m). We selected these depths range because in Andalusian coast the higher populations of this species are between 0 and 15 metres depth (Terrón-Sigler & León-Muez 2009-Section 5). Moreover, we chose a site with the same *Astroides calycularis* density coverage (between 50-75% cover), orientation and slope of the wall (North-eastern). Cebrián & Ballesteros (2004) described the zonation of the rocky benthic communities in our study area and concluded that the depth was the main axis of distribution variation. These authors observed differences among communities between lower (25m) and shallow infralittoral (5 m).

A total of 67 *Astroides calycularis* colonies were collected at three different depths: 22 colonies at 0 m; 23 colonies at 5 m and 22 colonies at 10 m. The colonies were covered *in situ* with plastic bags before they were removed, in order to keep all the associated fauna. The samples were fixed in 4% formalin and subsequently placed in 70% ethanol in the laboratory. The colonies were washed, the macrofauna sieved using a 0.5 mm mesh size, and the specimens collected were sorted and identified at the species level, when the specimens allowed it. We expressed the density of the macrofauna as the number of individuals per volume (1,000 mL) of colony. Volume of *A. calycularis* colonies was estimated as the difference between the initial and final volume when placed into a graduated cylinder with a fixed amount of water (see Pereira *et al.* 2006). Moreover, colony length ( $L_c$ , major axis of the colony) and colony width ( $W_c$ , minor axis of the colony) were measured, and colony area ( $A_c$ ) was calculated using the formula for an ellipse ( $A_c = \pi(L_c * W_c)/4$ ), according to Goffredo *et al.* (2011a). The number of polyps and the biovolume were also studied. We selected the colony area as the main biometric parameter for the colonies, because it is a more accurate and representative measure of colony size than colony length (Bak & Meesters 1998; Meesters *et al.* 2001; Vermeij & Bak 2002; Nozawa *et al.* 2008; Goffredo *et al.* 2011a).

All collected colonies reached 989 mL of biovolume, with a mean of 15 mL per colony. To standardize the individual abundance, because colonies had different biovolumes, we calculated the abundance for 1,000 mL of coral volume and we estimated a mean of 1,223 individuals based on a colony volume of 1,000 mL.

The abundance of the associated macrofauna was calculated for each colony and the total number of species (S) and the Shannon-Wiener diversity (Shannon & Weaver 1963) were obtained. To test whether the total number of species and diversity of macroinvertebrate assemblages were similar across depth (0, 5 and 10 m) and time, we

used a multifactor analysis of variance (ANOVA) with the following factors: time and depth, both factors are orthogonal to each other and fixed, with six levels to time (February, March, April, May, June and July) and three levels to depth; three replicates were collected for both factors. Prior to ANOVA, the heterogeneity of variance was tested via Cochran's C-test. Univariate analyses were conducted with GMAV5 (Underwood *et al.* 2002). When statistical differences were detected, *a posteriori* Student-Newman-Keuls test was applied.



**Figure 4.1:** Map of the study site showing Granada coast (Andalusia; Spain) and Marina del Este beach, where the colonies of *Astroides calycularis* were collected.

A permutational multivariate analysis of variance (PERMANOVA) was used to test differences in associated macrofaunal assemblages across depth and time, with the following factors: time and depth, both as fixed factor. Data were square-root-transformed and the similarity matrix was calculated using the Bray-Curtis index. The percentage similarity procedure (SIMPER) was then used to calculate the contribution of each species to the dissimilarity between depths and months. A permutational analysis of multivariate dispersions (PERMDISP) was carried out to test differences in the variation of the macrofaunal assemblages between depth and time. Multivariate analyses were carried out using the PRIMER v.6 + PERMANOVA package (Clarke 1993).

### **4.1.3 RESULTS**

#### *4.1.3.1 Biometric analyses*

Colony length, width, biovolume and number of polyps all correlated positively with colony area, whose variation explained 72.5–94.4% of the variance. Colony length, width, biovolume and colony area all correlated positively with the number of polyps, whose variation explained 63.3–73.0% of the variance (Figure 4.2).

Considering the correlations at each depth, colony length, width, biovolume and the number of polyps were also correlated positively with colony area for all depths. However, this correlation was greater for deeper sites. Thus, the variances were 50.5–89.9% at 0 m; 75.2–90.8% at 5 m; and 79.0–97.3% at 10 m. The best correlation was between length and width of colony and colony area at each depth, but these correlations were better for deeper sites: 89.1–89.9% at 0 m; 90.3–90.8% at 5 m; and 96.5–97.3% at 10 m. The worst correlation was between colony area and number of polyps at 0 m (50.5% of the variance).

For colony area, biovolume, length and colony width correlated positively with the number of polyps in all cases, differences in which explained 41.0–62.4% of the variance at 0 m; 61.5–75.2% at 5 m and 64.3–82.2% at 10 m. Hence, the correlations were better for deeper sites.

Globally, colony area had a better positive correlation with the other variables that the number of polyps of the colony (Figure 4.2).



#### 4.1.3.2 Associated fauna

##### Invertebrate community structure

A total of 81 species were identified out of 878 invertebrate specimens that were sorted and examined (Table 4.1): almost 80% were crustaceans, followed in importance by annelids (more than 16%), molluscs (2.85%), echinoderms (2.85%), cnidarians (0.11%) and platyhelminthes (0.11%). In this study, 38 different species of crustaceans were identified: 14 species of gammarids, seven decapods, six caprellids, four isopods, four tanaids, two cumacean and one barnacle. The most abundant crustacean group was gammarids (more than 68.5%), followed by isopods (20.8%). In fact, almost the 90% of the crustacean specimens belonged to both these taxa. The most common species collected were *Stenothoe cavimana* and *Lembos* spp. (gammarids) and *Janira maculosa* (isopod). These species comprised almost 77% of the crustacean abundance associated with *A. calycularis* colonies.

From the annelid group, 27 polychaete species were identified that belonged to four orders. The most representative order in terms of the number of species and specimens was Phyllodocida, with 16 species, the dominant species of which were *Nereis zonata* and *Syllis* spp. Both species represented 41% of the specimens belonging to this order. The order Sabellida was the second one in terms of abundance, with seven species and 15.6% of the specimens identified; *Vermiliopsis infundibulum* was the most abundant species. In the order Eunicida, three species were identified that contributed 12.1% to the annelid total; *Lumbrineris coccinea* was the dominant species. The order with the lowest species number was Spionida, with only one species identified.

The nine species of mollusks identified belonged to bivalves (three species), gastropods (five species) and polyplacophorans (one species); the bivalve *Gregariella semigranata* was the most common.

Echinoderms, cnidarians and platyhelminthes were rare and represented only 1.1% of the specimens identified. The specimens of the abovementioned groups were all juveniles.

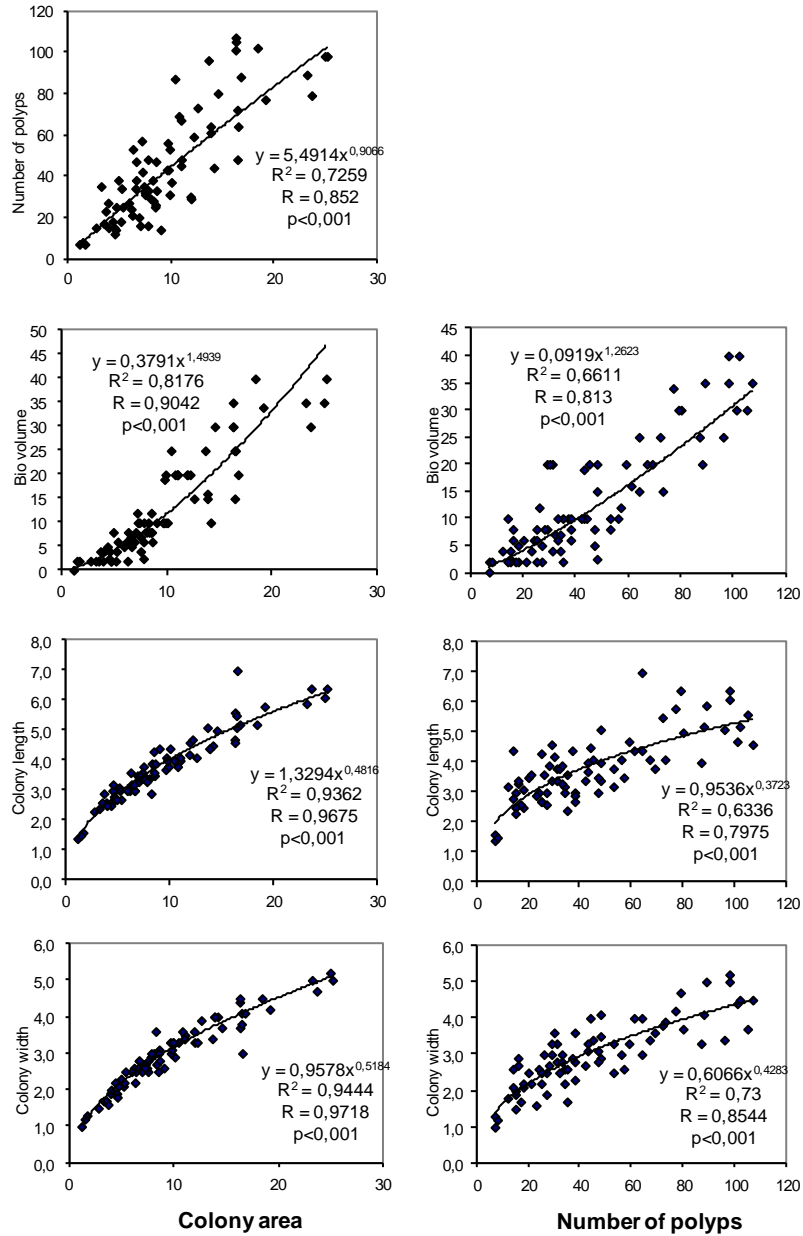


Figure 4.2: Biometric analyses based on pooled data for all depths.

**Table 4.1.** Mean abundance (ind/l) of the macroinvertebrate species associated with *Astroides calycularis*.

Individual/l	2010			2011							Depth		
	Ag	Sp	Oc	Jan	Fb	Mr	Ap	My	Jn	Jl	0 m	5 m	10 m
<b>ARTROPODA</b>													
<b>Isopoda</b>													
<i>Janira maculosa</i>	167	0	300	150	79	37	61	101	90	187	79	76	160
<i>Munna</i> sp.	0	0	0	0	0	0	0	0	0	19	0	7	0
<i>Paragnathia formica</i>	0	0	100	0	0	16	0	0	4	14	17	8	3
<i>Stenosoma capito</i>	0	0	0	0	0	0	0	0	0	2	0	1	0
<b>Amphipoda</b>													
Gammaridea													
<i>Amphilocheus spencebatei</i>	0	0	0	0	0	0	0	0	22	0	0	0	9
<i>Apocorophium acutum</i>	0	0	0	0	0	0	56	0	0	0	0	0	23
<i>Autonoe spiniventris</i>	0	0	0	0	0	0	0	7	47	2	0	11	12
<i>Elasmopus pocillimanus</i>	0	0	0	0	37	0	6	0	0	0	17	0	0
<i>Eusiroides dellavallei</i>	0	0	0	0	0	0	14	0	0	0	6	0	0
<i>Lembo</i> spp.	0	0	33	0	79	154	233	1304	0	381	41	177	658
<i>Lembo websteri</i>	0	0	0	50	64	30	11	0	22	2	6	20	33
<i>Leucothoe spinicarpa</i>	0	0	33	17	33	0	17	0	0	4	0	28	0
<i>Liljeborgia dellavallei</i>	0	0	0	0	0	0	6	0	7	0	5	0	0
<i>Liljeborgia psaltrica</i>	0	0	100	0	0	0	0	19	17	13	8	21	4
<i>Maera</i> sp.	42	0	0	0	0	0	0	0	0	0	8	0	0
<i>Microdeutopus armatus</i>	83	0	367	0	0	0	0	0	0	0	0	19	45
<i>Stenothoe cavimana</i>	0	0	633	100	267	517	222	354	244	132	389	300	108
<i>Stenothoe monoculoides</i>	0	0	0	100	37	0	0	0	0	3	1	13	15
Caprellidea													
<i>Caprella</i> sp.	0	0	0	0	6	0	0	0	0	0	0	2	0
<i>Caprella acanthifera</i>	42	0	0	0	0	0	22	0	6	0	0	18	0
<i>Caprella grandimana</i>	0	0	0	0	0	0	0	0	0	3	0	0	1
<i>Caprella penantis</i>	0	0	0	0	0	14	0	0	0	0	6	0	0
<i>Pseudoprotella phasma</i>	0	0	0	17	0	0	17	0	0	0	0	0	9
<i>Phtisica marina</i>	0	0	0	0	22	0	0	19	0	15	8	9	5
<b>Cumacea</b>													
<i>Cumella limicola</i>	0	0	0	0	0	0	0	0	4	9	0	2	2
<i>Cumella pygmaea</i>	0	0	33	0	0	0	0	0	0	0	0	4	0
<b>Tanaidacea</b>													
<i>Apeudes</i> sp.	0	0	0	0	0	0	0	3	0	6	0	2	1
<i>Apeudes talpa</i>	0	0	167	0	0	0	0	0	0	0	0	0	23
<i>Leptocheilia dubia</i>	42	0	0	17	0	0	0	59	0	18	36	1	4
<i>Tanais dulongii</i>	0	0	33	0	0	0	44	37	0	0	36	0	2
<b>Decapoda</b>													
<i>Alpheus glaber</i>	0	0	0	0	0	0	0	0	0	5	0	2	0
<i>Anapagurus laevis</i>	0	0	33	0	0	0	0	0	0	0	0	4	0
<i>Galathea nexa</i>	0	0	33	0	4	0	0	0	0	0	0	4	2
<i>Pilumnus hirtellus</i>	0	0	33	33	0	14	6	4	0	7	14	7	1
<i>Periclimenes</i> sp.	0	0	0	0	0	0	0	0	4	0	0	1	0
<i>Processa edulis</i>	0	0	67	17	48	0	0	0	0	0	15	13	2
<i>Pasiphaea sivado</i>	0	0	0	0	0	0	0	0	0	5	0	2	0
<b>Cirripeda</b>													
<i>Balanus</i> sp.	0	0	0	0	8	0	0	0	0	0	2	0	2
<b>Chelicerata</b>													
<i>Pygogonida</i> indet.	0	0	0	0	0	0	0	0	3	0	0	1	0
<b>MOLLUSCA</b>													
<b>Bivalvia</b>													
<i>Arca noae</i>	0	0	0	0	0	0	0	3	0	0	0	1	0
<i>Gregariella semigranata</i>	0	0	0	0	0	0	0	1111	31	17	3	4	467
<i>Modiolus barbatus</i>	42	0	0	0	0	0	0	0	0	0	0	7	0
<b>Gastropoda</b>													
<i>Coralliophila</i> sp.	0	0	0	0	0	0	0	7	3	7	0	4	3
<i>Diodora gibberula</i>	0	0	0	0	0	0	0	0	3	0	0	1	0
<i>Nassarius</i> sp.	0	0	0	0	0	0	0	7	0	3	0	3	1
<i>Nassarius incrassatus</i>	0	0	0	0	0	0	0	0	94	0	0	0	39
<i>Nassarius reticulatus</i>	0	0	0	0	0	0	0	0	28	2	0	1	11
<b>Polyplacophora</b>													
<i>Chiton</i> sp.	0	167	0	17	0	0	0	0	0	0	0	24	0
<b>CNIDARIA</b>													
<i>Actinia</i> sp.	0	0	0	17	0	0	0	0	0	0	2	0	0
<b>PLATYHELMINTHES</b>													
<i>Turbellaria</i> indet..	0	0	0	0	0	0	0	0	0	3	0	0	1
<b>ANNELIDA</b>													
<b>Eunicida</b>													
<i>Lumbrineris coccinea</i>	0	0	0	0	0	0	0	28	11	34	2	18	10
<i>Hilbigneris gracilis</i>	0	0	0	0	0	0	0	0	0	3	0	0	1

<i>Scoletoma</i> sp.	0	0	0	0	6	0	0	0	0	2	0	3	0
<b>Phyllococida</b>													
<i>Glycera</i> sp.	0	0	0	33	45	67	56	0	6	0	41	2	32
<i>Glycera tessellata</i>	0	0	167	0	0	0	0	0	0	0	0	0	23
<i>Lepidonotus clava</i>	0	0	0	0	4	19	0	9	0	3	0	7	7
<i>Neanthes irrorata</i>	0	0	33	0	0	0	0	0	0	0	0	4	0
<i>Nereis</i> sp.	0	0	0	0	0	0	0	0	0	2	0	1	0
<i>Nereis zonata</i>	0	0	0	0	0	0	0	19	19	60	16	8	15
<i>Perinereis</i> sp.	0	0	0	0	0	22	0	0	0	2	0	9	0
<i>Perinereis cultrifera</i>	42	167	0	0	4	0	28	0	4	0	25	9	11
<i>Pholoe minuta</i>	0	0	0	0	0	0	0	0	11	3	0	0	6
<i>Phyllodoce</i> sp.	0	0	0	0	0	0	0	0	3	2	0	2	0
<i>Platynereis dumerilii</i>	0	0	33	17	10	36	11	7	7	5	21	17	0
<i>Syllis</i> spp.	0	0	167	0	28	16	11	37	19	8	32	11	27
<i>Syllis gracilis</i>	42	0	33	33	0	0	0	0	0	16	17	3	4
<i>Syllis hyalina</i>	0	0	33	0	0	0	0	0	0	3	5	0	1
<i>Syllis variegata</i>	0	0	0	0	0	0	0	0	3	3	0	1	1
<i>Websterinereis glauca</i>	0	0	0	0	0	0	0	4	16	9	10	0	1
<b>Sabellida</b>													
<i>Branchiomma</i> sp.	0	0	0	0	0	0	0	0	0	3	0	0	1
<i>Hydroides pseudouncinatus</i>	0	0	0	0	0	0	11	0	0	0	0	4	0
<i>Protula tubularia</i>	0	0	0	0	11	0	0	0	0	0	0	4	0
<i>Sabella</i> sp.	0	0	0	0	0	0	0	0	56	7	3	0	23
<i>Serpula</i> sp.	0	0	0	0	0	19	0	0	0	6	0	7	2
<i>Serpula concharum</i>	0	0	0	0	26	0	0	0	0	0	0	9	2
<i>Vermiliopsis infundibulum</i>	0	167	67	50	0	22	11	0	0	0	18	30	2
<b>Spionida</b>													
<i>Spionidae</i> sp.	0	0	0	0	0	0	0	0	0	6	0	1	1
<b>ECHINODERMA</b>													
<b>Ophiuroidea</b>													
<i>Amphiura</i> sp.	0	0	0	0	6	0	0	0	0	0	0	2	0
<i>Ophiura ophiura</i>	0	0	0	0	0	0	28	4	0	12	2	4	13
<b>Holothuroidea</b>													
Holothuroidea indet.	0	0	0	0	0	0	11	0	0	0	5	0	0
<b>Equinoidea</b>													
<i>Paracentrotus lividus</i>	0	0	0	0	0	0	0	0	0	3	0	0	1

## Abundance

In colonies harvested at 0 m depth, we found 245 specimens, which mean represented 892 ind/L (24.2%), in terms of density for the whole volume estimated at this depth. Crustacean species were dominant (77.4% of the total group) and three species accounted for more than 56% of the crustacean specimen total; *Stenothoe cavimana* (gammarid), *Janira maculosa* (isopod) and *Lembos* spp. (gammarid). The second group in terms of density was annelids (21.2%), with one dominant species, *Perinereis cultrifera* (almost 6% of the total specimen density). Molluscs, cnidarians and echinoderms represented only 1.3% of the total.

At 5 m depth, we identified 408 specimens and estimated their abundance to be 957 ind/L (26% of the total). The crustacean group, which reached 78.9% of the fauna at 5 m depth, was the dominant group, with three principal species in density importance; *Stenothoe cavimana* (gammarid), *Lembos* spp. (gammarid) and *Janira maculosa* (isopod), respectively. These species comprised 57.9% of the total invertebrate assemblage. Annelids reached 15.7% of the total density. Furthermore, there were no dominant species among the 20 species identified, with all species having all the same representation. Molluscs and echinoderms accounted the remaining 5.5%.

At the deepest site (10 m), we found a total of 260 specimens, which reached 1,832 ind/L (49.8% of the total). Crustacean species accounted for 61.4% of this assemblage, including *Lembos* spp. (gammarid), *Janira maculosa* (isopod) and *Sthenothoe cavimana* (gammarid). Although there was one crustacean species that reached 1,000 ind/L (*Microdeutopus armatus*), this species appeared only in one sample. The remaining groups were molluscs (28.5%), annelids (9.3%), echinoderms (0.5%) and plathelminthes (0.1%).

In summary, we observed the greatest abundance at greater depth. At all three depths, crustaceans were the dominant group, with *Lembos* spp. (gammarid), *Janira maculosa* (isopod) and *Sthenothoe cavimana* (gammarid) being the most abundant species. After crustaceans, annelids and molluscs, appeared at all depths and were the most important fauna. Nevertheless, these taxa showed approximately the same proportion at each depth. Finally, echinoderms, cnidarians and plathelminthes were clearly present but did not appear at all depths.

On a monthly basis, the density of specimens was similar, although some months showed higher densities, such as October and May, whereas August and September showed lower densities. *Lembos* spp. (gammarid), *Janira maculosa* (isopod) and *Sthenothoe cavimana* (gammarid) were present in almost all months in a similar proportion.

Species richness (S) and Shannon-Wiener diversity (H')

The monthly species richness (S) and Shannon-Wiener diversity (H') showed significant differences (Table 4.2), increasing considerably in July (Figure 4.3) and being double the values for other months. No differences were detected for S and H' in terms of depth, but the greatest value was achieved at 5 m depth (S = 6.22 ± 1; H' = 1.3 ± 0.13) (Table 4.2, Figure 4.3). Additionally, there was no recorded interaction between the factors time and depth (Table 4.2).

**Table 4.2.** Results of the two-factor ANOVA for Shannon–Wiener diversity and species richness for the macrofauna assemblage. Df = degrees of freedom; MS = mean square; P = level of significance; \*\*\* = p < 0.001.

Source of variation	Df	Species richness (S)			Shannon diversity (H')		
		MS	F	P	MS	F	P
Time	5	2.9025	12.10	0.0000***	1.3708	5.85	0.0005***
Depth	2	0.4771	1.99	0.1516	0.2186	0.93	0.4030
Ti X Dep	10	0.3019	1.26	0.2896	0.3745	1.60	0.1471
RESIDUAL	36	0.2399			0.2345		
TOTAL	53						
Cochran's C-test		C=0.2673 NS			C=0.1653 NS		
Transformation		Sqrt			None		

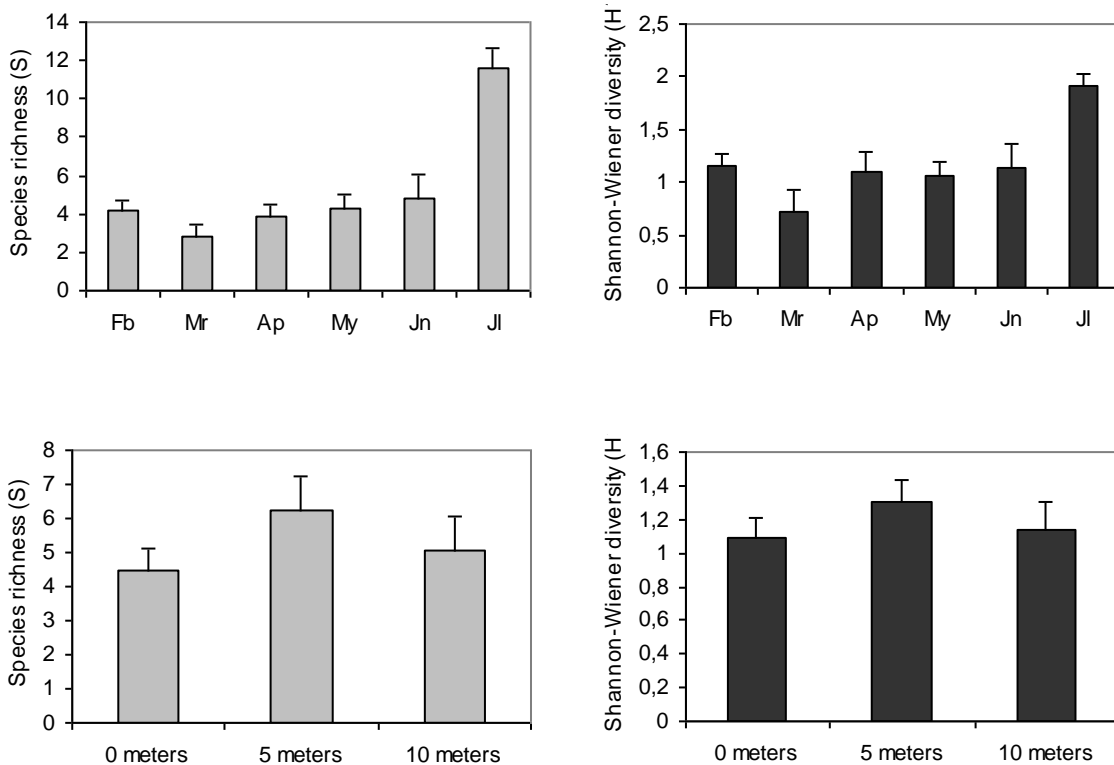
NS: not significant; \*\*\* P<0.001

However, PERMANOVA analyses showed significant differences in invertebrate assemblages associated with *Astroides calycularis* colonies for time and depth ( $p < 0.001$ ), whereas no interaction was observed between both factors (Table 4.3). PERMDISP results indicated that macrofaunal community did not showed a significant variation in depth ( $F_{2,61}=0.52$ ;  $p=0.632$ ) and time ( $F_{9,54}=2.67$ ;  $p=0.179$ ).

**Table 4.3.** Results of PERMANOVA analysis for macrofauna assemblages, based on Bray–Curtis dissimilarities of square root transformed data. Df = degrees of freedom; MS = mean square; P = level of significance; \* =  $P < 0.001$ .

Source of variation	Df	MS	F	P
Time	9	5760.9	2.1953	0.001*
Depth	2	6740.8	2.5687	0.001*
Ti X Dep	16	2968.9	1.1314	0.155
RESIDUAL	36	2624.1		
TOTAL	63			
Transformation		Sqrt		

NS: not significant; \*  $P < 0.001$



**Figure 4.3:** Temporal and bathymetric changes of Species richness (S) and Shannon-Wiener diversity (H') monthly and in depth of macrofauna associated to *Astroides calycularis*.

#### 4.1.4 DISCUSSION

In the Mediterranean Sea, there are no spatio-temporal studies on invertebrate communities associated with scleractinian corals. However, knowledge concerning these associated assemblages is crucial to understand the trophic web connections and health of the marine ecosystems and habitats. Though there are very few studies about some species associated to scleractinian corals in the Mediterranean Sea (Koukouras *et al.* 1998; Castellanos *et al.* 2003; Richter & Luque 2004; Conradi *et al.* 2006; Taviani *et al.* 2009; Antoniadou & Chintiroglou 2010), these contribute little about knowledge the communities associated spatio-temporal patterns and is needed more information. Accordingly, this is the first spatio-temporal study concerning the whole associated macroinvertebrate communities of *Astroides calycularis* (Scleractinian; Dendrophyllidae). Knowledge of this species will provide information about ecosystem health, as well as the Mediterranean habitats and species (García-Gómez 2007).

##### 4.1.4.1 Biometric analyses

Goffredo *et al.* (2011a) reported positive correlations between colony length, width or the number of polyps and colony area, and between colony length, width, or area and the number of polyps on colonies of *A. calycularis* from Italy at 7–10 m depth. Colony area and polyp number explained 74.7–91.6% and 68.7–90.9% of the variance, respectively. In our study, we record similar results, with 72.5–94.4% and 63.3–73.0% of the variance, respectively. Nevertheless, we show that these correlations are better at greater depths (0<5<10 m). Similarly to Goffredo *et al.* (2011a) with the same species and to findings of other authors who studied different scleractinian corals (e.g., Vermeij & Bak 2002; Nozawa *et al.* 2008), we report that the colony area, as a representative colony size parameter through the life history, is a good parameter for wildlife managers interested in understanding the dynamics of coral populations. Moreover, we demonstrate that it is necessary to take into account the depth of colonies, because the colony area changes according to physical (e.g., hydrodynamic, depth, sedimentation) or biological (e.g., symbiosis, competition, and predation) conditions (Zibrowius 1980; Chevalier & Beauvais 1987).

##### 4.1.4.2 Associated fauna

In the Mediterranean Sea, *Cladocora caespitosa* is the only one shallow waters scleractinian coral that may be considered as an ecosystem engineers (Peirano *et al.* 1999; Morri *et al.* 2001), forming large colonies with considerable interstitial space that could be generate a high diversified associated fauna (Koukouras *et al.* 1998). Indeed, in the Eastern Mediterranean studies about *C. caespitosa* have showed the high

zoobenthos biodiversity associated, reaching up 242 macrobentic species associated at this coral (Koukouras *et al.* 1998; Antoniadou & Chintiroglou 2010). Though the macrobentic species number was between 34 and 85 according to Koukouras *et al.* (1998) depending with the colonies size and 54 according to Antoniadou & Chintiroglou (2010). Globally, annelids were the dominants species followed by mollusc and crustaceans. But, these studies concur that the associated assemblages were probably differently structured among individual colonies and banks. In the present study, we have found 81 macroinvertebrate species associated with *Astroides calycularis* with clear differences between depth and season, where crustacean were the species dominant following by annelids and molluscs. Similarly, *Cladocora caespitosa* showed crustacean dominance in shallow waters while polychaetes were dominant in deeper waters (Koukouras *et al.* 1998). Moreover, in one study about the epifaunal assemblages associated with two gorgonians (*Eunicella gazelle* and *Leptogorgia lusitanica*) from the South of the Iberian Peninsula, the dominant group was arthropods, mainly crustaceans, followed by molluscs and annelids (Carvalho *et al.* 2014).

In the Western Mediterranean Sea, some studies have focused on macrofaunal assemblages associated with algae. Seaweeds have an important role as a spatial complex within the substratum, creating a three-dimensional habitat that increases species richness and diversity relative to unvegetated habitats (Dean & Connell 1987). Many studies deal with the associated fauna that inhabits algae throughout the Alborán Sea (Mediterranean area), and all showed the prevalence of crustaceans over other groups such as annelids, mollusks and echinoderms (Sánchez-Moyano & García-Gómez 1998; Sáchez-Moyano *et al.* 2000; Izquierdo & Guerra-García 2011; Pacios *et al.* 2011; Guerra-García *et al.* 2006; 2009; 2012), as also happens in *Astroides calycularis*. Guerra-García *et al.* (2011) identified 41 crustacean species on *Asparagopsis armata*, whereas for *Corallina elongata*, one of the most important algae in the intertidal ecosystem (Pérez-Cirera & Maldonado 1982; Guerra-García *et al.* 2006), a total of 78 crustacean species were found (Izquierdo & Guerra-García 2011). We have found 39 crustacean species, a similar value than those reported for algae species. Guerra-García *et al.* (2011) indicated that the different faunal composition on four algae species might be due to the differences in algal morphology. *C. elongata* and *A. armata* are native and invasive seaweeds, respectively, in the Mediterranean Sea that cohabit with *A. calycularis* in the study area and maintain a high diversity on peracarid crustacean assemblages (Pacios *et al.* 2011; Guerra-García *et al.* 2012). The most common associated species hosted by *C. elongata* are *Caprella penantis* (caprellid) and *Sthenotoe monoculoides* (gammarid) among others (Guerra-García *et al.* 2010). Alternatively, *A. armata* hosts *Aora spinicornis*, *Apherusa bispinosa*, *Dexamine spiniventris* (gammarids) and *Dynamene magnitorata* (isopod) as dominant species (Pacios *et al.* 2011; Guerra-García *et al.* 2012). *Janira maculosa* (isopods), *Lembos* spp. (gammarid) and *Sthenothoe cavimana* (gammarid) are the predominant species on *A.*



*calycularis* colonies. Therefore, peracarid species were different to those reported on both seaweeds and only the genera *Sthenothoe* (gammarid) is common between *A. calycularis* and *C. elongata*. So, macrofaunal assemblages seem to be host-dependent. Furthermore, the bright orange colour of the two dominant species observed in *A. calycularis* (*Caprella penantis* and *Sthenothoe cavimana*) suggests a relationship between coral and associated species (Figure 4.4). The nature of this relationship (obligated/facultative, see Castro 1976 or trophic/cryptic) still needs to be clarified.

On the other hand, both *Sthenothoe* genus and *Lembos websteri* were associated with *Cladocora caespitosa*, but none was dominant. *Leucothoe spinicarpa* and the genera *Liljeborgia*, *Microdeutopus* and *Maera* were common in both scleractinian species, being the last genus dominant in the *C. caespitosa* colonies from shallow waters (Koukouras *et al.* 1998).

Several species from genera *Gnathia* (Isopods) were described associated to *Astroides calycularis* in the South side of Alboran Sea, some of them being very abundant (Castellanos *et al.* 2003). However this genus did not appear in our study. A plausible hypothesis would be that these authors could have mixed species from adjacent substrates with those associated to *A. calycularis* due to the methods used to collect them. In fact, they removed the colonies directly, without using plastic bags to isolate the associated assemblages. Alternatively, the genus *Gnathia* is absent or rare on samples of many seaweeds (*Stypocaulon*, *Asparagopsis* and *Corallina*) along the North side of Alboran Sea (Guerra-García *et al.* 2009; Izquierdo & Guerra-García 2011; Pacios *et al.* 2011; Soler & Guerra-García 2011). Therefore, it can be expected that this genus will be also absent in *A. calycularis* colonies from this area.

Some genera of decapods have been found associated to *Astroides calycularis* in the present study (*Anapagurus*, *Galathea*, *Pilumnus*, *Periclemenes* and *Alpheus*). Furthermore, all of these genera have been also reported from coralligenous assemblages in the Alboran Sea (García-Muñoz *et al.* 2008). In the Eastern Mediterranean Sea, the genera *Galathea*, *Pilumnus*, *Periclemenes* and *Alpheus* were present associated with *Cladocora caespitosa*, but none was species dominant (Koukouras *et al.* 1998).

Annelids were the second most important group within the macrofaunal assemblages associated with the orange coral. Nevertheless, there were no dominant species; only *Perinereis cultrifera* (polychaeta) reached a high abundance at 0 m depth. In the Chafarinas Islands, López (1995) identified some polychaetes in dead colonies of *Astroides calycularis*, but no type of relationship was reported. Possibly, annelids are generalist habitat species and it is the physical and/or environmental factors (e.g. depth), which influence their preference to settle in different substrata. However, polychaetes were species dominant in deeper colonies of *C. caespitosa*, being common species

*Hydroides pseudouncinatus*; *Lepidonotus clava*; *Lumbrineris coccinea*; and *Syllis gracilis* (Koukouras *et al.* 1998).



**Figure 4.4:** A: *Sthenothoe cavimana*; B: *Caprella penantis*. Both species presents a strong orange color.

Scleractinian corals act as structural engineers (Jones *et al.* 1994), contributing to an increase in habitat complexity and surface topography, which promotes biodiversity by mediating competition or predation (Menge 1976; Holt 1987; Hixon & Menge 1991; Coker *et al.* 2009). The symbiotic associations with corals provide many benefits for invertebrate assemblages; corals provide a large surface area on and in which they can live, as well as refuges from predation, food in the form of coral tissue, mucus and its associated detritus and a hard skeleton used as a substratum by

specialised burrowers (Castro 1988). Taking this into account, the mollusks, echinoderms, plathelminthes and cnidarians found associated with *Astroides calycularis*, might use the coral skeleton as a refuge against potential predators. However, these groups are less abundant. Richter & Luque (2004) observed *Epitoniem dendrophylliae* (Gastropoda: Mollusca) feeding on *A. calycularis*. Nevertheless, these taxa were cited between 50 to 250 metres in depth and it is a rare species in shallow waters (Gofas *et al.* 2011). As a consequence, *E. dendrophylliae* did not appear in the present study, where depth ranged from 0 to 10 metres.

Univariate analyses (species-independent), showed that macrofaunal composition associated with *Astroides calycularis* colonies differed through time, with a greater diversity in summer. This pattern has been already observed in macrofaunal assemblages associated with seaweeds in the Mediterranean Sea (e.g., Guerra-García *et al.* 2010) and might be because some temperate species show peak abundances in summer and marked declines in winter (Keith 1971; Thom *et al.* 1995; Ashton 2006). Alternatively, the number of species and diversity did not show differences with depth. However, the PERMANOVA multivariate analysis (species-dependent), detected that faunal assemblages change with time and depth. Factors such as seawater temperature and wave action might be a seasonal reason of the vertical distribution of associated macrofauna (Neto 2000). In fact, at 0 m, the hydrodynamism is greater, due to strong wave action, whereas at deeper sites, this factor is greatly reduced, as showed Cebrián & Ballesteros (2004) in the same area. It has been pointed out that wave action affects patterns of distribution in the associated macrofauna at the intertidal (Chavanich & Wilson 2000). Oxygen concentration might be one additional factor that affects the patterns of macrofaunal assemblages associated with seaweeds (Guerra-García & Izquierdo 2010). Additionally, weather conditions, competition or predation might determine the associated macrofauna. More information concerning these factors and their interaction with macrofaunal assemblages is necessary.

The macrofauna community of *Astroides calycularis* was previously unknown, however, this study shows that this endangered and seascape-formation coral from the Western Mediterranean Sea maintains a diverse associated community that changes spatially and seasonally. Therefore, this could give us an idea about the potential role of *A. calycularis* on the thropic webs in the Mediterranean Sea rocky shore, although more studies are still necessary. Moreover, macrofaunal assemblages are essential tools as a baseline to evaluate disturbances due to natural and/or anthropogenic factors. In this context, this information is essential to the future conservation framework of the endangered coral *A. calycularis*.

**Plate I**

Artropoda

Isopoda

*Janira maculosa* (A)

*Munna* sp.(B)

*Paragnathia formica* (C)

Amphipoda-Gammaridea

*Autonoe spiniventris* (D)

*Elasmopus pocillimanus* (E)

*Eusiroides dellavallei* (F)

*Lembos* spp. (G)

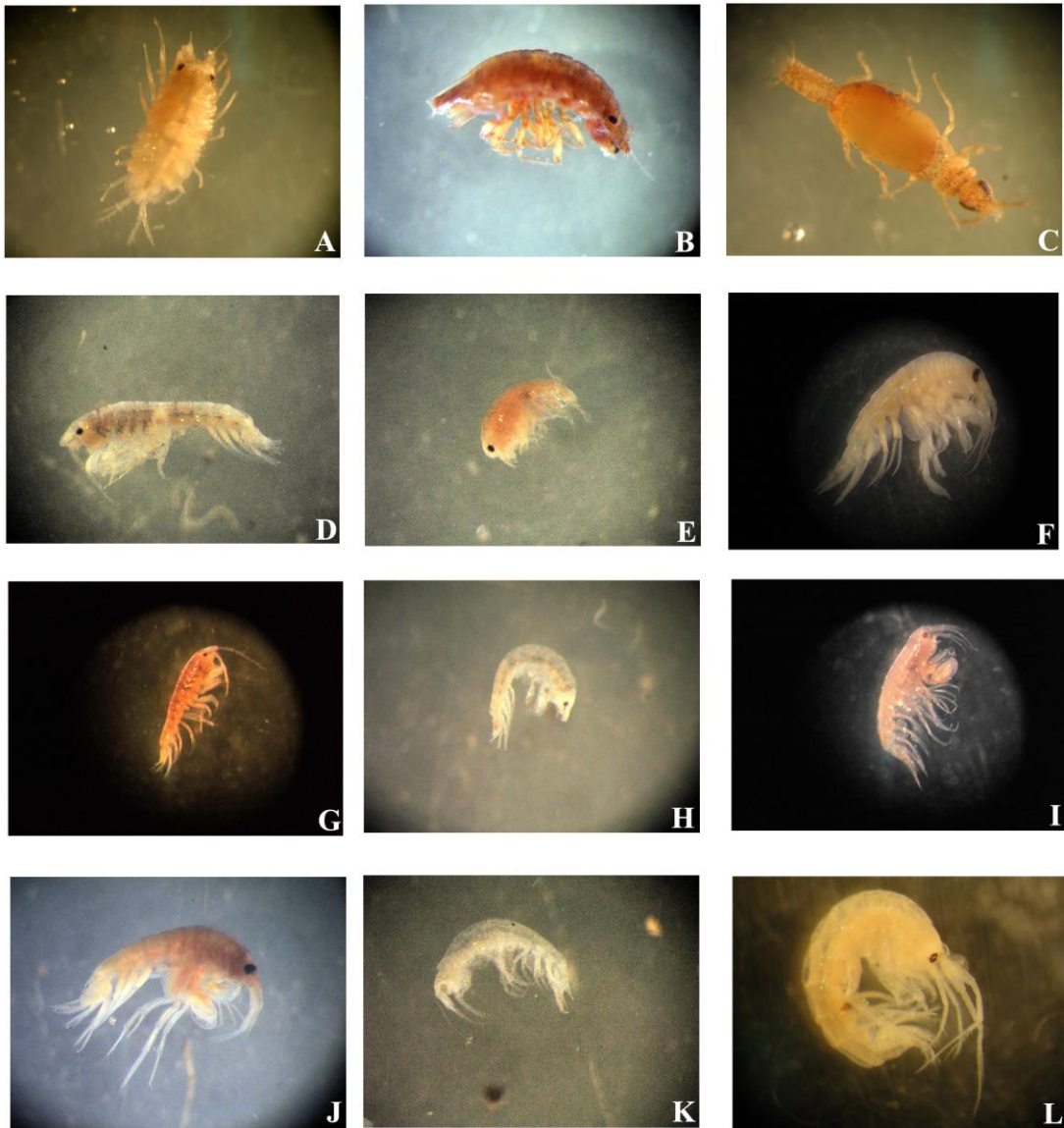
*Lembos websteri* (H)

*Leucothoe spinicarpa* (I)

*Liljeborgia dellavallei* (J)

*Liljeborgia psaltrica* (K)

*Maera* sp. (L)



## Plate II

### Artropoda

#### Amphipoda-Gammaridea

*Microdeutopus armatus* (A)

*Stenothoe cavimana* (B)

*Stenothoe monoculoides* (C)

#### Amphipoda-Caprellidea

*Caprella* sp. (D)

*Caprella acanthifera* (E)

*Caprella penantis* (F)

*Pseudoprotella phasma* (G)

*Phthisica marina* (H)

### Cumacea

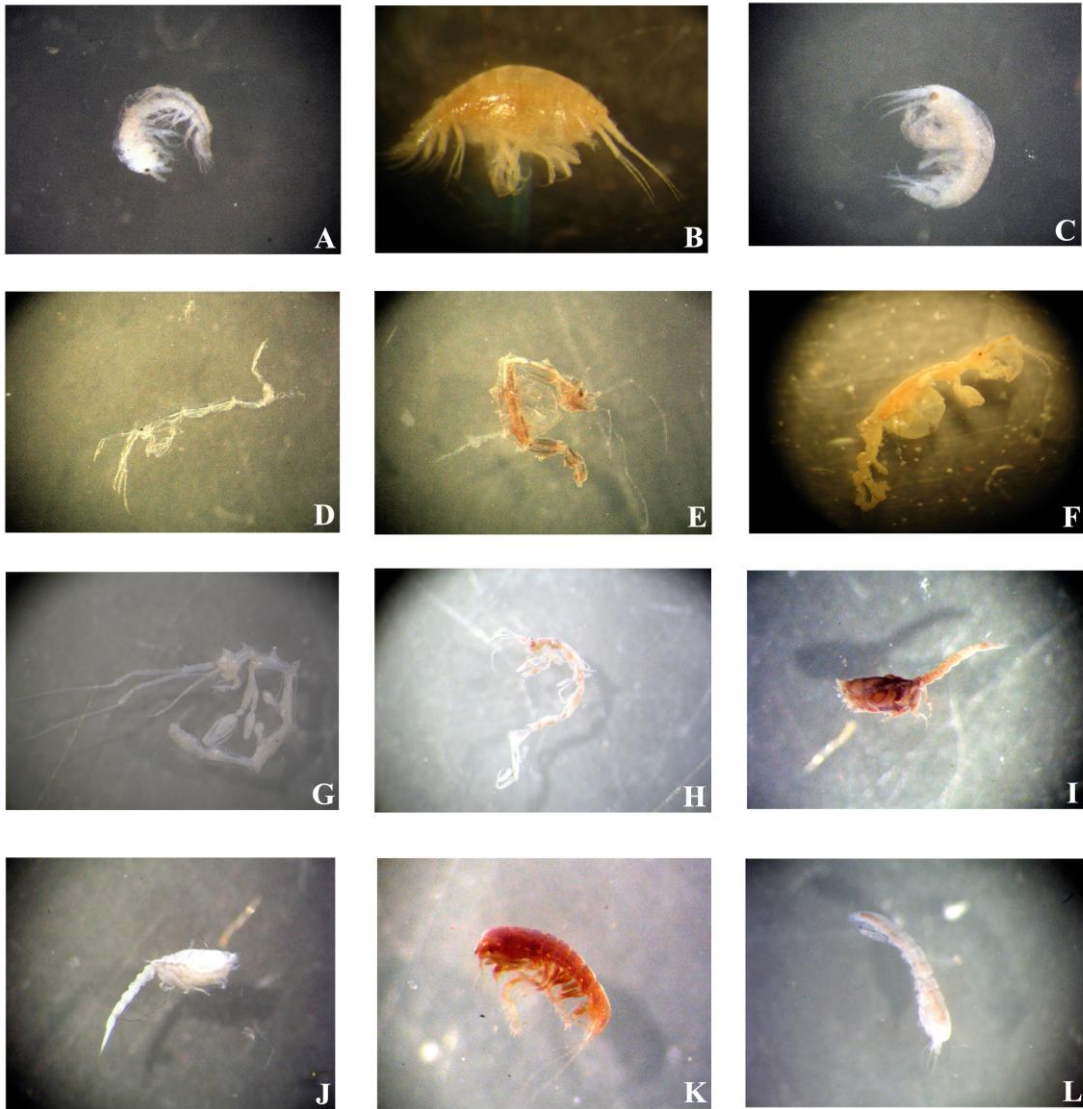
*Cumella limicola* (I)

*Cumella pygmaea* (J)

### Tanaidacea

*Apseudes* sp. (K)

*Apseudes talpa* (L)



**Plate III**

Artropoda

Tanaidacea

*Leptochelia dubia* (A)

*Tanais dulongii* (B)

Decapoda

*Galathea nexa* (C)

*Pilumnus hirtellus* (D)

Mollusca

*Modiolus barbatus* (E)

*Chiton* sp. (F)

Cnidaria

*Actinia* sp. (G)

Annelida

Phyllodocida

*Neanthes irrorata* (H)

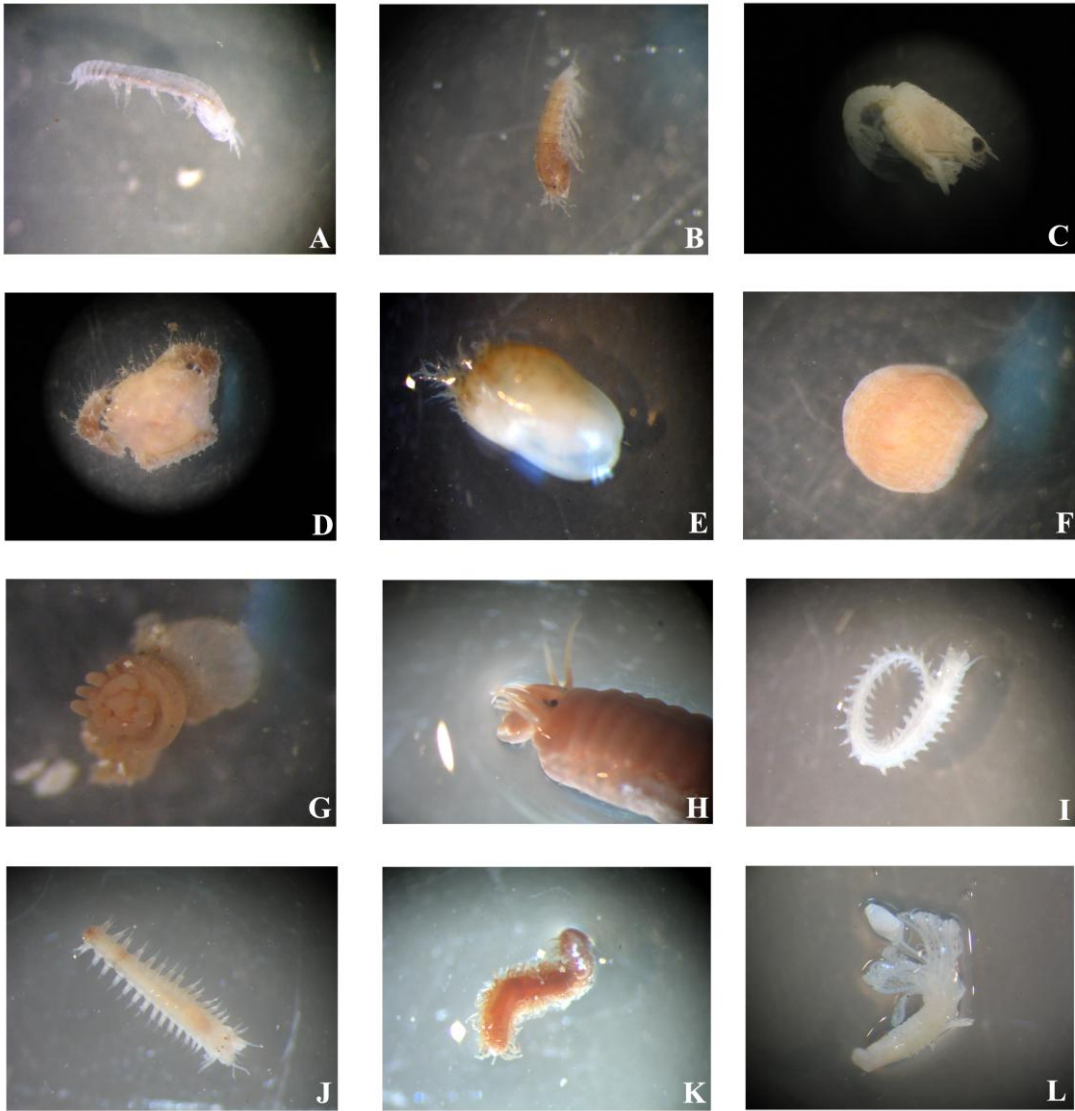
*Perinereis cultrifera* (I)

*Platynereis dumerilii* (J)

*Syllis* spp. (K)

*Vermiliopsis infundibulum* (L)









4.2 Diets of peracarid crustaceans associated with the orange coral *Astroides calycularis* in southern Spain

*Paragnathia formica* after immersion in Hertwig's liquid. Author: Terrón-Sigler A.

**Abstract**

The endangered and endemic orange coral (*Astroides calycularis*) hosts an important macrofaunal assemblage, mainly peracarid crustaceans, and some of them have a strong orange colour as a *Janira maculosa* (isopod), *Lembos* sp. (gammarid) and *Sthenothoe cavimana* (gammarid). The gut contents of the main peracarids associated with the orange coral was analysed. In total 161 specimens belonging to 11 species and 9 families were examined on the southern coast of the Iberian Peninsula. The gut content study was carried out introducing the specimens of each species in Hertwig's liquid. The analysis revealed that the peracarids assemblage associated with *A. calycularis* may include different strategies and the main food source was detritus. The results highlight that peracarids may depend on the host and the detritus that the coral produces.

**Resumen**

El coral naranja (*Astroides calycularis*), especie endémica y amenazada del mar Mediterráneo, alberga una importante comunidad de macro-invertebrados asociada, principalmente crustáceos peracáridos. Se ha observado que algunos de estos peracáridos presentan un fuerte color naranja, como por ejemplo el isópodo *Janira maculosa* o los gammáridos *Lembos* spp. y *Sthenothoe cavimana*. Para conocer qué tipo de asociación existe entre estas especies y su hospedador se ha analizado el contenido estomacal de los principales peracáridos asociados al coral. Fueron analizados un total de 161 individuos pertenecientes a 11 especies diferentes de 9 familias del sur de la Península Ibérica. La metodología para el estudio del contenido estomacal consistió en introducir los individuos en líquido de Hertwig. Los análisis revelaron que la comunidad peracárida asociada a *A. calycularis* presenta diferentes estrategias alimenticias y su principal fuente de alimento son los detritos. Los resultados obtenidos vislumbran la posibilidad de que esta comunidad asociada dependa del hospedador y del detrito que éste origina.

#### 4.2.1 INTRODUCTION

*Astroides calycularis* (Pallas 1766) is an azooxanthellate colonial scleractinian, with a calcium carbonate exo-skeleton (Zibrowius 1980) that inhabits the rocky shores from the surface to a depth of 50 m (Rossi 1971). It occupies both well-lit and dark habitats and appears to prefer environments with strong currents (Zibrowius 1978; 1995; Kružić *et al.* 2002). Its population density can be locally high, with colonies covering up to 90% of the sea bottom (Goffredo *et al.* 2011). Mainly, the populations have a limited geographical distribution in the Mediterranean Sea, restricted to the Southwestern area (Zibrowius 1980; 1995; Bianchi 2007). Though, the species has been quoted in Adriatic Sea (Grubelić *et al.* 2004). Andalusian rocky shores (southern Iberian Peninsula) host one of the highest densities of the orange coral populations (Terrón-Sigler *et al.* 2015-Section 6).

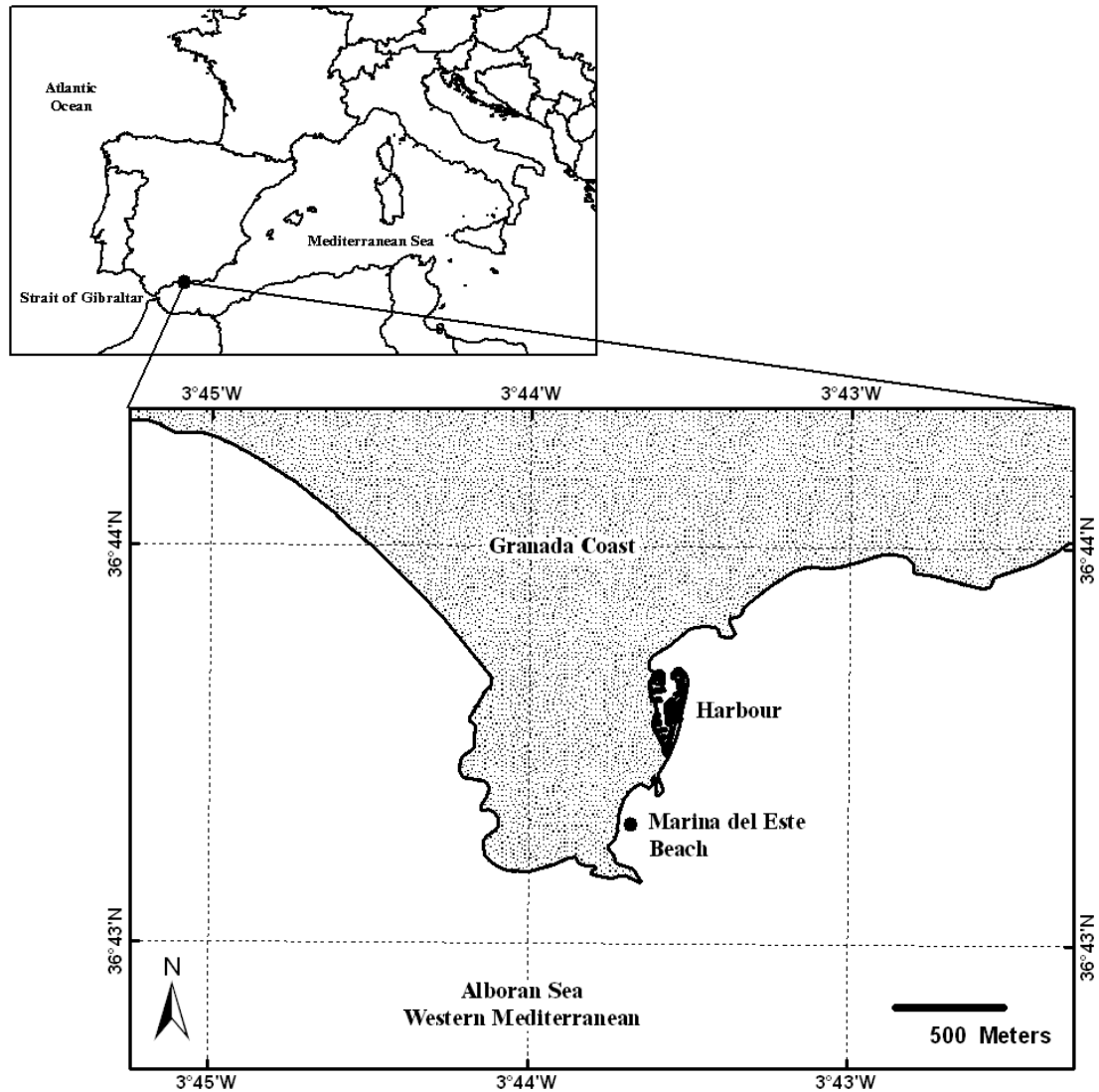
Terrón-Sigler *et al.* (2014a) observed that peracarids crustacean were the most prevalent species associated with the orange coral. Additionally, three of the peracarids associated with *Astroides calycularis* had a strong orange colour (i.e. *Janira maculosa*, *Sthenothoe cavimana*, and *Caprella penantis*) (Terrón-Sigler *et al.* 2014a). However, the nature of the association is unclear, whether it is obligate or facultative, and whether as a substrate (e.g. refuge, spawning, brood care or protection by crypsis) or as a feeding source (partial or totally).

Amphipods are the most diverse group of crustaceans with regard to lifestyle, trophic types and habitats (De Broyer & Jazdzewski 1996). Due to their population's characteristics, amphipods may play major roles in the ecology of their habitats (Conlan 1994). These amphipods are known to have versatile feeding strategies (i.e. Carrasco & Arcos, 1984; Sarvala & Uitto 1991) and show a wide range of feeding habits (Conradi & Cervera 1995). They also constitute an important food source for a large variety of marine predators (Vázquez-Luis *et al.* 2013); hence playing a key role in energy flow through food webs (Guerra-García *et al.* 2014). Consequently, the main objective of the present study is to explore if peracarids assemblage feed on tissues of *Astroides calycularis*. The results could highlight the nature of the association between peracarids and host.

#### 4.2.2 MATERIAL AND METHODS

There were collected 161 specimens of peracarids from 23 colonies of *Astroides calycularis* located in Marina del Este beach (Granada coast; Andalusia; Spain) during July of 2011 (Figure 4.5). Colonies were covered *in situ* with plastic bags before they were removed, in order to keep all the associated fauna. The samples were fixed in 4%

formalin and subsequently placed in 70% ethanol in the laboratory. The colonies were washed, the macrofauna sieved using a 0.5 mm size mesh, and the specimens collected were sorted and identified at the species level, when possible.



**Figure 4.5:** Map of the study site showing Granada coast (Andalusia; Spain) and Marina del Este beach, where the colonies of *Astroides calycularis* were collected (\* = 36°43'15" N; 3°43'43" W).

Diet study was analysed following the methodology proposed by Bello & Cabrera (1999) with slight variations. Recently, this method was successfully used in amphipods (i.e. Navarro-Barranco et al. 2013; Vázquez-Luis et al. 2013; Guerra-García et al. 2014). The method involves; specimens of each species were introduced in vials with Hertwig's liquid (consisting of 270 g of chloral hydrate, 19 ml of chloridric acid 1 N, 150 ml of distilled water and 60 ml of glycerin) and oven-heated at 65 °C for 4 to 6 hours depending on the cuticle thickness of the specimens. After this, samples were

mounted on slides for microscope study. The percentage of absolute gut content (i.e. total area occupied by the content in the whole digestive tract) and the relative gut content (i.e. area occupied by each component within the total gut content) were estimated using a microscope equipped with an ocular micrometer (at 40× or 100×). Mean and standard error of the mean were calculated.

### 4.2.3 RESULTS AND DISCUSSION

The collected specimens of paracarids belonged to 11 species and distributed in 9 families (Table 4.4), representing the dominant paracarids associated with *Astroides calycularis* in the South of the Iberian Peninsula. Gut contents of the studied amphipod species included detritus, crustaceans and macroalgae (Table 4.4). The dominant component was detritus, followed by crustacean pieces and macroalgal tissues (Figure 4.6). In a diet analysis of marine amphipods around the Iberian Peninsula, Guerra-García *et al.* (2014) concluded that detritivorous species had fuller guts than carnivorous species, where empty guts predominated. Nevertheless, trophic classifications may be subjective. In our study, three species namely *Liljeborgia psaltrica*, *Sthenothoe cavimana* and *Eusiroides dellavallei*, presented empty guts. Therefore, we could infer these species as carnivorous, following Guerra-García *et al.* (2014). Although, these three species might be considered carnivorous, the amphipods associated with *A. calycularis* colonies presented the majority of the gut content occupied by detritus, highlighting the importance of detritus for the amphipods already noted in the Iberian Peninsula by Guerra-García *et al.* (2014).

Gut content ranged from 16% in *Leucothoe spinicarpa* to 64.3% in *Janira maculosa*. In general, species with detritus had a higher area occupied by content in the digestive tract than other species.

Regarding caprellid species, our findings are in agreement with Iberian Peninsula and worldwide data, where the detritus was the dominant food source in seaweed-associated caprellids (Guerra-García & Tierno de Figueroa 2009; Guerra-García *et al.* 2014). In the present study, the two caprellids collected from orange coral colonies showed more than 98% detritus composition in their gut contents.

There is scarce information about the feeding habits of marine isopods (e.g. Navarro-Barranco *et al.* 2013). In our study, *Paragnathia formica* showed a 100% of detritus gut content, while *Janira maculosa* had a diverse gut content (Table 4.4).

The tanaid *Tanais dulongii* associated with *Fucus spiralis* in the South of the Iberian Peninsula is herbivorous with furoid algae its primary diet source (Torrecilla-Roca & Guerra-García 2012). The specimens associated with *A. calycularis* did not show this feeding habit; more than 98% of the gut content was detritus and the rest

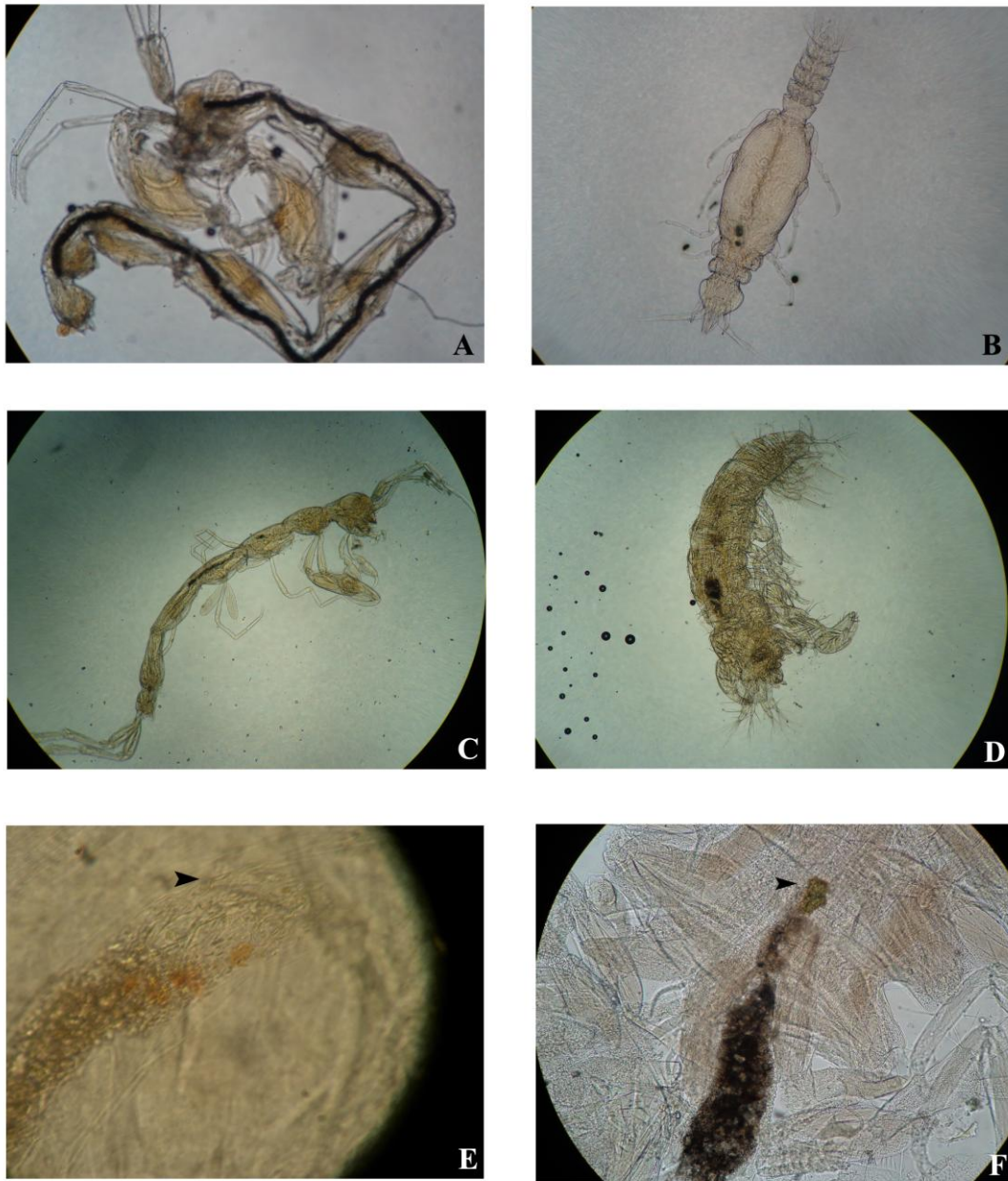


crustacean fragments. This finding highlights the plasticity in the feeding habits of peracarids species depending on the host or habitat where they inhabit.

Scleractinian corals function as structural engineers (Jones *et al.* 1994), contributing to an increase in habitat complexity and surface topography, which promotes biodiversity by mediating competition or predation (Menge, 1976; Holt, 1987; Hixon & Menge 1991; Coker *et al.* 2009). Corals provide a large surface area where invertebrate assemblages can live, as well as refuges from predator, food source in the form of coral tissue, mucus and its associated detritus, and a hard skeleton used as a substratum by specialized burrowers (Castro 1988). Taking all these factors into account, the peracarids associated with *Astroides calycularis* might use the coral skeleton as a refuge against potential predators (by hiding or crypsis) or as food source of detritus that the colonies generate. Commensalisms between amphipods and cnidarians has been documented worldwide (e.g. Vader, 1984; Vader & Krapp-Schickel 1996; Esquete *et al.* 2014), but there is little information about this association with corals. Therefore, more studies are necessary in this regard with scleractinian corals. The analysis of gut contents of peracarids associated with the intertidal algae *Fucus spiralis* revealed that the assemblage is dominated by species feeding mainly on this algae (Torrecilla-Roca & Guerra-García 2012), in contrast with other crustaceans inhabiting different seaweed assemblages which mainly feed on detritus (Guerra-García & Tierno de Figueroa 2009; Alarcón-Ortega *et al.* 2012). This could highlight that depending on the host where the peracarids are associated, the relationship could be host-dependent or not, but further research needs to be carried out in this regard.

**Table 4.4.** Gut contents of the studied peracarid species. N: number of specimens of each species examined, n: number of specimens with detected digestive contents. % Abs: total area occupied by the content in the whole digestive tract. Det: detritus, Crust: crustaceans, Malg: Macroalgae. Mean values with standard errors of the mean (in parentheses) are included.

PERACARIDS	N/n	% Abs	% Det	% Crust	% Malg	Feeding group
<i>Isopods</i>						
<i>Janira maculosa</i> Leach, 1814	22/22	64.3 (19.1)	93.9 (8.6)	5.7 (3.1)	0.5	Omnivorous
<i>Paragnathia formica</i> Hesse, 1864	12/10	27.5 (15.5)	100	--	--	Detritivorous
<i>Amphipods</i>						
<i>Lembos</i> sp. Bate, 1857	23/22	50.2 (30.7)	92.7 (6.3)	7.3 (5.2)	--	Omnivorous
<i>Autonoe spiniventris</i> Della Valle, 1893	9/8	59.4 (39)	100	--	--	Detritivorous
<i>Leucothoe spinicarpa</i> Abildgaard, 1789	19/17	16 (9.1)	98.1 (3.1)	1.9 (0.8)	--	Omnivorous
<i>Liljeborgia psaltrica</i> Krapp-Schickel, 1975	20/0	--	--	--	--	Carnivorous
<i>Sthenothoe cavimana</i> Chevreux, 1908	20/0	--	--	--	--	Carnivorous
<i>Eusiroides dellavallei</i> Chevreux, 1899	3/0	--	--	--	--	Carnivorous
<i>Caprella acanthifera</i> Leach, 1814	14/14	61.1 (3.7)	100	--	--	Detritivorous
<i>Phtisica marina</i> Slabber, 1769	11/8	19.4 (13.9)	98.7 (3.5)	1.3	--	Omnivorous
<i>Tanaids</i>						
<i>Tanais dulongii</i> Audouin, 1826	8/7	55.7 (28.7)	98.8 (3.7)	1.2	--	Omnivorous



**Figure 4.6:** A) *Caprella acantiphera* with almost 100% of the gut content fill of detritus. B) Gut content of *Paragnathia formica* with 40% filled. C) *Phtisica marina*. D) *Tanais dulongii* E) and F) Crustacean pieces.






## 5. THREATS

A fisher gear on *Astroides calycularis* colonies (Marina del Este beach, Almuñécar, Granada). Atuhor: Terrón-Sigler A.



An underwater photograph showing a diver in the upper left, partially obscured by a semi-transparent text box. The diver is wearing a mask and a scuba tank. The background is a deep blue sea. In the foreground and middle ground, there is a diverse coral reef. The reef is covered with various types of coral, including large, branching green corals and numerous bright orange, cup-shaped corals. The lighting is natural, coming from above, creating a clear view of the underwater environment.

**5.1** The effects of SCUBA diving on endemic Mediterranean coral *Astroides calycularis*

Recreative SCUBA diving in the Acantilados de Maro-Cerro Gordo Natural Park  
(Málaga-Granada). Athor: Terrón-Sigler A.

## Abstract

Many papers have dealt with the impact of diving activity, although most of them have been focussed on diver's physical contact and their equipment. Nevertheless, there are more factors that may be affecting the benthic community: environment, diver's behaviour, dive characteristics, or previous knowledge of the divers about the surrounding wildlife. In the present study, several factors have been studied that may affect the orange coral (*Astroides calycularis*) populations in the North Alborán Sea (Mediterranean Sea). It has been demonstrated that detached colonies are more common in an impacted station than a controlled station. However, bigger size detached colonies were found in the controlled station, this is probably due to the species growing without impact factors until they reach a size that they become detached by natural phenomena. Factors studied such as characteristics of dives, dive experience, environmental perception, or previous knowledge of divers are affecting the endangered orange coral, showing the characteristics of dives as a more noteworthy factor. But this, in synergy with the other factors, may be the cause of losing colonies. The results of this study are helpful to the managers of marine environment and MPAs, especially where sensitive species are present during diving activities. Therefore, essential diver education programmes must teach the environmental value and the fragility of different species. Protecting these populations should be a high priority of the environment managers to preserve our natural heritage.

## Resumen

Existe mucha información sobre el impacto que produce el buceo autónomo sobre las comunidades marinas, aunque la mayoría se centra en los daños que producen los buceadores mediante el contacto físico con las especies. Sin embargo, existen muchos factores que pueden estar afectando a la comunidad bentónica, como por ejemplo el propio ambiente, el comportamiento de los buceadores, las características de la inmersión o incluso el conocimiento previo que tienen los buceadores sobre la fauna marina que les rodea. El presente estudio trata de analizar varios factores que pueden estar afectando a las poblaciones de coral naranja (*Astroides calycularis*) en las zonas de buceo del norte del mar de Alborán (mar Mediterráneo). En este estudio se ha demostrado que las colonias desprendidas son más numerosas en las zonas con mayor actividad de buceo que en aquellas que la actividad está limitada. No obstante, cabe destacar que las colonias desprendidas son de mayor tamaño en las zonas con menor actividad, probablemente como consecuencia de que la especie crece sin interacción con los buceadores hasta alcanzar un tamaño considerable y se desprenden de forma natural. Se han estudiado algunos factores inherentes al buceador, como por ejemplo las

características de la inmersión realizada, la percepción del ambiente o el conocimiento previo de los buceadores sobre la biota marina local. Los resultados muestran que estos factores están afectando a este coral amenazado, siendo la característica de la inmersión el factor más relevante. Sin embargo, este factor en sinergia con los otros estudiados puede ser la causa de la pérdida de colonias en aquellas zonas donde la actividad de buceo es mayor. Los resultados aquí presentes son de gran utilidad para los gestores del medio marino, así como de Áreas Marinas Protegidas, sobre todo en aquellas zonas donde exista actividad de buceo que puedan interaccionar con especies sensibles a dicha actividad. Por este motivo, son necesarios programas educativos dirigidos a buceadores con el fin de mostrarles el valor ambiental del lugar en el que realizan su actividad y el de las especies más relevantes, sensibles y/o amenazadas. Proteger estas poblaciones debe ser una prioridad para los gestores marinos, ya que debemos preservar nuestro patrimonio natural.



### 5.1.1 INTRODUCTION

The increase in recreational diving is resulting in more frequent physical contact between diving tourists and habitats, causing habitat damage largely due to diver inexperience and ignorance (Davis & Tisdell 1995). Many scientific manuscripts have dealt with the impact of diving activities (e.g. Hawkins & Roberts 1997; Roupheal & Inglis 1997; 2001). Nevertheless, most of the studies focus on tropical regions, while temperate ones such as in the case of the Mediterranean Sea, is poorly studied (Di Franco *et al.* 2009; Luna *et al.* 2009). SCUBA divers may affect organisms both intentionally and unintentionally through physical contact with their hands, body, equipment, and fins (Tratalos & Austin 2001; Zakai & Chadwick-Furman 2002; Pulfrich *et al.* 2003; Uyarra & Côté 2007). These contacts are more frequent, for example, when the intensity of dive users is high (Hawkins *et al.* 1999; 2005); the dive time is longer (Di Franco *et al.* 2009; Luna *et al.* 2009); diver experience is lower (Roberts & Harriot, 1994); and/or the behaviour of divers is poor (Davis & Tisdell 1995; Roupheal & Inglis 2001; Barker & Roberts 2004). Nevertheless, the impact of recreational activities may be influenced more by the experience and behaviour of the divers than by the number of them (Davis & Tisdell 1995; Roupheal & Inglis 2001; Barker & Roberts, 2004). The diver's experience, the characteristic of the dive, and/or diver behaviour are factors that may determine the degree of impact that divers perform during their activity (Luna *et al.* 2009). However, the knowledge that divers have about the marine biota present around their diving has had less attention.

Moreover, the effects of recreational activities on marine fauna may be bigger when this activity focuses on Marine Protected Areas (MPAs). Over the last decade, a lot of MPAs have been designated around the world (Ballantine 1995; Agardy 2003). These are contributing, among other attributes, to the promotion of tourism activities along the coast (Badalamenti *et al.* 2000; Milazzo *et al.* 2002). Additionally, MPAs are an important component of the socio-economy of the area (Sorice *et al.* 2007; Parsons & Thur 2008). Recently, studies made in Mediterranean MPAs have highlighted this problem, and management programmes have been raised (Luna *et al.* 2009).

*Astroides calycularis* has a narrow distribution in the western Mediterranean due to its temperature tolerance (Zibrowius 1995) and its preference to unpolluted environments (Terrón-Sigler *et al.* 2015-Section 6). It is affected by siltation caused by high coastal urbanisation (Ocaña *et al.* 2009) because it is a suspension feeder (Cebrián & Ballesteros 2004). Moreover, orange coral is affected by human activities on the littoral, such as the constructions of recreational ports or beach regenerations that increase marine pollution and/or habitat destruction (Moreno *et al.* 2007). It has also been demonstrated that recreational activities like SCUBA diving have a negative effect on their populations because colonies can be damaged or removed by fins, hands, and

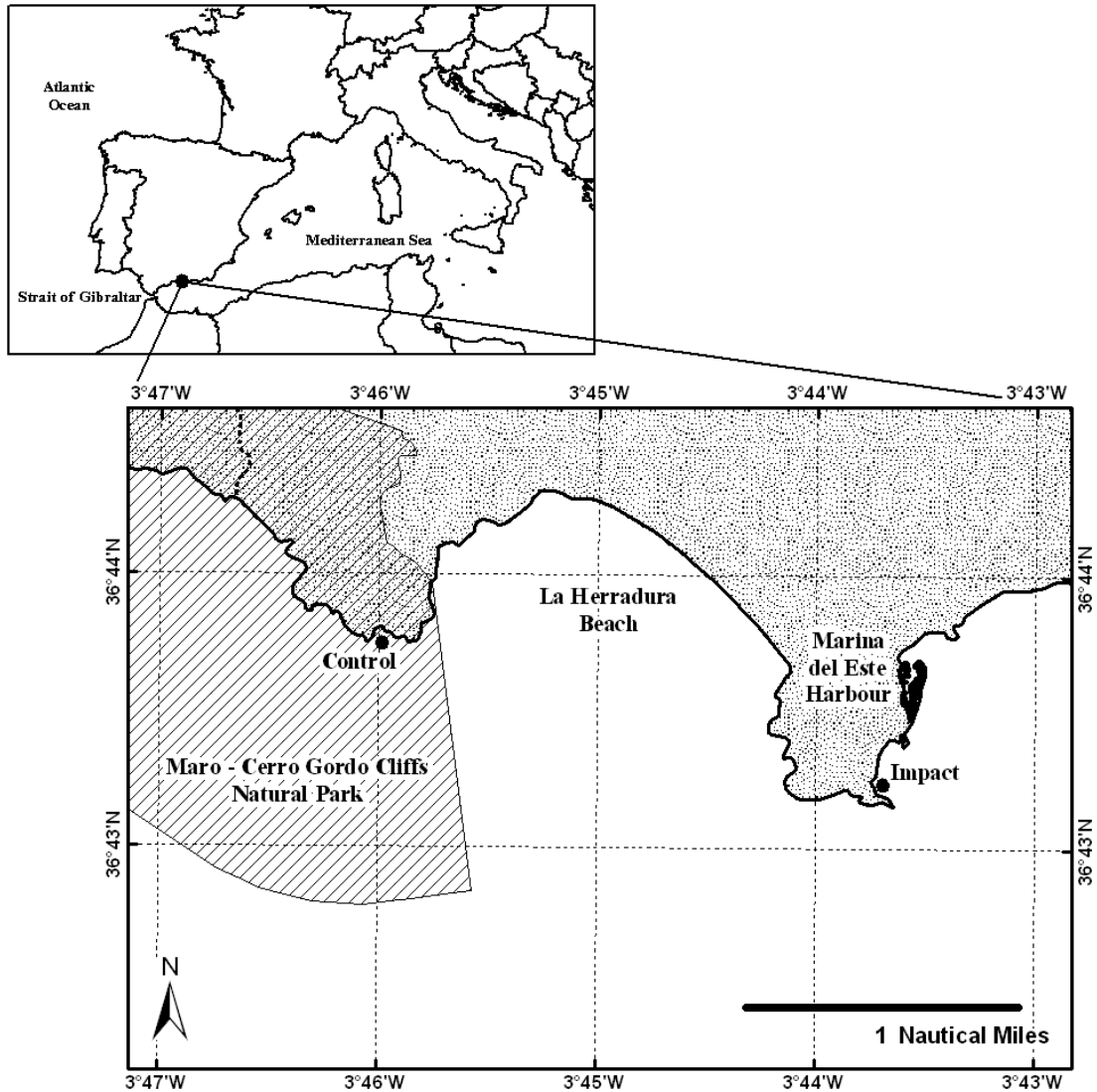
other diving equipment parts (Moreno *et al.* 2007; Di Franco *et al.* 2009; Terrón-Sigler & León-Muez 2009-Section 5). As a result, *A. calycularis* is presently protected by national and international organisations as an endangered species (i.e. the Bern and Barcelona Conventions and the Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES]).

Knowledge about the level of interaction between recreational activities and benthic communities and/or species is needed. This may be vital to apply the appropriate assessment measure for the conservation of marine benthic fauna. The aim of this study was to increase the knowledge about the impact of recreational activities on marine benthic species. Thus, the populations of the Mediterranean *Astroides calycularis* placed in the Southern Iberian Peninsula were studied, taking two populations as reference: one from a protected area and another one from a higher impacted area where recreational activities occur.

## **5.1.2 MATERIALS AND METHODS**

### *5.1.2.1 Study area*

The study was conducted at the coast of Granada (Southern Iberian Peninsula, Northern Alborán Sea, Western Mediterranean Basin), between April-October 2008. This area is a biodiversity hotspot in the region (Templado *et al.* 2006), and *A. calycularis* is the dominant coral between 0 and 12 metres depth (Terrón-Sigler *et al.* 2015-Section 6). In order to assess the impact of recreational activities on the orange coral, two stations were established: a control and an impacted station. The control station was placed in the MPA of the Acantilados de Maro-Cerro Gordo Natural Park (Figure 5.1). This MPA has marine recreational uses restrictions which include: anchoring; recreational fishing; spear fishing; SCUBA diving; and vehicle access by land. SCUBA diving is regulated by special permits to divers, which only allow a specific numbers of daily dives. The impacted station was placed at Marina del Este beach, a well-known dive site in the Andalusia coast, which is frequently used by recreational SCUBA divers (Terrón-Sigler & León-Muez 2009-Section 5), due to the easy accessibility by land (the vehicles may park very close to the beach); it is close to the recreational harbour (Marina del Este harbour, where the dive boats anchor); and high diversity of fauna is present, excellent water transparency and lastly, it is a sheltered beach. Moreover, it has been estimated that no more than 1,000 divers per year are in the MPA and between 7,000-8000 divers per year are in Marina del Este beach (Terrón-Sigler *et al.* 2008-Section 5). Therefore, for these reasons Marina del Este beach station was considered impacted relative to the MPA station.



**Figure 5.1:** Study site showing impact and control area in Granada coast (Andalusia, South of Spain).

Both stations were placed in an Easterly direction and sheltered from the western windstorms. However, following Howes *et al.* (1994), a fetch model index was developed for each station. This model provides good quantitative approximations of wave exposure in order to predict marine community patterns (e.g. Hill *et al.* 2010). This model relies on two indices of fetch: modified effective fetch and maximum fetch. A combination of the two indices allows for determining the wave exposure class of each area (Table 5.1) and is calculated using the following equation:

$$Fe = [\sum (\cos\theta_i) \times Fi] / \sum \cos\theta_i ,$$

where Fe is the effective fetch in km,  $\theta_i$  is the angle between the shore-normal and the direction ( $0^\circ$ ,  $45^\circ$  to the left and  $45^\circ$  to the right), and Fi is the fetch distance in km along the relevant vector. Maximum fetch is defined as the maximum fetch distance

in km measured from the point of interest. A value of 1000 km is conventionally used when open-ocean fetches occur. The mean values in km of the modified-effective fetch and maximum fetch (hereafter average fetch) for each station were used as continuous variables in subsequent analysis.

**Table 5.1.** Wave exposure classes based on the modified-effective fetch and maximum fetch matrix (after Howes *et al.* 1994). VP: very protected; P: protected; SP: semi-protected; SE: semi-exposed; E: exposed; n/a: No assessment.

Max Fetch (km)	Modified-effective fetch (km)				
	< 1	1-10	10-50	50-500	> 500
< 10	VP	P	n/a	n/a	n/a
10-50	n/a	SP	SP	n/a	n/a
50-500	n/a	SE	SE	SE	n/a
> 500	n/a	n/a	SE	E	E

#### 5.1.2.2 Recreational activities

On each station, the number of users of each activity such as SCUBA diving, snorkelling, spear fishing, recreational fishing, kayaking and anchoring, that have been seen as a potential to affect marine benthic invertebrates were counted two days per month, during the study period.

#### 5.1.2.3 Biometric and statistical analyses of detached colonies

Two days per month at each study station, all detached coral colonies from the rocky wall at an area of 5 x 2 metres (10 m<sup>2</sup>) were collected over seven months. The collected colonies were counted, weighted, measured, and returned to the environment.

Each colony area was calculated using the formula of the ellipse, according to Goffredo *et al.* (2011a):

$$A_c = \pi(L_c * W_c) / 4$$

Where  $A_c$  is the area of the colony;  $L_c$  is the colony length (major axis); and  $W_c$  is the colony width (minor axis).

In addition, the weight was measured to obtain data on the length–weight relationship of populations in areas with high and low pressure activities. The number of detached colonies between stations (impact vs. control) was analysed by one-way ANOVA test. Prior to ANOVA, the heterogeneity of variance was tested via Levene test. Weight data and the detached area colonies were analysed using a two-way

ANOVA test where the factors were time (fixed) and area (fixed and orthogonal with time). Prior to ANOVA, the heterogeneity of variance was tested via Cochran's C-test. Data from four months (June, July, August and September) and two areas (impact vs. control) in which there were a significant minimum number of replicates for each factor level ( $n = 9$ ) were analysed. In those months in which there were less than 9 collected data, they were eliminated in the analysis to balance all levels of each factor. Statistical analyses were performed using SPSS<sup>®</sup> 15.0 and GMAV5<sup>®</sup>.

#### 5.1.2.4 Divers' questionnaire

After the sampling described in section 5.1.2.2, divers were randomly selected and surveyed. Four dimensions were established as potential impacts of the SCUBA divers' activities on the orange coral in the study area: divers' experience, the characteristic of the dive, environmental perception, and orange coral knowledge (see Appendix I). For each dimension, factors likely to be affected by divers were identified, and a questionnaire was performed and addressed to target people with different perception and qualification levels (Table 5.2). For each factor, a question to ascertain the incidence on the target species was asked. This incidence may be positive, negative or neutral, depending on the diver's answer to each question. The questionnaire was pre-tested and adjusted and was given directly to each diver after the activity. The questionnaire was applied to all divers: diving's starting from boat or from the shore.

**Table 5.2.** Brief description of each factor from the dimensions selected.

DIMENSION	FACTORS	BRIEF DESCRIPTION
<b>Divers' experience</b>	A.- Age	The age range of users influences the type of diving. For example, younger divers are more impulsive and impatient, and can have a negative effect on the environment. The older the diver is the more considerate he/she is on the environment.
	B.- Number of dives	Higher number of dives improves buoyancy control and dexterity underwater.
<b>Characteristics of dive</b>	C.- Depth/time	Between 0-15 metres is where the most abundant populations of orange coral are present.
	D.- Area frequency	Continued use of the dive area increases pressure on the environment.
<b>Environmental perception</b>	E.- Environmental health	Perception of the divers on environmental quality. To find out the user perception about the environmental health; the questionnaire will give options regarding the state of the environment.
	F.- Control	Assessment of any access control or activity limitation.
<b>Orange coral knowledge</b>	G.- Orange coral	Knowledge about the endemic orange coral characteristics.
	H.- Protection	Diver's awareness about the orange corals level of endangerment.

Four hundred and sixty one questionnaires were given to the SCUBA divers ten minutes after their dives. During questionnaire analysis, answers were weighted with values: 5 if positive, 3 if neutral, and 1 if negative. However, after analysis, positive values were considered to be between 5 and 3.6; neutrals between  $>3.6$  and 2.3; and negative  $>2.3$  due to the mean of the weighted answers (following Ramos *et al.*, 2007; Ten Brink *et al.*, 1991). For each factor, the mean of all answers was taken and the sum of all answers of each factor was used to calculate the mean for each dimension.

To show the interactions of each factor and dimension, an AMOEBA plot was used, which is a graphical device that uses a 'radar' diagram. Though the approach is simplistic, it has the advantage of representing the intervention's impact on respondents in a clear and easily understandable manner (Ten Brink *et al.* 1991).

### **5.1.3 RESULTS**

#### *5.1.3.1 Wave exposure analysis*

For the control station, the maximum fetch was 368.05 km, and the calculated modified effective fetch was 179.48 km. Meanwhile, for the impacted station, the maximum fetch obtained was 343.14 km, and the modified effective fetch was 167.33 km. Therefore, following fetch model index (Table 5.1), both stations are considered as semi-exposed (SE), and present the same hydrodynamic conditions.

#### *5.1.3.2 Recreational activities*

Six different recreational activities with a potential to affect the orange corals in the study area have been surveyed: SCUBA diving, snorkelling, spear fishing, recreational fishing (from the shore), kayaking and anchoring (from recreational boat). In general, the activity with the highest user numbers was SCUBA diving (69.8%), followed by anchoring recreational boat (14.8%), snorkelling (6.9%), spear fishing (3.8%), kayaking (2.5%) and recreational fishing (2.2%). According to the monthly studies, SCUBA diving activity is the most common and has the highest user numbers every month. Nevertheless, anchoring is mainly present in summer time (June to August), and snorkelling had the same low frequency every month, as did the other activities.

#### *5.1.3.3 Detached colonies analysis*

The number of colonies (or fragments of them) was higher in the impact station (see Table 5.3), but there were no significant differences ( $F_{1,12}=2.33$ ;  $p=0.15$ ). This

occurs from April to September. Additionally, there were an even higher number of detached colonies between June and August (summer time), either in the control or impacted station when SCUBA divers and other users are more frequent. Therefore, recreational activities (SCUBA divers principally) have the most influence on the colonies of this coral. At the end of the summer season the numbers of detached colonies decreased.

Regarding the area and weight of the detached colonies, the results were higher in size and weight in control than impacted station (see Table 5.4). However, there were no significant differences through time. No interaction was observed between factors for both variables (see Table 5.4).

**Table 5.3.** Number of colonies detached (N); average and standard deviation (SD) of the area (cm<sup>2</sup>) and weight (gr.) per month and points samples.

	Control					Impacted				
	Area		Weight		Area		Weight			
	N	Average	SD	Average	SD	N	Average	SD	Average	SD
<b>April</b>	2	37.6	43.92	150	183.84	9	17.26	11.85	33.33	25.77
<b>May</b>	6	7.9	3.07	21.83	8.68	8	5.85	5.38	16.87	14.14
<b>June</b>	9	30.57	11.83	94.4	35.99	17	7.23	4.24	17.82	16.41
<b>July</b>	11	25.77	24.41	91.54	96.03	18	8.85	7.74	13.11	17.39
<b>August</b>	12	26.2	13.32	81.41	59.28	14	4.44	4.28	8.42	15.65
<b>September</b>	9	26.21	14.76	74.55	54.06	15	13.12	14.24	28.33	39.84
<b>October</b>			No data			7	5.48	6.85	9.57	13.83
<b>November</b>			No data			6	7.13	5.03	24.33	22.71

**Table 5.4.** Analysis of the *Astroides calycularis* colonies area and weight in impacted and control zones through four months (June, July, August, and September).

Source of variation	Colonies area					Colonies weight				
	SS	df	MS	F	P	SS	df	MS	F	P
<b>Time (Ti)</b>	113.7252	3	37.9084	0.2	n. s.	1980.0556	3	660.0185	0.26	n. s.
<b>Zone (Zo)</b>	5921.6217	1	5921.6217	30.63	***	89605.5556	1	89605.5556	35.09	***
<b>Ti x Zo</b>	428.963	3	142.9877	0.74	n. s.	4398.1111	3	1466.0370	0.57	n. s.
<b>Residual</b>	12373.8635	64	193.3416			259415.50000	64	2553.6215		
Cochran test (C=0.4451, p<0.01)					Cochran test (C=0.4950, p<0.01)					
n. s. = Not Significant, *** p<0.01					n. s. = Not Significant, *** p<0.01					

#### 5.1.3.4 Divers' questionnaire

SCUBA divers from 17 different countries were interviewed. Among those, more than 70% were divers from the different localities of the Andalusia region, 14.3% from the rest of Spain and less than 15% from other countries (for example, UK—

6.57%, Ireland—2.41% or USA—1.53%). This data can be used in conjunction with previous knowledge on orange coral and the local marine environment.

#### *5.1.3.5 Divers' experience*

Taking into account the age and previous dive numbers as an experience index, more than 60% of the divers were between 15 and 35 years old, while 30.4% were between 35 and 45. Finally, 8.1% were older than 46 years old. As a first assessment in this area, the majority of divers were below 35 years old.

On the other hand, this area is frequented by three diver categories. Firstly, 42.7% of divers were inexperienced (who are doing the certificate course) or with a little experience (less than 5 dives per year). Secondly, 37.3% of the SCUBA divers studied were experienced, with more than 20 dives per year since starting to dive. Finally, the rest of the divers surveyed were an intermediate level, with more than 5 dives per year.

#### *5.1.3.6 Dive characteristics*

Most divers' activities were performed between 0-15 metres depth (53.4%), and times of dives were  $44 \pm 22.38$  minutes. The divers whose mean depth was between 15 and 20 metres depth were diving around  $48 \pm 13.65$  minutes (38.4%). Lastly, only 7.8% of the divers surveyed were diving below 20 metres depth, and the activity lasted  $47 \pm 11.85$  minutes.

For 32.8% of interviewed divers, it was their first time in this area. Due to 67.2% were habitual divers in this area, a high percentage of divers could assess whether this area had been environmentally damaged.

#### *5.1.3.7 Environmental perception*

From divers who were regulars in this area, only 12.4% considered that there was not any damage, while 31.6% thought that this underwater landscape had been damaged. Finally, 56% of the regular divers asserted that the area was being damaged by SCUBA divers and other recreational marine activities.

Interestingly, 60% of the divers who thought that the area was damaged recommended that some control of access is necessary due to the number of divers being so high, especially during summertime. Nevertheless, 32% said that it is not necessary; this is probably due to the fact that they are afraid the dive point could be closed. Only 8% of users had no opinion about this question.



#### 5.1.3.8 Orange coral knowledge

From all divers surveyed, 63% confirmed that they have some knowledge about orange coral (*Astroides calycularis*). Nevertheless, when asked for a brief animal description, it was observed that some divers were confused with other marine species with the same colour as the sponge *Crambe crambe* or the false red coral *Myriapora truncata* (Bryozoan). The rest (27%) confirmed that did not know the species. In fact, most of them did not even know that there were corals in the Mediterranean.

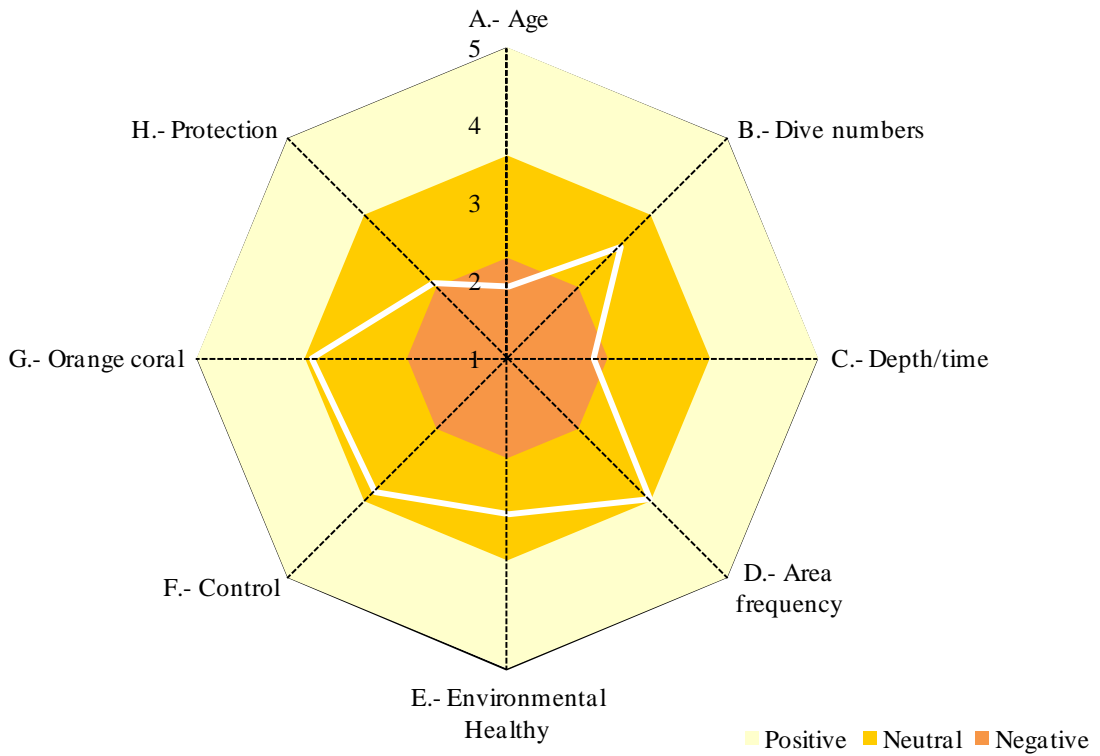
The divers who thought they knew the orange coral were asked if they knew that this species was endangered and protected by national and international conventions and/or laws. Only 27% of the divers answered affirmatively. Therefore, it is markedly shown that the regular divers in the area had no information about the species present in the underwater landscape.

#### 5.1.3.9 AMOEBA plots

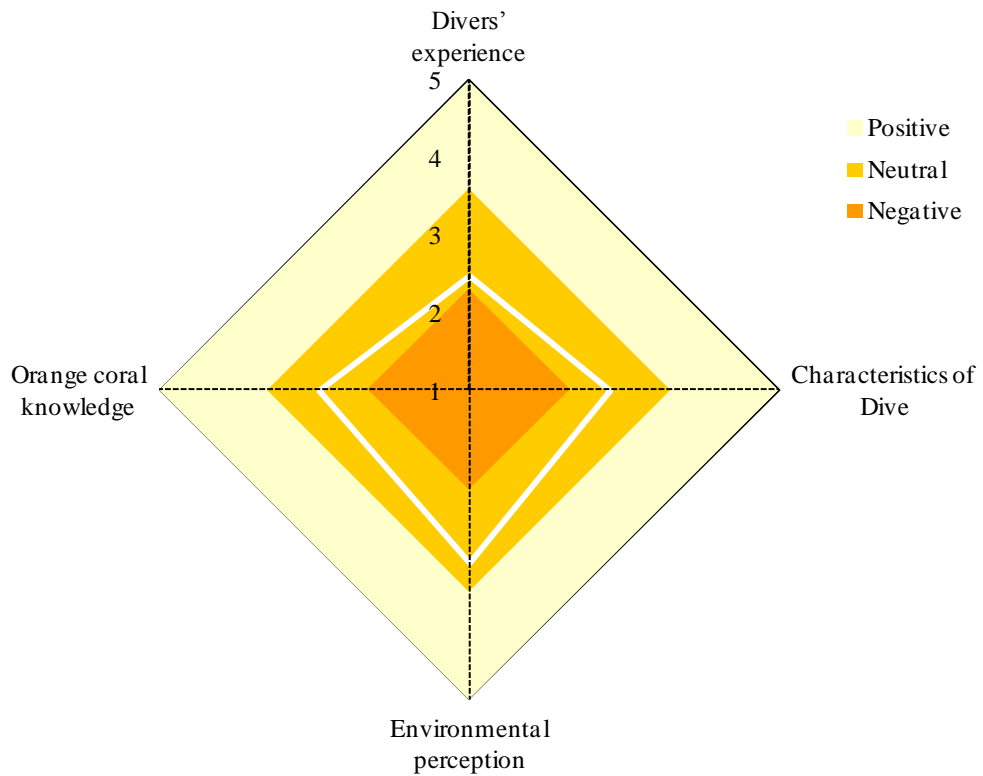
The desegregation of the dimensions by factor shows that each one of them has potential impact (Figure 5.2). After looking at all the SCUBA divers' results by factor, it appears that age and depth/time had the most negative impacts. Although the rest are not present in a positive range, greater values are found inside the neutral range in relation to dive numbers, frequency area, environmental health, control, species, and protection was the closest to the negative range.

The most positive values were regular divers, species knowledge, and that there should be some control in the area. Noteworthy is that previous knowledge of the divers on orange coral is not totally true, due to the divers confusing the species with others with the same colour. Therefore, this factor should be taken carefully into account. On the other hand, during the first assessment, regular divers could be considered as a negative impact, because these divers have a high probability of interacting with the coral colonies. Nevertheless, *a priori*, these divers have performed more dives, and their buoyancy and diving skills are better. Therefore, finally it has been assessed as a positive factor. Indeed, the assessment that there should be an access control in the area by the regular divers is very relevant.

To have a global point of view of which could be the principal cause of coral perturbation in the area by SCUBA divers, a dimension analysis has been conducted. Thus, it is showed that diver experience has the most negative impact, although this does not reach negative values. This dimension is followed by dive characteristics, orange coral knowledge, and environmental perception (Figure 5.3).



**Figure 5.2:** AMOEBA analyses of the different factors studies on the surveys.



**Figure 5.3:** AMOEBA analyses of the different dimensions studies on the surveys.

#### 5.1.4 DISCUSSION

All activities surveyed in the study area have shown an impact on sessile organisms (Dixon *et al.* 1993; Hawkins *et al.* 1999; Lloret *et al.* 2006; Coelho & Manfrino 2007), with SCUBA diving interaction being the most studied. Nevertheless, Di Franco *et al.* (2009) observed that one of the species most frequently damaged by diver contact was colonial species, and all contacts made on *Astroides calycularis* caused evident injuries to the colonies in the form of detaching one or more corallites.

In the Mediterranean Sea, Luna *et al.* (2009) demonstrated that divers' contact was caused by flapping and contact with fins, principally, as already shown in other seas around the world (e.g., Harriot *et al.* 1997; Rouphael & Inglis 2001; Barker & Roberts 2004). Moreover, with regard to any part of the body, the most frequent was contact with the hands (Luna *et al.* 2009). Additionally, divers make contact with the environment on average 2.52 times every seven minutes, and this contact is unintentional in 76% of all cases (Di Franco *et al.* 2009). Therefore, in places in which SCUBA diving is the principal recreational activity, interactions with the benthic macro-fauna are potentially greater than those where this activity is secondary or lower. This assumption is corroborated in our data because the number of detached colonies has been always higher in the impacted station.

Garrabou *et al.* (1998) demonstrated that *Pentapora fasciatis* colonies were detached as a consequence of intensive SCUBA diving at Medas Island (Western Mediterranean Sea). These authors observed that there were no differences between colony size and time.

The data of the present study supports that there is less detached colonies in the control station, but these have a larger size. Some works have showed that coral reef may present similar damage both in areas with high diver presence as low diver presence, and the conclusion was that external factors damaging the corals should exist (Riegl & Velimirov 1991; Hawkins & Roberts 1992; Hawkins *et al.* 1999). In the control station, the colonies loss may be explained because colonies grow without diver interference, and these colonies, when they are larger in size, they are more susceptible to being detached by environmental conditions as, for example, hydrodynamics or windstorms (pers. obs.). This factor may explain that in the control station, orange coral colonies are bigger and heavier than in the impact area, where the colonies are smaller, which may be caused by unintentional interactions between divers and colonies.

On the other hand, it has been shown that the previous lack of knowledge about the orange coral as an endangered and endemic species by the SCUBA divers could be an impact factor. This, in synergy with other impact factors supported by the other characteristics of diving activities, could be the causes of the decreasing orange coral coverage in the study area (Terrón-Sigler & León-Múez 2009-Section 5).

Impacts caused by recreational SCUBA diving may vary widely among different dive locations and individual divers (Rouphael & Inglis 2001). *A priori*, experienced divers should cause fewer impacts by contact with the substratum than inexperienced divers (Luna *et al.* 2009). Although this factor has been shown as a potential impact (Roberts & Harriott 1994), some authors did not observe it (Harriott *et al.* 1997; Rouphael & Inglis 2001). In the present study, diver experience dimension has been the most negative impact, age being the most important factor. Younger divers are more impatient and thus are more likely to inadvertently make contact with the coral colonies. According to Luna *et al.* (2009), diver experience may not be a good indicator as the only factor to determine whether a diver is qualified for diving at a site, and more factors must be studied and assessed.

In the present study, the frequency and the binomial depth-time as a dive characteristic and their potential impact on the orange coral have been studied. Generally, dives were done between 0 and 15 metres depth, where the *Astroides calycularis* is more abundant (Terrón-Sigler *et al.* 2015-Section 6) and there is more probability of interacting with the colonies. Moreover, the means of the time dives were high and similar in all range depths (more than 40 minutes). In an MPA from the Mediterranean Sea, it has been demonstrated that in 45 minutes of immersion, recreational divers may make up to 8 contacts with fragile organisms (Luna *et al.* 2009). These contacts may be unintentional in 76% of cases (Di Franco *et al.* 2009). During a ten minute immersion, SCUBA divers may cause serious damage to the environment (e.g. Uyarra & Côté 2007). This may be worse when divers have low experience. This occurs in the present study area; normally, the divers which have low experience and make their immersions at the depth where *A. calycularis* are predominantly found. Actually, binomial factor depth/time was negative, and the dimension values were the second lowest.

Diver experience, characteristics of the dive, and/or the behaviour of divers have been already studied as potential impact factors on the marine environment (Medio *et al.* 1997; Barker & Roberts 2004; Luna *et al.* 2009). Nevertheless, the divers' perceptions about environment degradation and their previous knowledge of the species present in the dive site have been scarcely studied. These in synergy with the above might be taken into account with future species, habitats, ecosystems and/or MPA conservation frameworks.

Here, it has been shown that divers perceive degradation of dive sites where the SCUBA activities are high. Moreover, habitual divers have not been afraid to affirm that some access control should be taken as a management measure, even when this might involve a restriction on their activity. On the other hand, there is little information addressed to divers about the singularity of the dive site as the species, habitats and/or ecosystems present. Therefore, one way of reducing damage is environmental education


programs to promote awareness about endangered species within the diving community (Barker & Roberts 2004). Actually, Medio *et al.* (1997) demonstrated that there were fewer interactions between divers and the underwater environment after a little briefing about the characteristics of the ecosystem and problems caused by human contact. Recently, Krieger & Chadwick (2012) demonstrated that divers who received pre-dive ecological briefings in dive centres caused significantly less coral damage than those who did not. Although this measurement may be a good tool, high levels of damage may be unavoidable if the group is composed of large number of divers (Barker & Roberts 2004; Luna *et al.* 2009). Therefore, the education must be combined with small diver groups, among other features.

The present study suggests that the divers' ignorance about *Astroides calycularis* is a potential impact factor combined with the diver experience and characteristic of the dive on the Granada coast. Perhaps this is not the most negative impact, but whether the diver knows the special characteristics of this species, and additionally, do they take care of the environment and avoid touching the colonies. This topic needs to be considered when adopting management strategies in dive areas, where these species are present, due to it having been demonstrated that *A. calycularis* may be one of the most frequently damaged species by divers (Di Franco *et al.* 2009).

To improve the management on MPAs which harbours endangered species and which permits recreational activities such as SCUBA diving, diver education programmes are essential to teach the environmental value and the fragility of different species, as well as to show the potential damage of diving activity and how to minimise the negative impact of scuba diving (Luna *et al.* 2009). The Andalusia region harbours one of the most abundance populations of *Astroides calycularis*, and these are mainly present in MPAs (Terrón-Sigler *et al.* 2015-Section 6). Protecting these populations should be a high priority for the environment managers to preserve the natural heritage.







5.2 Abundance and distribution of the rapid expansive coral *Oculina patagonica* in the Northern Alboran Sea (Western Mediterranean): potential threats on *Astroides calycularis*



*Oculina patagonica* colony recently located in the Marina del Este beach (Almuñécar, Granada). Author: Terrón-Sigler A.



## Abstract

We describe for the first time the distribution and abundance of *Oculina patagonica* along the coasts of the North Alboran Sea (Andalusia Region, Southern Iberian Peninsula), which corresponds to the southernmost region of the known distribution range of the coral. After surveying 693 km of the Andalusia coastline along three different depths, we showed that *O. patagonica* was restricted to the eastern shores of the Alboran Sea. It was only present in 7 out of 195 sampling stations in the eastern region along the studied coasts and on the depth range of 0-3 m. Moreover, we observed that the distribution of the species in the northern coasts of the Alboran Sea might be related to substrate availability and sea surface temperature. In the localities in which its presence was described, annual mean sea water temperature was in the range of 18-21°C. In relation to substrate availability, it has to be denoted that the distribution of hard substrata—ideal for *O. patagonica* settlement and growth—along the sampling area, is not uniform in the study area; this might affect the continuity of the distribution of the species. Local studies such as this one are of importance as a starting point for delineating species relation with its habitat, population boundaries and population ecology. Given the fast expansion of this species along the Mediterranean coasts, this study would serve as a basis for continuous monitoring of the spread of the species and its long-term effects on the ecosystem and/or on the endemic and endangered orange coral *Astroides calycularis*.

## Resumen

Se ha descrito por primera vez la distribución y abundancia del coral *Oculina patagonica* a lo largo del litoral norte del mar de Alborán (Andalucía, sur de la Península Ibérica). Esta zona corresponde a la región más al sur de la distribución conocida de este coral. Tras muestrear 693 km de costa andaluza a tres profundidades, mostramos que *O. patagonica* está localizada en las zonas más al este del mar de Alborán. La especie estuvo presente únicamente en 7 de los 195 puntos de muestreo y se localizó sólo entre 0 y 3 metros de profundidad. Además, se observó que la distribución de la especie puede estar relacionada con la disponibilidad de sustrato y la temperatura de la superficie del mar. En las localidades donde se encontró la especie, la media anual de la temperatura superficial osciló entre 18 y 21°C. En relación a la disponibilidad de sustrato, se ha observado que la distribución de los fondos rocosos (donde se asienta y crece *O. patagonica*) no son uniformes a lo largo de toda costa, y esto puede afectar a la continuidad en la distribución de la especie. Este tipo de estudios son de gran importancia como punto de partida para cartografiar potenciales especies competidoras con el coral naranja, conociendo el límite de las poblaciones y aspectos

demográficos. En este sentido, *O. patagónica* presenta una rápida expansión a lo largo de las costas mediterráneas. Por tanto, este estudio servirá como base para conocer la expansión de la especie a través de nuevos muestreos y sus efectos a largo plazo sobre los ecosistemas y/o sobre el coral naranja (*Astroides calycularis*) especie amenazada y endémica del mar Mediterráneo.

### 5.2.1 INTRODUCTION

A fundamental issue in marine ecology is explaining the patterns of a species' distribution and abundance, together with the processes that are associated with these patterns (Eriksson & Jakobsson 1998; Magurran *et al.* 2011). For this, local studies on species spatial distribution are needed as a starting point for delineating species relation with biotical and abiotical factors, population boundaries and population ecology (Gage 2004).

The Mediterranean is considered to be a biodiversity hot-spot with a high level of endemism, as well as an assortment of temperate and subtropical elements (Coll *et al.* 2010). This is mainly related to its narrow connection to the Atlantic Ocean, its west-east orientation and its geological history (Boudouresque 2004). Thus, the current biological diversity of the Mediterranean is due to the interactions between ecological factors, as well as historical processes that shaped the Mediterranean Basin throughout the course of history (Templado 2014). Within the generally high diversity found in the Mediterranean Sea, some studies suggest that the Alboran Sea, the westernmost basin of the Mediterranean Sea, is a regional hot-spot of biodiversity (e.g. the Alboran Sea) because of the coexistence of species from three marine biogeographic provinces (Mediterranean, Lusitanian and Mauritanian regions) and because of the endemic species restricted to this zone (Coll *et al.* 2010; Aguilar *et al.* 2011).

*Oculina patagonica* (De Angelis D'Ossat 1908) is a zooxanthellate scleractinian coral that can only be found in the Mediterranean Basin. In particular, it has been found in abundance in the Western Mediterranean Basin in Italy (Rodolfo-Metalpa *et al.* 2006), the Gulf of Lyon (Zibrowius 1974) and the coasts of the Iberian Peninsula, mostly in the southeastern and eastern coasts (Zibrowius 1980; Ramos 1985; Zibrowius & Ramos 1983; Fine *et al.* 2001; Serrano *et al.* 2013; Rubio-Portillo *et al.* 2014). In the Eastern Mediterranean Basin, it has been cited in abundance in the coasts of Lebanon (Bitar and Zibrowius 1997), Israel (Fine *et al.* 2001) and Greece (Salomidi *et al.* 2013). In less abundance, colonies have been found in Algeria, Tunisia (Sartoretto *et al.* 2008) and Turkey (Çinar *et al.* 2006). *O. patagonica* has been cited as a species that is non-native of the Mediterranean Sea (Zibrowius 1983). Transoceanic transport of planula of the species via the Strait of Gibraltar to the Western Mediterranean has been considered to be the most probable way of introduction (Zibrowius 1974). Nevertheless, the origin of the species is still dubious. The unsolved situation consists in that the original description of *O. patagonica* is based on fossil materials from Holocene deposits on the temperate coast of South America. However, nowadays, living specimens have not been found in Patagonia (Serrano *et al.* 2013).

*Oculina patagonica* is commonly found on rocky foreshores and in caves with 0-10 m depth. It is present in artificial habitats, though it can be found in natural habitats as well (Ramos-Esplà 1985; García-Raso *et al.* 1992; Fine *et al.* 2001). This coral has been recorded in natural substrate in which the population densities were generally scarce (Fine *et al.* 2001; Sartoretto *et al.* 2008; Coma *et al.* 2011). However, recently, a rapid expansion of the coral's populations has been recorded, and the coral has been cited as the main component of a natural community (Sartoretto *et al.* 2008; Coma *et al.* 2011, Serrano *et al.* 2013, Salomidi *et al.* 2013, Rubio-Portillo *et al.* 2014). *O. patagonica* has the ability to live and reproduce under varying and diversified environmental conditions, such as a wide range of water temperatures, salinity, UV radiation, turbidity and strong wave energy (Fine *et al.* 2001). Therefore, it has been considered as an "opportunistic dominant settler" that overgrows the calcareous structures of serpulids, vermetids, barnacles, etc. It also competes with algae and other soft organisms at its growing edge (Sartoretto *et al.* 2008). This coral has been cited as rapidly expanding along the Iberian coasts (Coma *et al.* 2011). Therefore, local studies are of importance in order to establish the habitat preferences of the species, as well as its abundance and habitat acquisition characteristics.

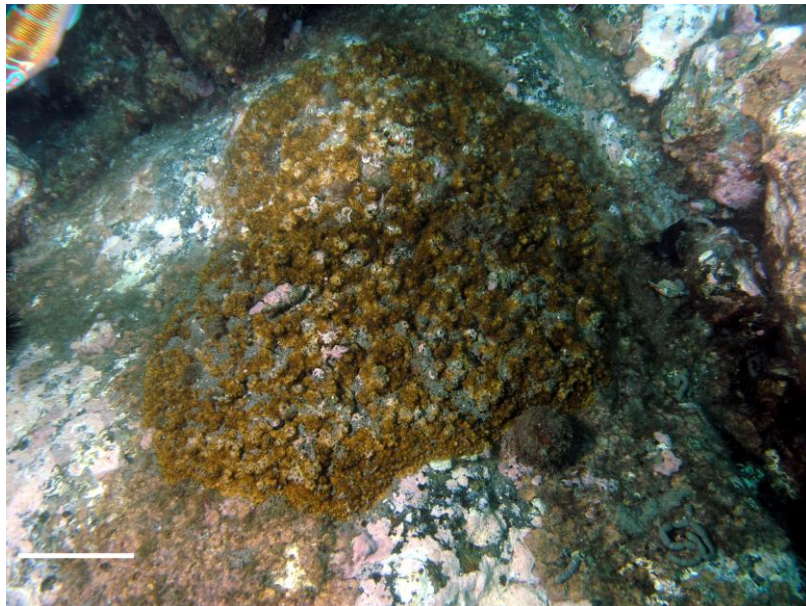
Competition among corals for space are known and reported on tropical reefs. These interspecific aggressive interactions are commonly seen where two colonies of different species grow close to each other (Sheppard *et al.* 2009). However, there is little information about these interactions among scleractinian corals from the Mediterranean Sea. In the Mediterranean Sea, *Astroides calycularis* is considered as an intermediate species along the r-K continuum (Goffredo *et al.* 2011a), while *O. patagonica* is typically r. Nevertheless, it is unknown whether the expansion of *O. patagonica* could affect the *A. calycularis* populations by competitive interaction.

Because of all of this, the main objective of our survey was to assess the distribution and abundance of *Oculina patagonica* in the Northern Alborán Sea (Andalusia Region, Southern Iberian Peninsula). This area, together with the coasts of Morocco (Northern African coasts, southern Alboran Sea), comprises the southernmost region of the Mediterranean Sea in which the species lives. Thus, there will be a baseline of *O. patagonica* populations that could be used to compare population's trends among species in future studies.

## 5.2.2 MATERIALS AND METHODS

The survey was performed along the rocky shores of the Andalusia coasts (Southern Iberian Peninsula, Northern Alborán Sea). The sampling area covered a total of 693 km of coastline between the province of Cádiz (36°38.38'N, 06°24.71'W) and the province of Almería (37°21.181'N, 1°39.303'W). In order to estimate the abundance and

distribution of *Oculina patagonica* (Figure 5.4), we sampled a total of 195 sites that were distant from each other 1 km (Figure 5.5). Of these sites, 10 were artificial substrate (breakwater) and 185 were natural habitats. We designed a systematic and stratified sampling following Benedetti-Cecchi *et al.* (1996) by using 1x1m quadrats, which were launched four times at 3 different depths: 0-3 m, 3-6 m and 6-12 m. Therefore, we considered a total 585 sampling stations. At each station, the presence and/or abundance of the species, the date, substrate composition and geographical location were pointed out. When colonies of *O. patagonica* were present, we estimated their abundance by visual observations of the percentage of coral coverage in the grid of quadrats (25cm x 25cm) in the sampling station; we also quantified the number of colonies found at each quadrat. Each time, two SCUBA divers independently performed the visual estimations in order to reduce the sampling bias.



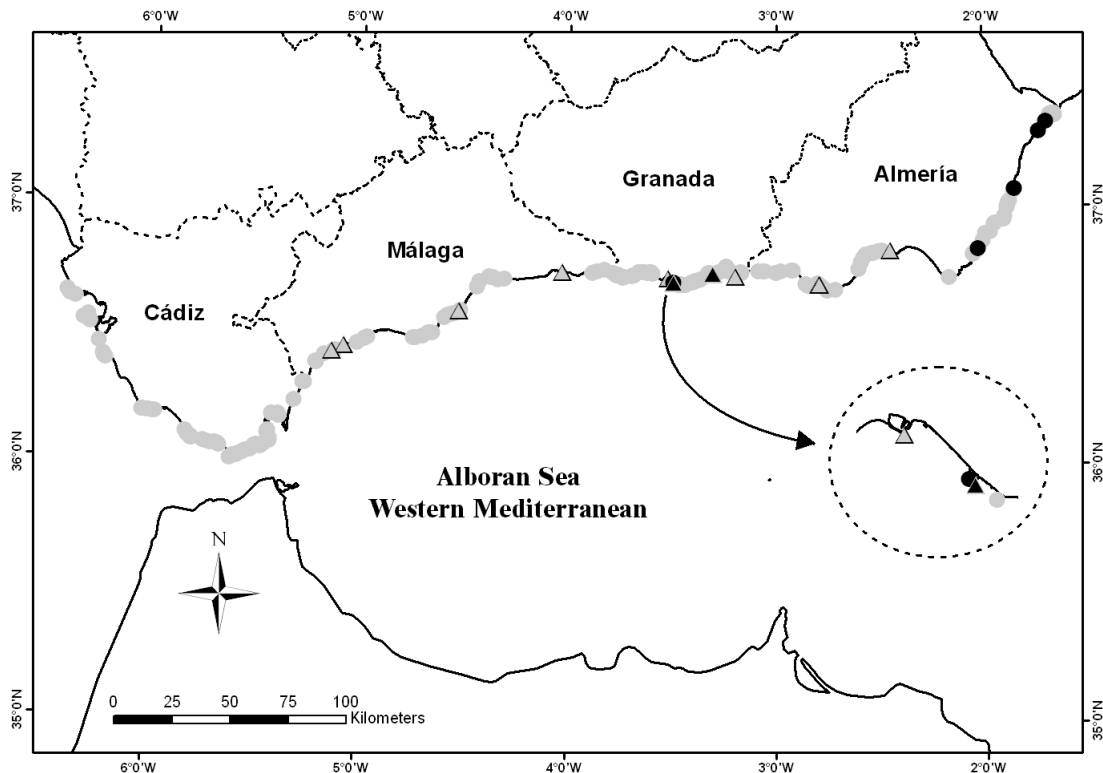
**Figure 5.4:** First record of *Oculina patagonica* from Granada coast at 7 meters depth (natural habitat). Scale bar: 10 cm.

### 5.2.3 RESULTS AND DISCUSSION

In this manuscript, we describe the first survey on the distribution and abundance of *Oculina patagonica* along the whole coasts of the North Alborán Sea (Andalusia Region, Southern Iberian Peninsula), which corresponds to the southernmost region of the known distribution range of the coral. In this area, previous studies have focused on the local presence of the species and/or abundance (CMA 2012; Serrano *et al.* 2012), but these studies focused only on some localities of the Andalusia region. Concerning other regions along the known distribution range of the species in the

Mediterranean, studies have been performed on the coasts of Catalonia and Valencia (NE and E of the Iberian Peninsula, respectively) (Serrano *et al.* 2013; Rubio-Portillo *et al.* 2014), as well as the Murcia coast (Algerian basin, SE Iberian Peninsula) (Coma *et al.* 2011).

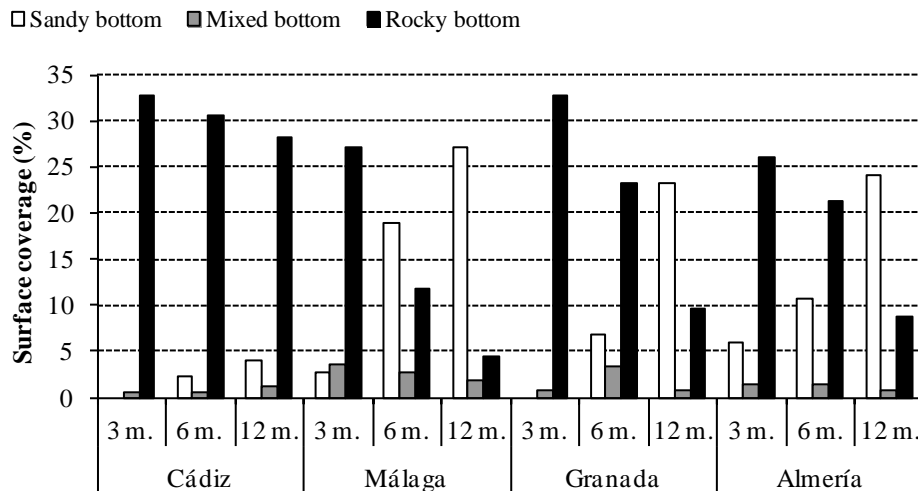
From the 693 km of the Andalusian coastline surveyed, *Oculina patagonica* was only present in 7 out of 195 sampling sites (Figure 5.5). These sites were located in the provinces of Granada and Almería, in the easternmost regions along the Andalusian coasts and in the depth range of 0-3 m. Therefore, it is possible to conclude that *O. patagonica* is restricted to the eastern shores of the Alborán Sea.



**Figure 5.5:** Cartography of *Oculina patagonica* presence along the shoreline studied. Gray circles show the points sampled of natural habitats; Gray triangles show the points sampled of artificial substratum; Black circles show the *Oculina patagonica* presence in natural habitats; and black triangles show *O. patagonica* presence in artificial

The distribution patterns of *Oculina patagonica* on the northern coasts of the Alborán Sea seem to depend mainly on hard substrate availability, as well as the sea surface temperature, which might play a secondary role in the distribution patterns of the species. In the stations in which the coral was found as present, annual mean sea water temperature was in the range of 18-21°C. Considering the availability of hard substrate, it has to be denoted that the distribution of rocky shores along the sampling area is not uniform. Given the need of the planulae of *O. patagonica* for a hard substrate in which it can settle down, the discontinuity found in rocky substrates is an important

factor to take into consideration for the distribution patterns of the species. Even though rocky shore bottoms were the most predominant substrata in all provinces, we observed the presence of sandy bottom and mixed bottoms (66.7%, 29.1% and 4.2% across the whole sampling region, respectively, as shown in Figure 5.6). The area of Cádiz (westernmost side of the study area) showed the greatest coverage of rocky bottoms, followed by Granada, Almería (easternmost sides of the study area) and, in a lesser extent, Málaga. Therefore, there is a continuum in the eastern side of the Andalusia coasts (Almería and Granada areas) that might facilitate the settlement of planulae in hard substrates. Mixed and sandy bottoms predominate the central area of Malaga, separating this region from the rocky shores on the western shores of the sampling area (Cadiz area). Rocky substrates were predominant at 3 m, in all provinces, followed by depths of 6 m and 12 m (Figure 5.6).



**Figure 5.6:** Percentage of bottom type per provinces and depth.

We found *Oculina patagonica* in two stations with artificial substrate (breakwater as a sandy shore defence), and five station with natural substrates (vertical and horizontal rocky shore). When the species was present, the colonies' abundance ranged between  $5.25 \pm 3.1$  colonies/m<sup>2</sup> and  $0.25 \pm 0.5$  colonies/m<sup>2</sup>, while the coverage surface ranged between 5% and 30%. Both measurements were higher in Almería than of those quantified in the Granada coastal line (Table 5.5). Therefore, both localities seem to correspond to the limit of distribution of the species in the Northern Alboran Sea. A decrease in species abundance within its distribution limits is a common feature in marine invertebrates and other passive dispersal species (Holt 2003; Astanei *et al.* 2005; Casado-Amezua *et al.* 2012). In contrast with other studies, most of the substrates for *O. patagonica* were found to correspond with natural rocky surfaces, whilst there were only two localities with artificial substrates in which the species was found (breakwater as a sandy shore barrier). *O. patagonica* has the characteristics of an

opportunistic species (Serrano *et al.* 2013), inhabits many natural sites and harbours along hundreds of kilometres of coastal line in southeast Spain. Moreover, it has been observed a high capacity of this species to settle on harbours and polluted areas (Zibrowius 1992). Therefore, artificial substrata might be another factor that supports dispersal (Serrano *et al.* 2013). This has already been observed by Rubio-Portillo *et al.* (2014); these authors asserted that a harbour could be a focal point of dispersion in the Valencia Region. Local studies on the Andalusian coasts showed that the species may colonize artificial reefs (CMA 2012). We also found the presence of this species in artificial reefs, although the species was in a lower abundance. This could be because the two stations that correspond to artificial reefs in which the species was found corresponded with the westernmost distribution range of the species, not only on the Andalusian coasts but also in the Mediterranean Sea.

**Table 5.5.** Sampling sites where colonies of *Oculina patagonica* were found (Gr= Granada; AL = Almería), Substrate type, Geographic location (ED 50; WGS84), number of colonies per square metre (including average and standard deviation), and coverage percentage.

Point	Substrate	Latitude	Longitude	Colonies/m <sup>2</sup> (average ± SD)	Coverage (%)
Gr 20	Breakwater	36°42.149 N	3° 29.540 W	0.25±0.5	8
Gr 28	Natural	36°42.093 N	3°24.429 W	0.25±0.5	5
Gr 40	Breakwater	36°44.315 N	3°18.598 W	0.5±0.58	10
Al 31	Natural	36°49.961 N	2°01.486 W	1±0.82	12
Al 44	Natural	37°03.870 N	1°51.103 W	5.25±3.1	30
Al 45	Natural	37°17.135 N	1°43.764 W	2±0.82	25
Al 46	Natural	37°19.512 N	1°41.663 W	1.75±1.71	25

Whilst high coverage and abundance of *Oculina patagonica* has been observed on artificial habitats, coverage of natural substrata is generally low (Fine *et al.* 2001; Sartoretto *et al.* 2008; Coma *et al.* 2011). However, recently, it has been reported in communities in which *O. patagonica* is the dominant species (Serrano *et al.* 2012), as it has observed in Torre Pirulico in SE Spain (37°4'70"N, 1°50'59"W). These communities may act as a focus for local dispersion. This local dispersion may have happened in Almería littoral, where these populations were observed, and close to this area, we have observed the highest abundance in natural substrates (point Al\_194- 37° 03.870 N, 1° 51.103 W). Coma *et al.* (2011) revealed a local-scale pattern of an increase in the abundance of coral colonies as a consequence of the opportunistic capacity of the species to colonize different types of substrata. Furthermore, these authors asserted that sea urchins' abundance, as grazers of macroalgae and thus creators of empty habitats, can play an important role regarding expansion of this opportunistic species.


In tropical coral reefs, the genus *Galaxea* is an aggressive species that competing by the space, but many coral families contain species showing both dominant and



subordinate species (Sheppard *et al.* 2009). The orange coral *Astroides calycularis* inhabit both well-lit and dark rocky substrata (e.g. Zibrowius 1995), due to be an azooxatellate species, while *Oculina patagonica* is a zooxanthellate species and inhabit only well-lit substrata. Nevertheless, currently there is no evidence that *O. patagonica* is competing by the substrata with *A. calycularis*. However, *O. patagonica* is expanding on one coast where the orange coral present an abundant populations (Andalusia, South of Spain), and it has already colonized the hot-spot Punta de la Mona (Almuñécar, Granda), first record in the present study. Therefore, the present baseline on *O. patagonica* populations in the North Alborán Sea may be useful as reference point on the knowledge of this possible competitive interaction.

Routine monitoring programs are required for people to gain better knowledge of the population dynamics of any species (Bramanti *et al.* 2011; Calvo *et al.* 2011). In the case of *Oculina patagonica*, these programmes would be useful for detecting a possible widespread at local or regional scales. Given the uncertain origin of the species, this is also helpful for disentangling ecological and taxonomical issues and even more for better understanding the long-term effects that this opportunistic species has on the ecosystem. These studies must be accompanied by temperature serial analyses and control stations in MPAs or additional strategic localities. All of these data collections, together with long serial data, can be evaluated and show evidences of changes in coastal areas.





**6. GEOGRAPHICAL DISTRIBUTION OF *ASTROIDES CALYULARIS* (SCLERACTINIA: DENDROPHYLLIIDAE) AS A BASELINE TO ASSESS FUTURE HUMAN IMPACTS ON THE SOUTHERN IBERIAN PENINSULA.**

Polyps of *Astroides calycularis* (Acantilados de Maro-Cerro Gordo Natural Park).  
Author: Terrón-Sigler A.

## Abstract

Human activities have increasingly affected biodiversity in the Mediterranean Sea. Data on the distribution and abundance of species allows researchers to assess the possible degradation of wild populations. These data could act as a baseline to assess the magnitude of the effects of human activities on a bioindicator species. The distribution and relative abundance of the south-western populations of the endemic *Astroides calycularis* in the South Iberian Peninsula were studied to establish a baseline for future studies. The rocky shoreline was studied at a depth range of 0 to 12 m, including more than 650 kilometers of Spain's Andalusian coastline. The species was present in 135 of the 585 dive points sampled. ANOVA analysis showed differences in depth in the four provinces studied, and there was no interaction between the two factors. As human activities on the Mediterranean coast are reducing the *A. calycularis* populations, a baseline on marine populations is greatly recommended for monitoring, assessment, and management studies, especially for endangered or bioindicator species. This baseline could be useful as a reference tool to assess the effects of human activities on marine biodiversity, including global change.

## Resumen

Las actividades humanas en el litoral están afectando a la biodiversidad del mar Mediterráneo. De esta manera, los datos sobre distribución y abundancia de especies permiten evaluar a los investigadores si las poblaciones de la fauna marina se están degradando. Además, estos datos pueden servir como punto de partida para valorar la magnitud de los efectos de las actividades humanas sobre una especie. Se ha estudiado la distribución y abundancia relativa de las poblaciones del coral endémico *Astroides calycularis* en el sur de la Península Ibérica, con el fin de establecer un estado inicial. Se han muestreado más de 650 kilómetros de litoral rocoso de la costa Andaluza (sur de España) en un rango de profundidad comprendida entre 0 y 12 metros. El coral naranja estuvo presente en 135 de los 585 puntos de muestreo. Los análisis ANOVA mostraron diferencias significativas entre las profundidades estudiadas y las cuatro provincias andaluzas objeto de estudio. Sin embargo, no hubo interacción entre ambos factores. Debido a que las actividades humanas sobre el litoral de la costa mediterránea están reduciendo las poblaciones de *A. calycularis*, establecer un estado inicial de dichas poblaciones es de gran utilidad para estudios de seguimiento, evaluación y gestión de la especie, sobre todo cuando se trata de una especie amenazada y bioindicadora. Por tanto, el estado inicial de las poblaciones de *A. calycularis* establecido en el presente estudio podrá ser de gran utilidad como punto de referencia para evaluar las actividades humanas sobre la biodiversidad marina, incluyendo el cambio global.



## 6.1 INTRODUCTION

Human activities have increasingly affected biodiversity in the Mediterranean Sea. Most of this biodiversity can be found in or near shallow shore waters, and the anthropogenic uses that threaten this biodiversity include, among other activities: land usage, which causes habitat destruction; water pollution; changes in nutrients and sedimentation; loss of coastal habitats (e.g. Airoidi & Beck 2007); fishing activities; changes in communities or habitat destruction (e.g. Coll *et al.* 2010); diving, which affects benthic communities (Garrabou *et al.* 1998; Wielgus *et al.* 2002; Coma *et al.* 2004; Linares *et al.* 2010); and mass mortality events related to climate change, which has already been observed in Mediterranean benthic invertebrates in the last decades (Gaino & Pronzato 1989; Bavestrello *et al.* 1994; Cerrano *et al.* 2000; Perez *et al.* 2000; Rodolfo Metalpa *et al.* 2000; Harvell *et al.* 2002; Garrabou *et al.* 2009; Vezzulli *et al.* 2010; Crisci *et al.* 2011; Kersting *et al.* 2013). These impacts and the synergies among them can greatly affect the distribution and survival of species (e.g. Coll *et al.* 2010; 2011; Micheli *et al.* 2013a; Serrano *et al.* 2013). Moreover, these threats may be endangering species that play a structural role in benthic assemblages, and their alteration may further threaten rich Mediterranean biodiversity (Cebrián *et al.* 2011; Kersting *et al.* 2013).

The evaluation of baseline conditions is the challenge to understand the causes of environmental change (Francour *et al.* 1994). The use of historical baselines allows researchers to assess possible degradations in wild populations and to guide conservation and management initiatives for good ecosystem conditions (Sala *et al.* 2012). In order to understand the effects of human impacts along the coastline, monitoring should be a routine activity (Coll *et al.* 2010), but a reference point is necessary.

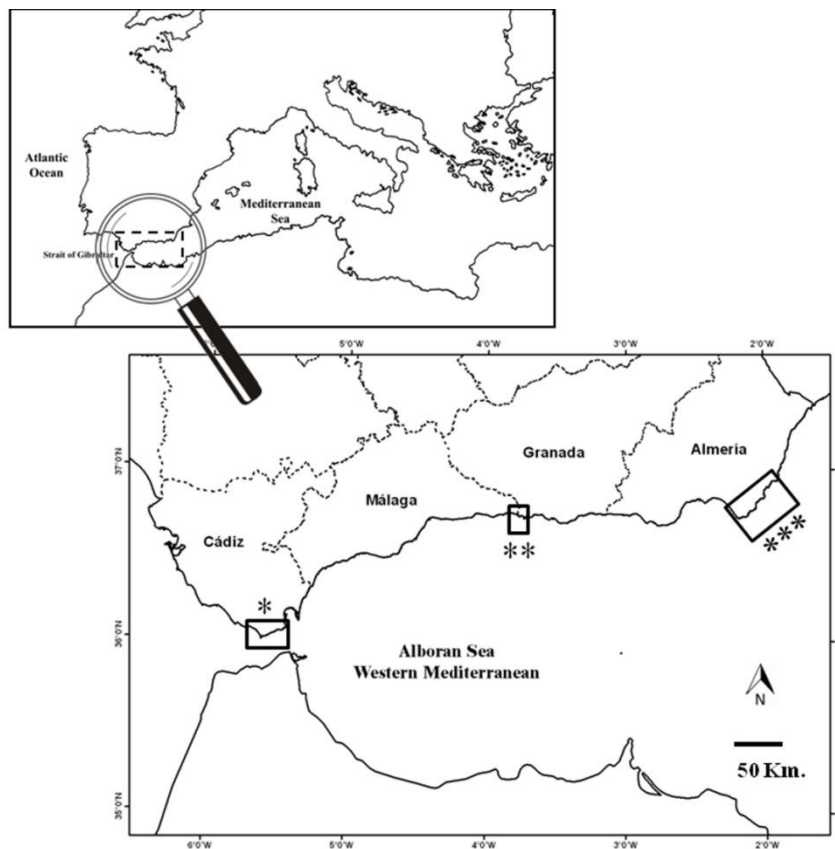
*Astroides calycularis* is considered a key species with high biomass values in our study area (Cebrián & Ballesteros 2004). Therefore, the aim of the present study is to establish a baseline of the distribution and abundance of *A. calycularis* in the southwestern Mediterranean Sea (the Andalusian coast). This baseline might be useful to identify changes in their populations resulting from human impact, and it could be used by management administrations, scientists, and/or MPA managers.

## 6.2 MATERIALS AND METHODS

The survey was conducted in the rocky and shallow bottoms of the Andalusian coastline in 2011. The Andalusian coastline studied comprises the provinces of Cádiz, Málaga, Granada, and Almeria (Figure 6.1). Monitoring sites were established at a

minimum distance of 0.5 nautical miles, depending on the presence of a rocky substrate. The area of study included three marine protected areas (MPA): the Natural Park of the Strait of Gibraltar (Cádiz), the Natural Reserve of Maro-Cerro Gordo Cliff (Málaga-Granada), and the Natural Park of the Cape of Gata-Níjar (Almería).

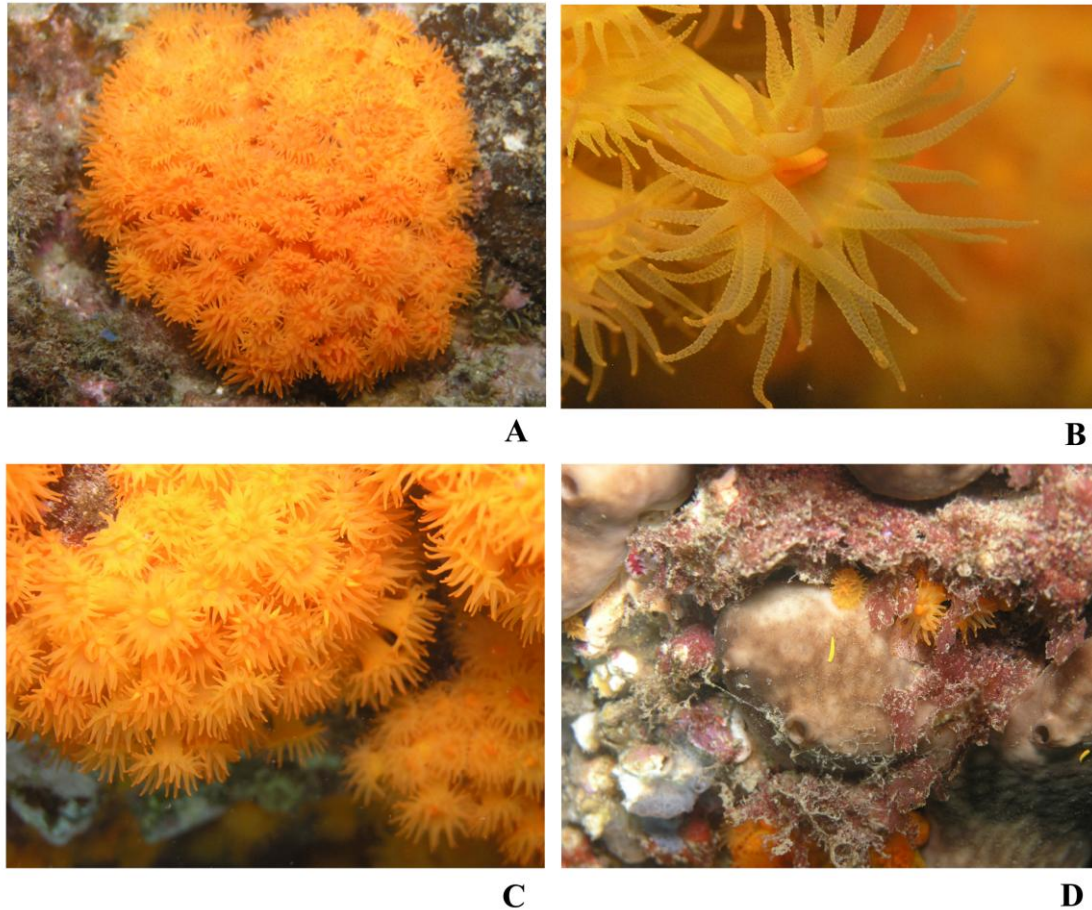
In order to study the distribution, a total of 195 monitoring sites were selected (59 in Cádiz, 37 in Málaga, 49 in Granada, and 50 in Almería shorelines) according to the substrata-type preferences of the orange coral. Following Benedetti-Cecchi *et al.* (1996), we studied the species' abundance in terms of cover percentage, as observed visually by SCUBA divers. The percentage of species covered may be estimated for sessile species such as corals, sponges, and encrusting bryozoans (Katsanevakis *et al.* 2012). At each monitoring sites, two SCUBA divers carried out three dives at different range depths (0-3 m, 3-6 m, and 6-12 m), meaning a total of 585 points were sampled. At each depth, the SCUBA divers localized the *Astroides calycularis* colonies (Figure 6.2) and took data relating to date, geographical position (ED 1950; WGS 84), depth, and substratum type. Four random replicate samples were collected using a 1x1-metre quadrant to assess the relative abundance in percentage of coverage. All data were registered in a database to perform statistical and spatial distribution analyses.



**Figure 6.1:** Map of the study area (Andalusian coastal line; Spain). \* Natural Park of the Strait of Gibraltar; \*\* Natural Reserve of Maro-Cerro Gordo; \*\*\* Natural Park of Cape of Gata.



Five hundred eighty-five dives were performed for the study and a total of 2,340 m<sup>2</sup> were visually estimated, obtaining data of the presence or absence of orange coral, its abundance, its depth, its geographical position, and the sea-bottom composition in the four provinces studied. For abundance, the mean and standard error of the mean were calculated.



**Figure 6.2:** The orange coral *Astroides calycularis*. A: Colony from Granada coast. B: Detail of a polyp extended. C: Colony with some polyps with larvae in the tentacles. D: Larvae on the sponge *Chondrosia reniformis*.

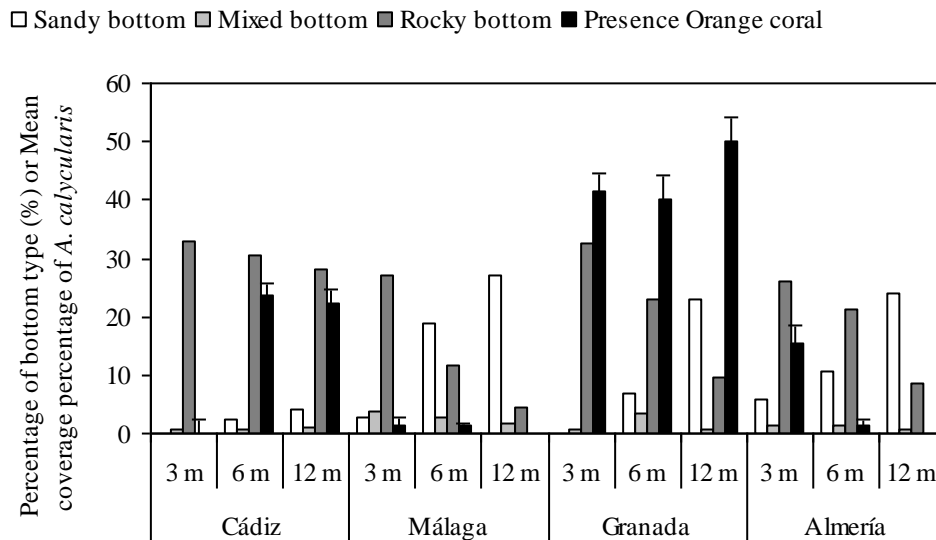
A multifactor analysis of variance (ANOVA) was used to test whether the relative abundance of *Astroides calycularis* was similar across provinces and depths, with the following factors: province as a fixed factor with four levels (Cádiz, Málaga, Granada and Almería); and depth as a fixed factor, orthogonal with province, including three levels (3, 6, and 12-m depth) where the species is typically found (Kružić *et al.* 2002). For ANOVA analysis, considering that Málaga was the province with the lowest number of sites (n=37), 37 sites were used as replicates for both factors, because a balanced ANOVA test is more suitable than an unbalanced ANOVA (Underwood 1997). Prior to ANOVA, the heterogeneity of variance was tested via a Cochran test.

Univariate analyses were conducted with GMAV5 (Underwood *et al.* 2002). When statistical differences were detected, an *a posteriori* Student-Newman-Keuls (SNK) test was applied.

Geospatial data from *Astroides calycularis* abundance were interpolated by means of raster analyses using a gridding of 1 m<sup>2</sup> and were represented spatially. We only interpolated the six points closest to the abundance of orange coral (no farther than 2 kilometers between the presence points) and used the bathymetric line of 15 metres of the marine cartography. Mean abundances were grouped into five categories (absent; scarce: >0-25% coverage; moderate: >25-50% coverage; abundant: >50-75% coverage; very abundant: >75% coverage) to perform SIG graphics in order to make the visualization of the map easier. ArcGIS 9.2 (<http://www.esri.com/>) software was used to represent and analyse the spatial distribution.

### 6.3 RESULTS

A rocky shore bottom was the most predominant substrata in all provinces, followed by a sandy bottom and, finally, a mixed bottom (66.7 %, 29.1 %, and 4.2 % respectively). Cádiz showed the greatest coverage for the rocky bottom, followed by Granada, Almería, and Málaga. The rocky substrata were predominant at 3 m in all provinces, followed by depths of 6 m and 12 m (Figure 6.3).



**Figure 6.3:** Percentage of bottom type per provinces and depth and data of that *A. calycularis* appeared in the provinces; depths and abundance (mean coverage percentage and standard error of the mean).

*Astroides calycularis* was present in 135 of the 585 monitoring points studied (Figure 6.3). Figure 6.4 shows the different abundances found in the 135 monitoring points where *A. calycularis* was found. Cádiz had a mean coverage of 23.11%, with similar values at the different depths studied. However, Granada showed higher abundances, with a mean coverage above 40% and maximum values observed at the deepest points (49.89%).

Almería and Málaga were the provinces with the lowest mean coverage: 12.6% and 1.31% respectively. Neither of them hosted any populations at 12 m. However, Málaga had similar population coverage at 3- and 6-m depths, while Almería showed a higher mean coverage at 3 m (15.43% for 3 m and 1.25% for 6 m).

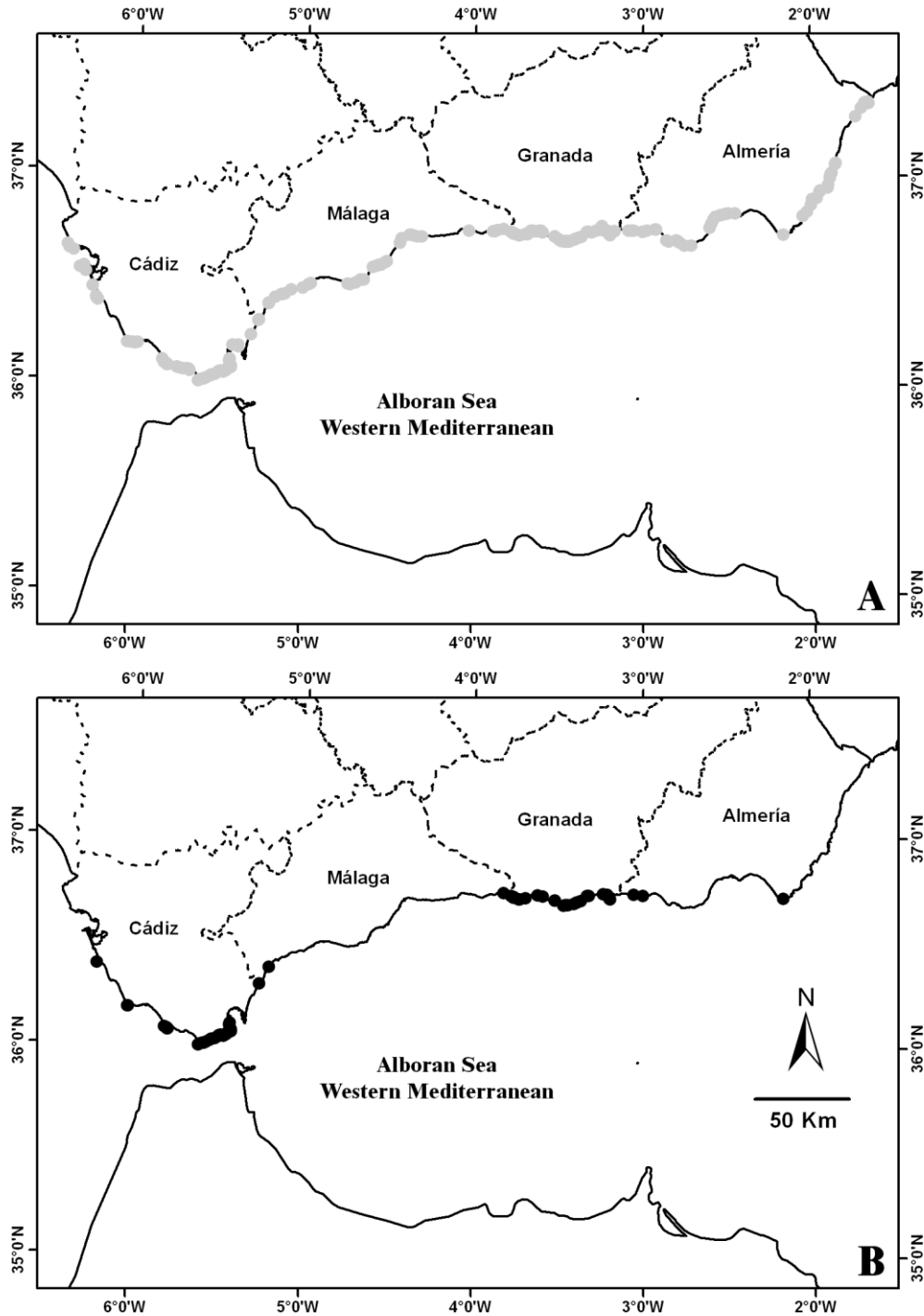
ANOVA analysis showed significant differences between provinces and depths regarding the abundance of *Astroides calycularis* coverage, but there were no interactions between the two factors (Table 6.1). Regarding depth, the highest presence of *A. calycularis* was found at 3 m, with the presence decreasing at lower depths. The SNK test showed variation between provinces (Table 6.1), with a break between Cádiz, Granada, and the group formed by Málaga and Almería. This test also showed variation between depths, grouping 0-3 m with 3-6 m and 3-6 m with 6-12 m. Finally, complete data for all the points sampled are provided as a supplementary table (Appendix II).

**Table 6.1.** Two-way ANOVA results for the influence of province and depth on the abundance of *Astroides calycularis*. Underlined provinces and depths corresponding with homogeneous groups based also on SNK test.

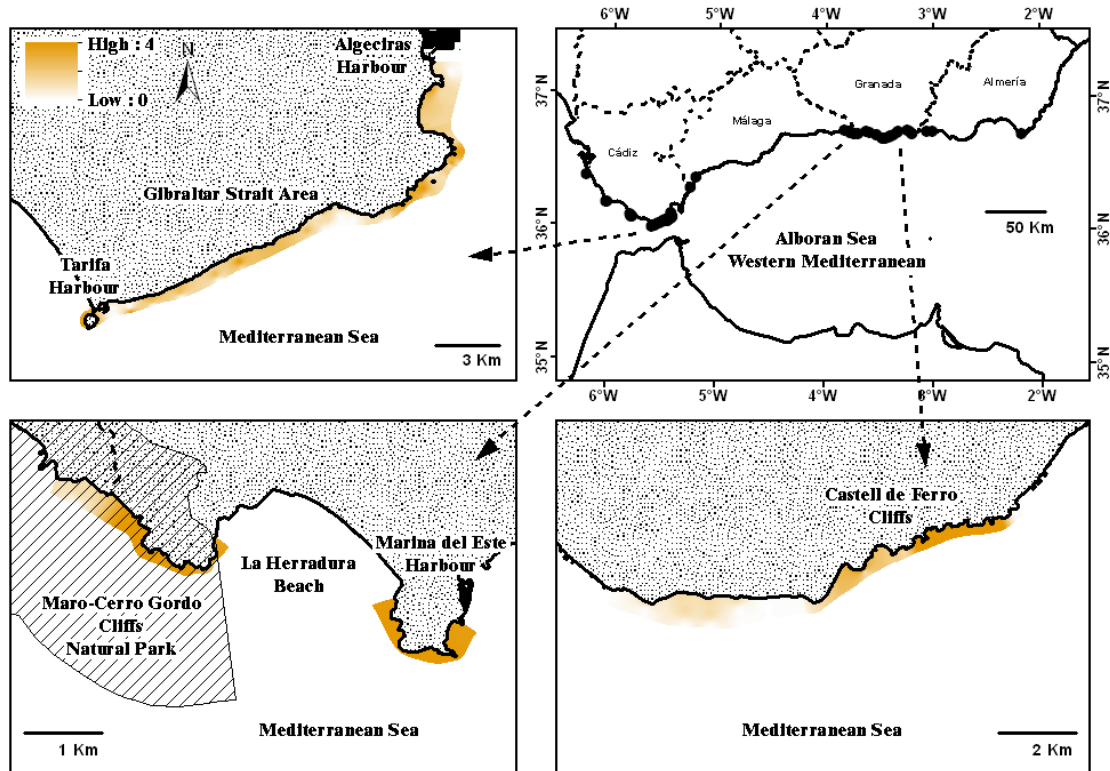
Source of variation	df	MS	F	P
Province	3	18.978	32.000	***
Depth	2	2.983	5.030	***
Pr * De	6	0.785	1.323	n. s.
Residual	432	0.5917		
Total	443			
Cochran's C-test		C=0.2178		
Transformation		None		
n.s. = not significant, *** P<0.01				
Ca>Gr>(Ma=Al)		0-6 m > 6-12 m		
<u>Ca</u> <u>Gr</u> <u>Ma</u> <u>Al</u>		<u>0-3 m; 3-6 m; 6-12 m</u>		

Raster analyses showed three areas where well-established populations could be found on the Andalusian coastline (Figure 6.5). The westernmost area was found to be in the Natural Park of the Strait of Gibraltar (Cádiz), which was also the biggest area. Here the population density was medium. Farther east, a small area (which includes the Natural Reserve of Maro-Cerro Gordo) between Málaga and Granada had the highest

abundance. The last area was found on the Castell de Ferro cliffs and the nearby area. This was not an MPA but had a high population density. The analysis showed that the population in the Natural Park of the Cape of Gata-Níjar was localized and had low density.



**Figure 6.4:** Presence of *Astroides calycularis* along the shoreline studied. A: gray circles show the points sampled; B: black circles show the *A. calycularis* presence.



**Figure 6.5:** Raster analyses of the three populations well-established on the Andalusian coastal line. Scale density: 0 = absence;  $> 0 < 1$  = scarce;  $> 1 < 2$  = moderate;  $> 2 < 3$  = abundant;  $> 3 < 4$  = very abundant.

## 6.4 DISCUSSION

This study has established a current assessment of the distribution and abundance of *Astroides calycularis* populations in the Iberian Peninsula (in particular, the Andalusian coast). The purpose was to identify a population baseline for future studies. Previous studies in the region have only focused on species distribution (Zibrowius 1980; López-González 1993; Ocaña *et al.* 2000; Moreno *et al.* 2008), and only a few have provided abundance data (Cebrián *et al.* 2000; Moreno *et al.* 2007). Furthermore, none have documented the entire Andalusian littoral zone, only some localities.

Our study area, the Alboran Sea, is a biodiversity hotspot in the Mediterranean Sea (Coll *et al.* 2010). However, harbors and enclosures of medium-high to very-high impact (Micheli *et al.* 2013a) create a fundamental problem of balancing human use with the conservation of nature (Micheli *et al.* 2013b). In this area, urbanization appears to be a high threat to marine ecosystems (Malvárez García *et al.* 2000; Costello *et al.* 2010). This phenomenon increases pollution, local eutrophication, and water turbidity (Malvárez García *et al.* 2000; Chapman 2003; Mangialajo *et al.* 2008). Shallow marine assemblages (up to 10 m depth) can be considered good indicators of environmental

change, because species living at shallow depths are particularly exposed to the impacts of coastal activities and thus tend to exhibit stronger responses to human pressure than assemblages from deeper habitats (Fraschetti *et al.* 2002). Ocaña *et al.* (2009) already reported that *Astroides calycularis* is also affected by siltation caused by high coastal urbanization. The fragmented distribution of *A. calycularis* populations in the Andalusian shores has been clearly demonstrated and is certainly an effect of high urbanization. Interestingly, the three MPAs along the studied coastline enclosed the majority of the populations. Marine reserves have become a highly advocated form of marine conservation (Allison *et al.* 1998). Given the high fecundity of many marine organisms, as well as evidence of limited larval dispersal distances, it is likely that reserves are able to maintain their own biodiversity while also servicing nearby non-reserve areas (Halpern & Warner 2003). Many invertebrate populations located within the MPAs can act as source populations, as reported by some authors (e.g. Goñi *et al.* 2003; Espinosa *et al.* 2013).

On the other hand, orange coral populations are well established in some coastal zones, such as eastern Granada. These populations are far from urbanized areas and/or present difficulties in terms of access for recreational activities. This distribution pattern could support the hypothesis that *Astroides calycularis* is affected by urbanization stress and also, potentially, by high levels of siltation. Furthermore, orange coral could be considered a good indicator of well-oxygenated waters (García-Gómez 2007; Casado-Amezúa 2012). As a consequence, it can be concluded that the species is being increasingly affected by coastal development. In fact, the extremely urbanized coastline of Malaga harbors scarce and isolated populations, suggesting that habitat fragmentation is a major threat to this species and could potentially lead to local extirpation of populations, thereby limiting the gene flow at different scales (Fauvelot *et al.* 2009; Bulleri & Chapman 2010).

It has already been demonstrated that recreational activities like scuba diving or fishing can have negative effects on *Astroides calycularis* populations, because colonies can be damaged or removed by the impact of fins, hands, and other diving equipment parts (Moreno *et al.* 2008). Lloret *et al.* (2006) claimed that orange coral had a middle-level population in a dive-perturbation assessment. However, in Andalusian MPAs, scuba diving is allowed, and prohibitions of certain human activities are not totally effective (Linares *et al.* 2011). In this regard, some regulations such as a maximum number of divers per day could be applied, although the paucity of data and turnover rates for most organisms does not allow a quantitative estimate to be made of diver capacity (Sala *et al.* 1996). These management measures could be greatly effective in preserving benthic invertebrate assemblages.

Moreno *et al.* (2007) reported *Astroides calycularis* abundances in some localities of the Andalusian coast. These authors observed the greatest abundances in

Cádiz and Granada, reaching densities of 80-85%. We have already observed the highest abundance in these provinces, showing densities of 90-95%, but in Málaga the populations are localized inside MPA (Natural Reserve of Maro-Cerro Gordo). Nevertheless, these authors observed an abundance that reached 70% in only one locality—in an MPA of Almería. In the same locality, we only found a maximum density of 50%. These populations are isolated and should be monitored to understand their population dynamics and possible threats.

In the last decades, shallow bottoms have been monitored in several studies to evaluate mass mortality events of invertebrate populations in the Mediterranean (e.g. Cebrian *et al.* 2011; Sala *et al.* 2012; Serrano *et al.* 2013). Following Goffredo *et al.* (2008), the demographic traits of coral populations may reveal relationships between the organisms and their environment and can be used to assess habitat stability and suitability. Therefore, the populations of this species should be studied in relation to the changes that could appear because of global changes.

Current observations suggest that an increase in the frequency of mass mortality events around the Mediterranean Sea is expected due to global climate change (Vargas-Yáñez *et al.* 2008). In fact, Maldonado *et al.* (2010) have already observed mass mortality events in marine invertebrates along the Andalusian coastline. Mortality associated with these global phenomena can result from increases of 1 to 2°C above the mean sea temperature in the summer (e.g. Bensoussan *et al.* 2010; Cebrian *et al.* 2011). Therefore, knowledge of abundance and distribution of *Astroides calycularis* as a baseline would be of great interest. In this sense, monitoring programs are an important tool (Calvo *et al.* 2011) that will enable scientists to assess the orange coral populations and detect the possible impacts of human activities on the local, regional, and global scale. In this way, environmental managers may be able to implement appropriate measures for species conservation.

Connectivity among environmental managers, researchers, NGOs, private agents, and citizens is required. Setting up volunteer webs could be a good management tool when the species being studied covers large areas (Bramanti *et al.* 2011). A simple qualitative and/or semi-quantitative sampling methodology will allow for routine collection of data about the targeted species' presence/abundance. These studies must be accompanied by temperature serial analyses by establishing control stations in MPAs or additional strategic localities. All of these data will show evidence of changes in coastal areas.

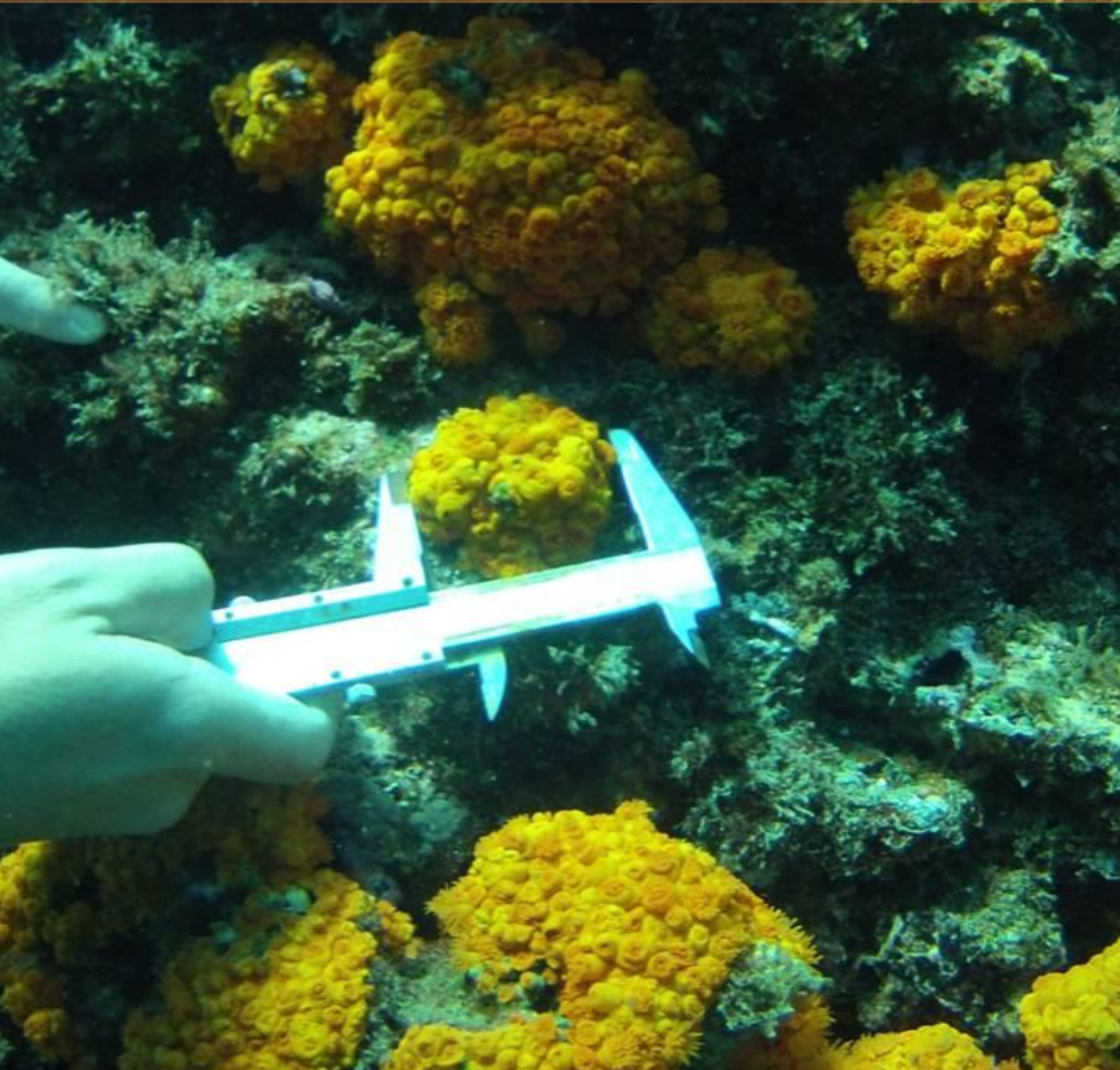
The knowledge of population dynamics through time and space is crucial, and therefore, baseline studies are also urgently required in order to implement management measures on the conservation, protection, and restoration of endangered habitats and ecosystems.







## 7. RESTORATION



A colony transplanted in the Punta de la Mona (Almuñécar, Granada). Author: Peñalver-Duque P.



An underwater photograph showing a diver in the foreground wearing a black CRESSI BAMBINO tank and a blue mesh net. The diver is holding a yellow and black tool. In the background, another diver is visible near a coral reef. The water is clear and blue.

7.1 A method for restoring populations of the endangered coral *Astroides calycularis*

View of a moment of one monitoring of the orange coral colonies transplanted on the Granada coast. Author: Peñalver-Duque P.

## Abstract

The endemic and endangered orange coral *Astroides calycularis* is a key species in the Mediterranean, where it serves as a microhabitat for several associated species and is highly attractive to divers. However, the increasing human impact in coastal areas could be affecting this endangered and endemic coral irreversibly. Therefore, management measures need to be developed in order to guarantee its conservation. The main aim of this study was to test a reliable attachment methodology for restoring places where the species is or will be affected by human activities. We chose an attachment technique using a marine quick-action epoxy resin, completing three treatments (control, transplanted, and translocated colonies) in two places with different hydrodynamic conditions. Control colonies were undisturbed colonies that were not manipulated; translocation colonies were dislodged and reattached in the same place; and transplantation colonies were dislodged and attached in a different habitat. Translocated colonies achieved a higher survival than did the transplanted ones, and the survival was also higher in environments with more hydrodynamism. Regarding growth, analysis of variance (ANOVA) analyses did not show significant differences among treatments, but significant differences between areas with different hydrodynamic conditions were noted. This study confirms that it is possible to transplant or translocate colonies of this coral with the technique proposed here, which could be used in future management plans for areas impacted by humans.

## Resumen

*Astroides calycularis* es una especie clave en el Mediterráneo, como sustrato para una diversa fauna macro-invertebrada que vive asociada al coral y como atractivo turístico para los buceadores recreativos. No obstante, el incremento de las actividades humanas que impactan en las áreas costeras está afectando a las poblaciones de esta especie endémica y amenazada. Por ello, son necesarias medidas de gestión encaminadas a garantizar la conservación de este emblemático coral. Así, el principal objetivo de este estudio ha sido probar una metodología de fijación eficaz para restaurar áreas donde la especie esté o se vea afectada por dichas actividades. Se ha seleccionado una técnica de fijación mediante el uso de resina epoxy de acción rápida. Para ello, se establecieron tres tratamientos para las colonias de coral naranja: colonias control; trasplantadas; y traslocadas, en dos zonas con diferentes condiciones hidrodinámicas. Las colonias control fueron aquellas que no se manipularon; mientras que las colonias trasplantadas fueron recolectadas de un hábitat y fijadas en otro diferente; y las colonias traslocadas se recogieron de un hábitat y fueron fijadas en el mismo. Las colonias traslocadas obtuvieron una mayor supervivencia que las trasplantadas, esta supervivencia fue mayor

también en los ambientes con mayor hidrodinamismo. Con respecto al crecimiento, los análisis ANOVA no mostraron diferencias significativas entre tratamientos, pero sí entre áreas con diferentes condiciones hidrodinámicas, mostrando un mayor crecimiento las colonias ubicadas en la zona con mayor hidrodinamismo, posiblemente debido al mayor aporte de alimento. El presente estudio confirma que es posible trasplantar y traslocar colonias de *A. calycularis* con la técnica aquí propuesta, la cual podría ser implementada en futuros planes de gestión de la especie para restaurar o potenciar la recuperación de aquellas poblaciones que hayan sido impactadas por alguna actividad humana.

### 7.1.1 INTRODUCTION

Scleractinian corals contribute to the complexity of the marine ecosystems in which they are present, creating shelter and refuge areas for other organisms (e.g. Rinkevich 1995; Jaap 2000; Gratwicke & Speight 2005). Moreover, the presence of healthy corals has important socio-economic consequences in the regions in which they are present (Moberg & Folke 1999; Jackson *et al.* 2001; Edwards & Gomez 2007), supporting fisheries, diving tourism and/or coastal protections (Moberg & Folke 1999; Hughes *et al.* 2003; Shaish *et al.* 2010). Thereby, these ecosystems are some of the most important and biodiverse around the world (Edwards & Gomez 2007) and must be conserved and/or restored if necessary. However, the dismal fate of coral reefs suggests the need for restoration measures (Epstein *et al.* 2003; Rinkevich 2008), and some authors have proposed that coral transplantation programmes are a major tool in active rehabilitation strategies (e.g. Raymundo 2001; Yap 2003; Rinkevich 2006).

Over the past few decades, several coral transplantation techniques have been used to improve degraded or damaged coral habitats, with excellent results (e.g. Epstein *et al.* 2003; Nishihira 2007). Mainly, these techniques have been carried out in tropical coral reefs, whereas temperate corals have received much less attention. In the Mediterranean Sea, the most important anthropogenic threats are habitat loss, degradation and pollution, overexploitation of marine resources, invasion of species and climate change (e.g. Coll *et al.* 2010). Furthermore, natural disturbances such as intense catastrophic environmental events are increasing, with negative effects on key species and habitats (e.g. Teixidó *et al.* 2013).

The Mediterranean endemic orange coral *Astroides calycularis* is an azooxanthellate scleractinian colony coral with a calcium carbonate exoskeleton (Zibrowius 1980) that inhabits the rocky shore from the surface to a 50-metre depth (Rossi 1971; Ocaña *et al.* 2000). Their population densities can be locally high, with colonies covering up to 90% of the sea bottom (Goffredo *et al.* 2011a), but the species has a limited geographical distribution in the Mediterranean Sea (Zibrowius 1995; Bianchi 2007). Along the Spanish coasts, the highest orange coral densities can be found on the Andalusian shores (Alboran Sea), where a high level of biodiversity associated with this species was recently discovered (Terrón-Sigler *et al.* 2014a-Section 4). The increasing human impacts on the coastal areas, such as marine pollution and/or habitat degradation, negatively affect this species (Moreno *et al.* 2008; Ocaña *et al.* 2009; Casado-Amezúa 2012). Furthermore, SCUBA diving has an impact on the coral's populations because colonies can be damaged or dislodged by the impact of fins, hands and other diving equipment parts (Moreno *et al.* 2008; Terrón-Sigler & León-Múez 2009-Section 5). Therefore, *A. calycularis* is protected by national and international organisations as an endangered species (i.e. the Bern and Barcelona Conventions and

the Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES]).

The aim of this study is to test a transplantation technique as well as explore the response of transplanted colonies to different levels of hydrodynamism. This technique could be used as a possible management tool in degraded areas, improving the conservation status of this endangered species.

## 7.1.2 MATERIAL AND METHODS

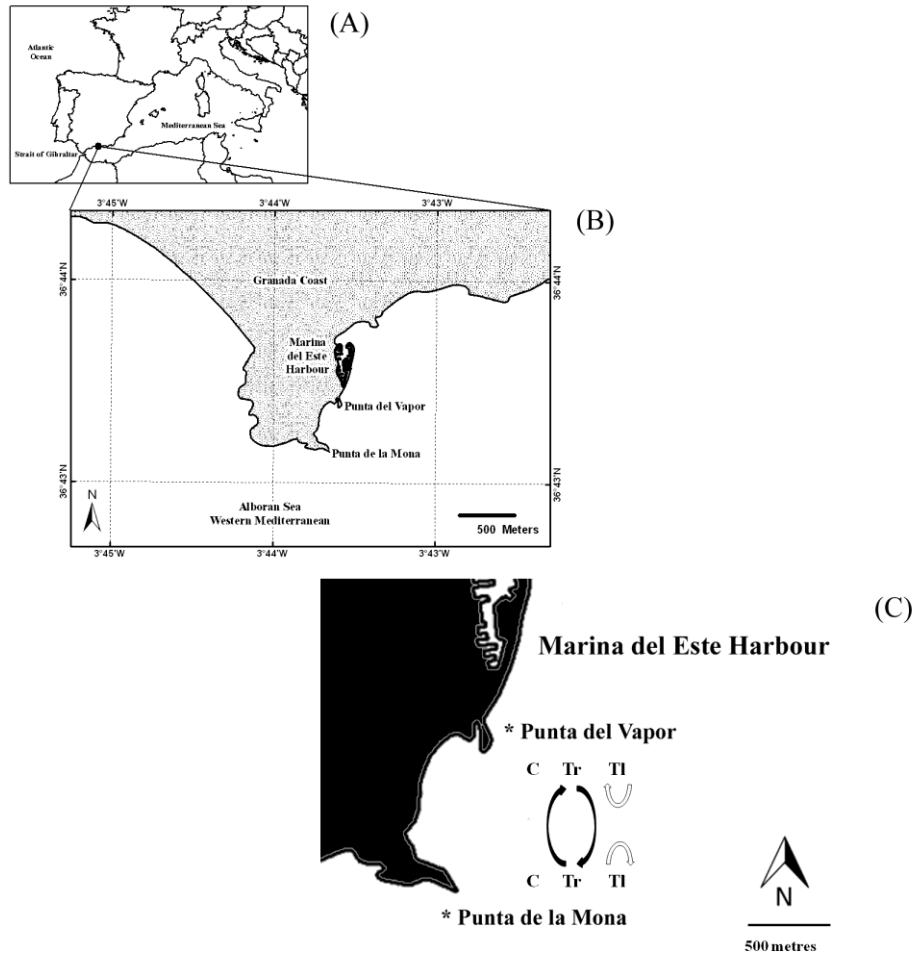
### 7.1.2.1 Study site

We conducted the experiment on the Granada coast, southern Iberian Peninsula, between July 2012 and July 2013 (Figure 7.1). The experiment was carried out on Marina del Este beach, an area frequently visited by recreational SCUBA divers (Terrón-Sigler & León-Muez 2009-Section 5). Two areas were selected: Punta de la Mona [PM] (36°43'08''N, 3°43'38''W) and Punta del Vapor [PV] (36°43'22''N, 3°43'35''W), both at a depth of 8 metres. PM is exposed to windstorms at the south, east and west, while PV is exposed to windstorms only at the east. In order to test the exposition difference, wave exposure analysis was calculated. Following Howes *et al.* (1994), a fetch model index was developed for each area. This model provides good quantitative approximations of wave exposure in order to predict marine community patterns (e.g. Hill *et al.* 2010). This model relies on two indices of fetch: modified effective fetch and maximum fetch. A combination of the two indices allows for determining the wave exposure class of each area (Table 7.1) and is calculated using the following equation:

$$Fe = [\sum (\cos\theta_i) \times Fi] / \sum \cos\theta_i ,$$

where  $Fe$  is the effective fetch in km,  $\theta_i$  is the angle between the shore-normal and the direction ( $0^\circ$ ,  $45^\circ$  to the left and  $45^\circ$  to the right), and  $Fi$  is the fetch distance in km along the relevant vector. Maximum fetch is defined as the maximum fetch distance in km measured from the point of interest. A value of 1000 km is conventionally used when open-ocean fetches occur. The mean values in km of the modified-effective fetch and maximum fetch (hereafter average fetch) for each area were used as continuous variables in subsequent analysis.





**Figure 7.1:** (A) Map of the study area (Andalusia coastal line; Spain). (B) Sites position in Granada littoral (Punta de la Mona and Punta del Vapor). (C) Graphic representation of experimental treatment. (C= Control; TL= Translocated colonies; TR= Transplanted colonies).

**Table 7.1.** Wave exposure classes based on the modified-effective fetch and maximum fetch matrix (after Howes *et al.* 1994). VP: very protected; P: protected; SP: semi-protected; SP: semi-exposed; E: exposed.

Max Fetch (km)	Modified-effective fetch (km)				
	< 1	1-10	10-50	50-500	> 500
< 10	VP	P	n/a	n/a	n/a
10-50	n/a	SP	SP	n/a	n/a
50-500	n/a	SE	SE	SE	n/a
> 500	n/a	n/a	SE	E	E

### 7.1.2.2 Experimental design

In each area (PM and PV), in order to distinguish between the effect of a new habitat (transplantation treatment: PM → PV or PV → PM) on the growth and survival of the coral and the effect of simply being unattached, manipulated and reattached, it

was also necessary to establish a translocation treatment within the habitat of origin (see Chapman 1986; Crowe & Underwood 1999). Per area, we established three treatments: control colonies (undisturbed colonies that were not manipulated); translocation colonies (dislodged and reattached in the same place); and transplantation colonies (dislodged and attached in a different habitat). Six colonies were used as replicates for each treatment (N=108; 54 for each area: PM and PV).

#### 7.1.2.3 Attachment methodology

We chose an attachment technique using a marine quick-action epoxy resin (five minutes for hardening) that had previously shown its usefulness in this species (see Terrón-Sigler *et al.* 2011-Section 3). As explained above, 18 colonies were transplanted from PV to PM, and vice versa. These colonies were collected via SCUBA diving and maintained in plastic containers filled with seawater. The colonies remained in the containers for fewer than 15 minutes before being transplanted to the new area.

In each area (PM and PV), the substratum was cleaned with a steel comb, scraped away the surface, and mashed and put the epoxy resin on the clean substrate. Finally, the colonies were attached firmly and observed until the resin had hardened. Each colony was labelled with a plastic tag inserted in the epoxy resin. In the case of control colonies, a piece of resin was placed in the vicinity of each colony in order to attach the plastic tags for labelling purposes.

#### 7.1.2.4 Monitoring coral survival and growth

The parameters of survival and growth are typically used as measures of colony transplantation (Raymundo 2001). In this study, survival is defined as the presence of the colony after the experiment has been started on each treatment, so the loss of colonies may be the result of the attachment methodology.

Growth was measured as an increase in the colony area and an increase in the number of polyps. We used the length ( $L_c$ , major axis of the colony) and width ( $W_c$ , minor axis of the colony) as biometric parameters. According to Goffredo *et al.* (2011a), the *Astroides calycularis* colony area ( $A_c$ ) must be calculated using the formula for an ellipse ( $A_c = \pi (L_c * W_c) / 4$ ). Colony area is a more accurate and representative measure of colony size than colony length is (Goffredo *et al.* 2011a), and it is a good parameter for understanding the dynamics of coral populations (Terrón-Sigler *et al.* 2014a-Section 4).

*Astroides calycularis* is a gonochoric (featuring male and female colonies) and brooding coral (Goffredo *et al.* 2010), but once the larva have been established on the bottom, the first polyp reproduces asexually by budding in order to create a colony

(Casado-Amezúa 2012). Therefore, the number of polyps in each colony was counted in the initial state and at each monitored time. As a result, growth in the colony area and in the number of polyps was estimated by the increase in each parameter between the initial state and the different monitored times.

We monitored the colonies at six and 12 months after the start of the experiment. Each time, we located the labelled colonies and measured the biometric parameters (Lc and Wc) as well as the number of polyps (Figure 7.2).



**Figure 7.2:** View with the three treatments in one site of PV. C=control colony; TL=translocate colony; and TR=transplanted colony. All colonies were labelled.

#### 7.1.2.5 Data analysis

In order to test whether or not the growth was similar between treatments and areas (PM: high hydrodynamism; PV: low hydrodynamism), we used a multifactor ANOVA with the following factors: treatment with three levels (control, translocation and transplantation) and hydrodynamism as an orthogonal with treatment and a fixed factor with two levels (high and low). Prior to ANOVA, the heterogeneity of variance was tested via Cochran's C test. Univariate analyses were conducted with GMAV5 (Underwood *et al.* 2002). When statistical differences were detected, a post-hoc Student-Newman-Keuls test was applied.

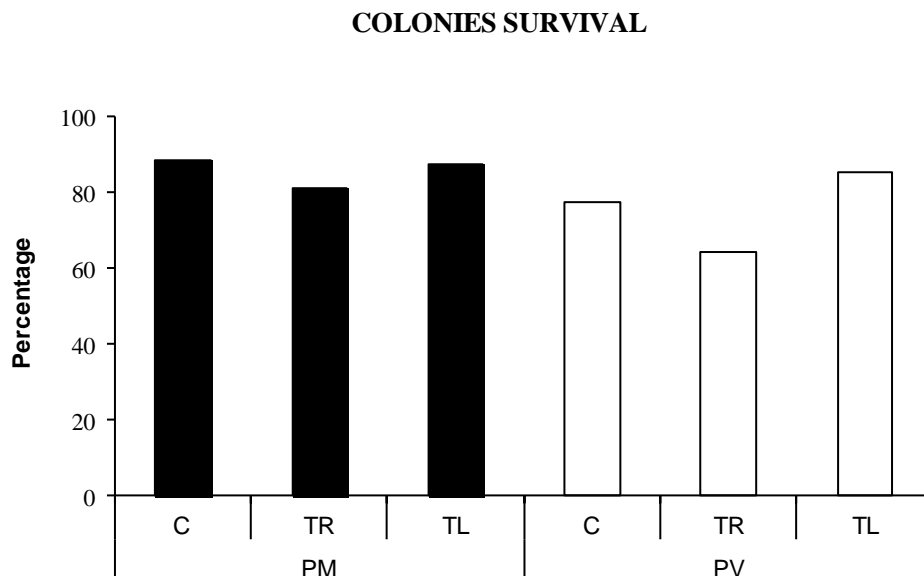
### 7.1.3 RESULTS

#### 7.1.3.1 Wave exposure analysis

For the PM area, the maximum fetch was 552.10 km, and the calculated modified effective fetch was 299.82 km. Meanwhile, for the PV area, the maximum fetch obtained was 379.58 km, and the modified effective fetch was 84.27 km. Thus, following Howes *et al.* (1994) fetch model index (Table 7.1), PM is considerate exposed (E), and PV is semi-exposed (SE). Therefore, the PM area has higher hydrodynamic conditions than does the PV area.

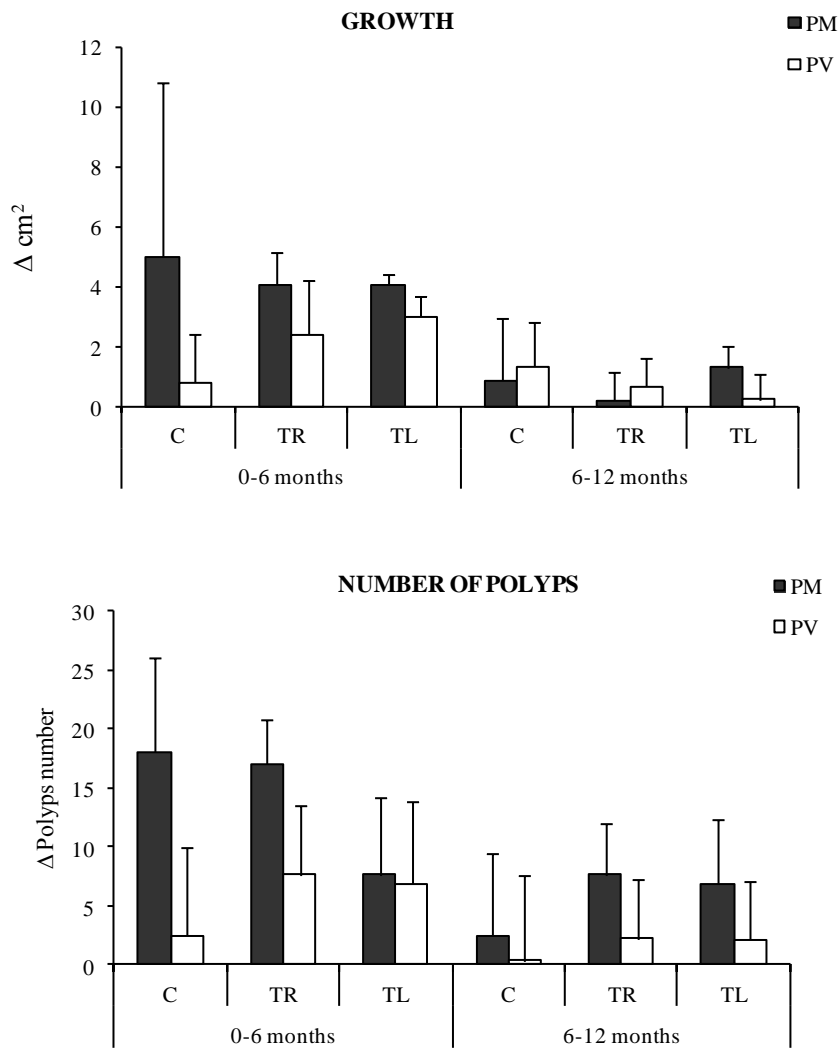
#### 7.1.3.2 Survival and growth

Translocated colonies obtained a higher survival than did transplant ones (Figure 7.3). For translocated colonies on PM, survival was slightly higher than it was on PV, with the two areas obtaining 87.5% and 85.7%, respectively (Figure 7.3). These differences between areas were also recorded for transplanted colonies, which showed a higher survival for PM (81.2%) than for PV (64.3%). Furthermore, survival in the control colonies was lower in PV than it was in PM (77.8% vs. 89.9%, respectively). It is important to note that all lost-colony treatments occurred in the first 6 months. In general, transplanted and translocated colonies showed a good aspect with a bright orange colour similar to the control colonies.



**Figure 7.3:** Percentage colonies survival per zone (PM= Punta de la Mona; PV= Punta del Vapor) and treatments (C= Control; TL= Translocation; TR= Transplantation).

Regarding the growth area of the colony (Figure 7.4), we detected high growth in all treatments within PM six months after the start of the experiment, ranging from 4 cm<sup>2</sup> for translocated and transplanted colonies to 5 cm<sup>2</sup> for control ones. However, this pattern was not observed for the PV area, where the growth was much higher in translocated and transplanted colonies than in control colonies. Generally, the growth was lower for all treatments and areas in the period of 6–12 months, without a clear pattern. Translocated and control colonies obtained higher values on PM, whereas for PV, the values were higher for control and transplanted colonies (Figure 7.4).



**Figure 7.4:** *Astroides calycularis* colonies growth per zone (PM= Punta de la Mona; PV= Punta del Vapor) and treatments (C= Control; TR= Transplantation; TL= Translocation) six and twelve months after the start of the experiment. Error bars are standard deviations.

The number of polyps increased notably during the first six months (Figure 7.4). In the environment with high hydrodynamic conditions (PM), this increment was similar for both control and transplantation treatments, but it was less for translocated treatment. Nevertheless, in low hydrodynamic conditions (PV), it was lower overall, and in these conditions, the control treatment yielded fewer polyps than did the other treatments. However, after 12 months, the increased number of polyps did not increase overall. Still, again, it was lower on PV than it was on PM for all treatments, and for inside treatments, control colonies presented a smaller increase in polyp numbers.

Regarding growth, ANOVA analyses did not show significant differences among treatments (Table 7.2), but differences were significant between areas with different hydrodynamic conditions. For PM (high hydrodynamic conditions), the mean and standard deviation values of the colony area were  $5.19 \text{ cm}^2 \pm 3.91$ , but for PV (low hydrodynamic conditions), these values were  $3.30 \text{ cm}^2 \pm 3.44$ . Regarding polyp numbers, ANOVA analyses did not show significant differences for any treatment or for hydrodynamic conditions (Table 7.2).

**Table 7.2.** Two-way ANOVA results for the influence of treatment and area on the growth (measured as change in polyps number and increment of area) of *Astroides calycularis* colonies after 12 months.

Source of variation	Df	Growth (cm <sup>2</sup> )			Polyps number Area (cm <sup>2</sup> )		
		MS	F	P	MS	F	P
<b>Treatment</b>	2	0.512	0.036	0.965	39.210	0.155	0.857
<b>Area</b>	1	61.491	4.344	0.041*	0.001	0.000	0.999
<b>Tr * A</b>	2	7.014	0.496	0.611	266.314	1.051	0.355
<b>RESIDUAL</b>	71	14.155			253.416		
<b>TOTAL</b>	77						
<b>Cochran's C-test</b>		C=0.1653 NS			C=0.2673 NS		
<b>Transformation</b>		None			None		

NS: not significant; \* P<0.05

#### 7.1.4 DISCUSSION

Little information is available about transplantation experiences associated with scleractinian corals in the Mediterranean Sea (Zibrowius 1995; Ocaña *et al.* 2009; Terrón-Sigler *et al.* 2011-Section 7). The survival and growth of transplanted and translocated colonies prove the success of these restoration techniques, which were used in this study for this coral species. Ocaña *et al.* (2009) used cement-like adhesive to transplant *Astroides calycularis* colonies in the Strait of Gibraltar, and less than 50% of colonies survived. Previously, Zibrowius (1995) tested transplanting the orange coral from the south of Spain to French Mediterranean waters, but he did not achieve success because recreational SCUBA divers harvested that strange orange coral. Recently,

Terrón-Sigler *et al.* (2011-Section 7) tested different epoxy resins as an adhesive material and concluded that quick epoxy resin (coraFix<sup>®</sup>) was better than other epoxy types. The selection of an appropriate attaching material is crucial depending on the different substrates and/or coral species (Dizon *et al.* 2008). Usually, massive corals have a higher potential for transplantation than do branching corals (Raymond *et al.* 2006; Omori 2011). However, previous experiences in tropical or subtropical areas showed that small massive colonies and fragments such as *A. calycularis* showed very low survival [from 2.2% to 23.3%] (Omori & Okubo 2004; Thongtham & Chansang 2008) in comparison to the high survivorship obtained in our study.

The survival of control colonies of *Astroides calycularis* decreased in both areas. Some studies involving tropical seas explain this mortality as a possible species strategy focused in a turnover, with dead and recruitment colonies (e.g. Yap *et al.* 1992; Raymond *et al.* 2006). The *A. calycularis* ecologic strategy should be studied further in the future.

Growth and survival should not be considered the sole criteria in the evaluation of transplantation efforts (Edinger *et al.* 2000; Raymond *et al.* 2006), as environmental conditions must also be taken into account (Yap & Gomez 1984). The interaction of physical and biological factors is also related to intrinsic physiological and behavioural characteristics of the species concerned (Gates & Edmunds 1999). Linares *et al.* (2008b) observed that environmental conditions did not affect the mortality of the red gorgonian (*Paramuricea clavata*) in transplantation experiments, though methodological failure rates have to be taken into consideration. The three treatments displayed significant differences between hydrodynamic conditions, showing higher survival and growth in the more exposed area of PM. High hydrodynamism promotes the suspension-feeder strategy (Zabala & Ballesteros 1989), explaining the higher growth observed on PM in comparison to PV. Thereby, *Astroides calycularis* shows different responses to different environmental constraints. However, this response does not have to be assigned to a single factor (Raymond *et al.* 2006) because all of the factors interact, producing complex patterns in the responses (Todd *et al.* 2004).

On the other hand, the attachment method can be a main factor for coral survival and/or growth. Some authors have found differences in the survivorship of colonies between epoxy resin and / or cyanoacrylate adhesive (Borneman & Lowrie 2001; Dizon *et al.* 2008). Nevertheless, Forrester *et al.* (2011) did not find influences of different attachment methods in the growth and survival of fragments, and they hypothesised that any method that keeps the coral firmly attached should be successful.

One factor for getting successful results is scraping away the point of attachment and cleaning organisms from the substrate where the colony will be placed (Dizon *et al.* 2008). This previous process is useful for the transplanted fragments; they can grow substantially larger when the substrate is cleared of surrounding algae (Forrester *et al.*

2011). In this sense, the orange coral seems to also benefit from this methodology, considering the values of growth and survival observed. This did not occur in the nearby area of the Strait of Gibraltar, where the colonies of the orange coral were colonised by the surrounding macroalgae (Ocaña *et al.* 2009). Thus, we recommend cleaning the surrounding algae and biological material in order to achieve the most effectiveness.

Finally, it is important to know the cost-efficiency of transplantation programs, including the cost of the attachment methodology in terms of the person-hours needed for coral transplantation (Dizon *et al.* 2008). In the coral restoration literature, few studies go into adequate detail on the costs involved, and those that do generally ignore some costs and resource implications altogether (Spurgeon 2001). In some cases, restoration costs can vary enormously; for example, some methods require a significant amount of labour and a complex approach to construction and substrate preparation, while others do not (Spurgeon 2001). Creating and maintaining a farming or nursery structure in a seabed is a laboriously and expensive method in comparison with other techniques, such as transplanting corals using fixed materials (Forrester *et al.* 2011). In the present study, an epoxy resin was selected as the fixed material because it is easy to use, is inexpensive, requires little labour, does not require the use of an artificial structure on the seabed, and is easy to monitor. Therefore, marine managers can easily implement this methodology scheme.

In conclusion, we have experienced a high level of success in the transplantation and translocation of orange coral colonies in both areas (high and low hydrodynamism), but it is crucial to take into account environmental conditions, methods (such as scraping) and attachment material type. When coral colonies are broken and fragmented either naturally by storms or by human activities, such as diving, anchoring, and boat grounding, as has already been demonstrated in our study area (Terrón-Sigler & León-Muez 2009-Section 5) using these fragments for restoration could be beneficial (Raymundo 2001). Therefore, this attachment methodology for transplanting *Astroides calycularis* colonies or fragments should be considered in conservation strategies for this species as a potential management tool in areas affected by local disturbances.



An underwater photograph showing a rocky seabed covered in various marine life, including green and brown seaweeds and yellowish-orange sponges. Several square, light-colored artificial plates are placed on the rocks. Two sea urchins are visible: one is dark purple and the other is light grey. The water is clear and blue.

7.2 Evaluation of artificial plates as a potential method for the restoring of *Astroides calycularis* populations

View of some plates installed on rocky shore from Punta del Vapor (Almuñécar, Granada). Author: Terrón-Sigler A.

## Abstract

*Astroides calycularis* is an endemic and endangered Scleractinian corals from Mediterranean Sea, which has an important function as microhabitat where their populations are presents. Orange coral populations are affected by several human and natural impacts that are damaging their populations. In the present study, we used artificial plates to test whether *A. calycularis* larvae may settle through carbonate plates installed in the rocky bottom as a new method to restore damaged populations. Plates with carbonate composition of 10 x 10 centimeters were selected. Thirty three plates were installed in a submarine rocky wall where orange coral was present, just two or three months before the species spawning. Plates have been covered by nineteen species belonging to Chlorophyta, Rhodophyta, Phaeophyta, Porifera, Cnidaria, Echinodermata, Arthropoda, Mollusca and Chordata (Ascidiacea). To assess the assemblage settled on plates we used the CPCe software. The sea urchin *Paracentrotus lividus*, *Arbacia lixula*, and *Sphaerochinus granularis* were present on and around plates every monitored time (T1, T2 and T3). Shannon-Weaver Index was higher in T1 ( $1.5 \pm 0.14$ ) than T2 and T3 ( $1.06 \pm 0.12$  and  $1.09 \pm 0.12$ , respectively), therefore it can be observed a decrease through time. No orange coral larvae were settled on plates, probably as a consequence of the sea urchin grazing. Future studies are required in order to assess the interaction between larvae settlement and sea urchin grazing.

## Resumen

*Astroides calycularis* cumple una importante función como microhábitat en aquellos lugares donde la especie está presente. Sin embargo, sus poblaciones se encuentran mermadas como consecuencia de impactos antropogénicos y naturales. En el presente estudio, se han instalado en sustrato rocoso placas de sustrato artificial con el fin de testar si las larvas del coral naranja pueden asentarse en ellas, y utilizar esta técnica para restaurar poblaciones de la especie que se encuentren mermadas. Se seleccionaron placas compuestas de carbonato cálcico de 10 x 10 centímetros. Treinta y tres placas fueron instaladas en una pared rocosa donde existía una población densa de *A. calycularis*, entre dos y tres meses antes del proceso de liberación de larvas de la especie. Las placas han sido colonizadas por diecinueve taxones distintos pertenecientes a los grupos Clorofitas, Rodofitas, Feofitas, Poríferos, Cnidarios, Equinodermos, Artrópodos, Moluscos y Cordados (Ascidas). Para evaluar la comunidad asentada en las placas se utilizó el programa CPCe. Los erizos de mar *Paracentrotus lividus*, *Arbacia lixula* y *Sphaerochinus granularis* estuvieron presentes sobre las placas y alrededor de ellas durante todos los muestreos de seguimiento realizados (T1, T2 y T3). El índice de diversidad Shannon-Weaver fue mayor en T1 ( $1,5 \pm 0,14$ ) que en T2 y T3

( $1,06 \pm 0,12$  y  $1,09 \pm 0,12$ , respectivamente), por tanto hubo un descenso de la diversidad a lo largo del tiempo. Ninguna larva de *A. calycularis* se asentó en las placas, probablemente como consecuencia del ramoneo de los erizos de mar. Para el futuro, se requieren más estudios para valorar la interacción entre el asentamiento de larvas y el ramoneo de los erizos de mar en las poblaciones del coral naranja.



### 7.2.1 INTRODUCTION

The orange coral (*Astroides calycularis*) is an endemic species with a carbonate calcium exoskeleton from Mediterranean Sea (Zibrowius 1980). Due to the biological and ecological characteristics the species has a narrow distribution into the Mediterranean (Zibrowius 1980), and in Southern Spain it is mainly found in Marine Protected Areas (MPA) in the North of Alborán Sea (Terrón-Sigler *et al.* 2015-Section 6). The orange coral inhabits the rocky shore from the surface to 50-metre depth, but is typically found in the shallow infralittoral zone (0 to 15-m depth), on vertical walls, or inside caves (Rossi 1971; Zibrowius 1978; Kružić *et al.* 2002), and it may reach population densities locally high, with colonies covering up to 90% of the sea bottom (Goffredo *et al.* 2011a; Terrón-Sigler *et al.* 2015-Section 6). This species is characterised as gonochoric, both at the polyp and colony level, and as a brooding species (Goffredo *et al.* 2010). Planula release may occur through different months along the Mediterranean coast depending on the sea surface temperature (SST). On Granada coast line, the realizing of planula has been observed from the middle of June to the end of July in Punta de la Mona, when SST was 18-23°C (Terrón-Sigler *et al.* 2011-Section 3; Casado-Amezúa 2012). While in Italy, it has been observed to take place between May and July between 19-25°C SST (Casado-Amezúa 2012). Moreover, recently, *A. calycularis* has been considered as an important habitat to macro-invertebrate, due to the high diverse assemblage community that harbours their colonies (Terrón-Sigler *et al.* 2014a-Section 4). Therefore, *A. calycularis* is protected by national and international organisations as an endangered species (Berna and Barcelona Conventions and CITES).

Nevertheless, the orange coral populations are affected by several human and natural impacts. Firstly, it has been documented that human activities on the littoral increase marine pollution and/or habitat destruction and it has been showed that these factors affect the species (Moreno *et al.* 2008). Moreover, siltation process affects the orange coral as a consequence of the high coastal urbanization (Ocaña *et al.* 2009). Secondly, the human recreational activities have a negative effect on the *A. calycularis* populations, among them, SCUBA diving has been the most studied. Thus, colonies can be damaged or dislodged by the impact of fins, hands and other diving equipment parts (Moreno *et al.* 2008; Terrón-Sigler & León-Múez 2009-Section 5). Other recreational activities may also affect the coral colonies, for example, recreational fishing, spearfishing, and anchoring (Terrón-Sigler & León-Múez 2009-Section 5). Secondly, natural conditions may affect the species too; storms; hydrodynamism; and inter-specific competition. Inter-specific competition may play an important role, especially when species with a high capacity to structure coastal habitats are present, as what takes place with the sea urchin in the Mediterranean Sea (Coma *et al.* 2011).

Different approaches have been used to restore the orange coral populations (Zibrowius 1995; Ocaña 2009; Terrón-Sigler *et al.* 2014b-Section 4). Nevertheless, all these techniques have used damaged colonies or dislodged in transplantation or translocation process. Thus, techniques based to capture larvae from the environment have received much less attention. In the Mediterranean, marble plates have been used to obtain recruitment estimates of red coral (*Corallium rubrum*), with excellent results in some Marine Protected Areas (Bramanti *et al.* 2005; Santangelo *et al.* 2012). Nevertheless, plates as a new colonization surface may favor other organisms not objective (Santangelo *et al.* 2012). Therefore, the aim of the present study was to test the usefulness of artificial plates placed in the rocky bottom as a technique to recruit larvae of *A. calycularis* for conservation programs.

## 7.2.2 MATERIAL AND METHODS

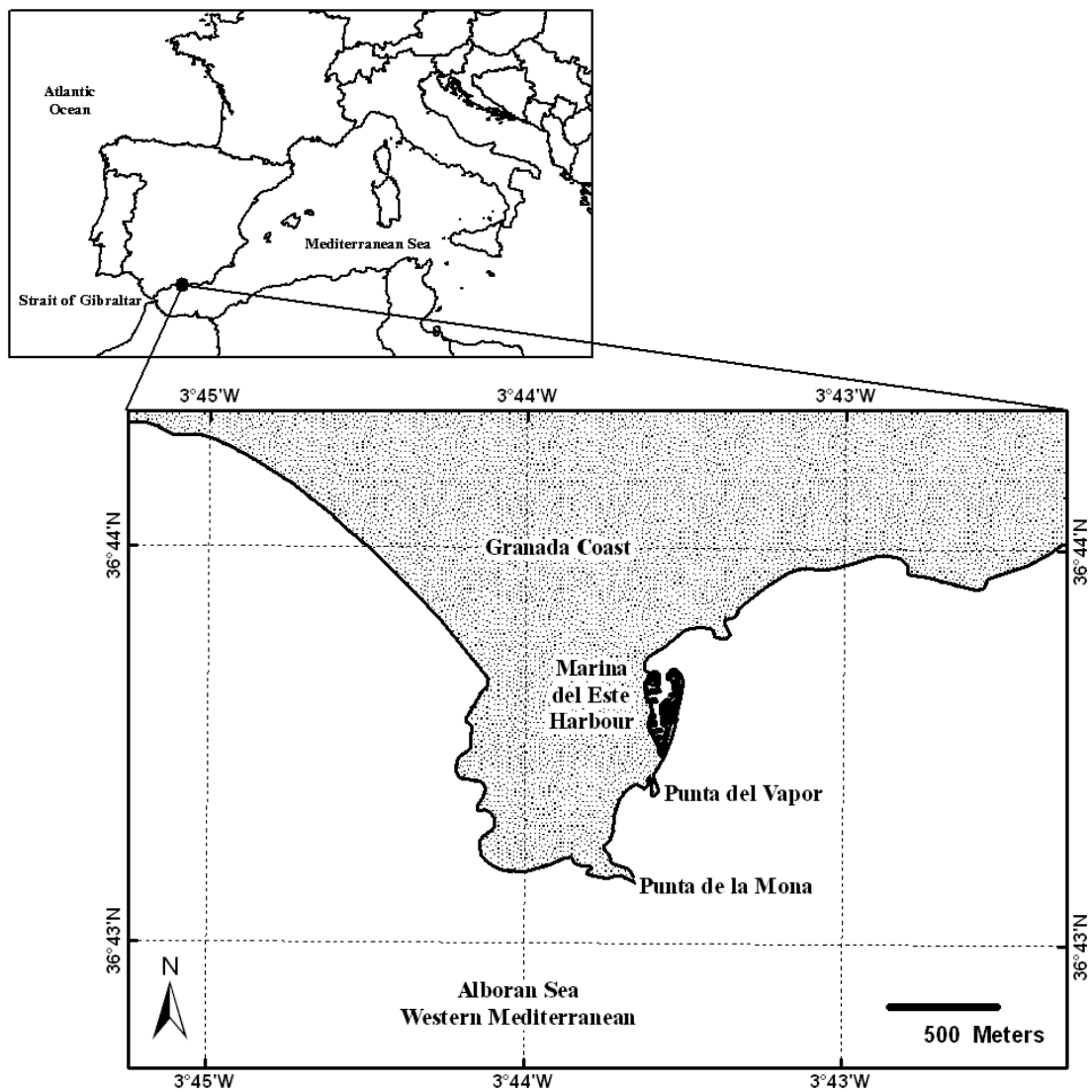
We conducted the experiment on the Granada coast, South of Iberian Peninsula, between April 2014 and June 2015 (Figure 7.5). This area is a biodiversity hotspot in the region (Templado 2011), and *Astroides calycularis* is the dominant coral between 0-12 metres depth (Terrón-Sigler *et al.* 2015-Section 6). The experiment was carried out in Marina del Este beach. One area was selected: Punta del Vapor –PV– (36°43'22''N, 3°43'35''W), between 5 and 8 metres depth, and the area is exposed to East windstorms.

To capture larvae, plates with carbonate composition of 10 x 10 centimeters were selected. The plates were installed in a submarine rocky wall where orange coral was very abundant. A total of thirty three plates were installed in April of 2014, just two or three months before the species spawning (Terrón-Sigler *et al.* 2011-Section 3).

Plates were monitored after four (T1), eight (T2) and fourteen months (T3) (Appendix III). The methodology consisted of monitoring all plates installed and taking a picture of each one. An underwater camera Olympus C-8080 was used. Plate analyses were carried out by CPCe software (Kohler *et al.* 2006). CPCe software consists of specifying a digital image, defining a frame border, overlaying random points (on this study case there were 20 points), identifying the species and/or substrate-type lying beneath each of the random points, and saving the data to file. After the images have been processed, the data can be automatically assembled into Excel spreadsheets for statistical analysis.

Temporal variability in total coverage, total number of species (S) and Shannon-Wiener diversity ( $H'$ ) (Shannon & Weaver, 1963) of assemblages settled on experimental plates was analysed using one-way repeated measures analysis of variance (RM-ANOVA). Mauchly's test of sphericity was used to test the assumption that variances of the differences between all possible pairs of groups are equal (data were

transformed when necessary). The factor “Time” has three levels (T1, T2 and T3). From 33 plates initially allocated, only 18 remained until the end of the monitoring period (14 months). Therefore, 18 replicates were used in the statistical analyses. The same design was considered to test any significant difference in assemblage composition by means of RM-permutational analysis of variance (RM-PERMANOVA) using binomial deviance dissimilarity. A permutational analysis of multivariate dispersions (PERMDISP) was carried out to test differences in the variation of the assemblages associated and time. Pairwise comparisons with Bonferroni correction were carried out in the case of significant differences with RM-ANOVA/PERMANOVA. All these analyses were performed using IBM SPSS Statistics software (Version 22) and PRIMER6 software (complete with PERMANOVA+ package) (Clarke & Gorley 2006).



**Figure 7.5:** Map of the study site showing Granada coast (Andalusia; Spain) and Punta del Vapor, where the plates were installed.

### 7.2.3 RESULTS

During the monitoring period, the plates have been covered by a diverse community made up of Chlorophyta, Rhodophyta, Phaeophyta, Porifera, Cnidaria, Echinodermata, Arthropoda, Mollusca and Chordata (Asciacea) (Figure 7.6). A total of nineteen taxa were present, but not all of them were present in the CPCe random analyses (Table 7.3).

**Table 7.3.** Mean coverage (%) and Standard deviation of assemblages settled on experimental plates.

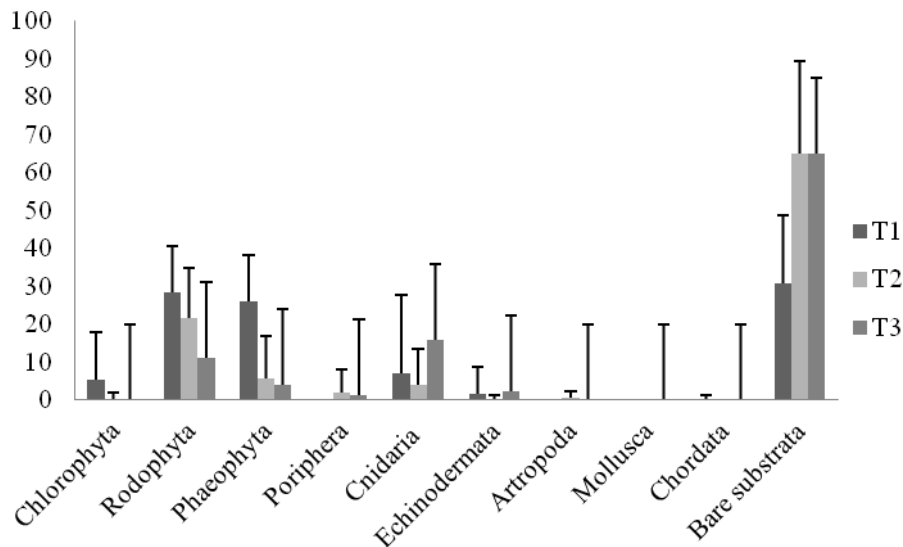
MONITORING DATE	After 4 months		After 8 months		After 14 months	
	28/08/2014		09/12/2014		18/06/2015	
	N=18		N=21		N=20	
	Mean	SD	Mean	SD	Mean	SD
<b>CHLOROPHYTA</b>						
Filament green seaweeds	5.28	12.66	0.48	1.50	0	0
<i>Ulva rigida</i>	0	0.00	0	0.00	0	0
<b>RHODOPHYTA</b>						
<i>Asparagopsis taxiformis</i>	0.28	1.18	3.1	10.06	2.5	7.86
<i>Corallina elongata</i>	0.28	1.18	0	0.00	0.25	1.12
<i>Lithophyllum incrustans</i>	23.06	13.08	18.57	12.56	8.5	15.05
<i>Peyssonnelia</i> sp.	5	12.13	0	0.00	0	0
<b>PHAEOPHYTA</b>						
<i>Pseudolithoderma adriaticum</i> *	0	0.00	0	0.00	0	0
Filament phaeophyta	26.11	12.19	5.71	11.10	4	7.88
<b>PORIFERA</b>						
<i>Cliona viridis</i>	0	0.00	2.14	5.82	1.25	2.75
<b>CNIDARIA</b>						
<i>Aglaophenia pluma</i>	7.22	20.60	4.05	9.44	12.75	23.87
<i>Clavularia crassa</i> *	0	0.00	0	0.00	0	0
<i>Sertularella</i> sp.	0	0.00	0	0.00	3.25	11.27
<b>ECHINODERMATA</b>						
<i>Arbacia lixula</i>	1.67	7.07	0.24	1.09	0	0
<i>Paracentrotus lividus</i> *	0	0.00	0	0.00	0	0
<i>Sphaerechinus granularis</i>	0	0.00	0	0.00	2.5	11.18
<b>ARTHROPODA</b>						
<i>Balanus</i> sp.	0	0.00	0.71	1.79	0	0
<b>MOLLUSCA</b>						
<i>Cirsotrema</i> sp.*	0	0.00	0	0.00	0	0
<i>Littorina</i> sp.*	0	0.00	0	0.00	0	0
<b>CHORDATA</b>						
<i>Diplosoma spongiforme</i>	0.28	1.18	0	0.00	0	0
<b>BARE SUSTRATA</b>						
Substrata	30.83	17.93	65	24.49	65	37.24
<b>Shannon-Wiener Index</b>						
	1.5	0.14	1.06	0.12	1.09	0.12

\* Taxa detected on plates but not in the CPCe analyses because any of twenty random points used to estimate coverage were allocated above.

In T1, plates were dominated by seaweeds with the 60 % of the surface covered, and being *Lithophyllum incrustans* and a filament phaeophyta the taxa more frequent (with coverage of 49%). The Cnidaria *Aglaophenia pluma* (Hydrozoan) obtained 7.22



% of coverage. Finally, the 30.83 % of the colonization surface was bare. Phaeophyta decrease towards 5.71 % coverage after T2 and *L. incrustans* was the dominant species (18.57%). *A. pluma* decreased until 4.05 % and bare surface increased until 65 %. On T3, all algae taxa achieved coverage of 15.25 % and *L. incrustans* remained as the most frequent species. *A. pluma* increased reach the 12.75 % cover and the bare surface remained at 65 % (see Figure 7.6).



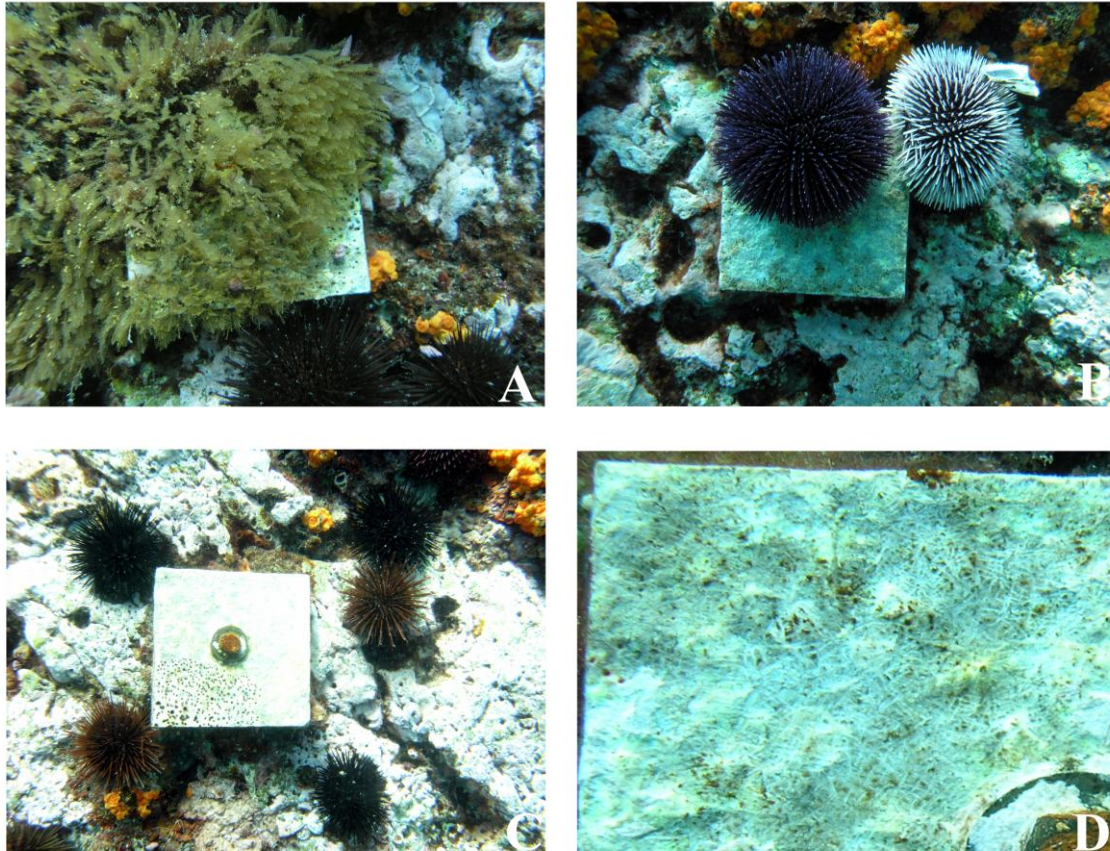
**Figure 7.6:** Mean coverage (%) and Standard deviation of different taxa recorded on experimental plates.

No orange coral larvae were detected on plates. It is important to take into account the high abundance of sea urchins on and around the plates while monitored (Figure 7.7), particularly the species *Paracentrotus lividus*, *Arbacia lixula*, and *Sphaerochinus granularis*. This high abundance has not been recorded by the CPCe analyses as a consequence of the mobility of these animals. Moreover, the action of sea urchins grazing was observed on the plates due to the marks of the Aristotle's lantern (Figure 7.7-D).

Shannon-Wiener Index was higher in T1 ( $1.5 \pm 0.14$ ) than T2 and T3 ( $1.06 \pm 0.12$  and  $1.09 \pm 0.12$ , respectively). RM-ANOVA analyses showed significant differences for coverage, species richness and Shannon diversity ( $p < 0.001$  for all variables) (Table 7.4). Moreover, pairwise comparison showed that  $T1 > (T2 = T3)$  for coverage and Shannon diversity; and  $(T1 > T3) = T2$  for species richness.

RM-PERMANOVA analyses showed significant differences in assemblages associated with plates for time ( $p < 0.0001$ ) (Table 7.5). Moreover, pair wise

comparisons demonstrated that T1 was different from T2 and T3 ( $p < 0.001$ ), but T2 and T3 were similar ( $p < 0.56$ ). PERMDISP results indicated that assemblages associated to the plates did not show a significant variation through time ( $F_{2,51}=2.22$ ;  $p=0.278$ ).



**Figure 7.7:** A: Plate with coverage of *Aglaophenia pluma*. B: Plate with two individuals of *Sphaerochinus granularis* grazing the surface. C: Plate flanked by three *Arbacia lixula* and two *Paracentrotus lividus* individuals. D: Detail of one plate with marks of sea urchin grazing by the aristotle's lantern.

**Table 7.4.** Result of one-way RM-ANOVA and pairwise comparison on coverage, species richness (S), and Shannon diversity (H') of community assemblage associated with plates measure from time (T1; T2; and T3). MS = mean square, df = degrees of freedom.

Source of variation	df	Coverage			S			H'		
		Pillai's Trace	F	p	Pillai's Trace	F	p	Pillai's Trace	F	p
Time	3	0.631	13.684	p<0.001	0.59	11.499	p<0.001	0.57	10.619	p<0.001
Residuals	51									
Mauchly's test	54		p<0.05			p<0.05			p<0.05	
Transformation			None			None			None	
Pairwise comparison		T1≠T2		p<0.001	T1=T2		p=0.084	T1≠T2		p<0.005
		T1≠T3		p<0.005	T1≠T3		p<0.001	T1≠T3		p<0.001
		T2=T3		p=1	T2=T3		p=0.489	T2=T3		p=0.986
		T1>(T2=T3)			(T1>T3)=T2			T1>(T2=T3)		

**Table 7.5.** Results of PERMANOVA analysis for community assemblages associated with plates, based on Bray-Curtis dissimilarities of square root transformed data. MS = mean square, df = degrees of freedom.

Source of variation	Df	MS	F	P
Time	2	244.14	8.0964	0.0001
Residual	51	30.154		
Total	53			
Transformation		Sqrt		
Pairwise comparison		T1≠T2		p<0.001
		T1≠T3		p<0.001
		T2=T3		p=0.056

## 7.2.4 DISCUSSION

The present study describes the community composition of experimental plates during two spawning periods of *Astroides calycularis*, to test whether these artificial substrata may be useful as a transplantation technique.

Nevertheless, it is necessary to take into account that in the Mediterranean coasts the sea urchins are the main benthic herbivore invertebrates. Among them, *Paracentrotus lividus* and *Arbacia lixula* are the dominant sea urchins in this temperate sea (Frantzis & Grémare 1992; Benedetti-Cecchi & Cinelli 1995). These species have important effects on the structure and dynamics of assemblages of species in coastal habitats (Valentine & Heck 1991; Chelazzi *et al.* 1997; Coma *et al.* 2011). They are often considered strong inter-actors because changes in their relative abundance can dramatically modify the composition and structure of marine communities (Paine 1992; Sala & Graham 2002), sometimes causing severe overgrazing events with complete remove of algal communities (Valentine & Heck 1999). In those areas where there are high densities of sea urchins, these can remove erect macrophytes, producing the 'barren habitat' dominated by encrusting corallines (e.g. Scheibling & Stephenson

1984). Moreover, the higher presence of sea urchins species may lean to one or other side of the community assemblage.

Marine assemblages of rocky bottoms have widely been studied in our study area, where *Paracentrotus lividus* and *Arbacia lixula* are very common in shallow waters (e.g. Ocaña *et al.* 2000). Moreover, these species cohabit with coralline algae *L. incrustans* together with the genus *Peyssonnelia* (in sheltered conditions), and *Asparagopsis* as erect algae (Cebrián & Ballesteros 2004). These genera were present on the plates studied here. Nevertheless, *A. lixula* had a strong influence on the abundance of several groups of algae, and the most drastic effects is on encrusting coralline and filamentous algae (Boudouresque & Verlaque 2001; Privitera *et al.* 2008). Although, studies about the *A. lixula* gut content showed that the filamentous algae are undigested by the species (Wangensteen *et al.* 2011), and the high presence in their gut proves its influence on benthic assemblages. Moreover, the genus *Lithophyllum* was one of the more abundant in the gut content of *A. lixula* on the South-eastern Mediterranean populations (Wangensteen *et al.* 2011). This feeding behaviour may explain the high presence and abundance of the seaweeds filaments (both Chlorophyta and Phaeophyta) and *L. incrustans* at the beginning of the study and their lower presence at the end of the monitoring period.

On the other hand, *Paracentrotus lividus* has preference by erect seaweeds and does not have effects on articulate coralline algae (Frantzis *et al.* 1988). Similarly, other algae species are affected by sea urchin grazing, similarly to the present study, the abundance of *Peyssonnelia* spp. was affected by the two species of sea urchins (*P. lividus* and *Arbacia lixula*) in several studies carried out in the Mediterranean (Bulleri *et al.* 1999; Wangensteen *et al.* 2011). Therefore, sea urchin grazing behaviour has affected the diversity of assemblages settled on experimental plates in a similar way that it has been previously demonstrated on the Mediterranean coasts (Bulleri *et al.* 2002; Privitera *et al.* 2008).

Plates could be unfavourable to orange coral larvae settlement, surely due to the sea urchins grazing. Additionally, sea urchin may prey on corals, especially *A. lixula* and *P. lividus* have showed presence of *Cladocora caespitosa* tissue in their gut content (Wangensteen *et al.* 2011). On the other hand, sea urchin may too contribute to the expansion of an invasive coral in the Mediterranean because sea urchins grazing activity indirectly facilitated the expansion of *Oculina patagonica* (Coma *et al.* 2011). Here the gut content of sea urchins were no analysed to test whether these species prey or not on *Astroides calycularis*, therefore further studies on this aspect are needed.

Sea urchin grazing also has an impact on many benthic invertebrates (Fletcher 1987; Andrew & Underwood 1993). Nevertheless, some plates were colonized by the Hydrozoan *Aglaophenia pluma* and no grazing activity was recorded on them. *A. pluma* is a very common species on the first 5 metres depth on Granada coast (Ocaña *et al.*

2000). Indeed, the plates with *A. pluma* showed a similar abundance and did not seem that they were affected by sea urchin grazing.

The results described herein highlight some clues to take into account to future studies on restoration of the orange coral. Plates have been used as substrata settlement favour to red coral in Mediterranean Sea (Bramanti *et al.* 2005; 2007), but the lower recruitment in some areas could be explained by local factors, such as inter-specific competition (Giannini *et al.* 2003) or predation (Rius & Zabala, 2008), and it may have a direct impact on coral settlers (Santangelo *et al.* 2012). No settlement of *Astroides calycularis* larvae was found in the present study, probably as a consequence of sea urchins plates grazing. Future studies must properly address the impact of sea urchin on settlement of *A. calycularis* and the potential use of plates in cage-excluding experiments as a conservational tool for orange coral.





An underwater photograph of a coral reef. The water is clear and blue. In the foreground, there are large, vibrant yellow and orange coral structures. In the background, a dark, rocky reef wall is visible. A single fish is swimming in the upper left, and a small group of fish is visible in the middle ground. The text '8. GENERAL DISCUSSION' is overlaid on a semi-transparent grey bar across the middle of the image.

## 8. GENERAL DISCUSSION

Rocky shores of the Acantilados de Maro-Cerro Gordo Natural Park (Málaga-Granada).

Author: Terrón-Sigler A.



## 8.1 *ASTROIDES CALYCULARIS* (PALLAS 1766)

*Madrepora calycularis*, Pallas 1766, Elench, zooph., p. 318; Linné 1767, Syst. nat., éd. 12, p. 1277; Cavolini 1785, Mem. per scrivere alla storia de' Polipi marittimi, p. 48, pl. 5, fig. 1 et 2; Esper 1791 (in Zibrowius 1980).

*Caryophyllia calycularis*, Lamarck 1816, Hist. des anim. sans vert., t. II, p. 226; Risso 1826 (in Zibrowius 1980); Milne Edwards 1849, Atlas du grand édit. du Règne Anim. de Cuvier, Zooph., pl. 83, fig. 2-2.

*Astroïdes lateus*, Quoy et Gaimard 1827 (in Zibrowius 1980).

*Astroïdes calycularis*, Blainville 1830, Dict. des se. nnt., t. LX, p. 332; M. Sars 1857; Milne Edwards 1860, Histoire Naturelle des Corallaires ou Polypes Proprement Dist., p. 131, Pl. E2, fig 1-1<sup>a</sup>; G. Cuvier 1869, Les Vers et Les Zoophytes Descrits et figurés d'après la classification, p. 70, fig 9-9a; Lacaze-Duthiers 1893 (in Zibrowius 1980); Carus, 1885 (in Zibrowius 1980); Döderlein 1913 (in Zibrowius 1980); Joubin, 1928 (in Zibrowius 1980); Riedl, 1956 (in Zibrowius 1980); Abel, 1959 (in Zibrowius 1980); Rossi, 1961 (in Zibrowius 1980); Pax and Müller, 1962 (in Zibrowius 1980); Schmidt, 1972 (in Zibrowius 1980); Zibrowius and Grieshaber, 1977; Zibrowius, 1978; 1980.

*Astrea calycularis*, Quoy et Gaimard 1833, Voy. de l'Astrolabe, Zooph., p. 200, pl. 15, fig. 16-23.

*Cladocora calycularis*, Ehrenberg 1834, Corall. des roth. Meeres. p. 68.

*Madrepora calycularis*, Delle Chiaje 1844, Anim. senza vert, del regno di Napoli, Pl. 153, fig. 7.

*Astroïtis calycularis*, Dana 1846, Zooph., p. 406.

## 8.2 Historical description

Throughout the history of the taxonomy of orange coral (*Astroïdes calycularis*) there have been several authors who have described the species, establishing them in different genera and/or families within the Zoophytes group (Kingdom of the Animals-Plants). Thus, the binomial nomenclature established by Linnaeus has changed to lock the species in the Order and Family where it stands today (Cnidaria: Anthozoa: Hexacorallaria: Scleractinia: Dendrophylliidae).

Linnaeus in 1758 makes a simple description of a *Madrepora* from fossil; “Colonial madrepora with starry concave calyxes and joined together” (page 796 of his work “*Systema Naturae*”, 1767) with this species called *Madrepora astroïtes* (madrepora moonstone). However, it was Peter Simon Pallas, zoologist and botanist of Berlin, who first described in 1766 in his “*Elenchus Zoophytorum*” the *Madrepora calycularis*. In general, Pallas described the species as: “Aggregate *Madrepora* (colonial) meeting in cylindrical shape, star-shaped crater eroded in the centre; mass without form, meeting in cylinders with similar thickness pumice; short calyxes that are tidy with some separation. With stars shaped crater terminals; the crown of stars tapering base. Skeleton has a greyish-white colour. Species located on the Mediterranean”. From this description, one can ascertain that the species that Pallas described came from Italy, where *Astroïdes calycularis* presents calyx’s circular and separate colonies, not present in the Alboran Sea (western Mediterranean).

Cavolini (1785) describes again the species as *Madrepora calycularis*. Filippo Cavolini, also known as Caulinus, was a great Italian scholar of natural history. In his “*Memorie per Servire alla Storia de Polipi Marini*”, he notes that “*Madrepora caliculare*” is common and abundant in Italian waters, can easily be seen in the entrance of the cave Lazzereto (Italy) up off the water’s surface, and can be differentiated by their bright colour “scarlet”. Cavolini also mentions that Italian fishermen call this species “*pietra preziosa*”. On page 75 of his book, Cavolini represents a living colony of *Madrepora caliculare*, the skeleton, where it can observe that colonies calyxes have circular structure. Moreover, polyps show six ovaries under microscopic observation. The latter is very difficult to differentiate (Figure 8.1).

In the early XIX century, Lamarck (1816) describes *Caryophyllia calycularis* as a madrepora inhabiting the Mediterranean with cylindrical, straight and short calyxes, with starry excavations and a pronounced centre (“*Histoire Naturelle des Animaux Sans Vertebrees*”, vol. 2). Blainville (1834) in his “*Manuel D`Actinologie ou Zoophytologie*” describes the genus *Astrée* or *Astreae*, as one consisting of short animals, roughly cylindrical, provided with a rounded mouth amid a disc covered with typically quite short and a few tentacles; contained in shallow calyxes, fitted radial septa and starry formed polyps; usually with an encrusting and sub-pipe structure. This one is further divided into several divisions with different calyx characteristics that are well defined. The first one has “round calyxes starry, often unrelated or non-contiguous”. Blainville gave this division one species that provides for “a fairly common species in the Mediterranean, and whose colony consists of round calyxes, often separated from each other, but sometimes together and “alveolus” shaped. It refers firstly to *Astroides calycularis* as genus and species (Figure 8.2).

During the middle of the XIX century, Milne-Edwards (1860) quotes the species with taxonomic characters quite well in Volume III of his work “*Historie Naturele ou des Coralliaires polypes proprement Dist*”, stating that the species has “four complete cycles, but the fourth rudimentary; septa not overflowing, very high; columella highly developed; widths calyxes, 7 or 8 mm; with a depth of 4 mm. Widespread polyps and are yellow-orange; lives in the Mediterranean” (Figure 8.3). A little later, George Cuvier (1869) in “*Les Zoophytes*” also describes *Astrea (Astroides) calycularis* as representing the outer surface of the colony and individual polyps (Figure 8.4).

In the XX century, Oskar Carlgren (1900) also cites the species and in 1940 provides some data on its cnidocysts composition. But “*Les Scléactiniaires de la Méditerranée et de l’Atlantique nord-oriental*” of Helmut Zibrowius (1980) is perhaps the most important work in this century about the Mediterranean Scleractinian corals. Zibrowius explains that the original description of *Madrepora calycularis* is based on material collected in the Mediterranean (unspecified), which has been lost. Moreover, the description of the species as *Astroides luteus* (Quoy & Gaimard 1827) is based on

samples from southern Spain. The species was in shallow waters near the port of Algeciras Bay (in Getares, Spain). Zibrowius says that this material should be in the National Museum of Natural History.

Thus, Zibrowius (1980) describes all the possible morphologies of colonies of the species, and, in general, *Astroides calycularis* is described as a colonial species with varied looks and extreme forms, which may present calyxes separate or joined by the *coenosteum* as roughly plentiful. In massive colonies, new calyxes are added to the periphery or are interspersed with existing ones. The colonies with separated calyxes make ramifications. The latter are composed of calyxes sub-cylindrical that can reach a height of 40 mm or more. Generally, the porous surface is fairly uniform.

Colonies can also be formed by calyxes attached on top; the cups are not too closing together and circular. They may also have cups that are closer together and polygonal. The species has great variability in the cups; in most cases the diameter of the cups are circular, lower than 10 mm, whereas polygonal cups are between 12 and 15 mm. The depth of the cup (groove around the columella and distance along the columella calyx) varies. Almost always the columella is well developed and fluffy and clearly stands out, is sometimes hemispheric and sometimes can be 2-3 times higher than it is wide. Sometimes the base of the columella is slightly narrow and is satisfied that the final part in turn tends to have a globular shape.

The species has 48 septa fairly regularly, but their development varies greatly from one colony to another, and even within the same colony. Often there are 12 septa (S1 and S2), which bind to the columella in the bottom of the cup. S3 is much closer and tends to join the S2. Finally, S4 is small, but more or less inclined to S3. In other calyxes, the bond between the S3 is broader, and S2 is more regular than S1 in a place near the columella.

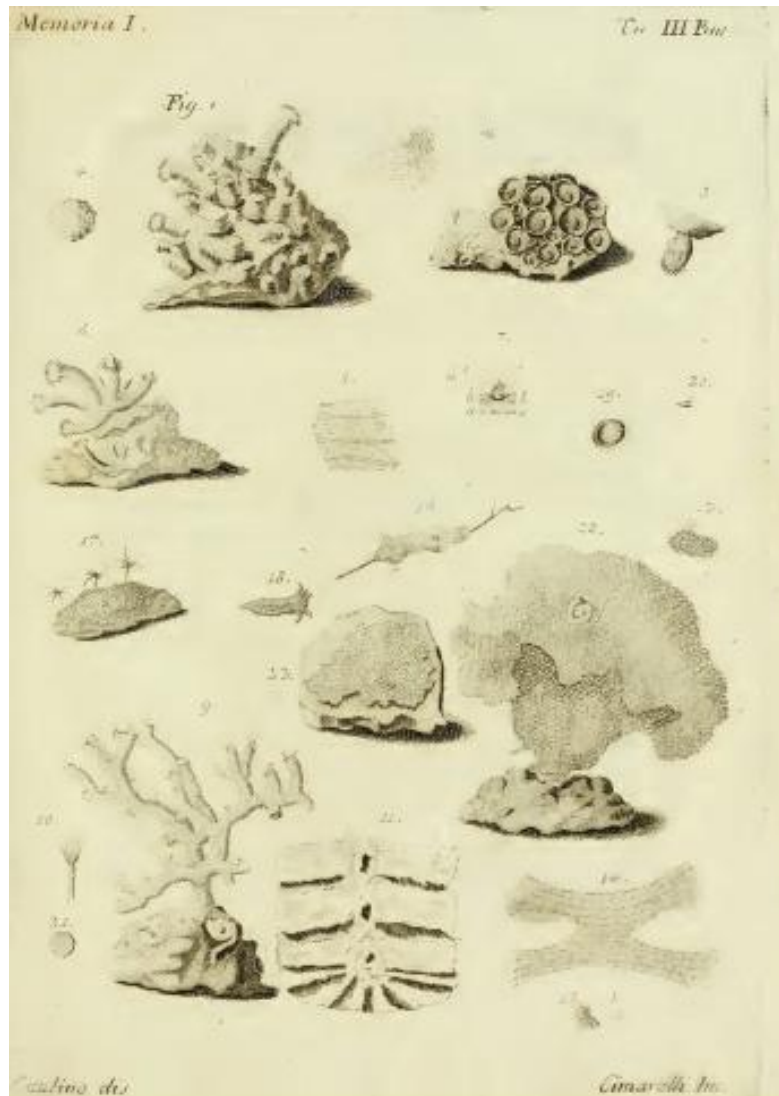
López-González (1993), in a study of Anthozoan taxonomy in the Strait of Gibraltar and nearby areas, describes the species from material that examined both the western part of the southern Iberian Peninsula and Ceuta (North Africa). This author describes massive colonies, which sometimes can be more or less arborescent. The calyx may be more or less together and tightened, as existing forms that have spaces between cups occupied by a spongy mass (*coenosteum*) separating some polyps of either 1 or 2 mm. In this case, they are circular in section cups. At other times, the polyps are located close together, adopting a polygonal section in which *coenosteum* is reduced to one line. Note that the diameter of the cups reaches 9 mm in those with 9-10 mm circular polygonal section which are presented. The species presents four cycles of septa; the first two are very similar in size and grow to the bottom of the cavity with its concave axial edge (joining the end with the columella). S3 and S4 are much less developed, and S4 is not always complete; when it is, it grows inclined to join S3. Septa do not have *pali*, and the columella is spongy and has great development.

This last description is very similar to that made by Zibrowius in 1980. However, already in the XXI century, Goffredo *et al.* (2011a), provides new information on “colony and polyp biometry and size structure in the orange coral *Astroides calycularis*”. These authors demonstrate that colony length, width, mass and number of polyps all showed marked relationships with colony area, confirming that this parameter is a powerful representation of colony size. Also, they find that the smaller size of peripheral polyps compared to central ones suggests that polyp budding occurs preferentially at the outskirts of the colonies. Moreover, larger colonies had polyps with smaller sizes than small and medium colonies, due to an over-representation of the size class containing polyp size at sexual maturity. Therefore, large colonies may invest energy in increasing polyp size up to the size at sexual maturity, rather than increasing the size of already mature polyps.

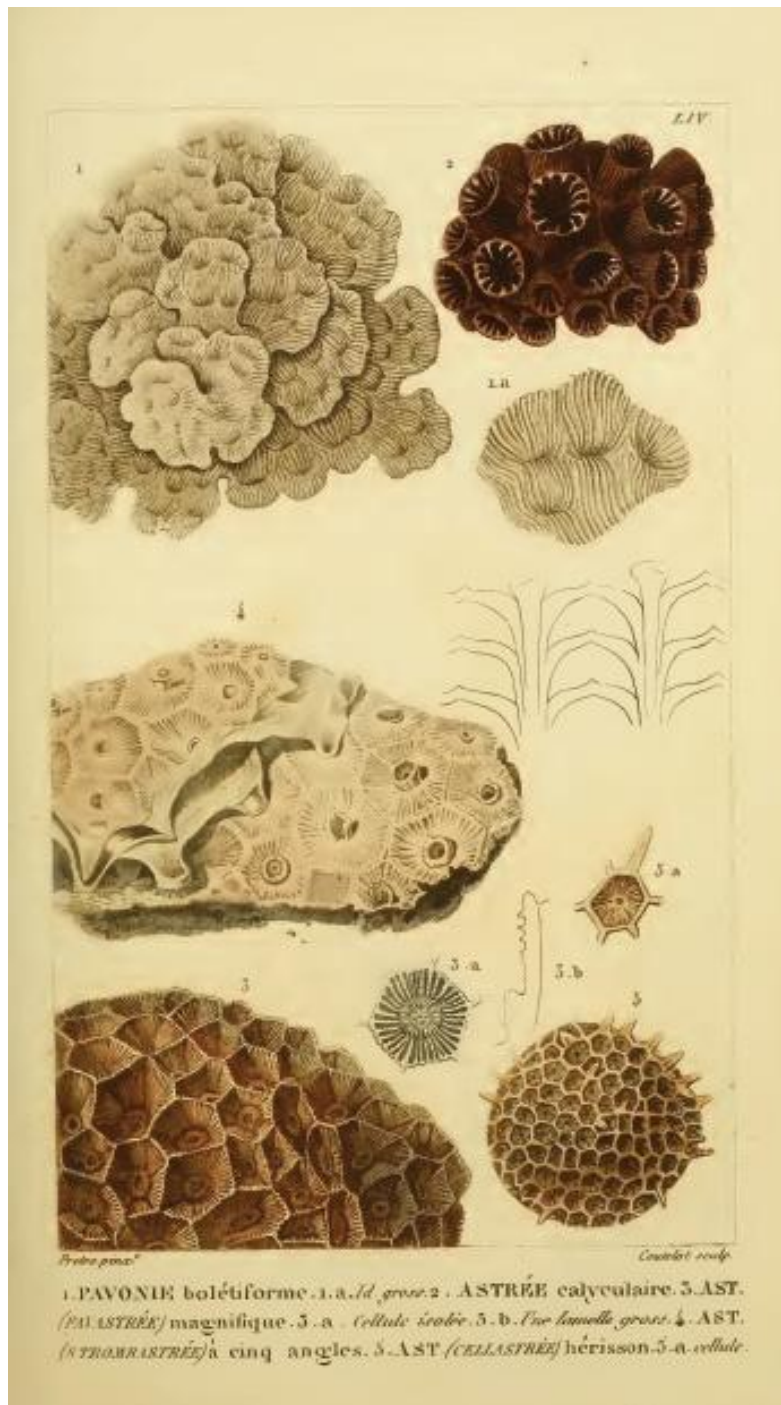
Lastly, it is appropriate to mention that during the bibliography search that has been reviewed in this study, many works of the authors discussed above and that describe the species acknowledge an author who is one of the most important of the centuries XVII-XVIII. It is Ferrante Imperato, a distinguished pharmaceutical-chemist and Neapolitan naturalist which Milne-Edwards in the first volume of his “*Historie Naturele des Coralliaires ou polypes proprement Dist*” from 1860 cites in his historical introduction. At that time, the most important work was definitely “*L’Histoire naturelle de la mer*”, which was published in 1599 by Ferrante Imperato and deals with the diversity of minerals, gems and other curiosities, with a variety of plants and animals until then undescribed. Ferrante Imperato could be the first to cite and describe the madrepora *Astroides calycularis*, due to Milne-Edwards cites that several species are described in the work of Ferrante Imperato under the name of corals and polyps, among which recognise a *Pocillopora*, *Stylophora digitata*, *Amphihelia oculata*, *Cladocora caespitosa*, *A. calycularis* and *Dendrophyllia ramea*. Furthermore, the consulted work of Cavolini (1785), “*Memorie per Servire Alla Storia de Polipi Marini*”, also named L’IMPERATO, describes a madrepora with spherical skeleton. Blainville (1834) also notes that it is one of the most interesting works on the natural history of Zoophytes where many new observations in animals, including madrepores and stony corals, are cited.

After an exhaustive search, it has been possible to consult the work “*Dell’Historia Naturale Ferrante Imperato Napolitano*” (1599). Page 717 notes a colonial madrepora having a common birth (will refer to each coralitos) and spongy tissue with a shared concavity for prominence in the centre (columella); and a star-shaped polyp of small size and of similar thickness from beginning to end, wrinkled, which presents a strong orange colour freshly extracted of the sea that will blacken as time passes out of the water. On page 721, Ferrante Imperato represents a sheet skeleton

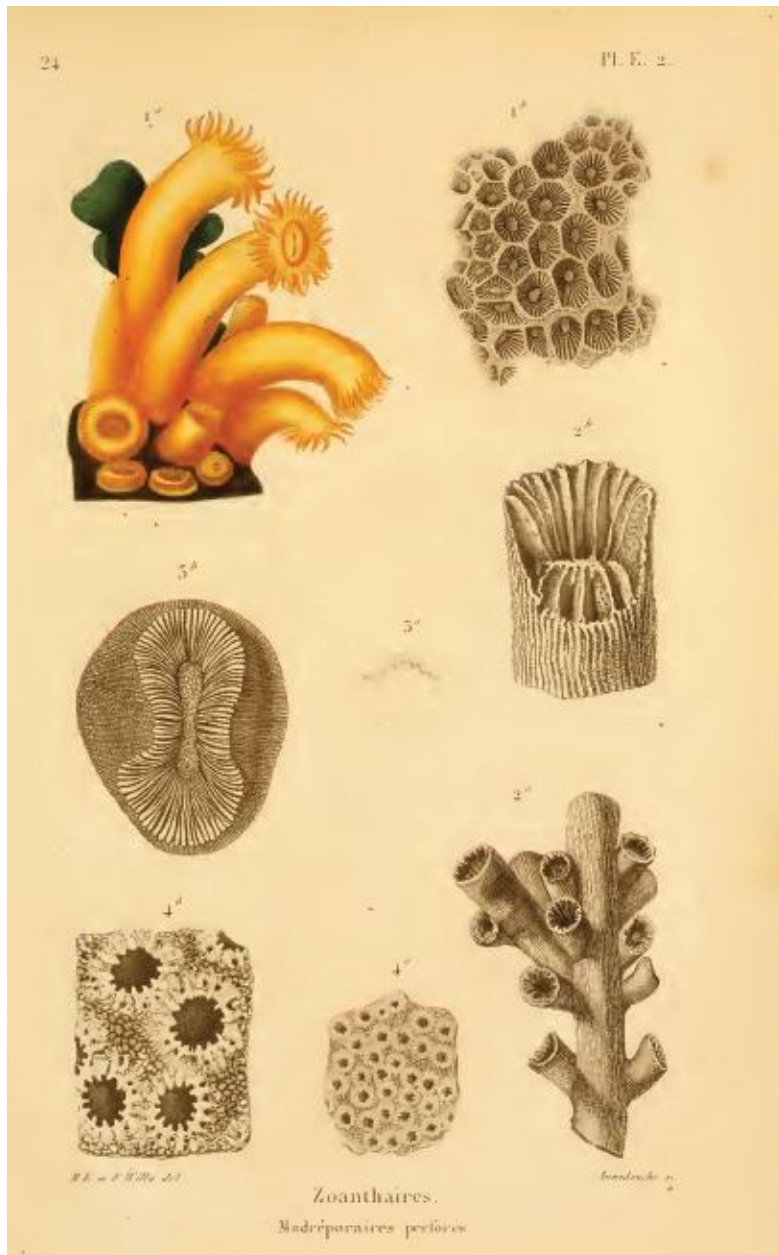
of madrepora (Figure 8.5), recalling the shape and structure representing the colonies of the eastern part of the geographical distribution of *Astroides calycularis*.



**Figure 8.1:** Plate exposed by Cavolini in 1785 in his work “*Memorie per Servire alla Storia de Polipi Marini*” (pág 74, fig. 1 y 2).

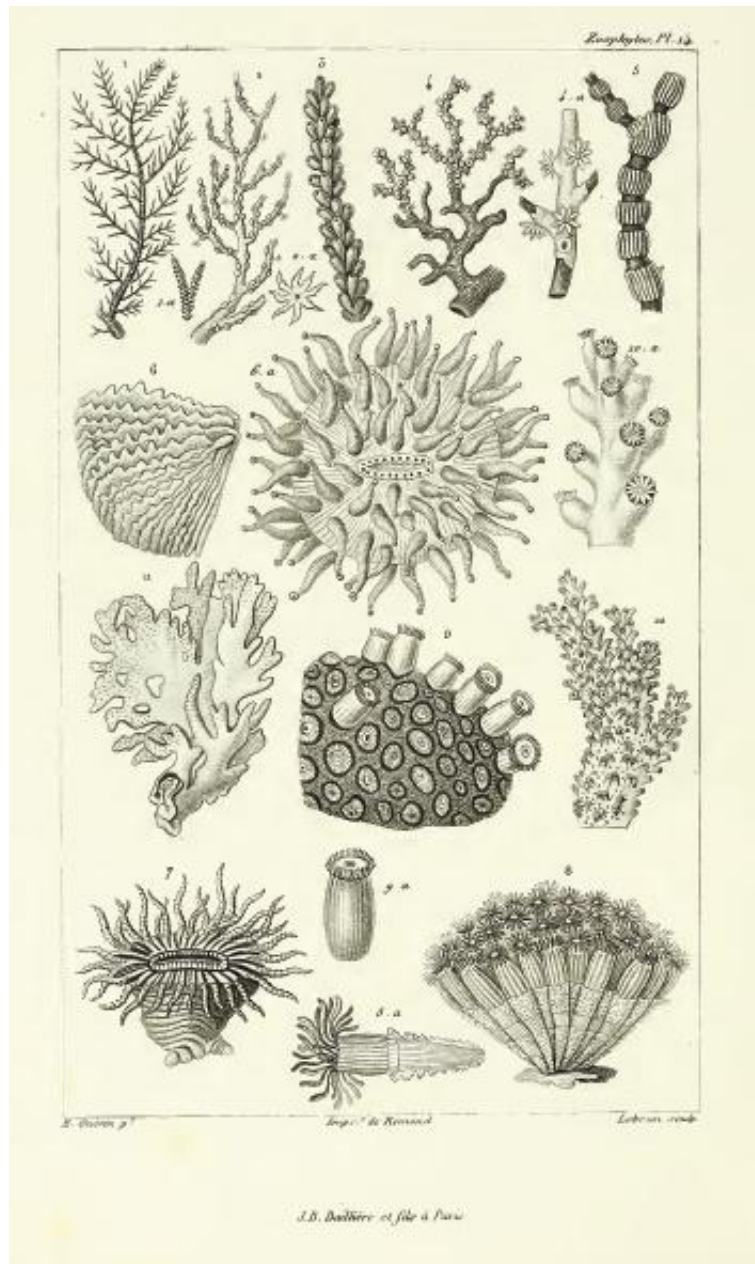


**Figure 8.2:** Plate 54 of the work “Manuel D'Actinologie ou de Zoophytologie” by Blainville 1834 (fig. 2).



**Figure 8.3:** Figure 1a, *Astroides calycularis* showing the polyps; figure 1b, skeleton. Plate of Milne Edwards (1857), “*Histoire Naturelle des Coralliaires Polypes Proprement Dits*”





**Figure 8.4:** George Cuvier in 1869 showed in his work “*Les Zoophytes*” the external surface of the colony of *Astroides calycularis* (fig. 9) and an isolate polyp (fig. 9a).





**Figure 8.5:** Madrepora showed by Ferrante Imperato in his work “*Dell’Historia Naturale*” published in 1599.

## 8.3 BIOLOGY AND ECOLOGY

### 8.3.1 Reproductive strategy

Most scleractinian corals are hermaphrodite, while gonochoric species are less than 25 %. However, reproductive strategy (broadcast or brooding) can vary within the species (Kruger & Schleyer 1998), or even populations that may be broadcast or brooding in different localities, as well as have different spawning and/or planulation season (e.g. Fautin 2002). Reproductive cycle of *Astroides calycularis* (gamete development in relation to environmental parameters, planulation timing, size at sexual maturity, fecundity, and sex ratio) is well studied in the Mediterranean Sea (Goffredo *et al.* 2010; 2011b; Casado-Amezúa *et al.* 2013). It is gonochoric and brooding species which has little difference on planulation release along geographic populations, surely due to temperature. Nevertheless, there were not studies about differences within populations on some aspect of their reproductive strategy, which could be essential to future conservation and management plans. The present work highlights intra-specific differences on populations from Northern of Alborán Sea (see Section 3), where there are significant differences on sex of the colonies depending on depth. Female colonies are present in shallow waters (between 0-5 metres depth) while male colonies are present deeper. Therefore, both field observations and experimental data indicated a

sexual segregation on space. These results are very important to take into account when a management tool was implemented such as reintroduction or reinforcement of populations. In this sense, it would be highly recommendable to collect colonies from different depths in order to guarantee a balanced sex-ratio.

### 8.3.2 Growth rate

Growth rate, demographic and age determination provide crucial information to species population dynamics. Moreover, these parameters are indispensable for future management and conservation plans (see Marchal *et al.* 2004). Nevertheless, this information has been widely studied in hermatypic corals species from tropical seas, as a consequence of the occurrence of the banding pattern (e.g. Knuston *et al.* 1972; Barnes & Lough 1993). However, this banding pattern has been also found in *Balanophyllia europaea* (Goffredo *et al.* 2004) and *Cladocora caespitosa* (Kersting & Linares 2012) both zooxanthellate species from Mediterranean Sea. These corals deposit two bands per year, a high density band in winter and a low density band in summer (e.g. Peirano *et al.* 1999: 2005; Goffredo *et al.* 2004; Kersting & Linares 2012). Some experiments suggest that sea temperature is not the only factor determining the coral growth, but feeding could have an important relation, due to the winter zooplankton is more abundant and could explain the high-density band deposition (Rodolfo-Metalpa *et al.* 2008). In the Section 7, it has been analyzed the growth rate of *Astroides calycularis* colonies with different experimental treatments (transplantation; translocation; and control). Generally, all treatments obtained a high growth rate in the months between July and January and lower between February and July. Nevertheless, the significant differences in growth rates were in the area, where the colonies that were in high hydrodynamics area grew more than those that were in the lower hydrodynamics area. Sebens *et al.* (2003) demonstrated that *Agaricia tenuifolia* grew more in high water flow habitats than which individuals that were on low water flow. Surely, it is because the high hydrodynamism support more food in the environment. These results would be in agreement with the results of the present study.

On the other hand, colonies of *Cladocora caespitosa* present a slow annual growth (Rodolfo-Metalpa *et al.* 2008). While the solitary coral *Balanophyllia europaea* is characterized for a rapid growth of younger polyps and a deceleration in the growth rate when the coral size is increasing (Goffredo *et al.* 2004). *Leptopsammia pruvoti*, an azooxanthellate solitary scleractinian coral, also reduces their growth rate with increasing age, whereas the growth rate have no relation with latitude (link with sea water temperature), due to *L. pruvoti* is more tolerant to temperature increases than *B. europaea*, probably because the absence of symbionts (Caroselli *et al.* 2012). Here, it is observed that the growth of *Astroides calycularis* is higher than previous data reported for *C. caespitosa* and *B. europaea*. However, there is no data about growth rate in

young colonies and over a long period, which it is crucial to understand the population dynamic of the orange coral. Thus, the age structure is also an important indicator to know the populations dynamic that must be studied.

### 8.3.3 Larval biology

Scleractinian corals are benthic organisms whose species with sexual reproduction have benthopelagic larvae as product, and present a free-living stage, as well as most benthic marine species (Pechenik 1999). Therefore, there is an inherent importance in the larval biology to know the species dispersion ability, and the potential recruitment in bare areas and capacity to maintain high levels of genetic diversity. Nevertheless, although there is basic information about larval biology of the species (Lacaze-Duthiers 1893; Duerdon 1902), there is little information about the response of the larvae to change in salinity, temperature, light availability and pollution (Richmond 1997). In this work, it is present a geographical distribution of *Astroides calycularis* in the North of the Alborán Sea (see Section 6), showing that the species are strictly located in MPAs or areas that are far from polluted urbanizations. Therefore, the larvae could have a little dispersion ability to colonize sites located outside of these MPAs or have a low tolerance to environmental factors, or a combination of them. Edmunds *et al.* (2001) studied the biology of larvae in *Porites astreoides* and concluded that strong increases in temperature significantly increased mortality larvae, and a similar trend was observed reducing the temperature, although this was not significant. Nevertheless, in the present study there are no significant geographical changes on temperature through the study area, hence temperature does not seem to be a relevant factor that would be influencing the larval dispersal at regional scale.

The tropical coral *Pocillopora damicornis* has free-living larvae that can settle and metamorphose after 103 days, allowing colonizing eastern Pacific waters from central Pacific populations (Richmond 1987). However, there is no data about the time that *Astroides calycularis* larvae may be living in the water column or sea bottom. Some authors suggest that this period is between two weeks and one month (Casado-Amezúa 2012). Nevertheless, field observations suggest that orange coral larvae are present more time in the water column or benthos. After the installation of artificial reef on November 2006 in Maro-Cerro Gordo Cliff Natural Park (Granada coast), in the first monitoring (April of 2007) appeared one *A. calycularis* colony, between 120 and 270 days after the spawning period of the species (Terrón-Sigler per. obs.). Therefore, more information must be obtained about the biology of the orange coral larvae and its interactions with abiotic and biotic factors, which will be useful to understand the dispersion ability, population's connectivity, and the role of MPAs as a reservoir of genetic diversity.

### 8.3.4 Environmental parameters

It has already been shown that environmental parameters as hydrodynamism and/or depth may have influence on certain aspects of the reproduction cycle, growth rate and larval biology (see above). Moreover, zooxanthellate corals depend on the illumination as well as water transparency and depth to growth (e.g. Wellington 1982; Barnes & Chalker 1990). In general, temperature, feeding, and light play an important role in the benthic suspension feeder's physiology (Ribes *et al.* 1999; Coma *et al.* 2000; 2002; Bavestrello *et al.* 2006). However, azooxanthellate corals have no light requirements, therefore their presence are controlled by other environmental parameters (depth, temperature, hydrodynamism, feed requirements, etc.) and/or inter-specific and intra-specific competition. *Astroides calycularis* inhabits dark and light substrata on vertical and horizontal rocky bottom between shallow waters to 50 meter depth (e.g. Zibrowius 1980; Ocaña *et al.* 2000; Kružić *et al.* 2002), but is typically found between 0 to 15 m depth in the North of Alborán Sea (see Section 6). Nevertheless, there is no information about different environmental requirements of the orange coral. Although this data could be crucial to know how the species respond to, for example, temperature changes, such as the consequences of global warming. Future studies should address the environmental requirements of the species. Thus, it allows to make inferences about its potential distribution.

### 8.3.5 *Astroides calycularis* as habitat

Coral reef, as marine ecosystem, host the most faunal diversity assemblages (Plaisance *et al.* 2011), however scleractinian corals by themselves can also host a high diverse associated community. In the Mediterranean Sea, *Cladocora caespitosa* is the sole scleractinian reef builder shallow water coral. This species is recognized as the main shallow water bioconstructors (see Kružić 2014), forming large colonies with considerable interstitial space that provides habitat for a high diversity of associated fauna (Koukouras *et al.* 1998). Although *Astroides calycularis* is not a reef builder, the high abundance in some places (more than 90% coverage; see Section 6) should be taken into account as habitat due to the faunal assemblage that host (see Section 4). This is one of the main outputs of the present work, enhancing the ecological value of the species due to the diverse fauna associated with the colonies. The results were surprising, hosting more than 80 different species, and indicating the ecological relevance of this Mediterranean scleractinian coral as habitat for many macrofaunal groups.

Invertebrates are the dominant group in association with corals habitat, and their relationship is very close due to food requirement, refuge use, and/or settlement

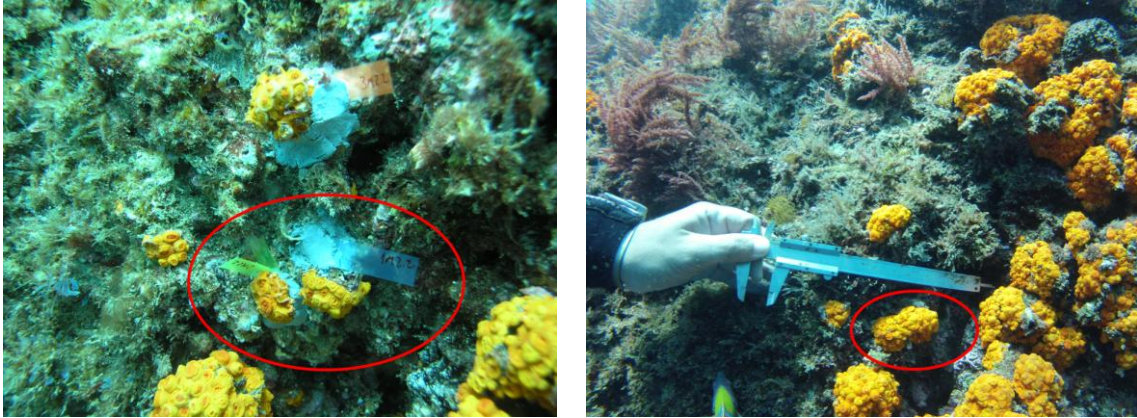
substrata (Stella *et al.* 2011). This relationship can be either obligate (must live on their coral host to survive) or facultative (may live on a coral host but does not have to for survival) (Castro 1976). The differences among one and other relationship can have ecological and/or conservational implications, due to with the disappearance of the host, facultative associates may persist, but obligate associates have greater risk of extinction (McKinney 1997). To increase the knowledge on *Astroides calycularis* colonies as habitat for macro-invertebrate, the interaction type between host and guest could be important to know the ecological relevance. Thus, the gut content of parts of the peracarids (arthropods) found in association with the species were analyzed (Section 4). In this way, were selected the commonest species and/or those that had a clearly orange colour. The results indicated that it is not a prey-predator relationship and many of the species found on the colonies of *A. calycularis* could use the coral as a refuge and/or source of detritus for feeding. In this sense, the polyps could produce the currents and facilitate siltation.

### 8.3.6 Coral behavior and predators

In regard to species behaviour, the present work contributes data about the intra-specific competition of *Astroides calycularis*. On the development of transplantation technique (Section 7), it was observed that two colonies from different treatments (control and transplantation) were fused after six months (Figure 8.6). This characteristic has not been described before in this species, but some studies have demonstrated that *Acropora* species may have different fusion types depending of the environmental stress. Thus, Nothdurft & Webb (2012) observed during a disturbance event the fusion of live *Acropora* branches dislodged with colonies no dislodge to complete soft tissue, but one species (dominant) overgrew the other (stressed species). Therefore, it seems that transplanted colony (stressed) was imbibed by control colony (without stress), however this is a hypothesis and more studies must be undertaken. This information might have important implications on the species biometry, behavior under stressful environment, and genetic studies.

Directly or indirectly, scleractinian corals are a food source for some species, either the tissues of the colony or the associated assemblages that may be prey for the predators (Halford *et al.* 2004). In tropical corals, many predators on colony tissues have been well documented. More than 130 fish consume live coral tissue (Cole *et al.* 2008) and can be highly specialized (Pratchett 2005). Moreover, it is well-known the attack of the Crown-of-thorns starfish (*Acanthaster planci*) on coral reef, that can exert enormous damage (e.g. Osborne *et al.* 2011). Nevertheless, it does not know any predator on the orange coral, only it was quoted that *Epitonium dendrophylliae* (Gastropoda: Mollusca) feeds on *Astroides calycularis* (Richter & Luque 2004). However, this taxon is a rare species in shallow waters (Gofas *et al.* 2011); therefore

this observation must be considered as punctual, and further studies have to be addressed to describe potential predators on orange corals and its response.



**Figure 8.6:** Left; inside the red circle two colonies with different treatments (control and transplantation). Right; Colonies fused after six months (inside the red circle).

## 8.4 THREATS

### 8.4.1 Human recreational activities

The main human recreational activities that interact with the benthic fauna are: SCUBA diving; snorkeling; and fishing. Indirectly, boating by anchoring action may have an important effect on biocenoses of the littoral (Medio & Ormond 1995; Leujak 2006). The western part of Granada coast is known for its clean water, rocky inlets, pleasant clima, and underwater macrofauna biodiversity, and therefore more than 25 years is an ideal place to dive and to practise nautical sports. In 1989 was declared the Maro-Cerro Gordo Cliffs Natural Park whose main objective is to preserve the biodiversity and regulate professional and recreational activities inside its area. Nevertheless, there are studies that demonstrate coral damage by divers and snorkellers in popular MPAs (Tilot *et al.* 2008), because the high coastal development near of these areas may be a threat, since a lot of recreational activities are associated therewith.

The studies about the effects of SCUBA divers or snorkellers on the marine biota have been addressed to show how the diver interacting with the species, habitats and/or ecosystems, whether the diver damages with the fins, and/or underwater camera, and/or other dive equipment (Pulfrich *et al.* 2003; Tratalos & Austin 2001; Uyarra & Côté 2007; Zakai & Chadwick-Furman 2002). However, the identification and quantification of threats will enable to take effective management measures. Therefore, our aim was the identification of those intrinsic aspects of the diver that may be



negatively interacting with the orange coral populations (Section 5). Among the factors inherent to divers, age of the divers and depth-time of dive as well as the minimum knowledge about the local fauna could be the main cause for the damage to the species. Moreover, it is showed the physical nature of the interaction between divers and colonies and the differences between not controlled areas and MPA. Thus, it is highlighting the value the importance of the awareness about the local biodiversity and relevant species, habitats and ecosystems of divers and others users.

#### 8.4.2 Alien species

The Mediterranean is considered to be a biodiversity hot-spot with a high level of endemism, and the Alborán Sea a regional hot-spot of biodiversity because of the coexistence of species from three marine biogeographic provinces (Mediterranean, Lusitanian and Mauritanian regions) and because of the endemic species restricted to this zone (Coll *et al.* 2010; Aguilar *et al.* 2011). Moreover, Atlantic waters usually describe an anticyclonic gyre that generates an upwelling of deep waters along the coasts of Málaga and Granada (southern Spain), this area is considered a hot spot biodiversity within Andalusian coast (Cebrián & Ballesteros 2004; Templado 2006). Nevertheless, due to its location, Alborán Sea is susceptible to harbor alien species, as a consequence of three possible entrances; the Suez Canal for species from the Red Sea; the Strait of Gibraltar for Atlantic species; and the Bosphorus–Dardanelles for Pontic species (Dulcic & Pallaoro, 2002). The number of exotic species that reach the Mediterranean increases year after year. 986 exotic species have been currently identified in the Mediterranean Sea including all the phyla (Zenetos *et al.*, 2012). Actually, during the period of this work, it has been found two new alien species for Andalusia region (Ibáñez-Yuste *et al.* 2012; Garrido *et al.* 2014). The Scleractinian coral *Oculina patagonica* was considered as a non native of Mediterranean Sea (Zibrowius 1983), but recently it is demonstrated the opposite (Leydet & Hellberg 2015). However, the species is having a rapid expansion along the Mediterranean coast line being considered as a main component of a natural community (Salomidi *et al.* 2013; Serrano *et al.* 2013; Rubio-Portillo *et al.* 2014). Therefore, the widespread pattern of the species may affect the orange coral *Astroides calycularis* by inter-specific competition. Therefore, baselines of alien and/or rapid expansion species distribution are crucial to know the behaviour pattern and future interactions. In the Section 5, it is reported a baseline distribution of *O. patagonica* populations on shallow waters along all Alborán Sea within the Andalusian region. Thus, marine managers have a benchmark of these populations. Indeed, over the last decade *O. patagonica* had not been observed in the hot-spot area Punta de la Mona (Granada coast), and as of June 2015 was found one colony at 6-7 metres depth which measures were 42 cm length and 40 cm width (pers. obs.).

On the other hand, it is being observed that the invasive alien algae *Asparagopsis taxiformis* may affect *Astroides calycularis* by friction when the species shows the spring bloom (Figure 8.7). These phenomena could explain punctual mortalities of *A. calycularis* along the coast line. Kersting *et al.* (2014) studied the interactions between two invasive macrophytes (*Lophocladia lallemandii* and *Caulerpa racemosa*) and the coral *Cladocora caespitosa*. Nevertheless, these authors did not observe any lethal or sublethal effects of these invasive algal species, perhaps, as a consequence of the toxic activity that *C. caespitosa* has. Future studies should be addressed to know the type of interactions between the orange corals and alien or invasive species, and the response of this endangered species.



**Figure 8.7:** View of one *Astroides calycularis* colony damaged by *Asparagopsis taxiformis* friction.

### 8.4.3 Global change

Global environmental changes have been identified as a major threat for scleractinian corals, which will not be able to physiologically adapt at the current rates of environmental changes (Walther *et al.* 2002; Hughes *et al.* 2003; Hoegh-Guldberg *et al.* 2007). Such changes may be more sensitive for the biological communities from temperate seas such as Mediterranean. The mankind global warming can be the reason for the local extinction of several populations (Garrabou *et al.* 2001; Bramantini *et al.* 2005; Santangelo *et al.* 2007), or low recruitment rate (Coma *et al.* 2001; 2004; Linares *et al.* 2007), or limit the dispersal of species due to the negative effects in their reproduction (Linares *et al.* 2008c). Indeed, an example could be the mass mortality



events occurred in the Mediterranean Sea (Gaino & Pronzato 1989; Bavestrello *et al.* 1994; Cerrano *et al.* 2000; Perez *et al.* 2000; Rodolfo), which are affecting an increasing number of marine species (Metalpa *et al.* 2000; Harvell *et al.* 2002; Garrabou *et al.* 2009; Vezzulli *et al.* 2010; Crisci *et al.* 2011; Kersting *et al.* 2013), and where engineer species were the most affected taxa (Garrabou *et al.* 2001; Coma *et al.* 2005), as well as suspension-feeding species (Días-Almeda *et al.* 2007). Negative effects on temperate Scleractinian corals have already been documented on *Cladocora caespitosa* and *Balanophyllia europaea* (Cerrano *et al.* 2000; Rodolfo-Metalpa *et al.* 2000). In fact, traces of mass mortality events in marine invertebrates have already been observed (Maldonado *et al.* 2010). But there are no studies about these events on the *Astroides calycularis*. Nevertheless, the results reported in the present study could be a reference point for detecting changes of the distribution and abundance of *A. calycularis* populations in the North of the Alborán Sea (see Section 6). However, additional experiments must be conducted to know the resilience of *A. calycularis* growth, reproduction and survival and environmental under environmental changes (focused on temperature).

On the other hand, it has been confirmed that global change rise of CO<sub>2</sub> and more recently, recognized effects of atmospheric CO<sub>2</sub> on ocean acidification will have even more profoundly detrimental long term effects on scleractinian corals (Veron *et al.* 2009). Ocean acidification may have negative effects on growth of scleractinian corals by decreasing of the calcification rate. For the Mediterranean Sea, it is expected a decreasing of 0.1 pH units owing to anthropogenic CO<sub>2</sub> (Touratier & Goyet 2011) and it has already been demonstrated detrimental effects on calcification rates of deep corals (Maier *et al.* 2012). Therefore, more studies including pH should be done to test any influence on *Astroides calycularis* growth rate in response to climate change scenarios.

## **8.5 MANAGEMENT**

### **8.5.1 Baseline**

Marine managers need baselines to take decisions about endangered species. However, few studies have been carried out in such a way as to provide the point of reference required for effective monitoring programs on corals (Tilot *et al.* 2008). The use of historical baselines allows researchers to assess possible degradations in wild populations and to guide conservation and management initiatives for good ecosystem conditions (Sala *et al.* 2012). For example, these degradations can come from human activities as the above described (human recreational activities and/or global change). Indeed, human activities have increasingly affected biodiversity in the Mediterranean Sea. Thus, in order to understand the effects of human impacts along the coastline,

monitoring should be a routine activity (Coll *et al.* 2010), but a reference point is necessary. In this way, the present work establishes the first baseline of the *Astroides calycularis* population from the North of Alborán Sea (Section 6). In fact, this is the first baseline of this species in the Mediterranean basin. This represents a point of references for the orange coral populations in the littoral zone particularly exposed to the impacts of coastal activities (0-10 metres depth) (Fraschetti *et al.* 2002). This baseline may be used by management administrators, scientists and MPA managers. Moreover, the knowledge of population dynamics through time and location is crucial in order to implement management measures on the conservation, protection and restoration of endangered habitats and ecosystems. In the future, monitoring stations must be well established on the littorals where the species threatens as well as on those places that can be used such as control areas (MPAs). Current monitoring stations are only focused in control areas (MPAs). In this context, international cooperation in the framework of pilot programs such as EMBOS (European Marine Biodiversity Observatory System- <http://www.embos.eu/>) would be of great interest to study endangered species in a long term. Finally, this baseline type must be implemented along the distribution of *Astroides calycularis* populations to test or monitor potential impacts in other areas and their consequences.

### 8.5.2 Marine Protected Areas (MPAs)

Perhaps, marine protected areas (MPAs) are the best management tool for the conservation of marine biota. MPAs may benefit corals by controlling human extractive activities and directly prevent destructive fishing practices, anchor damage and/or urbanization (Selig & Bruno 2010). However, several studies in tropical areas have shown that MPAs do not prevent coral loss and other forms of reef degradation (Kramer & Heck 2007; McClanahan 2008). Indeed, there are many MPAs or protected areas, but few are effective (Sheppard *et al.* 2009). There are many controversies about the effectiveness of the MPAs. Several authors assert that the effectiveness in preventing coral loss was strongly dependent on the duration of protection (Osenberg *et al.* 2008; Edgar *et al.* 2009; Selig & Bruno 2010). On the other hand, several authors uphold the idea that MPAs network may be more effectiveness than MPAs alone, but good monitoring plans are needed to corroborate this assertion (Grorud-Colvert *et al.* 2014). Moreover, Guidetti *et al.* (2014) demonstrated that no-take MPAs are more effective than partially-protected MPAs.

In the *Astroides calycularis* populations census (Section 6) it was highlighted that the most abundant and well established populations are found within the three MPAs of the study. Moreover, the population of Castell de Ferro cliff with similar characteristics was located within an area so far from urbanized shores and inaccessible by tourists, even though it is not a MPA. Therefore, with regard to orange coral

populations it seems that MPAs from Andalusian littoral are effective. Nevertheless, connectivity among populations could be limited, since there is no abundant populations between MPAs (except the above mentioned). This hypothesis must be tested through genetic studies in the future. Moreover, if it was true, it must be assessed the possibility of designing micro-reserves areas on the littoral that connect the bigger MPAs. This concept has already been proposed with other marine endangered species (García-Gómez *et al.* 2010).

### 8.5.3 Restoration

When the corals populations are so damaged the only possible management tool is restoration (e.g. Epstein *et al.* 2003; Rinkevich 2008). A single important action in coral restorations is the rescue of damaged part of colonies as rapidly as possible to transplant them back in the environment. Thus, transplantating actions should be considered in coral restoration to benefit recruitment, accelerate recovery, and improve the visual perspective (e.g. Jaap 2000). However, this management tool has been widely tested on tropical corals but less information has been highlighted in temperate seas. Some studies have been conducted with *Astroides calycularis*, but previous experiments did not have good results (Zibrowius 1995; Ocaña *et al.* 2009). In Section 7, it was used and proved useful, effective, cheap, and easy to implement of the transplantation and translocations technique of the colonies of orange coral. This technique could be used as a possible management tool in degraded areas by human activities (Section 7), improving the conservation status of this endangered species.





## 9. CONCLUSIONS

Colony of *Astroides calycularis* (Punta de la Mona, Almuñécar, Granada). Author:  
Terrón-Sigler A.

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## CONCLUSIONS

- The orange coral *Astroides calycularis* is a brooding and gonochoric species, which release the larvae coinciding with the full moon between June-July when the sea surface temperature reaches 18-23°C.
- In the populations of the North of Alborán Sea, the reproductive strategy of *Astroides calycularis* shows differences due to environmental factors. Thus, female colonies are present in shallow waters while male colonies are deeper.
- The area of the orange coral is the most representative colony size parameter through the life history, and is a good parameter for wildlife managers interested in understanding the dynamics of coral populations. But this parameter can vary, obtaining better correlations at greater depths.
- The *Astroides calycularis* colonies supporting high diverse and abundant macrofaunal assemblages. It is important to note the relevance of the orange coral as structural engineer for macro-invertebrate community and the possible role of this species in marine trophic webs when the populations reach high densities.
- The peracarid community associated with *Astroides calycularis* does not feed the coral tissues. However, peracarids found associated with *A. calycularis* might use the coral skeleton as a refuge against potential predators or as food source of detritus that the colonies generate.
- Divers' ignorance about *Astroides calycularis* must be taken into account as a potential impact factor on the detachment of orange coral colonies, and combined with the diver experience and characteristics of the dive are affecting the populations of this endangered species.
- High hydrodynamism conditions could induce the colonies detachment when they are large in size.
- The widespread expansion of *Oculina patagonica* can have negative consequences on the *Astroides calycularis* populations, especially on the Granada and Almería coasts, due to the ecological characteristics of this species. Recently, *O. patagonica* has already reached Punta de la Mona (Almuñécar-Granada), where the species had not been quoted before and where there is one of the most abundant *A. calycularis* populations of the Alborán Sea.
- In Andalusian coastal line, the highest abundances of *Astroides calycularis* are located in Cádiz and Granada, with abundance reaching up to 95% coverage. Lower abundances are located in Almería and Málaga. Additionally, the

populations are placed mainly in shallow waters (0-6 metres depth), whereas Granada province showed the most abundant population in deeper sites.

- In the studied area, the main populations are inside Marine Protected Areas or places that are far from urbanization, highlighting the seawater requirements of the species and its potential as a pollution bioindicator.
- It has been implemented as a restoration tool based on transplantation of *Astroides calycularis* colonies. The technique has a high survival rate of colonies and it uses epoxy resin of quick action as a fixing material, being cheap and easily usable.
- In the future restoration programs for the species, the environmental conditions, methods (such as scraping) and attachment material type, must be take into account.
- Transplantation of recruits using artificial plates could be useful when grazers were excluded.

### **General conclusion**

Andalusian coasts harbor one of the most abundant populations of the endangered and endemic coral *Astroides calycularis* from the Alborán Sea. Moreover, this littoral is characterized by sun and beach tourism and host a high level of recreational marine activities. These activities are affecting the populations. Nevertheless, the orange coral could be key for those habitats where their populations are abundant or where the species is dominant. The information collected in the present thesis will be useful to develop future regional programs or local plans to protect this emblematic species.



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## CONCLUSIONES

- El coral naranja (*Astroides calycularis*) es una especie gonocórica, que mantiene sus larvas en el interior y cuya liberación coincide con la luna llena de los meses de junio o julio y cuando la temperatura de la superficie del norte del mar de Alborán alcanza los 18-23°C.
- La estrategia reproductiva de las poblaciones de *Astroides calycularis* del norte del mar de Alborán muestran diferencias debido a factores ambientales. Las colonias femeninas se encuentran presentes a profundidades más someras que las colonias masculinas.
- El área de la colonia del coral naranja es el parámetro más representativo de su ciclo de vida y un buen parámetro para aquellos gestores marinos interesados en entender la dinámica de las poblaciones de este coral. Sin embargo, este parámetro puede variar con respecto a la profundidad, obteniéndose mejores correlaciones a mayor profundidad.
- Las colonias de *Astroides calycularis* albergan una elevada diversidad y abundancia de macrofauna asociada. Es importante tener en cuenta la relevancia de este coral como especie bioconstructora para otras comunidades de macroinvertebrados y su posible rol en las redes tróficas marinas cuando las poblaciones alcanzan elevadas densidades.
- La comunidad de peracáridos asociada a *Astroides calycularis* no parece alimentarse de los tejidos del coral. Sin embargo, los peracáridos podrían utilizar el esqueleto del coral como refugio ante depredadores potenciales o, indirectamente, como fuente de alimento de los detritos que las colonias originan y/o atrapan.
- El desconocimiento de los buceadores sobre *Astroides calycularis* debe ser tenido en cuenta como un factor potencial de impacto en el desprendimiento de las colonias de esta especie y, en sinergia con la falta de experiencia de los buceadores y determinadas características de las inmersiones, está afectando negativamente a las poblaciones de esta especie amenazada.
- El elevado hidrodinamismo podría incidir en el desprendimiento de las colonias cuando éstas alcanzan un gran tamaño.
- Debido a las características de *Oculina patagonica*, la expansión de este coral puede tener consecuencias negativas sobre las poblaciones de *Astroides calycularis*, especialmente en las costas de Granada y Almería. Recientemente, *O. patagonica* ha alcanzado la Punta de la Mona (Almuñécar, Granada), donde

la especie no había sido observada antes y donde se encuentra una de las poblaciones más abundantes de *A. calycularis* del mar de Alborán.

- En el litoral andaluz, las mayores abundancias de *Astroides calycularis* se localizan en las provincias de Cádiz y Granada, con abundancias que pueden alcanzar el 95% de cobertura. Por el contrario, los valores más bajos se encuentran en Almería y Málaga. Además, las poblaciones se encuentran principalmente a profundidades comprendidas entre 0 y 6 metros de profundidad. En el litoral de la provincia de Granada se encuentran las poblaciones más abundantes en profundidad.
- En el litoral andaluz, las principales poblaciones de coral naranja se encuentran dentro de Áreas Marinas Protegidas o alejadas de zonas urbanizadas, vislumbrándose los requerimientos ambientales de la especie y su potencial como bioindicador de contaminación.
- Ha sido implementada una metodología como herramienta de restauración para el trasplante de colonias de *Astroides calycularis*. La técnica presenta una elevada tasa de supervivencia de las colonias y el uso de resina epoxy de acción rápida como material de fijación es barata y fácil de utilizar.
- Para futuros programas de restauración de la especie se deben tener en cuenta las condiciones ambientales, el método y el tipo de material de fijación.
- El uso de placas artificiales para la captación de larvas de coral naranja como método de restauración podría ser útil si se excluyen las especies ramoneadoras.

### **Conclusión general**

La costa andaluza, enclavada al norte del mar de Alborán, presenta una de las poblaciones más abundantes de coral naranja (*Astroides calycularis*), especie endémica y amenazada. Por otro lado, Andalucía se caracteriza por su turismo de sol y playa, y alberga un elevado desarrollo turístico dirigido a actividades recreativas en el mar que está afectando a las poblaciones de *A. calycularis*. Sin embargo, el coral naranja podría ser una especie clave en aquellos hábitats donde sus poblaciones son abundantes o donde la especie es dominante. Por tanto, la información recogida en la presente tesis doctoral será de gran ayuda para desarrollar futuros programas regionales o planes locales para proteger a este emblemático coral.

## Publication status associated to the present thesis

### 3. Influencing factors on the reproductive biology of the endangered coral *Astroides calycularis*

- Terrón-Sigler A., Peñalver P. y D. León-Muez. 2011. Detectan la reproducción sexual del coral naranja, una especie amenazada. *Quercus*, 307: 61.

### 4. *Astroides calycularis* as habitat

- Terrón-Sigler A., León-Muez D., Peñalver-Duque P., Espinosa Torre F. Under revision. Feeding habits of the peracarid crustaceans associated with the orange coral *Astroides calycularis*. *Mediterranean Marine Science*.
- Terrón-Sigler A., Peñalver-Duque P., León-Muez D., Espinosa Torre F. 2014. Spatio-temporal macrofaunal assemblages associated with the endangered orange coral *Astroides calycularis* (Scleractinia: Dendrophylliidae). *Aquat. Biol.*, 21: 143–154. doi: 10.3354/ab00577.

### 5. Threats

- Terrón-Sigler A., León-Muez D., Peñalver P., Espinosa Torre F. Under revision. SCUBA diving effects on an emblematic and endemic Mediterranean coral: the orange coral *Astroides calycularis*. *Ocean and Coastal Management*.
- Terrón-Sigler A., Casado-Amezúa P., Espinosa Torre E. 2015. Abundance and distribution of the rapid expansive coral *Oculina patagonica* in the Northern Alboran Sea (Western Mediterranean). *Marine Biodiversity Records.*, 8; e45: 1-5. doi:10.1017/S1755267215000238
- Terrón-Sigler A., León-Muez D. 2009. CORAL NARANJA: Los Impactos del Buceo. *Quercus*. 281: 28-33.

### 6. Geographic distribution of *Astroides calycularis* (Scleractinia: Dendrophylliidae) as a baseline to assess future human impacts on the Southern Iberian Peninsula.

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## **7. Restoration**

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An underwater photograph of a coral reef. The left side of the image shows a large, vibrant yellow-orange coral structure, possibly a brain coral or a similar species. The rest of the image is filled with a dense field of smaller, darker blue-green corals. The water is clear, and the lighting is bright, highlighting the textures and colors of the marine life.

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View of a rocky shore where the orange coral is the dominant species (Punta de la Mona, Almuñécar, Granada). Author: Terrón-Sigler A.



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APPENDIX I: QUESTIONARY

Two divers on rocky shores from Granada coast. Author: Terrón-Sigler A.



PROYECTO DE CONSERVACIÓN DEL CORAL NARANJA EN EL MEDITERRÁNEO

Fecha: \ \ 2008

ZONA DE INMERSIÓN:

¿CUÁL ES TU CCAA DE PROCEDENCIA? (EN EL CASO DE LA CCAA DE ANDALUCÍA INDICAR PROVINCIA)

\_\_\_\_\_

¿CUÁL ES TU CLASE DE EDAD? 15-25  25-35  35-45  45-55  55-65  65 o MÁS

¿CUÁL ES TU EXPERIENCIA COMO BUCEADOR (Nº INMERSIONES)? 1-20  20-40  40-60  MÁS DE 60

¿CUÁLES HAN SIDO TU TIEMPO / PROFUNDIDAD EN LA INMERSIÓN? \_\_\_\_\_ min \_\_\_\_\_ metros

¿ES LA PRIMERA VEZ QUE VIENES A ESTA ZONA DE BUCEO? SI  NO  \_\_\_\_\_ VEZ

¿CONSIDERAS QUE ESTA ZONA ESTÁ DETERIORADA AMBIENTALMENTE?

NADA  POCO  BASTANTE  MUCHO

¿Crees que esta zona de buceo debería someterse a algún tipo de control de acceso?

SI  NO  NO SE NO CONTESTO

¿CONOCES LA EXISTENCIA DE ALGÚN ESPACIO MARINO PROTEGIDO POR LA ZONA?

SI  ¿CUÁL? \_\_\_\_\_ NO

¿CONOCES EL CORAL NARANJA? SI  NO

¿SABÍAS QUE ES UNA ESPECIE QUE SOLO VIVE EN EL SUR DE LA PENÍNSULA? SI  NO

¿SABÍAS QUE ES UNA ESPECIE PROTEGIDA POR CONVENIOS INTERNACIONALES? SI  NO

Gracias por tu colaboración



Con el patrocinio de:







APPENDIX II: COMPLETE DATA ABOUT POINTS  
SAMPLED OF *ASTROIDES CALYCULARIS*

Rocky shore of the Acantilados de Maro-Cerro Gordo Natural Park (Málaga-Granada).  
Author: Terrón-Sigler A.

Transect	Location	Depth	Latitude	Longitude	Abundance	R1	R2	R3	R4	Mean
CA_1	Cádiz	3	36°38,38N	6°24,71W	Absent	0,00	0,00	0,00	0,00	0,00
CA_1	Cádiz	6	36°38,32N	6°24,22W	Absent	0,00	0,00	0,00	0,00	0,00
CA_1	Cádiz	12	36°38,25N	6°24,35W	Absent	0,00	0,00	0,00	0,00	0,00
CA_2	Cádiz	3	36°37,44N	6°23,49W	Absent	0,00	0,00	0,00	0,00	0,00
CA_2	Cádiz	6	36°37,37N	6°23,56W	Absent	0,00	0,00	0,00	0,00	0,00
CA_2	Cádiz	12	36°37,31N	6°23,66W	Absent	0,00	0,00	0,00	0,00	0,00
CA_3	Cádiz	3	36°37,06N	6°21,79W	Absent	0,00	0,00	0,00	0,00	0,00
CA_3	Cádiz	6	36°36,73N	6°22,09W	Absent	0,00	0,00	0,00	0,00	0,00
CA_3	Cádiz	12	36°36,74N	6°22,06W	Absent	0,00	0,00	0,00	0,00	0,00
CA_4	Cádiz	3	36°32,283N	6°18,103W	Absent	0,00	0,00	0,00	0,00	0,00
CA_4	Cádiz	6	36°32,393N	6°18,190W	Absent	0,00	0,00	0,00	0,00	0,00
CA_4	Cádiz	12	36°32,486N	6°18,260W	Absent	0,00	0,00	0,00	0,00	0,00
CA_5	Cádiz	3	36°31,721N	6°19,204W	Absent	0,00	0,00	0,00	0,00	0,00
CA_5	Cádiz	6	36°31,766N	6°19,575W	Absent	0,00	0,00	0,00	0,00	0,00
CA_5	Cádiz	12	36°31,839N	6°19,585W	Absent	0,00	0,00	0,00	0,00	0,00
CA_6	Cádiz	3	36°31,839N	6°19,585W	Absent	0,00	0,00	0,00	0,00	0,00
CA_6	Cádiz	6	36°31,560N	6°18,560W	Absent	0,00	0,00	0,00	0,00	0,00
CA_6	Cádiz	12	36°31,560N	6°18,560W	Absent	0,00	0,00	0,00	0,00	0,00
CA_7	Cádiz	3	36°31,491N	6°18,519W	Absent	0,00	0,00	0,00	0,00	0,00
CA_7	Cádiz	6	36°30,865N	6°17,561W	Absent	0,00	0,00	0,00	0,00	0,00
CA_7	Cádiz	12	36°30,842N	6°17,591W	Absent	0,00	0,00	0,00	0,00	0,00
CA_8	Cádiz	3	36°30,808N	6°17,655W	Absent	0,00	0,00	0,00	0,00	0,00
CA_8	Cádiz	6	36°26,466N	6°14,860W	Absent	0,00	0,00	0,00	0,00	0,00
CA_8	Cádiz	12	36°26,487N	6°14,960W	Absent	0,00	0,00	0,00	0,00	0,00
CA_9	Cádiz	3	36°26,574N	6°15,108W	Absent	0,00	0,00	0,00	0,00	0,00
CA_9	Cádiz	6	36°23,449N	6°13,496W	Absent	0,00	0,00	0,00	0,00	0,00
CA_9	Cádiz	12	36°23,525N	6°13,597W	Absent	0,00	0,00	0,00	0,00	0,00
CA_10	Cádiz	3	36°23,577N	6°13,680W	Absent	0,00	0,00	0,00	0,00	0,00
CA_10	Cádiz	6	36°22,927N	6°13,370W	Presence	1,00	0,00	0,00	0,00	0,25
CA_10	Cádiz	12	36°22,967N	6°13,456W	Absent	0,00	0,00	0,00	0,00	0,00
CA_11	Cádiz	3	36°22,718N	6°13,155W	Absent	0,00	0,00	0,00	0,00	0,00
CA_11	Cádiz	6	36°22,711N	6°13,107W	Absent	0,00	0,00	0,00	0,00	0,00
CA_11	Cádiz	12	36°22,699N	6°13,029W	Absent	0,00	0,00	0,00	0,00	0,00
CA_12	Cádiz	3	36°10,805N	6°02,205W	Presence	5,00	1,00	0,00	5,00	2,75
CA_12	Cádiz	6	36°10,805N	6°02,205W	Presence	20,00	15,00	10,00	30,00	18,75
CA_12	Cádiz	12	36°10,805N	6°02,205W	Presence	15,00	25,00	20,00	20,00	20,00
CA_13	Cádiz	3	36°10,776N	6°01,905W	Presence	20,00	15,00	15,00	10,00	15,00
CA_13	Cádiz	6	36°10,776N	6°01,905W	Presence	20,00	10,00	30,00	15,00	18,75
CA_13	Cádiz	12	36°10,776N	6°01,905W	Presence	1,00	15,00	10,00	35,00	15,25
CA_14	Cádiz	3	36°10,901N	6°00,616W	Absent	0,00	0,00	0,00	0,00	0,00
CA_14	Cádiz	6	36°10,893N	6°00,614W	Absent	0,00	0,00	0,00	0,00	0,00
CA_14	Cádiz	12	36°10,633N	6°00,633W	Absent	0,00	0,00	0,00	0,00	0,00
CA_15	Cádiz	3	36°10,612N	5°59,188W	Absent	0,00	0,00	0,00	0,00	0,00
CA_15	Cádiz	6	36°10,603N	5°59,207W	Absent	0,00	0,00	0,00	0,00	0,00
CA_15	Cádiz	12	36°10,570N	5°59,188W	Absent	0,00	0,00	0,00	0,00	0,00
CA_16	Cádiz	3	36°10,771N	5°59,970W	Absent	0,00	0,00	0,00	0,00	0,00
CA_16	Cádiz	6	36°10759N	5°59,966W	Absent	0,00	0,00	0,00	0,00	0,00
CA_16	Cádiz	12	36°10,643N	5°59,990W	Absent	0,00	0,00	0,00	0,00	0,00
CA_17	Cádiz	3	36°10,583N	5°58,479W	Absent	0,00	0,00	0,00	0,00	0,00
CA_17	Cádiz	6	36°10,584N	5°58,477W	Absent	0,00	0,00	0,00	0,00	0,00
CA_17	Cádiz	12	36°10,605N	5°58,477W	Absent	0,00	0,00	0,00	0,00	0,00
CA_18	Cádiz	3	36°06,135N	5°49,571W	Absent	0,00	0,00	0,00	0,00	0,00
CA_18	Cádiz	6	36°06,135N	5°49,571W	Absent	0,00	0,00	0,00	0,00	0,00
CA_18	Cádiz	12	36°06,135N	5°49,571W	Absent	0,00	0,00	0,00	0,00	0,00
CA_19	Cádiz	3	36°04,824N	5°48,054W	Presence	1,00	15,00	25,00	30,00	17,75
CA_19	Cádiz	6	36°04,854N	5°48,066W	Presence	20,00	25,00	15,00	25,00	21,25
CA_19	Cádiz	12	36°04,887N	5°48,125W	Presence	15,00	20,00	25,00	20,00	20,00
CA_20	Cádiz	3	36°04,628N	5°47,796W	Abundant	65,00	70,00	70,00	75,00	70,00
CA_20	Cádiz	6	36°04,621N	5°47,793W	Abundant	75,00	70,00	70,00	65,00	70,00
CA_20	Cádiz	12	36°04,635N	5°47,870W	Abundant	65,00	65,00	60,00	70,00	65,00
CA_21	Cádiz	3	36°04,606N	5°47,660W	Presence	1,00	5,00	25,00	5,00	9,00
CA_21	Cádiz	6	36°04,493N	5°47,600W	Presence	5,00	5,00	10,00	1,00	5,25

Conservation biology of the endangered orange coral *Astroides calycularis*

CA_21	Cádiz	12	36°04,510N	5°47,682W	Presence	5,00	15,00	20,00	1,00	10,25
CA_22	Cádiz	3	36°04,501N	5°47,906W	Presence	1,00	1,00	1,00	15,00	4,50
CA_22	Cádiz	6	36°04,501N	5°47,906W	Presence	5,00	15,00	1,00	1,00	5,50
CA_22	Cádiz	12	36°04,501N	5°47,906W	Presence	20,00	10,00	15,00	5,00	12,50
CA_23	Cádiz	3	36°05,208N	5°48,826W	Presence	1,00	1,00	25,00	1,00	7,00
CA_23	Cádiz	6	36°05,208N	5°48,826W	Presence	15,00	20,00	20,00	15,00	17,50
CA_23	Cádiz	12	36°05,208N	5°48,826W	Presence	15,00	25,00	1,00	20,00	15,25
CA_24	Cádiz	3	36°04,174N	5°25,260W	Presence	5,00	5,00	1,00	5,00	4,00
CA_24	Cádiz	6	36°04,174N	5°25,260W	Presence	5,00	10,00	5,00	10,00	7,50
CA_24	Cádiz	12	36°04,174N	5°25,260W	Presence	15,00	25,00	1,00	5,00	11,50
CA_25	Cádiz	3	36°04,060N	5°44,421W	Absent	0,00	0,00	0,00	0,00	0,00
CA_25	Cádiz	6	36°04,044N	5°44,431W	Absent	0,00	0,00	0,00	0,00	0,00
CA_25	Cádiz	12	36°03,986N	5°44,471W	Absent	0,00	0,00	0,00	0,00	0,00
CA_26	Cádiz	3	36°03,803N	5°43,743W	Absent	0,00	0,00	0,00	0,00	0,00
CA_26	Cádiz	6	36°03,797N	5°43,790W	Absent	0,00	0,00	0,00	0,00	0,00
CA_26	Cádiz	12	36°03,755N	5°43,858W	Absent	0,00	0,00	0,00	0,00	0,00
CA_27	Cádiz	3	36°03,476N	5°42,386W	Absent	0,00	0,00	0,00	0,00	0,00
CA_27	Cádiz	6	36°03,476N	5°42,386W	Absent	0,00	0,00	0,00	0,00	0,00
CA_27	Cádiz	12	36°03,423N	5°42,384W	Absent	0,00	0,00	0,00	0,00	0,00
CA_28	Cádiz	3	36°03,523N	5°40,669W	Absent	0,00	0,00	0,00	0,00	0,00
CA_28	Cádiz	6	36°03,523N	5°40,779W	Absent	0,00	0,00	0,00	0,00	0,00
CA_28	Cádiz	12	36°03,495N	5°40,826W	Absent	0,00	0,00	0,00	0,00	0,00
CA_29	Cádiz	3	36°03,412N	5°40,210W	Absent	0,00	0,00	0,00	0,00	0,00
CA_29	Cádiz	6	36°03,250N	5°40,193W	Absent	0,00	0,00	0,00	0,00	0,00
CA_29	Cádiz	12	36°03,288N	5°40,217W	Absent	0,00	0,00	0,00	0,00	0,00
CA_30	Cádiz	3	36°03,332N	5°39,886W	Absent	0,00	0,00	0,00	0,00	0,00
CA_30	Cádiz	6	36°03,277N	5°39,836W	Absent	0,00	0,00	0,00	0,00	0,00
CA_30	Cádiz	12	36°03,013N	5°39,875W	Absent	0,00	0,00	0,00	0,00	0,00
CA_31	Cádiz	3	36°04,725N	5°25,397W	Abundant	65,00	45,00	50,00	55,00	53,75
CA_31	Cádiz	6	36°04,725N	5°25,397W	Abundant	75,00	45,00	50,00	55,00	56,25
CA_31	Cádiz	12	36°04,666N	5°25,360W	Abundant	65,00	50,00	55,00	75,00	61,25
CA_32	Cádiz	3	36°00,117N	5°36,782W	Abundant	45,00	60,00	60,00	45,00	52,50
CA_32	Cádiz	6	36°00,117N	5°36,782W	Abundant	60,00	60,00	65,00	45,00	57,50
CA_32	Cádiz	12	36°00,117N	5°36,782W	Abundant	60,00	75,00	65,00	70,00	67,50
CA_33	Cádiz	3	36°00,616N	5°35,917W	Presence	15,00	20,00	10,00	10,00	13,75
CA_33	Cádiz	6	36°00,581N	5°35,893W	Absent	0,00	0,00	0,00	0,00	0,00
CA_33	Cádiz	12	36°00,529N	5°35,898W	Absent	0,00	0,00	0,00	0,00	0,00
CA_34	Cádiz	3	36°00,682N	5°35,193W	Absent	0,00	0,00	0,00	0,00	0,00
CA_34	Cádiz	6	36°00,669N	5°35,199W	Absent	0,00	0,00	0,00	0,00	0,00
CA_34	Cádiz	12	36°00,593N	5°35,372W	Abundant	50,00	50,00	50,00	55,00	51,25
CA_35	Cádiz	3	36°00,764N	5°34,726W	Abundant	40,00	50,00	75,00	45,00	52,50
CA_35	Cádiz	6	36°00,640N	5°34,664W	Not abundant	35,00	30,00	20,00	20,00	26,25
CA_35	Cádiz	12	36°00,665N	3°34,665W	Absent	0,00	0,00	0,00	0,00	0,00
CA_36	Cádiz	3	36°01,069N	5°33,875W	Not abundant	25,00	20,00	30,00	40,00	28,75
CA_36	Cádiz	6	36°01,033N	5°33,870W	Not abundant	30,00	25,00	25,00	35,00	28,75
CA_36	Cádiz	12	36°01,040N	5°33,858W	Absent	0,00	0,00	0,00	0,00	0,00
CA_37	Cádiz	3	36°01,207N	5°33,396W	Presence	1,00	5,00	1,00	10,00	4,25
CA_37	Cádiz	6	36°01,562N	5°32,801W	Not abundant	30,00	25,00	25,00	25,00	26,25
CA_37	Cádiz	12	36°01,478N	5°32,789W	Absent	0,00	0,00	0,00	0,00	0,00
CA_38	Cádiz	3	36°01,927N	5°32,022W	Presence	1,00	1,00	1,00	1,00	1,00
CA_38	Cádiz	6	36°01,941N	5°31,631W	Abundant	35,00	30,00	25,00	20,00	27,50
CA_38	Cádiz	12	36°01,851N	5°32,072W	Presence	10,00	10,00	15,00	10,00	11,25
CA_39	Cádiz	3	36°02,193N	5°30,896W	Not abundant	20,00	30,00	25,00	25,00	25,00
CA_39	Cádiz	6	36°02,139N	5°30,958W	Not abundant	30,00	40,00	25,00	20,00	28,75
CA_39	Cádiz	12	36°02,121N	5°30,882W	Not abundant	40,00	30,00	20,00	25,00	28,75
CA_40	Cádiz	3	36°02,575N	5°30,021W	Presence	10,00	1,00	5,00	1,00	4,25
CA_40	Cádiz	6	36°02,764N	5°29,888W	Not abundant	25,00	25,00	20,00	30,00	25,00
CA_40	Cádiz	12	36°02,575N	5°29,931W	Presence	10,00	15,00	5,00	10,00	10,00
CA_41	Cádiz	3	36°03,017N	5°29,575W	Absent	0,00	0,00	0,00	0,00	0,00
CA_41	Cádiz	6	36°02,855N	5°29,643W	Presence	1,00	1,00	1,00	1,00	1,00
CA_41	Cádiz	12	36°02,930N	5°29,600W	Absent	0,00	0,00	0,00	0,00	0,00
CA_42	Cádiz	3	36°02,999N	5°29,419W	Not abundant	25,00	30,00	40,00	15,00	27,50
CA_42	Cádiz	6	36°02,999N	5°29,419W	Presence	10,00	1,00	1,00	1,00	3,25

CA_42	Cádiz	12	36°03,003N	5°29,403W	Absent	0,00	0,00	0,00	0,00	0,00
CA_43	Cádiz	3	36°03,078N	5°28,822W	Presence	1,00	5,00	1,00	1,00	2,00
CA_43	Cádiz	6	36°03,106N	5°28,860W	Absent	0,00	0,00	0,00	0,00	0,00
CA_43	Cádiz	12	36°03,107N	5°28,891W	Absent	0,00	0,00	0,00	0,00	0,00
CA_44	Cádiz	3	36°02,986N	5°28,536W	Presence	1,00	1,00	1,00	1,00	1,00
CA_44	Cádiz	6	36°03,068N	5°28,561W	Absent	0,00	0,00	0,00	0,00	0,00
CA_44	Cádiz	12	36°03,016N	5°28,519W	Absent	0,00	0,00	0,00	0,00	0,00
CA_45	Cádiz	3	36°02,949N	5°27,991W	Absent	0,00	0,00	0,00	0,00	0,00
CA_45	Cádiz	6	36°02,844N	5°27,896W	Presence	1,00	1,00	1,00	1,00	1,00
CA_45	Cádiz	12	36°02,844N	5°27,896W	Presence	1,00	1,00	1,00	1,00	1,00
CA_46	Cádiz	3	36°03,056N	5°27,606W	Not abundant	15,00	25,00	30,00	30,00	25,00
CA_46	Cádiz	6	36°03,056N	5°27,606W	Presence	1,00	5,00	1,00	1,00	2,00
CA_46	Cádiz	12	36°02,962N	5°27,682W	Absent	0,00	0,00	0,00	0,00	0,00
CA_47	Cádiz	3	36°03,302N	5°27,025W	Abundant	55,00	50,00	50,00	60,00	53,75
CA_47	Cádiz	6	36°03,302N	5°27,025W	Abundant	50,00	50,00	50,00	55,00	51,25
CA_47	Cádiz	12	36°03,387N	5°27,022W	Not abundant	35,00	30,00	40,00	35,00	35,00
CA_48	Cádiz	3	36°03,702N	5°26,570W	Abundant	50,00	60,00	65,00	50,00	56,25
CA_48	Cádiz	6	36°03,811N	5°26,250W	Abundant	65,00	65,00	70,00	75,00	68,75
CA_48	Cádiz	12	36°03,774N	5°26,439W	Presence	10,00	15,00	15,00	25,00	16,25
CA_49	Cádiz	3	36°03,902N	5°25,992W	Absent	0,00	0,00	0,00	0,00	0,00
CA_49	Cádiz	6	36°03,934N	5°25,969W	Presence	10,00	10,00	10,00	10,00	10,00
CA_49	Cádiz	12	36°03,934N	5°25,969W	Presence	10,00	10,00	15,00	10,00	11,25
CA_50	Cádiz	3	36°04,415N	5°25,630W	Abundant	70,00	65,00	60,00	50,00	61,25
CA_50	Cádiz	6	36°04,415N	5°25,630W	Abundant	50,00	75,00	50,00	50,00	56,25
CA_50	Cádiz	12	36°04,394N	5°25,645W	Presence	5,00	5,00	1,00	1,00	3,00
CA_51	Cádiz	3	36°04,994N	5°25,522W	Presence	1,00	5,00	0,00	1,00	1,75
CA_51	Cádiz	6	36°04,992N	5°25,502W	Presence	10,00	5,00	1,00	5,00	5,25
CA_51	Cádiz	12	36°04,990N	5°25,494W	Presence	1,00	1,00	1,00	0,00	0,75
CA_52	Cádiz	3	36°06,177N	5°26,055W	Abundant	50,00	45,00	60,00	45,00	50,00
CA_52	Cádiz	6	36°06,186N	5°26,051W	Presence	1,00	1,00	1,00	0,00	0,75
CA_52	Cádiz	12	36°06,177N	5°26,055W	Presence	5,00	0,00	0,00	10,00	3,75
CA_53	Cádiz	3	36°06,894N	5°25,829W	Presence	1,00	0,00	0,00	1,00	0,50
CA_53	Cádiz	6	36°06,594N	5°25,829W	Absent	0,00	0,00	0,00	0,00	0,00
CA_53	Cádiz	12	36°06,594N	5°25,829W	Absent	0,00	0,00	0,00	0,00	0,00
CA_54	Cádiz	3	36°10,612N	5°24,951W	Absent	0,00	0,00	0,00	0,00	0,00
CA_54	Cádiz	6	36°10,612N	5°24,951W	Absent	0,00	0,00	0,00	0,00	0,00
CA_54	Cádiz	12	36°10,612N	5°24,951W	Absent	0,00	0,00	0,00	0,00	0,00
CA_55	Cádiz	3	36°10,647N	5°22,967W	Absent	0,00	0,00	0,00	0,00	0,00
CA_55	Cádiz	6	36°10,647N	5°22,967W	Absent	0,00	0,00	0,00	0,00	0,00
CA_55	Cádiz	12	36°10,636N	5°22,981W	Absent	0,00	0,00	0,00	0,00	0,00
CA_56	Cádiz	3	36°10,111N	5°22,880W	Absent	0,00	0,00	0,00	0,00	0,00
CA_56	Cádiz	6	36°10,111N	5°22,880W	Absent	0,00	0,00	0,00	0,00	0,00
CA_56	Cádiz	12	36°10,111N	5°22,880W	Absent	0,00	0,00	0,00	0,00	0,00
CA_57	Cádiz	3	36°13,855N	5°18,680W	Absent	0,00	0,00	0,00	0,00	0,00
CA_57	Cádiz	6	36°13,801N	5°18,694W	Absent	0,00	0,00	0,00	0,00	0,00
CA_57	Cádiz	12	36°13,750N	5°18,568W	Absent	0,00	0,00	0,00	0,00	0,00
CA_58	Cádiz	3	36°17,915N	5°16,028W	Absent	0,00	0,00	0,00	0,00	0,00
CA_58	Cádiz	6	36°17,915N	5°16,028W	Absent	0,00	0,00	0,00	0,00	0,00
CA_58	Cádiz	12	36°17,915N	5°16,028W	Absent	0,00	0,00	0,00	0,00	0,00
CA_59	Cádiz	3	36°18,063N	5°15,850W	Presence	1,00	1,00	0,00	0,00	0,50
CA_59	Cádiz	6	36°18,178N	5°15,809W	Absent	0,00	0,00	0,00	0,00	0,00
CA_59	Cádiz	12	36°18,043N	5°15,680W	Absent	0,00	0,00	0,00	0,00	0,00
MA_1	Málaga	3	36°22,977N	5°12,376W	Absent	0,00	0,00	0,00	0,00	0,00
MA_1	Málaga	6	36°22,698N	5°12,471W	Presence	1,00	0,00	5,00	0,00	1,50
MA_1	Málaga	12	36°22,900N	5°12,552W	Absent	0,00	0,00	0,00	0,00	0,00
MA_2	Málaga	3	36°22,733N	5°12,427W	Absent	0,00	0,00	0,00	0,00	0,00
MA_2	Málaga	6	36°22,733N	5°12,427W	Absent	0,00	0,00	0,00	0,00	0,00
MA_2	Málaga	12	36°22,733N	5°12,427W	Absent	0,00	0,00	0,00	0,00	0,00
MA_3	Málaga	3	36°24,860N	5°10,206W	Absent	0,00	0,00	0,00	0,00	0,00
MA_3	Málaga	6	36°24,760N	5°10,200W	Absent	0,00	0,00	0,00	0,00	0,00
MA_3	Málaga	12	36°24,555N	5°10,167W	Absent	0,00	0,00	0,00	0,00	0,00
MA_4	Málaga	3	36°25,682N	5°07,053W	Absent	0,00	0,00	0,00	0,00	0,00
MA_4	Málaga	6	36°25,650N	5°06,997W	Absent	0,00	0,00	0,00	0,00	0,00



Conservation biology of the endangered orange coral *Astroides calycularis*

MA_4	Málaga	12	36°25,614N	5°06,896W	Absent	0,00	0,00	0,00	0,00	0,00
MA_5	Málaga	3	36°25,665N	5°07,435W	Absent	0,00	0,00	0,00	0,00	0,00
MA_5	Málaga	6	36°25,545N	5°07,485W	Absent	0,00	0,00	0,00	0,00	0,00
MA_5	Málaga	12	36°25,449N	5°07,439W	Absent	0,00	0,00	0,00	0,00	0,00
MA_6	Málaga	3	36°28,609N	4°58,639W	Absent	0,00	0,00	0,00	0,00	0,00
MA_6	Málaga	6	36°28,537N	4°58,604W	Absent	0,00	0,00	0,00	0,00	0,00
MA_6	Málaga	12	36°28,385N	4°58,592W	Absent	0,00	0,00	0,00	0,00	0,00
MA_7	Málaga	3	36°27,503N	5°00,637W	Absent	0,00	0,00	0,00	0,00	0,00
MA_7	Málaga	6	36°27,385N	5°00,625W	Absent	0,00	0,00	0,00	0,00	0,00
MA_7	Málaga	12	36°27,312N	5°00,634W	Absent	0,00	0,00	0,00	0,00	0,00
MA_8	Málaga	3	36°28,912N	4°57,739W	Absent	0,00	0,00	0,00	0,00	0,00
MA_8	Málaga	6	36°28,902N	4°57,732W	Absent	0,00	0,00	0,00	0,00	0,00
MA_8	Málaga	12	36°28,813N	4°57,697W	Absent	0,00	0,00	0,00	0,00	0,00
MA_9	Málaga	3	36°28,945N	4°44,569W	Absent	0,00	0,00	0,00	0,00	0,00
MA_9	Málaga	6	36°28,945N	4°44,569W	Absent	0,00	0,00	0,00	0,00	0,00
MA_9	Málaga	12	36°28,710N	4°44,619W	Absent	0,00	0,00	0,00	0,00	0,00
MA_10	Málaga	3	36°29,284N	4°41,928W	Absent	0,00	0,00	0,00	0,00	0,00
MA_10	Málaga	6	36°29,284N	4°41,928W	Absent	0,00	0,00	0,00	0,00	0,00
MA_10	Málaga	12	36°29,144N	4°41,894W	Absent	0,00	0,00	0,00	0,00	0,00
MA_11	Málaga	3	36°29,124N	4°43,616W	Absent	0,00	0,00	0,00	0,00	0,00
MA_11	Málaga	6	36°29,013N	4°43,704W	Absent	0,00	0,00	0,00	0,00	0,00
MA_11	Málaga	12	36°28,712N	4°43,778W	Absent	0,00	0,00	0,00	0,00	0,00
MA_12	Málaga	3	36°30,165N	4°40,257W	Absent	0,00	0,00	0,00	0,00	0,00
MA_12	Málaga	6	36°30,072N	4°40,219W	Absent	0,00	0,00	0,00	0,00	0,00
MA_12	Málaga	12	36°29,957N	4°40,193W	Absent	0,00	0,00	0,00	0,00	0,00
MA_13	Málaga	3	36°30,122N	4°40,069W	Absent	0,00	0,00	0,00	0,00	0,00
MA_13	Málaga	6	36°30,122N	4°40,069W	Absent	0,00	0,00	0,00	0,00	0,00
MA_13	Málaga	12	36°30,003N	4°40,070W	Absent	0,00	0,00	0,00	0,00	0,00
MA_14	Málaga	3	36°30,296N	4°39,062W	Absent	0,00	0,00	0,00	0,00	0,00
MA_14	Málaga	6	36°30,205N	4°39,050W	Absent	0,00	0,00	0,00	0,00	0,00
MA_14	Málaga	12	36°30,051N	4°39,070W	Absent	0,00	0,00	0,00	0,00	0,00
MA_15	Málaga	3	36°34,408N	4°34,760W	Absent	0,00	0,00	0,00	0,00	0,00
MA_15	Málaga	6	36°34,283N	4°34,720W	Absent	0,00	0,00	0,00	0,00	0,00
MA_15	Málaga	12	36°34,106N	4°34,641W	Absent	0,00	0,00	0,00	0,00	0,00
MA_16	Málaga	3	36°34,623N	4°33,785W	Absent	0,00	0,00	0,00	0,00	0,00
MA_16	Málaga	6	36°34,600N	4°33,742W	Absent	0,00	0,00	0,00	0,00	0,00
MA_16	Málaga	12	36°34,366N	4°33,529W	Absent	0,00	0,00	0,00	0,00	0,00
MA_17	Málaga	3	36°34,576N	4°34,074W	Absent	0,00	0,00	0,00	0,00	0,00
MA_17	Málaga	6	36°34,529N	4°34,091W	Absent	0,00	0,00	0,00	0,00	0,00
MA_17	Málaga	12	36°34,278N	4°34,002W	Absent	0,00	0,00	0,00	0,00	0,00
MA_18	Málaga	3	36°34,719N	4°33,195W	Absent	0,00	0,00	0,00	0,00	0,00
MA_18	Málaga	6	36°34,680N	4°33,154W	Absent	0,00	0,00	0,00	0,00	0,00
MA_18	Málaga	12	36°34,454N	4°32,950W	Absent	0,00	0,00	0,00	0,00	0,00
MA_19	Málaga	3	36°34,776N	4°33,021W	Absent	0,00	0,00	0,00	0,00	0,00
MA_19	Málaga	6	36°34,687N	4°32,993W	Absent	0,00	0,00	0,00	0,00	0,00
MA_19	Málaga	12	36°34,443N	4°32,880W	Absent	0,00	0,00	0,00	0,00	0,00
MA_20	Málaga	3	36°35,219N	4°31,638W	Absent	0,00	0,00	0,00	0,00	0,00
MA_20	Málaga	6	36°35,218N	4°31,637W	Absent	0,00	0,00	0,00	0,00	0,00
MA_20	Málaga	12	36°35,090N	4°31,472W	Absent	0,00	0,00	0,00	0,00	0,00
MA_21	Málaga	3	36°35,386N	4°30,954W	Absent	0,00	0,00	0,00	0,00	0,00
MA_21	Málaga	6	36°35,356N	4°30,963W	Absent	0,00	0,00	0,00	0,00	0,00
MA_21	Málaga	12	36°35,338N	4°30,991W	Absent	0,00	0,00	0,00	0,00	0,00
MA_22	Málaga	3	36°40,865N	4°26,453W	Absent	0,00	0,00	0,00	0,00	0,00
MA_22	Málaga	6	36°40,865N	4°26,453W	Absent	0,00	0,00	0,00	0,00	0,00
MA_22	Málaga	12	36°40,779N	4°26,356W	Absent	0,00	0,00	0,00	0,00	0,00
MA_23	Málaga	3	36°42,130N	4°25,689W	Absent	0,00	0,00	0,00	0,00	0,00
MA_23	Málaga	6	36°42,132N	4°25,689W	Absent	0,00	0,00	0,00	0,00	0,00
MA_23	Málaga	12	36°42,132N	4°25,689W	Absent	0,00	0,00	0,00	0,00	0,00
MA_24	Málaga	3	36°43,167N	4°22,930W	Absent	0,00	0,00	0,00	0,00	0,00
MA_24	Málaga	6	36°43,161N	4°22,936W	Absent	0,00	0,00	0,00	0,00	0,00
MA_24	Málaga	12	36°43,130N	4°22,969W	Absent	0,00	0,00	0,00	0,00	0,00
MA_25	Málaga	3	36°43,139N	4°22,367W	Absent	0,00	0,00	0,00	0,00	0,00
MA_25	Málaga	6	36°43,107N	4°22,360W	Absent	0,00	0,00	0,00	0,00	0,00



MA_25	Málaga	12	36°43,053N	4°22,367W	Absent	0,00	0,00	0,00	0,00	0,00
MA_26	Málaga	3	36°43,047N	4°21,601W	Absent	0,00	0,00	0,00	0,00	0,00
MA_26	Málaga	6	36°42,993N	4°21,641W	Absent	0,00	0,00	0,00	0,00	0,00
MA_26	Málaga	12	36°42,961N	4°21,641W	Absent	0,00	0,00	0,00	0,00	0,00
MA_27	Málaga	3	36°42,708N	4°20,111W	Absent	0,00	0,00	0,00	0,00	0,00
MA_27	Málaga	6	36°42,651N	4°20,107W	Absent	0,00	0,00	0,00	0,00	0,00
MA_27	Málaga	12	36°42,571N	4°20,237W	Absent	0,00	0,00	0,00	0,00	0,00
MA_28	Málaga	3	36°42,684N	4°18,579W	Absent	0,00	0,00	0,00	0,00	0,00
MA_28	Málaga	6	36°42,654N	4°18,581W	Absent	0,00	0,00	0,00	0,00	0,00
MA_28	Málaga	12	36°42,620N	4°18,587W	Absent	0,00	0,00	0,00	0,00	0,00
MA_29	Málaga	3	36°44,387N	3°53,075W	Absent	0,00	0,00	0,00	0,00	0,00
MA_29	Málaga	6	36°44,384N	3°53,084W	Absent	0,00	0,00	0,00	0,00	0,00
MA_29	Málaga	12	36°44,287N	3°53,163W	Absent	0,00	0,00	0,00	0,00	0,00
MA_30	Málaga	3	36°44,907N	3°51,689W	Absent	0,00	0,00	0,00	0,00	0,00
MA_30	Málaga	6	36°44,863N	3°51,689W	Absent	0,00	0,00	0,00	0,00	0,00
MA_30	Málaga	12	36°44,670N	3°51,689W	Absent	0,00	0,00	0,00	0,00	0,00
MA_31	Málaga	3	36°44,615N	3°52,503W	Absent	0,00	0,00	0,00	0,00	0,00
MA_31	Málaga	6	36°44,516N	3°52,513W	Absent	0,00	0,00	0,00	0,00	0,00
MA_31	Málaga	12	36°44,436N	3°52,540W	Absent	0,00	0,00	0,00	0,00	0,00
MA_32	Málaga	3	36°44,359N	3°47,061W	Presence	5,00	0,00	0,00	1,00	1,50
MA_32	Málaga	6	36°44,359N	3°47,061W	Presence	1,00	1,00	0,00	0,00	0,50
MA_32	Málaga	12	36°44,309N	3°46,922W	Absent	0,00	0,00	0,00	0,00	0,00
MA_33	Málaga	3	36°45,079N	3°49,575W	Absent	0,00	0,00	0,00	0,00	0,00
MA_33	Málaga	6	36°45,079N	3°49,575W	Presence	5,00	1,00	0,00	1,00	1,75
MA_33	Málaga	12	36°45,063N	3°49,575W	Absent	0,00	0,00	0,00	0,00	0,00
MA_34	Málaga	3	36°44,450N	4°01,887W	Absent	0,00	0,00	0,00	0,00	0,00
MA_34	Málaga	6	36°44,440N	4°01,889W	Absent	0,00	0,00	0,00	0,00	0,00
MA_34	Málaga	12	36°44,401N	4°01,880W	Absent	0,00	0,00	0,00	0,00	0,00
MA_35	Málaga	3	36°26,992N	5°04,691W	Absent	0,00	0,00	0,00	0,00	0,00
MA_35	Málaga	6	36°26,885N	5°04,695W	Absent	0,00	0,00	0,00	0,00	0,00
MA_35	Málaga	12	36°26,787N	5°04,695W	Absent	0,00	0,00	0,00	0,00	0,00
MA_36	Málaga	3	36°25,627N	5°08,043W	Absent	0,00	0,00	0,00	0,00	0,00
MA_36	Málaga	6	36°25,439N	5°08,030W	Absent	0,00	0,00	0,00	0,00	0,00
MA_36	Málaga	12	36°25,361N	5°08,028W	Absent	0,00	0,00	0,00	0,00	0,00
MA_37	Málaga	3	36°33,740N	4°35,898W	Absent	0,00	0,00	0,00	0,00	0,00
MA_37	Málaga	6	36°33,740N	4°35,898W	Absent	0,00	0,00	0,00	0,00	0,00
MA_37	Málaga	12	36°33,612N	4°35,785W	Absent	0,00	0,00	0,00	0,00	0,00
GR_1	Granada	3	36°44,010N	3°46,390W	Very Abundant	80,00	80,00	75,00	90,00	81,25
GR_1	Granada	6	36°44,002N	3°46,391W	Very Abundant	85,00	90,00	85,00	70,00	82,50
GR_1	Granada	12	36°43,995N	3°46,391W	Very Abundant	80,00	85,00	90,00	80,00	83,75
GR_2	Granada	3	36°43,744N	3°46,095W	Very Abundant	90,00	90,00	85,00	90,00	88,75
GR_2	Granada	6	36°43,744N	3°46,095W	Very Abundant	80,00	90,00	85,00	85,00	85,00
GR_2	Granada	12	36°43,744N	3°46,095W	Very Abundant	70,00	85,00	80,00	75,00	77,50
GR_3	Granada	3	36°43,780N	3°45,793W	Not abundant	35,00	45,00	45,00	45,00	42,50
GR_3	Granada	6	36°43,780N	3°45,793W	Not abundant	40,00	25,00	25,00	20,00	27,50
GR_3	Granada	12	36°43,780N	3°45,793W	Not abundant	20,00	35,00	20,00	30,00	26,25
GR_4	Granada	3	36°43,860N	3°45,773W	Abundant	80,00	50,00	75,00	40,00	61,25
GR_4	Granada	6	36°43,860N	3°45,763W	Abundant	65,00	40,00	40,00	55,00	50,00
GR_4	Granada	12	36°43,859N	3°45,748W	Abundant	60,00	55,00	50,00	50,00	53,75
GR_5	Granada	3	36°43,279N	3°44,079W	Very Abundant	85,00	90,00	80,00	80,00	83,75
GR_5	Granada	6	36°43,273N	3°44,108W	Very Abundant	70,00	75,00	80,00	75,00	75,00
GR_5	Granada	12	36°43,269N	3°44,128W	Very Abundant	75,00	80,00	70,00	75,00	75,00
GR_6	Granada	3	36°43,346N	3°43,686W	Abundant	60,00	40,00	65,00	40,00	51,25
GR_6	Granada	6	36°43,346N	3°43,686W	Abundant	65,00	40,00	50,00	45,00	50,00
GR_6	Granada	12	36°43,346N	3°43,686W	Abundant	65,00	60,00	55,00	55,00	58,75
GR_7	Granada	3	36°43,742N	3°41,279W	Absent	0,00	0,00	0,00	0,00	0,00
GR_7	Granada	6	36°43,742N	3°41,279W	Absent	0,00	0,00	0,00	0,00	0,00
GR_7	Granada	12	36°43,700N	3°41,269W	Absent	0,00	0,00	0,00	0,00	0,00
GR_8	Granada	3	36°43,723N	3°40,945W	Absent	0,00	0,00	0,00	0,00	0,00
GR_8	Granada	6	36°43,727N	3°40,992W	Absent	0,00	0,00	0,00	0,00	0,00
GR_8	Granada	12	36°43,677N	3°40,983W	Absent	0,00	0,00	0,00	0,00	0,00
GR_9	Granada	3	36°43,573N	3°41,708W	Presence	15,00	20,00	20,00	10,00	16,25
GR_9	Granada	6	36°43,573N	3°41,708W	Presence	10,00	20,00	30,00	15,00	18,75

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GR_9	Granada	12	36°43,573N	3°41,708W	Presence	10,00	10,00	15,00	30,00	16,25
GR_10	Granada	3	36°44,570N	3°39,698W	Absent	0,00	0,00	0,00	0,00	0,00
GR_10	Granada	6	36°44,569N	3°39,712W	Absent	0,00	0,00	0,00	0,00	0,00
GR_10	Granada	12	36°44,582N	3°39,701W	Absent	0,00	0,00	0,00	0,00	0,00
GR_11	Granada	3	36°44,660N	3°39,711W	Absent	0,00	0,00	0,00	0,00	0,00
GR_11	Granada	6	36°44,603N	3°39,633W	Absent	0,00	0,00	0,00	0,00	0,00
GR_11	Granada	12	36°44,576N	3°39,596W	Absent	0,00	0,00	0,00	0,00	0,00
GR_12	Granada	3	36°44,669N	3°39,000W	Absent	0,00	0,00	0,00	0,00	0,00
GR_12	Granada	6	36°44,590N	3°38,998W	Absent	0,00	0,00	0,00	0,00	0,00
GR_12	Granada	12	36°44,548N	3°38,992W	Absent	0,00	0,00	0,00	0,00	0,00
GR_13	Granada	3	36°44,519N	3°38,300W	Absent	0,00	0,00	0,00	0,00	0,00
GR_13	Granada	6	36°44,517N	3°38,300W	Absent	0,00	0,00	0,00	0,00	0,00
GR_13	Granada	12	36°44,511N	3°38,300W	Absent	0,00	0,00	0,00	0,00	0,00
GR_14	Granada	3	36°44,480N	3°37,960W	Absent	0,00	0,00	0,00	0,00	0,00
GR_14	Granada	6	36°44,482N	3°37,961W	Absent	0,00	0,00	0,00	0,00	0,00
GR_14	Granada	12	36°44,485N	3°37,966W	Absent	0,00	0,00	0,00	0,00	0,00
GR_15	Granada	3	36°44,690N	3°39,314W	Presence	20,00	25,00	15,00	15,00	18,75
GR_15	Granada	6	36°44,446N	3°37,333W	Absent	0,00	0,00	0,00	0,00	0,00
GR_15	Granada	12	36°44,428N	3°37,325W	Absent	0,00	0,00	0,00	0,00	0,00
GR_16	Granada	3	36°44,581N	3°36,703W	Absent	0,00	0,00	0,00	0,00	0,00
GR_16	Granada	6	36°44,576N	3°36,699W	Absent	0,00	0,00	0,00	0,00	0,00
GR_16	Granada	12	36°44,546N	3°36,697W	Absent	0,00	0,00	0,00	0,00	0,00
GR_17	Granada	3	36°44,629N	3°36,204W	Absent	0,00	0,00	0,00	0,00	0,00
GR_17	Granada	6	36°44,629N	3°36,202W	Absent	0,00	0,00	0,00	0,00	0,00
GR_17	Granada	12	36°44,589N	3°36,183W	Absent	0,00	0,00	0,00	0,00	0,00
GR_18	Granada	3	36°44,510N	3°38,276W	Presence	25,00	10,00	15,00	10,00	15,00
GR_18	Granada	6	36°44,124N	3°35,635W	Absent	0,00	0,00	0,00	0,00	0,00
GR_18	Granada	12	36°44,124N	3°35,635W	Absent	0,00	0,00	0,00	0,00	0,00
GR_19	Granada	3	36°44,480N	3°37,986W	Presence	25,00	25,00	20,00	20,00	22,50
GR_19	Granada	6	36°43,041N	3°31,353W	Absent	0,00	0,00	0,00	0,00	0,00
GR_19	Granada	12	36°43,041N	3°31,353W	Absent	0,00	0,00	0,00	0,00	0,00
GR_20	Granada	3	36°42,152N	3°29,521W	Absent	0,00	0,00	0,00	0,00	0,00
GR_20	Granada	6	36°42,144N	3°29,527W	Absent	0,00	0,00	0,00	0,00	0,00
GR_20	Granada	12	36°42,041N	3°29,562W	Absent	0,00	0,00	0,00	0,00	0,00
GR_21	Granada	3	36°41,786N	3°28,289W	Absent	0,00	0,00	0,00	0,00	0,00
GR_21	Granada	6	36°41,786N	3°28,989W	Absent	0,00	0,00	0,00	0,00	0,00
GR_21	Granada	12	36°41,752N	3°29,014W	Absent	0,00	0,00	0,00	0,00	0,00
GR_22	Granada	3	36°42,222N	3°29,640W	Absent	0,00	0,00	0,00	0,00	0,00
GR_22	Granada	6	36°42,208N	3°29,646W	Absent	0,00	0,00	0,00	0,00	0,00
GR_22	Granada	12	36°42,161N	3°29,733W	Absent	0,00	0,00	0,00	0,00	0,00
GR_23	Granada	3	36°44,124N	3°35,635W	Presence	20,00	25,00	20,00	20,00	21,25
GR_23	Granada	6	36°41,564N	3°28,217W	Absent	0,00	0,00	0,00	0,00	0,00
GR_23	Granada	12	36°41,535N	3°28,238W	Absent	0,00	0,00	0,00	0,00	0,00
GR_24	Granada	3	36°43,041N	3°31,353W	Presence	15,00	30,00	10,00	15,00	17,50
GR_24	Granada	6	36°43,041N	3°31,353W	Presence	10,00	10,00	25,00	20,00	16,25
GR_24	Granada	12	36°43,041N	3°31,353W	Presence	20,00	20,00	15,00	25,00	20,00
GR_25	Granada	3	36°41,633N	3°27,117W	Absent	0,00	0,00	0,00	0,00	0,00
GR_25	Granada	6	36°41,622N	3°27,116W	Absent	0,00	0,00	0,00	0,00	0,00
GR_25	Granada	12	36°41,514N	3°27,065W	Absent	0,00	0,00	0,00	0,00	0,00
GR_26	Granada	3	36°41,653N	3°26,606W	Absent	0,00	0,00	0,00	0,00	0,00
GR_26	Granada	6	36°41,640N	3°26,608W	Absent	0,00	0,00	0,00	0,00	0,00
GR_26	Granada	12	36°41,537N	3°26,575W	Absent	0,00	0,00	0,00	0,00	0,00
GR_27	Granada	3	36°41,655N	3°26,188W	Absent	0,00	0,00	0,00	0,00	0,00
GR_27	Granada	6	36°41,644N	3°26,185W	Absent	0,00	0,00	0,00	0,00	0,00
GR_27	Granada	12	36°41,545N	3°26,088W	Absent	0,00	0,00	0,00	0,00	0,00
GR_28	Granada	3	36°42,160N	3°24,372W	Absent	0,00	0,00	0,00	0,00	0,00
GR_28	Granada	6	36°42,160N	3°24,372W	Absent	0,00	0,00	0,00	0,00	0,00
GR_28	Granada	12	36°42,160N	3°24,372W	Absent	0,00	0,00	0,00	0,00	0,00
GR_29	Granada	3	36°41,711N	3°27,509W	Presence	15,00	20,00	5,00	10,00	12,50
GR_29	Granada	6	36°41,710N	3°27,517W	Presence	5,00	1,00	5,00	5,00	4,00
GR_29	Granada	12	36°42,375N	3°23,728W	Absent	0,00	0,00	0,00	0,00	0,00
GR_30	Granada	3	36°41,622N	3°27,116W	Presence	5,00	5,00	10,00	15,00	8,75
GR_30	Granada	6	36°41,622N	3°27,116W	Presence	10,00	1,00	1,00	1,00	3,25

GR_30	Granada	12	36°42,474N	3°23,359W	Absent	0,00	0,00	0,00	0,00	0,00
GR_31	Granada	3	36°41,653N	3°26,606W	Presence	5,00	5,00	5,00	1,00	4,00
GR_31	Granada	6	36°42,795N	3°22,151W	Absent	0,00	0,00	0,00	0,00	0,00
GR_31	Granada	12	36°42,795N	3°22,151W	Absent	0,00	0,00	0,00	0,00	0,00
GR_32	Granada	3	36°42,977N	3°21,804W	Absent	0,00	0,00	0,00	0,00	0,00
GR_32	Granada	6	36°42,951N	3°21,790W	Absent	0,00	0,00	0,00	0,00	0,00
GR_32	Granada	12	36°42,950N	3°21,782W	Absent	0,00	0,00	0,00	0,00	0,00
GR_33	Granada	3	36°42,093N	3°24,429W	Very Abundant	75,00	70,00	80,00	75,00	75,00
GR_33	Granada	6	36°42,093N	3°24,429W	Presence	15,00	25,00	20,00	20,00	20,00
GR_33	Granada	12	36°42,093N	3°24,429W	Presence	10,00	5,00	15,00	25,00	13,75
GR_34	Granada	3	36°42,375N	3°23,728W	Not abundant	35,00	45,00	40,00	45,00	41,25
GR_34	Granada	6	36°42,375N	3°23,728W	Presence	15,00	25,00	10,00	15,00	16,25
GR_34	Granada	12	36°42,375N	3°23,728W	Presence	10,00	10,00	5,00	15,00	10,00
GR_35	Granada	3	36°42,474N	3°23,359W	Very Abundant	85,00	90,00	80,00	70,00	81,25
GR_35	Granada	6	36°42,474N	3°23,359W	Very Abundant	75,00	70,00	85,00	75,00	76,25
GR_35	Granada	12	36°42,474N	3°23,359W	Very Abundant	85,00	80,00	90,00	80,00	83,75
GR_36	Granada	3	36°42,795N	3°22,151W	Very Abundant	80,00	70,00	75,00	80,00	76,25
GR_36	Granada	6	36°42,795N	3°22,151W	Very Abundant	80,00	75,00	80,00	80,00	78,75
GR_36	Granada	12	36°42,795N	3°22,151W	Very Abundant	85,00	80,00	75,00	80,00	80,00
GR_37	Granada	3	36°42,827N	3°21,970W	Abundant	55,00	70,00	65,00	45,00	58,75
GR_37	Granada	6	36°44,285N	3°19,773W	Absent	0,00	0,00	0,00	0,00	0,00
GR_37	Granada	12	36°44,282N	3°19,773W	Absent	0,00	0,00	0,00	0,00	0,00
GR_38	Granada	3	36°42,953N	3°21,774W	Abundant	75,00	70,00	70,00	70,00	71,25
GR_38	Granada	6	36°44,463N	3°19,132W	Absent	0,00	0,00	0,00	0,00	0,00
GR_38	Granada	12	36°44,400N	3°19,141W	Absent	0,00	0,00	0,00	0,00	0,00
GR_39	Granada	3	36°44,373N	3°19,550W	Absent	0,00	0,00	0,00	0,00	0,00
GR_39	Granada	6	36°44,370N	3°19,548W	Absent	0,00	0,00	0,00	0,00	0,00
GR_39	Granada	12	36°44,346N	3°19,544W	Absent	0,00	0,00	0,00	0,00	0,00
GR_40	Granada	3	36°44,240N	3°18,288W	Absent	0,00	0,00	0,00	0,00	0,00
GR_40	Granada	6	36°44,174N	3°18,269W	Absent	0,00	0,00	0,00	0,00	0,00
GR_40	Granada	12	36°44,133N	3°18,235W	Absent	0,00	0,00	0,00	0,00	0,00
GR_41	Granada	3	36°44,809N	3°16,505W	Absent	0,00	0,00	0,00	0,00	0,00
GR_41	Granada	6	36°44,736N	3°16,497W	Absent	0,00	0,00	0,00	0,00	0,00
GR_41	Granada	12	36°44,697N	3°16,497W	Absent	0,00	0,00	0,00	0,00	0,00
GR_42	Granada	3	36°45,014N	3°15,972W	Absent	0,00	0,00	0,00	0,00	0,00
GR_42	Granada	6	36°44,948N	3°15,921W	Absent	0,00	0,00	0,00	0,00	0,00
GR_42	Granada	12	36°44,866N	3°15,914W	Absent	0,00	0,00	0,00	0,00	0,00
GR_43	Granada	3	36°45,174N	3°15,173W	Absent	0,00	0,00	0,00	0,00	0,00
GR_43	Granada	6	36°45,123N	3°15,178W	Absent	0,00	0,00	0,00	0,00	0,00
GR_43	Granada	12	36°45,060N	3°15,179W	Absent	0,00	0,00	0,00	0,00	0,00
GR_44	Granada	3	36°45,043N	3°14,348W	Absent	0,00	0,00	0,00	0,00	0,00
GR_44	Granada	6	36°45,047N	3°14,409W	Absent	0,00	0,00	0,00	0,00	0,00
GR_44	Granada	12	36°45,972N	3°14,406W	Absent	0,00	0,00	0,00	0,00	0,00
GR_45	Granada	3	36°44,990N	3°13,979W	Absent	0,00	0,00	0,00	0,00	0,00
GR_45	Granada	6	36°44,947N	3°13,705W	Absent	0,00	0,00	0,00	0,00	0,00
GR_45	Granada	12	36°44,768N	3°13,678W	Absent	0,00	0,00	0,00	0,00	0,00
GR_46	Granada	3	36°44,315N	3°18,598W	Presence	5,00	10,00	5,00	0,00	5,00
GR_46	Granada	6	36°44,682N	3°12,639W	Absent	0,00	0,00	0,00	0,00	0,00
GR_46	Granada	12	36°44,645N	3°12,640W	Absent	0,00	0,00	0,00	0,00	0,00
GR_47	Granada	3	36°44,705N	3°10,189W	Absent	0,00	0,00	0,00	0,00	0,00
GR_47	Granada	6	36°44,672N	3°10,211W	Absent	0,00	0,00	0,00	0,00	0,00
GR_47	Granada	12	36°44,596N	3°10,190W	Absent	0,00	0,00	0,00	0,00	0,00
GR_48	Granada	3	36°44,611N	3°09,868W	Absent	0,00	0,00	0,00	0,00	0,00
GR_48	Granada	6	36°44,567N	3°09,826W	Absent	0,00	0,00	0,00	0,00	0,00
GR_48	Granada	12	36°44,499N	3°09,872W	Absent	0,00	0,00	0,00	0,00	0,00
GR_49	Granada	3	36°44,958N	3°13,996W	Presence	0,00	0,00	5,00	1,00	1,50
GR_49	Granada	6	36°44,949N	3°14,000W	Presence	0,00	0,00	1,00	1,00	0,50
GR_49	Granada	12	36°44,912N	3°14,157W	Absent	0,00	0,00	0,00	0,00	0,00
AL_1	Almería	3	36°44,780N	3°05,052W	Absent	0,00	0,00	0,00	0,00	0,00
AL_1	Almería	6	36°44,777N	3°05,047W	Absent	0,00	0,00	0,00	0,00	0,00
AL_1	Almería	12	36°44,723N	3°05,047W	Absent	0,00	0,00	0,00	0,00	0,00
AL_2	Almería	3	36°44,757N	3°03,849W	Absent	0,00	0,00	0,00	0,00	0,00
AL_2	Almería	6	36°44,752N	3°03,785W	Absent	0,00	0,00	0,00	0,00	0,00

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AL_2	Almería	12	36°44,752N	3°03,785W	Absent	0,00	0,00	0,00	0,00	0,00
AL_3	Almería	3	36°44,752N	3°03,309W	Presence	1,00	1,00	0,00	0,00	0,50
AL_3	Almería	6	36°44,750N	3°03,182W	Absent	0,00	0,00	0,00	0,00	0,00
AL_3	Almería	12	36°44,750N	3°03,182W	Absent	0,00	0,00	0,00	0,00	0,00
AL_4	Almería	3	36°44,520N	3°00,010W	Absent	0,00	0,00	0,00	0,00	0,00
AL_4	Almería	6	36°44,750N	3°03,182W	Presence	5,00	0,00	0,00	0,00	1,25
AL_4	Almería	12	36°44,352N	2°59,991W	Absent	0,00	0,00	0,00	0,00	0,00
AL_5	Almería	3	36°44,488N	3°00,116W	Absent	0,00	0,00	0,00	0,00	0,00
AL_5	Almería	6	36°44,460N	3°00,125W	Absent	0,00	0,00	0,00	0,00	0,00
AL_5	Almería	12	36°44,357N	3°00,118W	Absent	0,00	0,00	0,00	0,00	0,00
AL_6	Almería	3	36°44,270N	2°58,563W	Absent	0,00	0,00	0,00	0,00	0,00
AL_6	Almería	6	36°44,191N	2°58,599W	Absent	0,00	0,00	0,00	0,00	0,00
AL_6	Almería	12	36°44,759N	2°58,579W	Absent	0,00	0,00	0,00	0,00	0,00
AL_7	Almería	3	36°45,029N	2°55,725W	Absent	0,00	0,00	0,00	0,00	0,00
AL_7	Almería	6	36°45,029N	2°55,725W	Absent	0,00	0,00	0,00	0,00	0,00
AL_7	Almería	12	36°45,027N	2°55,725W	Absent	0,00	0,00	0,00	0,00	0,00
AL_8	Almería	3	36°44,991N	2°55,232W	Absent	0,00	0,00	0,00	0,00	0,00
AL_8	Almería	6	36°44,991N	2°55,232W	Absent	0,00	0,00	0,00	0,00	0,00
AL_8	Almería	12	36°44,991N	2°55,232W	Absent	0,00	0,00	0,00	0,00	0,00
AL_9	Almería	3	36°41,850N	2°51,474W	Absent	0,00	0,00	0,00	0,00	0,00
AL_9	Almería	6	36°41,850N	2°51,474W	Absent	0,00	0,00	0,00	0,00	0,00
AL_9	Almería	12	36°41,842N	2°51,503W	Absent	0,00	0,00	0,00	0,00	0,00
AL_10	Almería	3	36°41,680N	2°50,706W	Absent	0,00	0,00	0,00	0,00	0,00
AL_10	Almería	6	36°41,544N	2°50,702W	Absent	0,00	0,00	0,00	0,00	0,00
AL_10	Almería	12	36°41,535N	2°50,700W	Absent	0,00	0,00	0,00	0,00	0,00
AL_11	Almería	3	36°41,976N	2°47,980W	Absent	0,00	0,00	0,00	0,00	0,00
AL_11	Almería	6	36°41,976N	2°47,980W	Absent	0,00	0,00	0,00	0,00	0,00
AL_11	Almería	12	36°41,976N	2°47,980W	Absent	0,00	0,00	0,00	0,00	0,00
AL_12	Almería	3	36°41,666N	2°47,730W	Absent	0,00	0,00	0,00	0,00	0,00
AL_12	Almería	6	36°41,687N	2°47,717W	Absent	0,00	0,00	0,00	0,00	0,00
AL_12	Almería	12	36°41,660N	2°47,714W	Absent	0,00	0,00	0,00	0,00	0,00
AL_13	Almería	3	36°41,600N	2°47,540W	Absent	0,00	0,00	0,00	0,00	0,00
AL_13	Almería	6	36°41,600N	2°47,541W	Absent	0,00	0,00	0,00	0,00	0,00
AL_13	Almería	12	36°41,545N	2°47,550W	Absent	0,00	0,00	0,00	0,00	0,00
AL_14	Almería	3	36°41,222N	2°46,879W	Absent	0,00	0,00	0,00	0,00	0,00
AL_14	Almería	6	36°41,222N	2°46,879W	Absent	0,00	0,00	0,00	0,00	0,00
AL_14	Almería	12	36°41,222N	2°46,879W	Absent	0,00	0,00	0,00	0,00	0,00
AL_15	Almería	3	36°40,674N	2°45,222W	Absent	0,00	0,00	0,00	0,00	0,00
AL_15	Almería	6	36°40,474N	2°45,253W	Absent	0,00	0,00	0,00	0,00	0,00
AL_15	Almería	12	36°40,406N	2°45,242W	Absent	0,00	0,00	0,00	0,00	0,00
AL_16	Almería	3	36°40,863N	2°42,841W	Absent	0,00	0,00	0,00	0,00	0,00
AL_16	Almería	6	36°42,562N	2°42,788W	Absent	0,00	0,00	0,00	0,00	0,00
AL_16	Almería	12	36°40,468N	2°42,778W	Absent	0,00	0,00	0,00	0,00	0,00
AL_17	Almería	3	36°45,422N	2°36,165W	Absent	0,00	0,00	0,00	0,00	0,00
AL_17	Almería	6	36°45,422N	2°36,165W	Absent	0,00	0,00	0,00	0,00	0,00
AL_17	Almería	12	36°45,422N	2°36,165W	Absent	0,00	0,00	0,00	0,00	0,00
AL_18	Almería	3	36°46,759N	2°35,592W	Absent	0,00	0,00	0,00	0,00	0,00
AL_18	Almería	6	36°46,731N	2°35,560W	Absent	0,00	0,00	0,00	0,00	0,00
AL_18	Almería	12	36°46,731N	2°35,560W	Absent	0,00	0,00	0,00	0,00	0,00
AL_19	Almería	3	36°47,959N	2°34,406W	Absent	0,00	0,00	0,00	0,00	0,00
AL_19	Almería	6	36°47,949N	2°34,674W	Absent	0,00	0,00	0,00	0,00	0,00
AL_19	Almería	12	36°47,949N	2°34,674W	Absent	0,00	0,00	0,00	0,00	0,00
AL_20	Almería	3	36°48,647N	2°34,016W	Absent	0,00	0,00	0,00	0,00	0,00
AL_20	Almería	6	36°48,647N	2°34,016W	Absent	0,00	0,00	0,00	0,00	0,00
AL_20	Almería	12	36°48,647N	2°34,016W	Absent	0,00	0,00	0,00	0,00	0,00
AL_21	Almería	3	36°49,110N	2°32,339W	Absent	0,00	0,00	0,00	0,00	0,00
AL_21	Almería	6	36°49,110N	2°32,339W	Absent	0,00	0,00	0,00	0,00	0,00
AL_21	Almería	12	36°49,110N	2°32,339W	Absent	0,00	0,00	0,00	0,00	0,00
AL_22	Almería	3	36°49,099N	2°31,976W	Absent	0,00	0,00	0,00	0,00	0,00
AL_22	Almería	6	36°49,099N	2°31,976W	Absent	0,00	0,00	0,00	0,00	0,00
AL_22	Almería	12	36°49,099N	2°31,976W	Absent	0,00	0,00	0,00	0,00	0,00
AL_23	Almería	3	36°49,457N	2°30,516W	Absent	0,00	0,00	0,00	0,00	0,00
AL_23	Almería	6	36°49,457N	2°30,516W	Absent	0,00	0,00	0,00	0,00	0,00

AL_23	Almería	12	36°49,457N	2°30,516W	Absent	0,00	0,00	0,00	0,00	0,00
AL_24	Almería	3	36°49,656N	2°29,514W	Absent	0,00	0,00	0,00	0,00	0,00
AL_24	Almería	6	36°49,656N	2°29,514W	Absent	0,00	0,00	0,00	0,00	0,00
AL_24	Almería	12	36°49,656N	2°29,514W	Absent	0,00	0,00	0,00	0,00	0,00
AL_25	Almería	3	36°49,537N	2°27,141W	Absent	0,00	0,00	0,00	0,00	0,00
AL_25	Almería	6	36°49,537N	2°27,141W	Absent	0,00	0,00	0,00	0,00	0,00
AL_25	Almería	12	36°49,537N	2°27,141W	Absent	0,00	0,00	0,00	0,00	0,00
AL_26	Almería	3	36°43,578N	3°11,809W	Not abundant	30,00	20,00	25,00	25,00	25,00
AL_26	Almería	6	36°43,578N	3°11,809W	Absent	0,00	0,00	0,00	0,00	0,00
AL_26	Almería	12	36°43,578N	3°11,809W	Absent	0,00	0,00	0,00	0,00	0,00
AL_27	Almería	3	36°43,345N	3°11,630W	Not abundant	35,00	25,00	20,00	25,00	26,25
AL_27	Almería	6	36°43,345N	3°11,630W	Absent	0,00	0,00	0,00	0,00	0,00
AL_27	Almería	12	36°43,345N	3°11,630W	Absent	0,00	0,00	0,00	0,00	0,00
AL_28	Almería	3	36°43,317N	2°10,023W	Presence	5,00	15,00	10,00	10,00	10,00
AL_28	Almería	6	36°43,317N	2°10,023W	Absent	0,00	0,00	0,00	0,00	0,00
AL_28	Almería	12	36°43,317N	2°10,023W	Absent	0,00	0,00	0,00	0,00	0,00
AL_29	Almería	3	36°48,703N	2°02,976W	Absent	0,00	0,00	0,00	0,00	0,00
AL_29	Almería	6	36°48,703N	2°02,976W	Absent	0,00	0,00	0,00	0,00	0,00
AL_29	Almería	12	36°48,703N	2°02,976W	Absent	0,00	0,00	0,00	0,00	0,00
AL_30	Almería	3	36°48,802N	2°02,937W	Absent	0,00	0,00	0,00	0,00	0,00
AL_30	Almería	6	36°48,802N	2°02,937W	Absent	0,00	0,00	0,00	0,00	0,00
AL_30	Almería	12	36°48,802N	2°02,937W	Absent	0,00	0,00	0,00	0,00	0,00
AL_31	Almería	3	36°49,961N	2°01,486W	Absent	0,00	0,00	0,00	0,00	0,00
AL_31	Almería	6	36°49,961N	2°01,486W	Absent	0,00	0,00	0,00	0,00	0,00
AL_31	Almería	12	36°49,961N	2°01,468W	Absent	0,00	0,00	0,00	0,00	0,00
AL_32	Almería	3	36°51,796N	2°00,165W	Absent	0,00	0,00	0,00	0,00	0,00
AL_32	Almería	6	36°51,788N	2°00,124W	Absent	0,00	0,00	0,00	0,00	0,00
AL_32	Almería	12	36°51,803N	2°00,075W	Absent	0,00	0,00	0,00	0,00	0,00
AL_33	Almería	3	36°53,464N	1°59,533W	Absent	0,00	0,00	0,00	0,00	0,00
AL_33	Almería	6	36°53,559N	1°59,533W	Absent	0,00	0,00	0,00	0,00	0,00
AL_33	Almería	12	36°53,565N	1°59,534W	Absent	0,00	0,00	0,00	0,00	0,00
AL_34	Almería	3	36°53,871N	1°58,015W	Absent	0,00	0,00	0,00	0,00	0,00
AL_34	Almería	6	36°53,871N	1°58,015W	Absent	0,00	0,00	0,00	0,00	0,00
AL_34	Almería	12	36°53,871N	1°58,015W	Absent	0,00	0,00	0,00	0,00	0,00
AL_35	Almería	3	36°55,642N	1°56,929W	Absent	0,00	0,00	0,00	0,00	0,00
AL_35	Almería	6	36°55,642N	1°56,929W	Absent	0,00	0,00	0,00	0,00	0,00
AL_35	Almería	12	36°55,642N	1°56,929W	Absent	0,00	0,00	0,00	0,00	0,00
AL_36	Almería	3	36°55,978N	1°56,619W	Absent	0,00	0,00	0,00	0,00	0,00
AL_36	Almería	6	36°55,978N	1°56,619W	Absent	0,00	0,00	0,00	0,00	0,00
AL_36	Almería	12	36°55,978N	1°56,619W	Absent	0,00	0,00	0,00	0,00	0,00
AL_37	Almería	3	36°56,790N	1°53,946W	Absent	0,00	0,00	0,00	0,00	0,00
AL_37	Almería	6	36°56,790N	1°53,946W	Absent	0,00	0,00	0,00	0,00	0,00
AL_37	Almería	12	36°56,790N	1°53,946W	Absent	0,00	0,00	0,00	0,00	0,00
AL_38	Almería	3	36°57,638N	1°53,888W	Absent	0,00	0,00	0,00	0,00	0,00
AL_38	Almería	6	36°57,638N	1°53,888W	Absent	0,00	0,00	0,00	0,00	0,00
AL_38	Almería	12	36°57,638N	1°53,888W	Absent	0,00	0,00	0,00	0,00	0,00
AL_39	Almería	3	36°59,608N	1°53,065W	Absent	0,00	0,00	0,00	0,00	0,00
AL_39	Almería	6	36°59,608N	1°53,065W	Absent	0,00	0,00	0,00	0,00	0,00
AL_39	Almería	12	36°59,608N	1°53,065W	Absent	0,00	0,00	0,00	0,00	0,00
AL_40	Almería	3	36°59,668N	1°53,182W	Absent	0,00	0,00	0,00	0,00	0,00
AL_40	Almería	6	36°59,668N	1°53,182W	Absent	0,00	0,00	0,00	0,00	0,00
AL_40	Almería	12	36°59,668N	1°53,182W	Absent	0,00	0,00	0,00	0,00	0,00
AL_41	Almería	3	37°00,515N	1°52,949W	Absent	0,00	0,00	0,00	0,00	0,00
AL_41	Almería	6	37°00,519N	1°52,906W	Absent	0,00	0,00	0,00	0,00	0,00
AL_41	Almería	12	37°00,519N	1°52,906W	Absent	0,00	0,00	0,00	0,00	0,00
AL_42	Almería	3	37°01,043N	1°52,660W	Absent	0,00	0,00	0,00	0,00	0,00
AL_42	Almería	6	37°01,050N	1°52,616W	Absent	0,00	0,00	0,00	0,00	0,00
AL_42	Almería	12	37°01,050N	1°52,481W	Absent	0,00	0,00	0,00	0,00	0,00
AL_43	Almería	3	37°03,493N	1°51,300W	Absent	0,00	0,00	0,00	0,00	0,00
AL_43	Almería	6	37°03,479N	1°51,263W	Absent	0,00	0,00	0,00	0,00	0,00
AL_43	Almería	12	37°03,476N	1°51,208W	Absent	0,00	0,00	0,00	0,00	0,00
AL_44	Almería	3	37°03,870N	1°51,103W	Absent	0,00	0,00	0,00	0,00	0,00
AL_44	Almería	6	37°03,863N	1°51,075W	Absent	0,00	0,00	0,00	0,00	0,00

*Conservation biology of the endangered orange coral Astroides calycularis*

AL_44	Almería	12	37°03,859N	1°50,974W	Absent	0,00	0,00	0,00	0,00	0,00
AL_45	Almería	3	37°17,135N	1°43,764W	Absent	0,00	0,00	0,00	0,00	0,00
AL_45	Almería	6	37°17,135N	1°43,764W	Absent	0,00	0,00	0,00	0,00	0,00
AL_45	Almería	12	37°17,135N	1°43,764W	Absent	0,00	0,00	0,00	0,00	0,00
AL_46	Almería	3	37°19,512N	1°41,663W	Absent	0,00	0,00	0,00	0,00	0,00
AL_46	Almería	6	37°19,512N	1°41,663W	Absent	0,00	0,00	0,00	0,00	0,00
AL_46	Almería	12	37°19,512N	1°41,663W	Absent	0,00	0,00	0,00	0,00	0,00
AL_47	Almería	3	37°21,058N	1°40,595W	Absent	0,00	0,00	0,00	0,00	0,00
AL_47	Almería	6	37°21,106N	1°40,603W	Absent	0,00	0,00	0,00	0,00	0,00
AL_47	Almería	12	37°21,003N	1°40,471W	Absent	0,00	0,00	0,00	0,00	0,00
AL_48	Almería	3	37°21,314N	1°40,077W	Absent	0,00	0,00	0,00	0,00	0,00
AL_48	Almería	6	37°21,314N	1°40,077W	Absent	0,00	0,00	0,00	0,00	0,00
AL_48	Almería	12	37°21,263N	1°40,027W	Absent	0,00	0,00	0,00	0,00	0,00
AL_49	Almería	3	37°20,867N	1°39,056W	Absent	0,00	0,00	0,00	0,00	0,00
AL_49	Almería	6	37°20,867N	1°39,056W	Absent	0,00	0,00	0,00	0,00	0,00
AL_49	Almería	12	37°20,867N	1°39,056W	Absent	0,00	0,00	0,00	0,00	0,00
AL_50	Almería	3	37°21,181N	1°39,303W	Absent	0,00	0,00	0,00	0,00	0,00
AL_50	Almería	6	37°21,181N	1°39,303W	Absent	0,00	0,00	0,00	0,00	0,00
AL_50	Almería	12	37°21,181N	1°39,303W	Absent	0,00	0,00	0,00	0,00	0,00



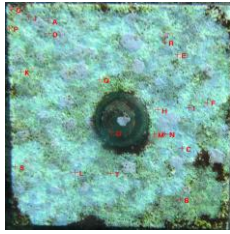
An underwater photograph showing several sea urchins on a rock. The urchins have spines that are dark purple, reddish-brown, and light blue. In the background, there are yellowish-orange coral-like structures. A semi-transparent brown horizontal band is overlaid across the middle of the image, containing the text 'APPENDIX III: PLATES' in white, serif, all-caps font.

**APPENDIX III: PLATES**

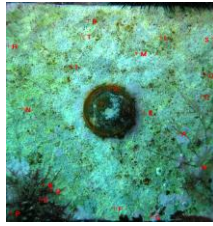
One plate installed on Granada coast. Author: Terrón-Sigler A.



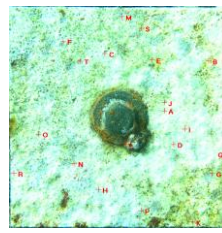
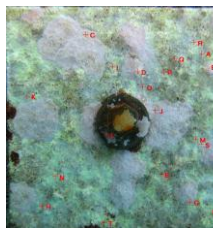
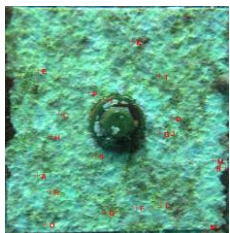
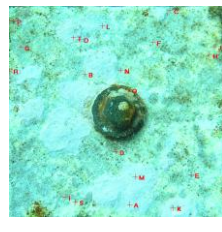
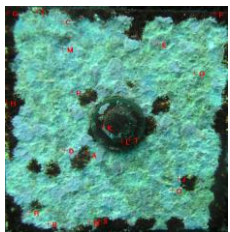
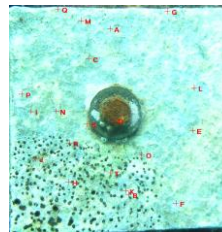
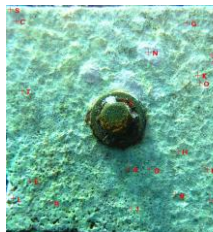
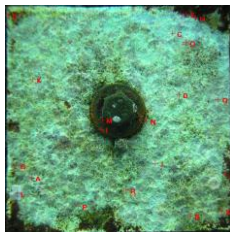
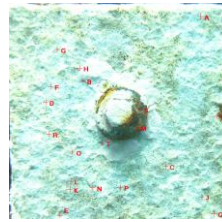
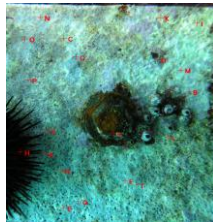
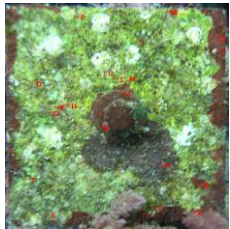
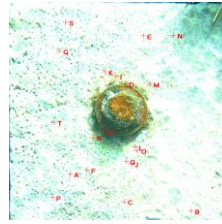
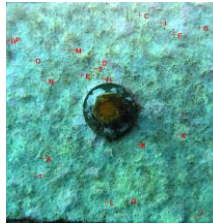
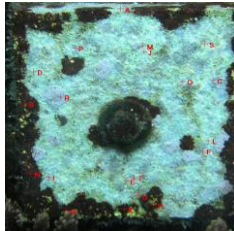
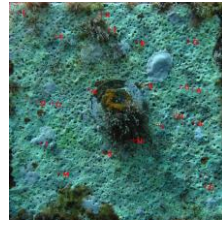
**T1**



**T2**



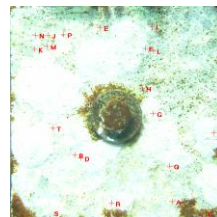
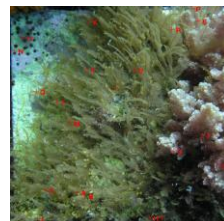
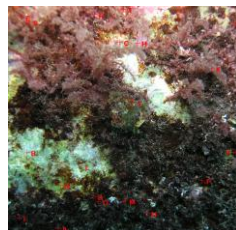
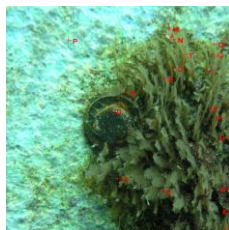
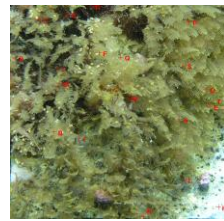
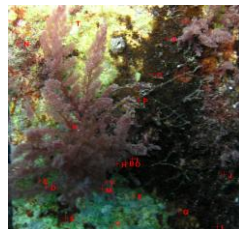
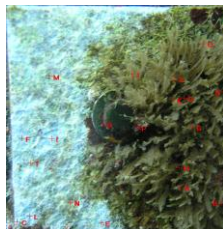
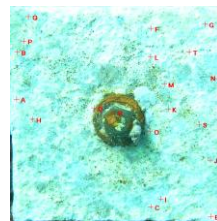
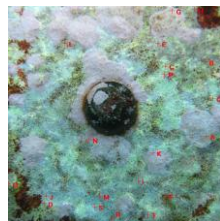
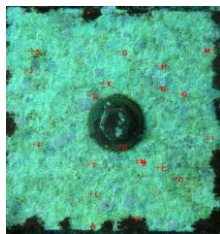
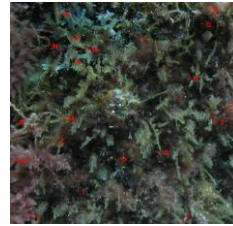
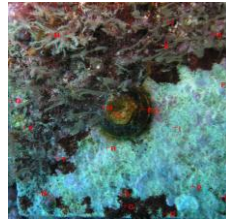
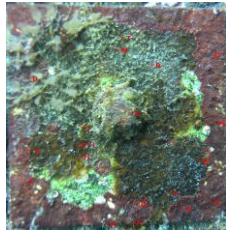
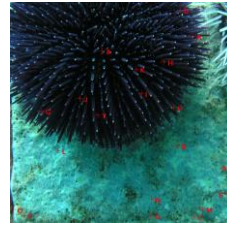
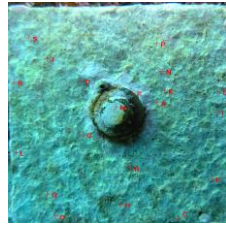
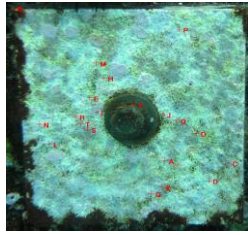
**T3**



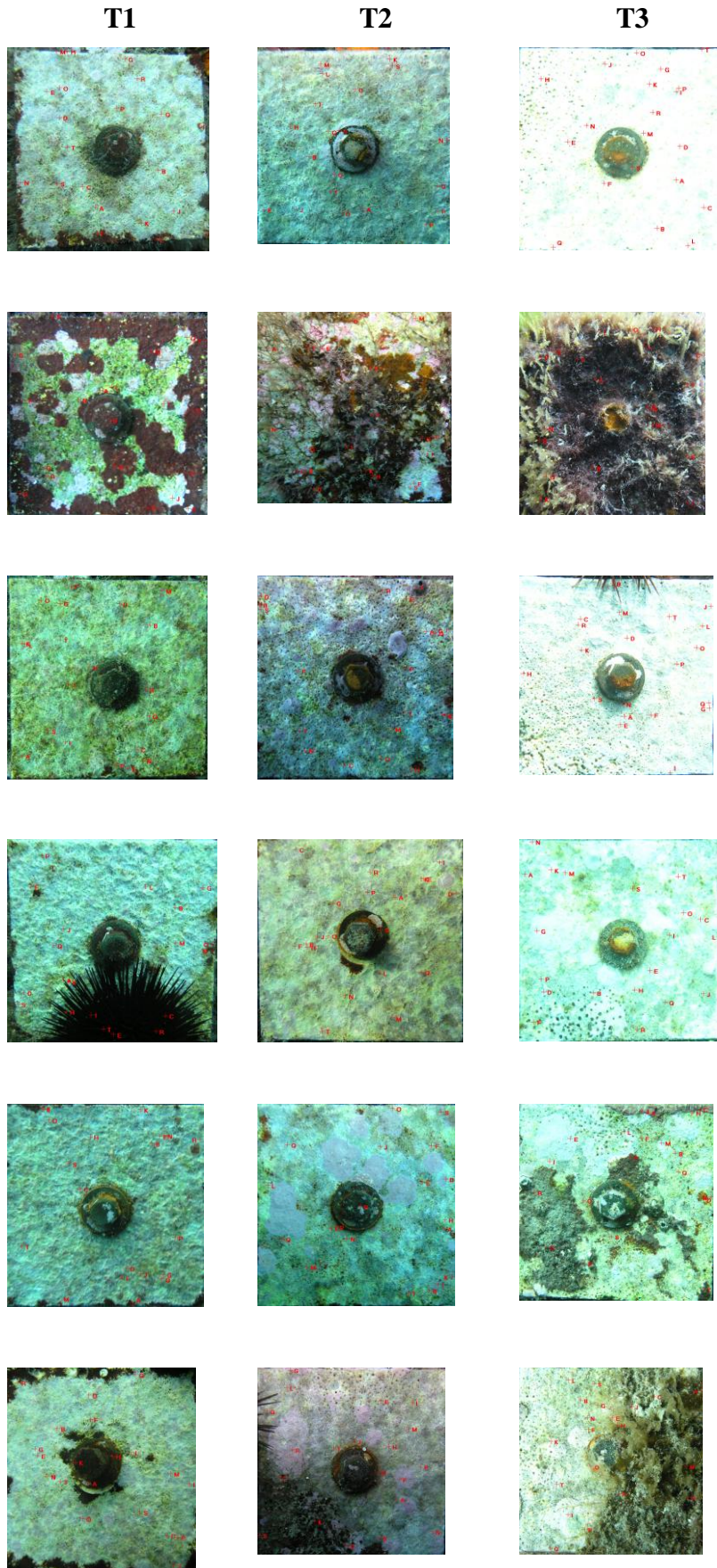
**T1**

**T2**

**T3**













A través de la lectura de todas las referencias que han sido consultadas para el desarrollo de la presente tesis doctoral, me entristeció leer en una de ellas que ya no existen hábitats de coral prístinos en el planeta. No sé si esto es realmente cierto, espero que no, pero hace que tenga un poco más de sentido el presente trabajo. *Astroides calycularis* (Pallas 1766) se distribuye principalmente en el mar Mediterráneo sur-occidental debido a los requerimientos ambientales de la especie, sobre todo en temperatura y aguas transparentes y bien oxigenadas. La especie habita fondos rocosos desde la superficie del mar hasta los 50 metros de profundidad y localmente se pueden encontrar poblaciones muy abundantes. Este emblemático coral se encuentra afectado por las actividades humanas desarrolladas en el litoral, incluyéndose en catálogos regionales y nacionales y en anexos internacionales como los convenios de Berna y Barcelona y la convención CITES. En la costa andaluza se encuentra el límite de distribución más occidental del coral naranja del norte del mar de Alborán (sur de la Península Ibérica). Por otro lado, Andalucía se caracteriza por un elevado desarrollo costero dirigido a cubrir las necesidades turísticas. Este hecho hace que las poblaciones presentes en este litoral se encuentren sometidas a diversos impactos. Por ello, el objetivo principal de la presente tesis doctoral es avanzar en el conocimiento de la biología y ecología de *A. calycularis* en el norte del mar de Alborán desde la perspectiva de la conservación.