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THERMOTOLERANCE EXPERIMENTS AND TRANSPLANTATION PROCEDURES OF FOUR GORGONIAN SPECIES FROM THE SOUTH COAST OF PORTUGAL, ALGARVE

UNIVERSIDADE DO ALGARVE
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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

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Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Sara Maria da Costa Pereira

(Sara Maria da Costa Pereira)

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Resumo

Gorgónias são organismos coloniais da classe Anthozoa, subclasse Octocorallia e ordem Alcyonacea, caracterizados pelo seu lento crescimento, pela sua longevidade e por possuírem um esqueleto interno como suporte. Os seus pólipos são geralmente pequenos, uniformes e simples, e conhecidos por possuírem oito tentáculos monomórficos que é um aspeto característico destes organismos. Estes encontram-se unidos por um tecido designado de cenênquima, que consiste numa camada de mesogeleia constituída por cálices e espículas. A sua distribuição pode ser aleatória, uniforme ou bilateral que varia consoante a espécie. As gorgónias são um grupo de organismos cnidários bênticos, mundialmente conhecidos pela sua importância para os fundos marinhos e para as comunidades bênticas. A sua estrutura tridimensional possui a capacidade de alterar o habitat físico que as rodeia, interferindo com a velocidade das correntes e processos de sedimentação. Deste modo, são frequentemente abundantes em locais de baixo relevo e de substrato compacto (duro) com fluxo de água relativamente abundante. Estas podem ser simbióticas, ou seja, apresentam simbiose com *zooxanthellae* (dinoflagelados do género *Symbiodiunium*) ou no caso contrário, assimbióticas, sendo que a maioria das espécies do Mediterrâneo é assimbiótica. Em Portugal continental, existem oito espécies conhecidas pertencentes a quatro géneros, nomeadamente (*Eunicella* (4 espécies), *Ellisella* (1 espécie), *Leptogorgia* (2 espécies), *Paramuricea* (1 espécie), encontradas a profundidades entre cinco e mais de cem metros.

Comparando com as espécies do Mediterrâneo, as espécies de gorgónias da costa Sul de Portugal, ainda estão pouco estudadas. Atualmente, as comunidades de gorgónias encontram-se vulneráveis a impactos naturais e de índole humana e encontram-se pouco protegidas. O aquecimento global, a acidificação dos oceanos e o aumento da temperatura dos oceanos são dos principais impactos naturais que afetam as comunidades de gorgónias a nível mundial. Por outro lado, relativamente aos impactos antropogénicos, estes podem ter origem na poluição, pesca excessiva ou pesca de arrasto e mergulho recreativo, por exemplo. Como consequência, verifica-se um declínio de comunidades de corais a nível mundial, culminando com perda de biodiversidade, perda de estrutura dos recifes, função e crescimento. Durante as últimas duas décadas, têm-se verificado danos nas populações costeiras de gorgónias ao largo da costa de Portugal continental. Consequentemente este estudo aborda experiências de

termotolerância face aos impactos naturais acima referidos bem como medidas de restauração das comunidades de gorgónias da costa Sul de Portugal continental, nomeadamente através da transplantação de gorgónias para efeitos de repopulação. Deste modo, foi efetuado um estudo de longa exposição com quatro espécies de gorgónias (*Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata*) capturadas a baixas profundidades (12-16 m) no Porto da Baleeira em Sagres, Sul de Portugal, com o objetivo de compreender a resposta fisiológica das gorgónias ao aumento da temperatura dos oceanos. Foram realizadas experiências de termotolerância em aquários, onde as amostras foram sujeitas a quatro regimes de temperatura, nomeadamente, 18ºC (controlo), 22ºC, 24ºC e temperatura ambiente da Ria Formosa ao longo de 134 dias. Foram quantificadas três variáveis: atividade dos pólipos, necrose dos tecidos e crescimento dos fragmentos. Com base em estudos anteriores e semelhantes com espécies de gorgónias temperadas, estabelecemos as seguintes hipóteses: (1) Como a temperatura e o metabolismo estão relacionados, previmos que elevadas temperaturas terão um impacto negativo, resultando no decréscimo da atividade dos pólipos e um aumento de necrose dos tecidos a elevadas temperaturas; (2) As taxas de crescimento serão mais elevadas nos tratamentos de mais baixas temperaturas (18ºC) e menos elevadas nos tratamentos de temperaturas mais elevadas, e (3) as respostas à temperatura diferem entre espécies. Tentámos ainda responder a outras questões relacionadas com a resposta fisiológica dos fragmentos em estudo ao aumento da temperatura. Os resultados demonstraram que a atividade dos pólipos era dependente da temperatura e diferia consoante a espécie, demonstrando maior atividade ao tratamento de temperatura ambiente da Ria Formosa para a espécie *Paramuricea clavata*. Também se verificaram diferenças interespecíficas com o aumento da temperatura. Relativamente ao grau de necrose, a espécie mais afetada foi a *Leptogorgia sarmentosa* com cerca de 21% de necrose. Considerando o crescimento dos fragmentos, este não foi afetado pela temperatura e não diferiu entre espécies. Como consequência, verificou-se que amostras provenientes de baixas profundidades apresentam maior termotolerância a elevadas temperaturas e menor grau de stress. Este trabalho teve ainda como objetivo testar métodos de restauração de gorgónias, pois devido à sua relativamente recente aplicação, as práticas de restauração ainda necessitam de ser melhoradas de modo a obter resultados eficientes e positivos. Apesar das ameaças conhecidas, praticamente ainda não tinham sido aplicadas medidas de restauração às populações de gorgónias em Portugal. Deste modo, foram testados

procedimentos de transplantação de gorgónias no Parque Marinho Professor Luiz Saldanha, no Parque Natural da Arrábida, Setúbal. Os fragmentos utilizados foram os mesmos utilizados nas experiências de termotolerância, acima referidos. O objetivo global da transplantação consiste na repopulação de zonas degradadas do Parque Marinho Professor Luiz Saldanha mas neste trabalho apenas se pretendeu testar a técnica de transplantação denominada "Raw technique", que consiste em colar o fragmento diretamente ao substrato. Deste modo, sessenta e sete fragmentos saudáveis de gorgónias foram transplantados (14 *Paramuricea clavata*, 13 *Leptogorgia sarmentosa*, 21 *Eunicella verrucosa* and 19 *Eunicella labiata*) numa nova localização no Parque Marinho Professor Luis Saldanha. Com base em estudos anteriores e semelhantes formulámos a seguinte hipótese: com a técnica de transplante escolhida, espera-se observar sobrevivência e crescimento dos fragmentos nas novas condições do local de transplante. Os resultados demonstraram o sucesso do método de transplante, pois as colónias que permaneceram agarradas ao substrato sobreviveram saudáveis por 4 meses. Houve o desaparecimento de algumas colónias devido a eventos naturais, que não refletem falha de sobrevivência ou de condição fisiológica mas apenas descolamento do substrato. Não foi possível quantificar crescimento dos fragmentos devido a este ser lento para registar nos primeiros meses e devido a eventos naturais que resultaram no desaparecimento total dos fragmentos no ano seguinte por competição com macroalgas que recrutaram massivamente na zona de estudo.

Palavras-chave: Gorgónias, aumento da temperatura dos oceanos, experiências de termotolerância, métodos de transplantação de gorgónias

Abstract

Currently, coral communities are exposed to both environmental and human pressures and are poorly protected. The major natural events responsible for negatively impact the coral assemblages are global climate change, ocean acidification and rising sea surface temperatures. Gorgonians are colonial organisms which have slow growth, long life span, and the structural support of a skeleton. This group of benthic cnidarians is recognized worldwide by its ecological role for marine rocky bottoms and benthic assemblages. During the last two decades, there has been an increased awareness of damages induced on coastal gorgonian populations along the Portuguese continental coastline. To understand the physiological response to increasing seawater temperature, a long-time exposure study was conducted with four gorgonian species (*Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata*) collected from shallow depths (12-16 m) at Sagres on the South Coast of Portugal. Thermotolerance experiments were carried out in aquaria where samples were subjected to four temperature regimes for a duration of 134 days. The results showed that polyp activity was temperature dependent and differed among species, with higher activity at ambient seawater temperatures for *P. clavata*. Interspecific differences in polyp activity increased with increasing temperature. *L. sarmentosa* was the species with higher percentage (21%) of necrosis. Fragment growth was not affected by temperature and did not differ among species. The response variable results indicate that samples from shallow depths had greater thermotolerance of elevated seawater temperatures and less stress. After the thermotolerance experiments, transplantation procedures were carried out in Marine Park Luiz Saldanha (MPLS) with the aim to repopulate the area and to test the “Raw technique” of transplantation from Linares et al., (2008). Sixty-seven healthy fragments of gorgonians were transplanted on a new location site in MPLS. The results showed high success of transplantation in the initial four months, although some colonies got dislodged. However, after one year no colonies were left on the site due to a novel recruitment of a canopy of seaweeds that outcompeted the slow growing gorgonians.

Keywords: Gorgonians, sea surface temperature increasing, thermotolerance experiments, gorgonian transplantation procedures

Table of contents

Index of Figures

CHAPTER 1: OCTOCORALLIA INTRODUCTION

CHAPTER 2: THERMOTOLERANCE OF TEMPERATE GORGONIAN POPULATIONS IN SOUTH COAST OF PORTUGAL, ALGARVE

Index of Tables

CHAPTER 2: THERMOTOLERANCE OF TEMPERATE GORGONIAN POPULATIONS IN SOUTH COAST OF PORTUGAL, ALGARVE

List of Abbreviations

BEE - Biogeographical Ecology and Evolution

CCMAR – Centro de Ciências do Mar

El – *Eunicella labiata*

ENSO - El Niño–Southern Oscillation

Ev – *Eunicella verrucosa*

IUCN - International Union for Conservation of Nature

Ls – *Leptogorgia sarmentosa*

MPLS – Marine Park Luiz Saldanha

NW – Northwest

Pc – *Paramuricea clavata*

POC – Particulate Organic Carbon

UK – United Kingdom

CHAPTER 1: OCTOCORALLIA INTRODUCTION

The subclass Octocorallia is one of the monophyletic lineages comprised in the class Anthozoa (Daly et al., 2007). This class comprises gorgonians (sea fans and sea whips), soft corals, sea pens and the blue coral (Daly et al., 2007). As the name suggests, the main characteristics of the subclass are the eight tentacles and eight mesenteries of octocoral polyps (Daly et al., 2007). Moreover, although absent in several taxa, the presence of pinnules (lateral extensions) on the tentacles is also considered diagnostic (Daly et al., 2007). All octocorals have colonial polyps and it is currently estimated that Octocorallia includes approximately 3,000 species (Daly et al., 2007). Cnidarians are a group significantly represented in benthic communities by their ecological role, diversity, abundance, interspecific relationships and beauty (Pérez et al., 2016). Octocorals are distributed in all marine environments and are represented by several growth forms, from encrusting, filiform and membranous to more complex structures with elaborate "arborescent architectures" (Pérez et al., 2016) which compartmentalize the benthic boundary layer by creating microhabitats and nursery areas for other organisms (e.g. invertebrates and fishes) (Mosher and Watling, 2009; Neira et al., 1992; Patton, 1972; Ramos, 1995; Vreeland and Lasker, 1989). Furthermore, where they form dense stands, they interfere with light penetration, thereby affecting sessile photoautotrophic organisms and hydrodynamic gradients (Wainwright et al., 1976), which in turn influences water exchange and material cycles (Fréchette et al., 1989; González-Ortiz et al., 2014; Irlandi, 1996; Vogel, 1994).

Generally, these organisms are conspicuous and diverse in shallow tropical reefs such as the Red Sea (Benayahu and Loya, 1981), the Indo-West Pacific (Fabricius, 1997), and the Mediterranean Sea (e.g., (Ros et al., 1985)) to deep-sea habitats (Williams and Cairns, 2013) and colder regions such as the North Atlantic (e.g., Migné and Davoult (1997)), southern Africa (e.g., Benayahu (1993); Williams (1992)), the Pacific Northwest coast of North America (McFadden and Hochberg, 2003), and Antarctica (e.g., Slattery et al. (1995)). They can be found inhabiting both reef environments and soft bottoms from the intertidal zone to depths up to 6,400 m (Williams, 2011). Albeit being common in all oceans, their distribution is influenced by several factors e.g. current intensity and also its presence/absence, suspended organic matter and distance from coast (Fabricius and Alderslade, 2001) to name a few, and only few species are cosmopolitan (Williams, 2011).

Octocorals can be symbiotic (have symbiosis with zooxanthellae, which are dinoflagellates of the genus *Symbiodinium*) and azooxanthellate, and most of symbiotic species are from Alcyonacea (Schubert et al., 2017). In fact, symbiotic and azooxanthellate species are globally distributed, however their proportions vary depending on the region (Schubert et al., 2017). Azooxanthellate species are dominant in Indo-Pacific and Mediterranean, whereas symbiotic species are more frequent in Eastern Pacific, Caribbean, Australia and Red Sea (Figure 1.1) (Schubert et al., 2017). The Mediterranean has only azooxanthellate species with an exception of *Eunicella singularis* which is a facultative symbiotic species (Schubert et al., 2017). The presence/absence of zooxanthellae is a fact to have in consideration, since it plays a crucial role concerning the bathymetric distribution of octocorals and other physical environmental parameters, such as light, flow rates and sedimentation (e.g., Kinzie and Robert (1973); Sánchez (1999)) (Schubert et al., 2017). For instance, the vertical distribution of symbiotic species is mainly affected by light availability (Kinzie and Robert, 1973), consequently, most of symbiotic species are found in shallow and/or less turbid areas (<50m) and are autotrophic due to their photosynthetic activity that is influenced by polyp size, tentacle morphology and tissue surface area, which vary among octocoral species (Schubert et al., 2017). In contrast, azooxanthellate species are found in deeper areas where light is more limited, in this case, water flow is the main factor that affect these organisms and their energetic demands depend on suspended particulate food from the surrounding water (Schubert et al., 2017).

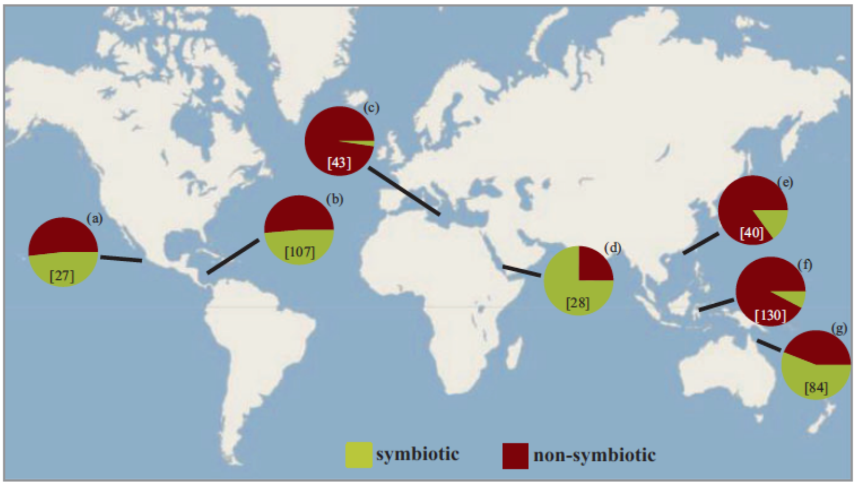

Figure 1.1. Relative numbers of zooxanthellate and azooxanthellate octocoral species in different regions. Numbers indicate the total number of reported species and letters indicate literature cited (Schubert et al., 2017).

Concerning taxonomy and systematic relationships, currently classification of most octocorals remains problematic and uncertain (McFadden et al., 2010). This uncertainty is mainly due to poor original descriptions and due to a paucity of taxonomic studies (Haverkort-Yeh et al., 2013). Presently it is very difficult to estimate the precise number of Octocorallia species, since most of the genera have never been revised and several regions of the world are still unexplored (Pérez et al., 2016). Additionally, there is a limited number of taxonomists and the literature is still not well integrated (Bayer, 1981; Cairns, 2007). However, although octocorals were divided into four orders (Alcyonacea, Gorgonacea, Stolonifera, Telestacea) in the past by Hickson (1930), they were later merged into one order called Alcyonacea by Bayer (1981) which was recognized and embraced by modern taxonomists (Daly et al., 2007). The order Alcyonacea includes "30 families of soft corals (octocorals without a supporting skeletal axis) and gorgonians (octocorals with a supporting skeletal axis of scleroproteinous gorgonin and/or calcite)" (Bayer, 1981). The most relevant characters used to distinguish genera and species of octocorals, are the form and distribution of sclerites (microscopic calcite crystals embedded in the coenenchymal tissue and polyps) (Daly et al., 2007). Phylogenetic studies were supported by the development of molecular techniques that started to be employed in the study of octocorals since1990, although there is still no unanimity regarding the relationships among groups bellow the subclass level (McFadden et al., 2010). Despite that, the fast development of new molecular markers was significant to improve the resolution of molecular techniques in taxonomy of octocorals (Pérez et al., 2016) and so far, the most complete phylogeny published for Octocorallia is from McFadden et al. (2006a).

Gorgonians: Biology and Ecology

Gorgonians (Octocorallia: Alcyonacea) are colonial organisms characterized by a supportive skeleton axis, slow growth and long life span (Cúrdia et al., 2012). Gorgonian polyps (models) always have eight monomorphic pinnate tentacles, which are capable of diverse physiological functions (i.e. reproduction, feeding, excretion, defense, etc.) (Sanchez, 2004). Their polyps "are immersed in coenenchyme, a mesoglear layer mix of calcareous sclerites and the non-cellular portion of the middle layer" (Sanchez, 2004). The distribution of polyps can be random, uniform or bilateral and varies between species (Sanchez, 2004), but higher complexity occurs in the process of branching, where over of 20 patterns have been identified (Bayer et al., 1983). Their arborescent structure is formed by a continued connection between the polyps and branches along their axis (Sánchez and Lasker, 2004) and the polyps are indeed connected by a network of stem canals (Bayer, 1973).

This group of benthic cnidarians is recognized worldwide by its ecological role on marine rocky benthic communities (Ballesteros, 2006; Coma et al., 2006; Cupido et al., 2008; Linares et al., 2008). Gorgonians are considered foundation species, providing the framework for the community through the creation of habitat cascades (Bruno et al., 2003; Cúrdia et al., 2012; Thomsen et al., 2010). In fact, gorgonians are among the most important suspension feeders of benthic communities (Weinberg, 1976). Their three-dimensional structure may modify the physical habitat, in the sense that it interferes with current velocity, reducing it, and may also help stabilizing soft substrata, enhancing local accumulation of fine particles and sedimentation (Idjadi and Edmunds, 2006; Norling and Kautsky, 2007). Octocorals are relatively abundant in low relief hard substrate with preference for high water motion areas (Kinzie and Robert, 1973; Yoshioka and Yoshioka, 1989). Their distribution depends on environmental factors such as light, water motion and sediment transport (Yoshioka and Yoshioka, 1989). These benthic organisms have a significant role in shaping the communities by providing shelter and forming nursery areas for other marine organisms (Gili and Coma, 1998). Besides that, they also interfere with the carbon and nitrogen cycles by either capturing planktonic prey and filtering large amounts of dissolved organic matter and particulate matter, and through the release of metabolic wastes to seawater (Gili and

Coma, 1998; Wild et al., 2004). Concerning their nutrition acquisition, gorgonians feed on a wide spectrum of food types, including picoplankton, nanoplankton, microplankton, zooplankton, particulate organic matter (POM) (Coma et al., 1994; Ribes et al., 1999) and organic matter from re-suspended sediment (Anthony and Fabricius, 2000; Mills and Sebens, 2004; Orejas et al., 2001).

These modular marine invertebrates reproduce both sexually (e.g. free-swimming larvae) and asexually (e.g. asexual budding, fission, partial disintegration), which endow them with the flexibility to develop a variety of ecological strategies (Hughes and Jackson, 1980; Jackson, 1977). In colonial organisms, reproduction is often associated with growth and demographic factors, such as space occupation/competition. For example, in highly space saturated benthic habitats, in which modular organisms, such as gorgonians, are prevalent, asexual reproduction is sufficient to maintain the populations (Hughes, 1985; Jackson, 1977). However, when subjected to mass mortality events or overharvesting, the persistence of the populations is assured by sexual reproduction (Linares and Doak, 2010; Linares et al., 2007), in particular, recruitment rate, which depends on reproductive output (determined by fertility, sex ratio, fecundity, age/size structure and population density), larval settlement and settler survival (Santangelo et al., 2003). Data on the sexual reproduction of these organisms are crucial for the study of genetic structure and connectivity of populations and consequently, its effects on populations dynamics and resilience face to natural or anthropogenic disturbances (Rossi and Gili, 2009). Gorgonians have low recruitment rates and suffer high mortality rates as recruits and juvenile colonies (Coma Bau et al., 2001) and display high longevity and low natural mortality as adults (Coma et al., 1998; Lasker, 1991; Yoshioka, 1994).

Shallow gorgonian populations at the Algarve coast, Portugal

In continental Portugal, there are eight known species of gorgonians, belonging to four genera (*Eunicella* (4 species), *Ellisella* (1), *Leptogorgia* (2), *Paramuricea* (1)), found between 5 and >100 m depth (Cúrdia et al., 2012; Rodrigues, 2008). When compared to the Mediterranean, gorgonian populations from southern Portugal are still poorly described (Cúrdia et al., 2012). The most recent studies on gorgonians from the Algarve coast reported 10 species caught as by-catch by bottom gill nets (Vieira, 2008) and quantified the occurrence and abundance of the main gorgonian species in the shallow rocky bottoms (0–30 m) of the western Algarve coast (Cúrdia et al., 2012); (Figure 1.2).

The gorgonian species present in Lagos Bay showed no signs of segregation (Cúrdia et al., 2012). *Eunicella labiata* was the most abundant and frequent species and in total *Eunicella labiata*, *Eunicella verrucosa*, *Eunicella gazella* and *Leptogorgia sarmentosa* were responsible for 96,6% of total abundance (Cúrdia et al., 2012). *Eunicella singularis* showed low abundance and frequency values as in the Mediterranean (Cúrdia et al., 2012). There is a clear depth pattern in the distribution of gorgonians in Lagos Bay with an evident turning point at 15 m that can be related mostly with higher irradiance above this depth (Cúrdia et al., 2012). Moreover, there is evidence of an increasing of abundance of gorgonian species with depth in Lagos Bay, showing an association with sponges (Porifera), another suspension feeding taxa (Cúrdia et al., 2012).

Phylum Cnidaria Hatschek, 1888

Class Anthozoa Ehrenberg, 1834

Subclass Octocorallia Haeckel, 1886

Order Alcyonacea Lamouroux, 1816

Suborder Holaxonia Studer, 1887

Family Gorgoniidae Lamouroux, 1812

Genus Leptogorgia Milne Edwards & Haime, 1857

Leptogorgia lusitanica Stiasny, 1937

Leptogorgia sarmentosa (Esper, 1791)

Genus Eunicella

Eunicella verrucosa (Pallas, 1766)

Eunicella gazella (Studer, 1901)

Eunicella labiata (Thomson, 1927)

Family Plexauridae Gray, 1859

Genus Paramuricea Kölliker, 1865

Paramuricea clavata (Risso, 1826)

Figure 1.2. Taxonomy of the main gorgonian species in the shallow rocky bottoms of the western Algarve coast. Adapted from Rodrigues (2008).

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CHAPTER 2: THERMOTOLERANCE OF TEMPERATE GORGONIAN POPULATIONS IN THE SOUTH COAST OF PORTUGAL, ALGARVE

ABSTRACT

Gorgonian populations have a fundamental ecological role as habitat-forming species that provide a complex three-dimensional structure to rocky reefs. They are currently threatened by direct and indirect anthropogenic impacts such as intense diving, bottom fishing and anchoring, pollution and warming waters that cause necrosis and/or epiphyte and bacterial proliferation, and ocean acidification. During the last two decades, there has been an increased awareness of damages induced on coastal gorgonian populations along the Portuguese continental coastline. To understand the physiological response to increasing seawater temperature, a long-time exposure study was conducted with four gorgonian species (*Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata*) collected from shallow depths (12-16 m) at Sagres on the South Coast of Portugal. Thermotolerance experiments were carried out in aquaria where samples were subjected to four temperature regimes: 18ºC (control), 22ºC, 24ºC and ambient seawater from Ria Formosa for a duration of 134 days. Three biological response variables were used to evaluate the effects of the treatments: growth estimates, polyp activity and coenenchyme necrosis. The results showed that polyp activity was temperature dependent and differed among species, with higher activity at ambient seawater temperatures for *P. clavata*. Interspecific differences in polyp activity increased with increasing temperature at 22ºC between *P. clavata* and *E. verrucosa* ($p=0.006$) and at 24ºC between *P. clavata* and *E. verrucosa* ($p<0.001$), *L. sarmentosa* and *E. verrucosa* ($p<0.001$) and *P. clavata* and *E. labiata* ($p=0.008$). *E. verrucosa* and *E. labiata* did not show any signs of necrosis at any temperature, whereas *L. sarmentosa* was the species with higher percentage (21%) of necrosis. Fragment growth was not affected by temperature and did not differ among species. The response variable results indicate that samples from shallow depths had greater thermotolerance of elevated seawater temperatures and less stress. Therefore, it is important to invest in future research efforts using thermotolerance studies to understand better the Portuguese temperate gorgonians population behaviour and responses to climate change temperature impacts.

INTRODUCTION

Our oceans have a massive significance to our planet, without them, life as we know it would be unthinkable; a concept which should be present on a daily basis to lead sustainable and thoughtful human actions in a way and ultimately conserving the future of Earth. Covering approximately 71% of Earth's surface, the oceans are considered the main reservoirs of both water and O_2 (Roff and Zacharias, 2011). The oceans have very interesting particularities such as to modulate and regulate Earth's conditions, being responsible for homeostatic effects and consequently attenuating the adverse effects that are the result of human impacts (Roff and Zacharias, 2011). However, there is still evidence of continuing degradation of marine ecosystems (Roff and Zacharias, 2011) and with it is the necessity to protect and conserve our oceans.

Human activities are the primary source of noticeable alterations to the Earth's oceans via warming, deoxygenation, and acidification (Hoegh-Guldberg and Bruno, 2010). Consequently, the uptake of anthropogenic CO_2 by the oceans will result in drastic changes for the next centuries and beyond (Solomon, 2009), such as increasing sea surface temperatures. Since temperature and metabolism are strongly linked (Arrhenius, 1889; Van't Hoff, 1896), temperature alterations have a strong impact, for example, on animal metabolism. Global warming certainly will lead to a profound impact on marine life, particularly in benthic communities since the upper threshold for temperature of sessile organisms may be exceeded (Previati et al., 2010). Moreover, with increasing temperatures oxygen solubility decreases (Carpenter, 1966; Truesdale et al., 1955) and therefore limiting its availability. Another outcome is the alteration of the equilibrium of dissolved inorganic carbon, thus decreasing the pH and calcium carbonate saturation (Lunden et al., 2014). All of these consequences manifest themselves in different levels such as the ecosystems, communities, populations, and individuals (Walther et al., 2002) resulting in a drastic reduction in biodiversity. However, adaptation and acclimation in some cases may be possible (Lunden et al., 2014). Among the consequences, there is evidence of variation in species distribution, increase of disease and ultimately, mortality outbreaks of a wide range of organisms (reviewed in Harley et al. (2006); Harvell et al. (1999)).

Currently, coral reefs are exposed to both environmental and human pressures and are poorly protected, therefore an estimated 20% of the world's coral reefs have been permanently damaged, 24% are in danger of collapse due to human threats and

26% are under threat of long-term degradation according to The Status of Coral Reefs of the World (2004) report (Ammar, 2009; Precht and Robbart, 2006). Regarding environmental threats, the major natural events responsible for negatively impact on coral reefs are cyclones, hurricanes, tsunamis, coral disease, severe ENSO events, global climate change, ocean acidification and rising sea surface temperatures (Hoegh-Guldberg et al., 2007; Jaap, 2000; Precht and Robbart, 2006). Although the last three phenomena are natural events, their causes are related to human activities. The anthropogenic activities that affect these reefs, can impact (1) directly the reefs such as dredging, anchor damage, fishing (i.e. overfishing, trawls, dynamite blasting, use of toxic chemicals) and ship groundings to name a few (Alcala and Gomez, 1987; Eldredge, 1987; Precht and Robbart, 2006); and (2) indirectly through pollution, sedimentation due to coastal exploration activities and terrestrial runoff (Precht and Robbart, 2006).

As a consequence, coral communities declined worldwide over the past decades, resulted in the loss of biodiversity, decrease in reef structure, function, growth, and coastal buffering (Alvarez-Filip et al., 2009; Bruckner, 2003). Nonetheless, coral reefs have successfully persisted over evolutionary timescales (Lugo et al., 2000), essentially due to mechanisms of resistance and structural repair (Epstein et al., 2003). Depending on the level of degradation and the health state of the reef, there is the possibility of natural recovery to the pre-disturbance state with success (Omori and Fujiwara, 2004). However, it must be recognized that natural restoration may take prolonged periods (decades or centuries) (Dobson et al., 1997; Rinkevich, 1995). In contrast, the persistence of human-induced chronic stress factors leads to severe damage and degradation and also can switch to an alternate and undesired state (Ammar, 2009).

Over the last decades, gorgonian populations have been affected by drastic, rapid and lasting disturbances either of natural (Coma et al., 2006; Garrabou et al., 2009; Linares et al., 2005) or anthropogenic origin (Cerrano and Bavestrello, 2008; Chiappone et al., 2005; Linares et al., 2008b). Additionally, gorgonian dominated reefs are recognized as "a habitat whose conservation requires the designation of Special Areas of Conservation" under Annex 1 of the EC Habitats Directive (UK Marine SAC.s Project, 2003). Hence, it is clear that there is the urgent need to protect and preserve these marine resources through the implementation of restoration strategies and management measures (Pratt, 1994). There is still an unbalance regarding research into the effects of climate change on tropical corals and temperate reef communities, and the latter have been "neglected" to a certain point (Munro, 2003). Gorgonians are temperature sensitive, and it is believed that temperature plays a strong role in the distribution of many temperate-water gorgonian species (Munro, 2003). Their sensibility is probably due to (1) a narrow temperature tolerance of adult colonies and (2) influences of temperature on the survival and settlement of gorgonian larvae (Munro, 2003). The synchronization of spawning events of these organisms is significantly influenced by water temperature (Munro, 2003). Spatial distribution of gorgonian species is also linked with water temperature (Munro, 2003). Therefore, there is the need to study the impacts of climate change on these organisms due to their significant role on marine ecosystems and also since temperature is a main factor in determine their distribution, survival, spawning events and larvae settlement (Munro, 2003).

Over the last decades, in the Mediterranean Sea, a significant increase of mortality outbreaks has been detected in marine ecosystems, affecting marine communities, among them Cnidarians (Torrents et al., 2008). At the end of the summers of 1999 and 2003, two large-scale mortality events have affected marine rocky benthic communities in the NW area of Mediterranean Sea and have been related to positive temperature anomalies (Torrents et al., 2008). However, as previously mentioned, research on thermotolerance experiments on temperate gorgonian species is still scarce, particular in the NW Mediterranean rocky subtidal communities (Torrents et al., 2008). It is clear that the effects of climate change are already visible in the marine ecosystems. Hence, it is essential to study their impact on marine organisms, especially corals due to their ecological role and as these organisms are impacted by bleaching and mortality events (Pivotto et al., 2015). These studies can inform us about the ability of these organisms to face the global warming (Pivotto et al., 2015). Moreover, Mediterranean octocorals provide good ecological models, however experimental studies of the levels of thermotolerance in Mediterranean octocorals have provided contrasting results (Pivotto et al., 2015).

The aim of this study is to test the physiological response of four of the most common gorgonian species from South Portugal, namely *Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata* to elevated seawater temperatures, by analysing their growth, polyp activity and necrosis signs under common garden conditions. Consequently, we attempt to answer the following questions:

Does increasing seawater temperature affect temperate gorgonian growth, polyp activity and tissue necrosis?

Are there interspecific (*Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata*) differences in physiological response to sea surface temperature increasing?

Based on other similar studies with temperate gorgonian species we establish the following hypotheses: (1) Since temperature and metabolism have been related (Arrhenius, 1889; Van't Hoff, 1896) we predict that elevated temperature will have a negative impact, decreasing polyp activity and increasing of necrosis signs with higher temperatures, especially at 24°C; (2) Growth rates will be higher around 18ºC and lower and possibly negative at higher temperatures, such as 24°C; (3) Responses to temperature will vary among species. To test these hypotheses, we have performed temperature experiments with the four most common Algarvian gorgonians *Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata.*

MATERIALS AND METHODS

Species description

***Eunicella verrucosa* (Pallas, 1976)** is a representative gorgonian of temperate to cold waters, found in both the Atlantic Ocean, from northern Ireland to North-West Africa, and in the Mediterranean basin (Carpine, 1963; Carpine, 1975; Hayward and Ryland, 2017; Mistri, 1995; Vafidis, 2009; Weinberg, 1976). This species inhabits rocky bottoms ranging from 2 to 60 m depths and is considered a major heritage species (Hiscock, 2003; Lafargue, 1969). Generally, the colonies are widely dispersed, but in some areas, they can form "forests" thus creating complex structures with elevated and intricate architectures, up to 30 cm tall. In the northern part of the western Mediterranean, *E. verrucosa* is mostly found on hard substrates between 35 to 200 m depths (Carpine, 1975; Grasshoff, 1992) and its presence at shallower depths is scarce (Cocito et al., 2002; Rossi, 1959), although it has been observed in shallow bathymetric range (25 - 35 m depth) with high colony density in the region of Marseilles (south-east France) (Sartoretto and Francour, 2011). Habitats of *E. verrucosa* are described in the

European Nature Information System marine habitat typology and in the Marine Habitat Classification for Britain and Ireland (Connor et al., 2004).

Regarding biology and ecology, *E. verrucosa* exhibits a slow growth rate (approximately 1 cm per year) (Bunker, 1986) and a long-life span, hence indicating that their population might be renewed very slowly (Coz et al., 2012). Although there is no specific information on reproduction of this species, the production and settlement of larvae are successful in occasional years in South-West Britain, where their larvae are presumably lecithotrophic and short lived (Weinberg and Weinberg, 1979). In addition, this species is highly sensitive to abrasion and to substratum loss (Hiscock, 2007). For all these reasons, it was classified as "Vulnerable" on the IUCN red list of threatened species in 1996 (Coz et al., 2012).

Eunicella verrucosa colonies are characterized by heterogeneous surfaces with *verrucae* and usually larger in size than *E. gazella*; their colour varies from white to cream and pale orange with polyps varying from white to orange; usually have irregular branching in one dimension resembling a fan form (Cúrdia et al., 2012).

***Leptogorgia sarmentosa* (Linne, 1758)** is a representative suspension feeder and an ubiquitous member of the Mediterranean benthic fauna community (Gili and Ros, 1985; Mistri, 1995; Rossi, 2001; Weinberg, 1978) that is mostly found between 20 and 200 m depth (Carpine, 1963; Weinberg, 1976), albeit, in some locations it can be found in depths shallower than 10 m (Ribes et al., 2003). Additionally, there are records of this species in the Atlantic region, from Biscay to Guinea Gulf (Grasshoff, 1992). This azooxanthellate gorgonian lives on soft bottom-gravel boulders (Rossi and Gili, 2009) in a wide range of current velocities, preferably strong ones (Carpine, 1975; Weinberg, 1976). Habitat, distribution and morphology differs from the other Mediterranean gorgonians (Rossi and Gili, 2009), for example at a diet level basis, that is based on detrital POC, small-low motile invertebrates, bivalve larvae (Ribes et al., 2003; Rossi et al., 2004), nanoeukaryotes, dinoflagellates, diatoms, ciliates and microplankton (Ribes et al., 2003). It inhabits areas with high resuspension events (Gili and Ros, 1985; Weinberg, 1978), low colony density (Mistri, 1995; Rossi, 2001) except in specific areas, and it is mostly always orientated perpendicular to the substrate (Rossi, 2001; Weinbauer and Velimirov, 1998; Weinberg, 1978). The structural features of *L. sarmentosa* are similar to *Eunicella cavolini*, with a higher number of thin branches per unit surface area and a higher number of small polyps per branch (Carpine, 1975; Weinbauer and Velimirov, 1998; Weinberg, 1976).

The colonies of *Leptogorgia sarmentosa* have a bush-like ramification in one dimension with a distribution of ramets in different directions with polyps present in all surfaces of their thinner branches; their colour is usually uniform but variable (red, yellow, brick orange, rarely white and rarely green) (Cúrdia et al., 2012). In this work, we considered the species *Leptogorgia lusitanica* as *Leptogorgia sarmentosa* since there are discussions regarding the taxonomic validity of *L. lusitanica*.

Eunicella labiata **(Thomson, 1927)** is a common gorgonian species along African West Coast from Morocco to Angola, including Cape Verde islands, and is mostly found at shallow depths (e.g. 12-18 m in Mauritania) throughout that region (Altuna, 1993). Moreover, there is evidence of several specimens along the coast of Malaga at 9-12 m depths and also in Biscay Gulf and in Iberian Peninsula, recording its existence in European waters (Altuna, 1993).

The colonies of *Eunicella labiata* are divided in two main branches after the base of the colony, with fewer branches than *E. gazella* and *E. verrucosa*; the branches are homogeneous and usually in one dimension; conspicuous *verrucae* are observed all over the colony and are usually lighter; their colour is usually darker, ranging from cream to dark brown (Cúrdia et al., 2012).

Paramuricea clavata **(Risso, 1826)** also known as the red gorgonian, is one of the key species of Mediterranean coralligenous assemblages and is considered an ecosystem engineer, playing an important role in the maintenance of structural complexity and biomass in these habitats (Ballesteros, 2006; True, 1970). This species is widely distributed in the western Mediterranean and Adriatic Sea (Carpine, 1975) exhibiting a discontinuous distribution, and, is less commonly found in Atlantic Ocean (Mokhtar - Jamai et al., 2011). *P. clavata* extends its bathymetric distribution from 5 m to beyond 200 m (Mokhtar - Jamai et al., 2011) and can be found on rocky substrates subjected to strong currents (Weinberg, 1991). The red gorgonian is a long-lived species (50-100 years) and a slow-growing gorgonian (Coma et al., 1998) with discrete colonies, assuming a two-dimensional arborescent form, reaching heights up to 1.5 m (Linares et al., 2007). The assemblages shaped by this species constitute one of the most beautiful seascapes in the Mediterranean Sea (Harmelin and Marinopoulos, 1994).

The red gorgonian is dioecious with a sex ratio of 1:1 in the studied populations, displays delayed sexual maturity, spawning is synchronous and occurs twice each year and fertilization is external with embryogenesis taking place on the surface of the colony (Ribes et al., 1995). Even though it displays a great annual investment in sexual reproduction, there is evidence of low recruitment rates (Coma Bau et al., 2001; Ribes et al., 1995) which would be enough to ensure the persistence of *P. clavata* populations under low mortality rates observed in natural conditions (Coma et al., 2004). After being released, their planula larvae exhibit negative phototaxis behavior (Linares et al., 2008c) with little potential to disperse and mostly settle near the parental colony (Linares et al., 2007). Moreover, high post-settlement mortality rates have been observed for *P. clavata* (Linares et al., 2008a).

Paramuricea clavata is characterized by bush-like colonies with a rigid structure in one dimension; with prominent polyps very abundant around the branches with salient sclerites; usually their colour is uniform, often yellow in Portuguese populations but can be wine red/purple and also can have a combination of two colours purple and yellow (Cúrdia et al., 2012).

Sampling and aquarium maintenance

On 19th March 2017, a total of 135 apical fragments (about 6 – 10 cm high) of four species of gorgonians*:* *Eunicella verrucosa* (n=58), *Leptogorgia sarmentosa* (n=28), *Eunicella labiata* (n=25) and *Paramuricea clavata* (n=24) were randomly collected from Porto da Baleeira, Sagres, South coast of Portugal between 12- 16 m depth using SCUBA diving by the CCMAR Scientific Diving Team (Figure 2.1). During the sampling, the fragments were collected in sampling nets per species, and were placed per species in plastic zip lock bags filled with seawater from the collection site and kept at 14ºC inside a cooler box during transportation. Fragments were transported to the Biogeographical Ecology and Evolution (BEE) laboratory in two hours and kept in a walk-in climate chamber until the next day, when they were transferred to the Ramalhete Marine Station.

At the Marine Station, the gorgonians were transferred for acclimation to a provisory tank while the maintenance system was prepared. The system consisted of an assemblage of three plexiglass square plates with several tubes intercalated to avoid shading, where the fragments were inserted. The second device consisted in a rectangular net with several tubes inserted with plastic pipette tips as a cap also intercalated to avoid shading. The pipette tips were longitudinal cut to insert the fragments. Subsequently, the two devices were screwed into a 500 L tank where they were maintained for 1 month of acclimation. The tank had a water flow through system and a pump to generate a circular current and the fragments were fed every day with natural food (copepods and *Artemia nauplii*) and a gorgonian powder mixture food (UltraMinf).

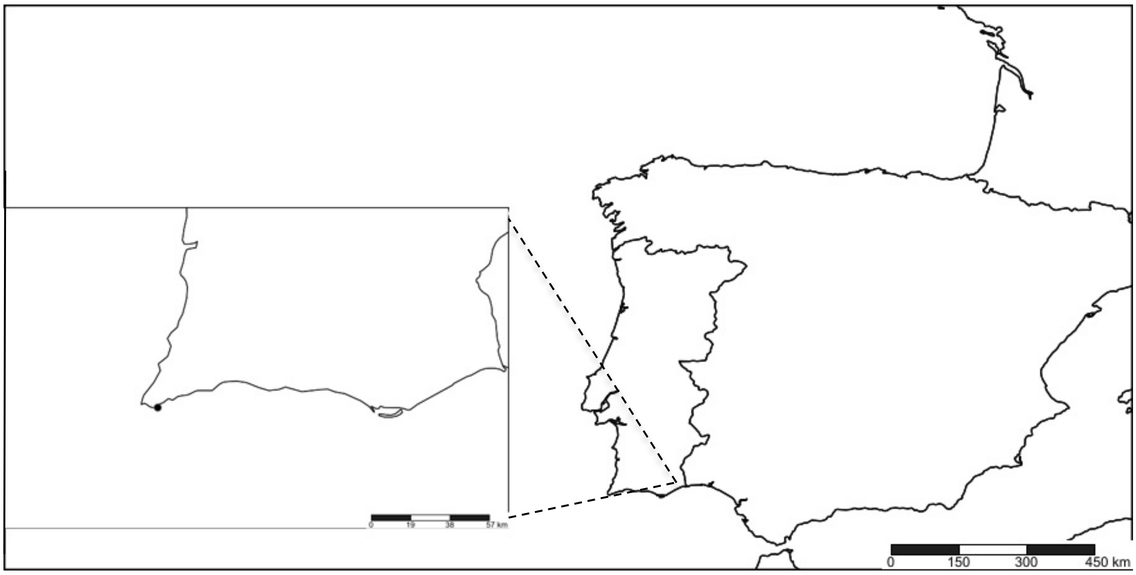

Figure 2.1. Sampling site, Porto da Baleeira, Sagres, South Portugal.

Temperature regime experiments

Experiments were carried out with 96 healthy fragments, 24 from each of the four coral species collected: *Eunicella verrucosa* (n=24), *Eunicella labiata* (n=24), *Leptogorgia sarmentosa* (n=24) and *Paramuricea clavata* (n=24).

After four weeks of acclimation, the fragments were attached to circular epoxy discs. First, a small proportion of their base was scraped using a scalpel and then, each fragment was attached to an epoxy disc previously labelled with a code (species initials and number) with a hot glue gun. After this procedure, the fragments were distributed into the experimental aquarium set that consisted of four aquariums (70 cm long x 40 cm wide x 32 cm deep) set at: 18 (control set), 22, and 24 °C and ambient seawater temperature (temperature treatments). Each tank contained 24 randomly dispersed fragments, 6 per species mimicking the natural conditions. Long-time exposure to high temperature experiments were carried out for four months, to identify the upper thermal limits of these species. The constant temperatures were obtained using submersible resistance heaters and coolers and the temperatures were recorded on each aquarium using temperature loggers. The aquarium set was monitored with a temperature control system from AQUATRONICA composed by one controller ACQKIT115, one POWER UNIT ACQ08 and five temperature sensors acq001s. All the aquariums received water from Ria Formosa, that was previously aerated in tanks and then distributed to the aquariums. All the systems were open and had the same renovation rate and the controlled temperature aquariums had recirculation of water (Figure 2.2).

To control seawater quality in the aquariums, salinity and dissolved oxygen were daily measured and both salinity and dissolved oxygen values were constant during all the experiments i.e. about 38 ‰ and 90%, respectively. The fragments were daily fed with natural food (copepods and *Artemia nauplii*) or with a gorgonian powder mixture food, every morning. All tanks were siphoned regularly to eliminate food deposits and to minimize sponge growth and adherence to the walls and fragments. The experiments began on May 11th 2017 and ended on August 28th 2017.

The remaining fragments (39) were placed in small 1,5L aquaria and were subjected to daily temperature fluctuations from the Ria Formosa lagoon. All the fragments were weighted using a digital weight balance (KERN, model PCB) with 0.01 g precision.

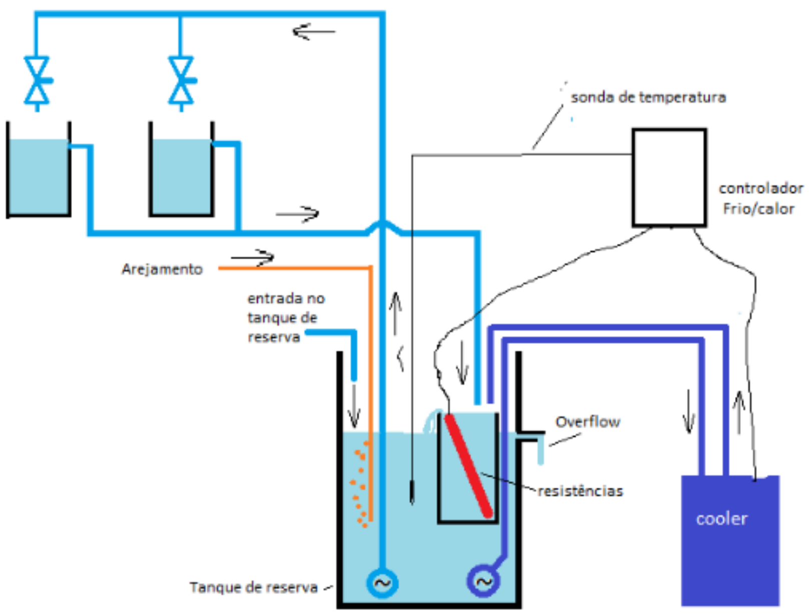

Figure 2.2. Experimental design showing the aquarium set used to carry out the thermotolerance experiments performed in this study. Designed by Dr. João Reis from CCMAR.

Polyp activity and coenenchyme necrosis

Polyp activity was daily estimated during the morning after feeding through photography in the first 3 weeks of the experiment and then monthly estimated, where each fragment was photographed with a Canon Reflex 600D camera with a EF-S 18-55 mm and a EF-S 55- 250 mm lens by a defined order for each temperature. Two different states of expansion were recorded: (1) expanded which included fully and semi-expanded polyps and (2) retracted polyps. Consequently, the polyp activity was calculated through the ratio between open polyps and fragments size.

Coenenchyme necrosis is preceded by polyp retraction and then necrosis followed by detachment from the skeleton. This phenomenon was visually detected as the change of coenenchyme colour and the appearance of skeleton axis. The percentage of coenenchyme necrosis was calculated as the percentage of necroded fragment length.

Growth estimates

In the beginning and at the end of the experiment, each fragment was photographed individually with a Canon Reflex 600D camera with a EF-S 55- 250 mm at the same distance with a scale and with the same zoom (100 mm) to estimate growth. Subsequently, digital images were analysed by Image J software. Each branch of the different species of gorgonians was measured using Image J software.

Statistical analysis

Parametric two-way ANOVA tests were performed using Rstudio software to test polyp activity and growth estimates, after testing normality and homogeneity of variances. Regarding polyp activity, it was also performed a pairwise comparison of the interaction of the temperature and species using Holm-Sidak method. For growth estimates, permutation tests were also performed, namely a Permutational Multivariate Analysis of Variance (PERMANOVA) to compare patterns among temperature treatments and species.

RESULTS

Polyp activity and coenenchyme necrosis

Polyp activity differed among species and was temperature dependent (Table 2.1). Temperature did not affect polyp activity of *L. sarmentosa*, *E. verrucosa* and *E. labiata*, but did so in *P. clavata* at 24ºC compared to the ambient seawater treatment ($p=0.001$). Interspecific differences in polyp activity were found at 22ºC between *P. clavata* and *E. verrucosa* ($p=0.006$) and at 24ºC between *P. clavata* and *E. verrucosa* ($p<0.001$), *L. sarmentosa* and *E. verrucosa* ($p<0.001$) and *P. clavat*a and *E. labiat*a ($p=0.008$). *P. clavata* showed higher polyp activity compared with the other species, reaching the highest activity at ambient seawater temperatures. *E. labiata* and *E. verrucosa* showed higher polyp activity at 24ºC and *L. sarmentosa* at 22ºC (Figure 2.3).

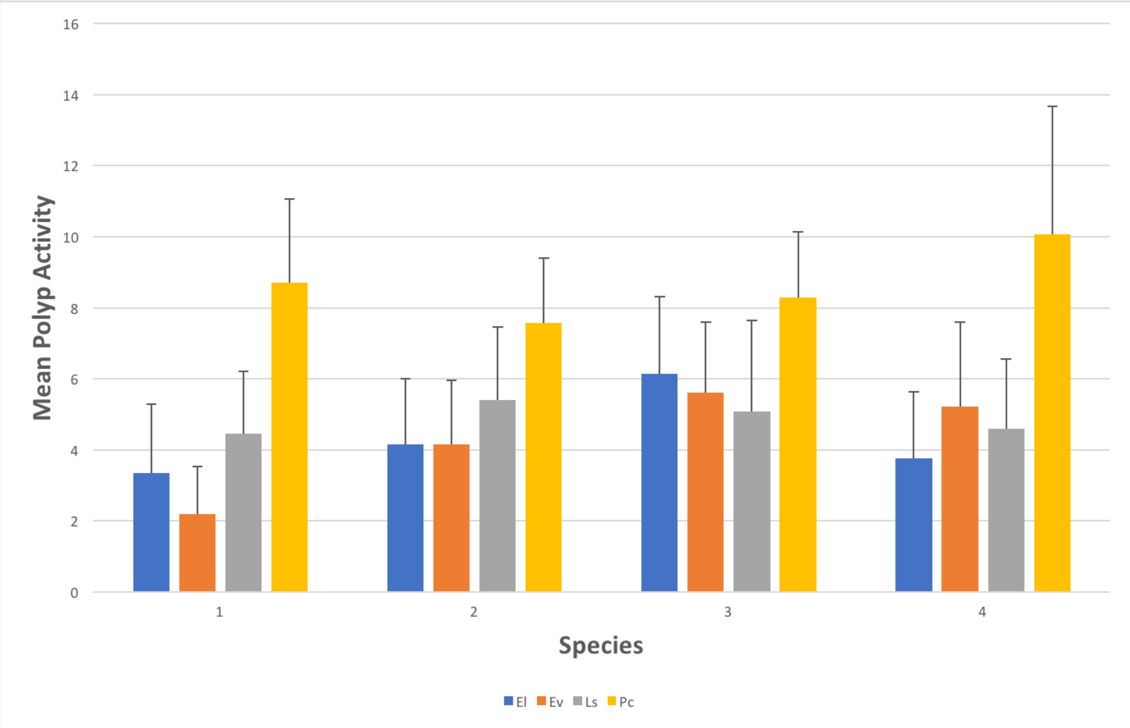

Figure 2.3. Mean polyp activity of the temperate gorgonians *Eunicella labiata* (El)*, Eunicella verrucosa* (Ev)*, Leptogorgia sarmentosa* (Ls) *and Paramuricea* clavata (Pc) after 134 days at 18ºC,22ºC, 24ºC and ambient seawater temperature.

E. verrucosa and *E. labiata* did not show any signs of necrosis at any temperature, albeit *L. sarmentosa* was the species with higher percentage of necrosis (Figure 6) *P. clavata* also showed necrosis signs, but only 8% of necrotic tissue at ambient seawater treatment (Figure 2.4).

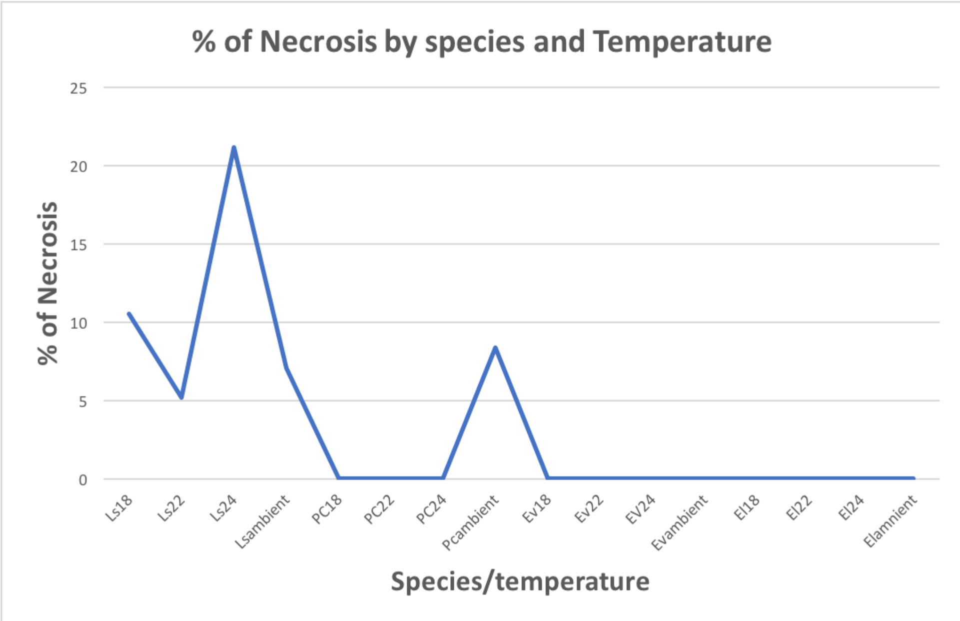

Figure 2.4. Mean relative necrosis of temperate gorgonians *Leptogorgia sarmentosa* (Ls), *Paramuricea clavata* (Pc), *Eunicella verrucosa* (Ev) and *Eunicella labiata* (El) at different temperature regimes: 18ºC, 22ºC, 24ºC and ambient seawater after 134 days of exposure.

Tabel 2.1. Analysis of variance for polyp activity on different temperatures (18ºC, 22ºC, 24ºC and ambient seawater) (df = degrees of freedom; SS = Sum of Squares).

Source of Variation	*df*	SS	Mean Square	F	P
Species	3	274,606	91,535	7,109	<0,001
Temperature	3	98,741	32,914	2,556	0,061
Species x temperature	9	330,368	36,708	2,851	0,006
Residual	76	978,520	12,875		
Total	91	1659,786	18,239		

Growth estimates

Fragment growth was not affected by temperature and did not differ among species (2-way ANOVA; p>0.397). Growth was mainly negatively among species and treatments. *L. sarmentosa* showed the highest negative growth (with a maximum of -14,76 cm) and *E. labiata* showed the highest positive growth with a maximum of 1.26 cm (Figure 2.5).

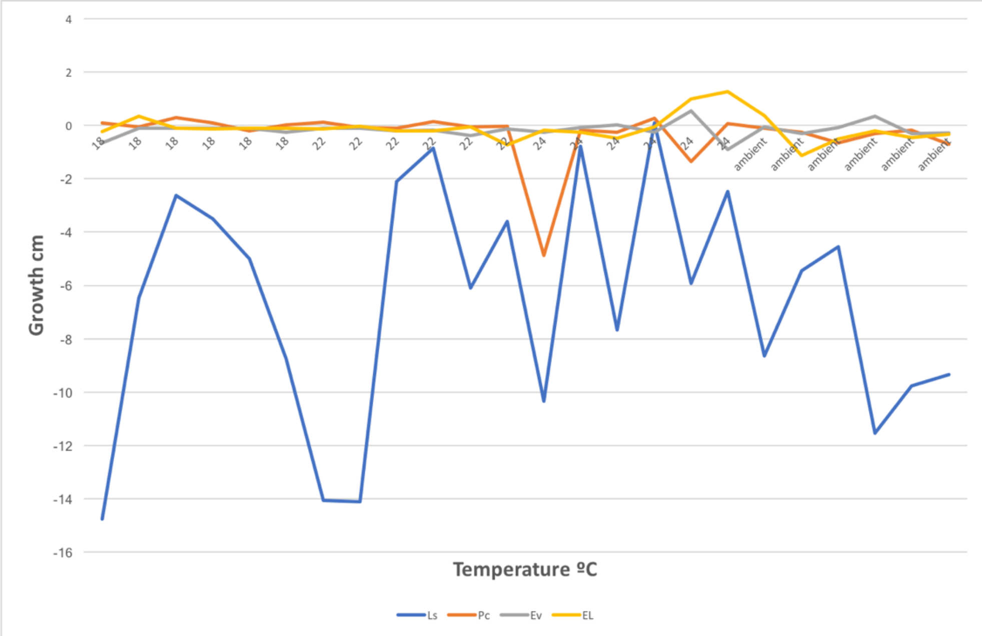

Figure 2.5. Growth estimates of four temperate gorgonian species *L. sarmentosa* (Ls), *P. clavata* (Pc), *E. verrucosa* (Ev) and *E. labiata* (El) for at 18, 22, 24 and ambient seawater temperature.

DISCUSSION

This study inferred the upper thermotolerance levels for four gorgonian species from the South Coast of Portugal, namely *Eunicella labiata*, *Eunicella verrucosa*, *Leptogorgia sarmentosa* and *Paramuricea clavata*.

Polyp activity and coenenchyme necrosis

Long exposure to 24ºC and to ambient seawater temperature affected *Paramuricea clavata* colonies. At higher temperatures *P. clavata* and *L. sarmentosa* suffered from coenenchyme necrosis. In general, there was no trend on polyp activity of all the species among the temperature treatments, which contrasts with others thermotolerance experiments with temperate gorgonians, which showed elevated temperatures increase coenenchyme necrosis and decrease of polyp activity. In this study, the maximum of mean polyp activity was obtained at 24ºC and ambient seawater that can reach 26ºC for *P. clavata* colonies. So, seawater temperature affects polyp activity and necrosis of temperate gorgonian populations of the South Coast of Portugal and both factors vary within species. In this case, the polyp activity increased with the increasing temperature, in contrast with similar studies of thermotolerance experiments with temperate gorgonians. This may be an indicator, that the shallow populations of the South Coast of Portugal are less sensitive to thermal stress than the Mediterranean gorgonian species. This inference is in agreement with the literature, if we compare with studies on these and other Mediterranean species (see below).

The four species studied showed slightly different thermal tolerance patterns, with *L. sarmentosa* the most sensitive and *E. verrucosa* and *E. labiata* the most tolerant to high temperatures. Previati et al. (2010) studied the effects of temperature increase on the physiology of four octocorals species of the Mediterranean (*Paramuricea clavata*, *Eunicella singularis*, *Eunicella cavolinii* and *Coralium rubrum*) by analyzing oxygen consumption, polyp activity and necrosis signs under controlled aquarium conditions. The four species studied showed slightly different thermal tolerance patterns. *P. clavata* proved to be the most sensitive to high temperatures, *E. cavolinii* did not show signs of necrosis, even after being exposed for three days to 25 °C and *E. singularis* appears to be the most tolerant to high temperatures. *P. clavata* and *E. singularis* showed increasing metabolic activity between 14 °C and 22 °C, *E. cavolinii* between 14 °C and 20 °C, and *C. rubrum* between 16 °C and 20 °C. It was confirmed that the metabolism of the four species studied decreases substantially with high temperature, above a definite threshold and also a relation between temperature and mass mortality.

The potential effects of warming in the NW Mediterranean were assessed for Mediterranean red coral *Corallium rubrum* by Torrents et al. (2008). Four types of thermotolerance experiments were carried out at the same time of the year over two consecutive years. The results showed that exposure to 24 °C for 24 days caused a beginning of mortality only for the deep population, and to 25 °C for between 9 and 14 days caused mass mortality of both sample groups. Samples from the shallow population had greater thermotolerance of elevated seawater temperatures than the deep samples. Their results suggest that temperature played a major role (direct or indirect) in mass mortality events occurrence in red coral populations. Hence, there is possible to verify that shallow populations are more resistant to temperature effects.

Pivotto et al. (2015) performed a thermotolerance study of the non-symbiotic gorgonian *Eunicella cavolini*, which demonstrated the existence of highly thermotolerant individuals They analysed the rate and risk of necrosis and almost no sign of necrosis was observed in the control condition at 16ºC. After 28 days of exposure at 27.5ºC, only 15–20% of the fragments displayed less than 5% necrosis levels.

Comparing our study with the studies above, we conclude that Portuguese gorgonian populations in the Atlantic seem less sensitive to higher temperatures than Mediterranean gorgonians, as our colonies showed higher polyp activity at higher temperatures (above 24ºC), no mortality and only a maximum of 21% of necrosis after 4 months of exposure, similar to the thermotolerance studies of Pivotto et al., (2015) with the gorgonian *Eunicella cavolini*.

Growth estimates

There were no significant growth differences among species with increasing seawater temperature, contradicting our hypothesis. There was prevalence of negative growth versus positive growth independently of the temperature. *L. sarmentosa* showed higher values of negative growth than other species.

Gorgonians have slow growth rates of only a few millimetres per year, but this study was only performed during four months. This possibly explains the low values of positive growth, given the lack of time, since the majority of growth studies with gorgonians have a duration of two years, at least. Hence, we can conclude that time was an interfering factor in terms of growth estimates.

Conclusion

This study demonstrated that increasing seawater temperature has the potential to affect South Coast gorgonian populations of Portugal, particularly above 24ºC. However, based on our results a difference degree of impact among species can be expected based on interspecific thermotolerances observed. *Eunicella verrucosa* and *Eunicella labiata* showed more thermotolerance resistance showing no signs of necrosis and mortality and higher growth values than other species in the study. Concerning growth estimates, there is the need for a more complete experimental duration, at least two years to reach to valid conclusions, since the duration of study was very low for this type of experiments. Our results differed in a certain part from the expected results and hypothesis which could lead to new interpretations and conclusions, e.g. higher polyp activity and growth at higher temperatures. Therefore, we think that is necessary to invest in future research efforts using thermotolerance studies and compare shallow versus deep populations and include ecological and genetic factors as well to understand better the Portuguese temperate gorgonians population behaviour and responses to climate change temperature impacts.

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CHAPTER 3: CONSERVATION STRATEGIES - Gorgonian Transplantation in Marine Park Luíz Saldanha, Setúbal, Portugal

ABSTRACT

Restoration strategies (for example, transplanting target species to damaged areas) are a management tool of conservation that has only been applied to marine communities in the last decades. Due to its relatively recent application, restoration practices still need to be improved to obtain efficient and successful outcomes. The majority of marine restoration efforts have been tried on tropical coral reefs and seagrasses, but temperate corals, such as gorgonians (Anthozoa, Cnidaria) are also subjected to intense disturbances and the focus of conservation policies (e.g., red coral and red gorgonian in the Mediterranean Sea, pink sea fan in the UK). Yet, despite the raise in concern, there has never been an attempt to restore impacted gorgonian sites or to develop techniques to transplant and recover these vulnerable species in Portugal. Transplantation procedures were carried out in Marine Park Luiz Saldanha (MPLS) with four species of temperate gorgonians (*Eunicella verrucosa*, *Eunicella labiata*, *Leptogorgia sarmentosa* and *Paramuricea clavata*) collected from shallow depths (12-16 m) of South Coast of Portugal, Sagres, Algarve, previous subjected to thermotolerance experiments in controlled aquaria. The goal of this transplantation is to test the transplantation method known as the "Raw technique" of transplantation from Linares et al., (2008), that consists in gluing the fragments directly to the substratum. Sixty-seven healthy fragments of gorgonians were transplanted (14 *Paramuricea clavata*, 13 *Leptogorgia sarmentosa*, 21 *Eunicella verrucosa* and 19 *Eunicella labiata*) on a new location site in MPLS. The results showed no success of transplantation with no data about fragments growth, mainly due to both technical failures e.g. planning and the choice of the location site and also due to natural events. Therefore, this type of studies need to be well planned and executed with successfully transplantation techniques and regular monitoring campaigns.

INTRODUCTION

Ecosystem restoration can be defined as "activities designed to restore an ecosystem to an improved condition" (Baird, 2005). However, this definition does not account that the desirable final state is achieved; instead it only implies that a better state than the previous damage one is reached (Ammar, 2009). On that account, the definition suggested by Bradshaw (2002) is more accurate: "the process of assisting the recovery and management of ecological integrity".

An implicit assumption is that the involved entities such as scientists and managers understand the ecological dynamics of restoration processes, yet a great number of restoration efforts established to date have fallen short of the desirable goals (Precht and Robbart, 2006). Thus, further understanding of the terms, strategies and approaches and their application is required to meet the aspired goals (Elliott et al., 2007). Furthermore, information on preimpact area and its species composition/structure, management legislation, financial resources and guidance on design implementation of restoration measures is also vital for the successful establishment of a project (Precht and Robbart, 2006). But important, is an effective management of anthropogenic stressors that negatively impact the coral communities (Edwards and Gomez, 2007).

The field of coral reef restoration has grown substantially over the past decades (Lirman and Schopmeyer, 2016; Young et al., 2012). A recent review conducted a literature search to quantify the increasing interest in this field around the world (Lirman and Schopmeyer, 2016). A total of 268 papers were identified and the year 2015 recorded the most publications, to be more precise 36 publications were registered (Lirman and Schopmeyer, 2016). However, restoration efforts on temperate regions weren't considered in this review. In addition to that, the number of citations, projects and programs had also increased (Lirman and Schopmeyer, 2016). Nonetheless, this is a relatively recent field that is in a continuous evolvement and there is still the need to formulate, evaluate and promulgate effective and cost-effective methodologies and strategies (Young et al., 2012).

Until this moment, there is no record of publications concerning temperate corals transplantation, in particularly gorgonians, through Portugal's Coast and in fact, there is evidence of natural and anthropogenic impacts on gorgonian populations of the South Coast of Portugal, especially in the Marine Park Luíz Saldanha, Arrábida Natural

Park, Setúbal (Rodrigues, 2008). Rodrigues (2008) studied the characterization and evaluated the gorgonian populations of Marine Park Luíz Saldanha and obtained data about gorgonian community based on density data, population structure and ecologic state and verified the presence of locations with damaged gorgonians populations, mainly in Jardim das Gorgónias. There were identified six species of gorgonians in MPLS, with more prevalence of *Leptogorgia sp.* and *Eunicella sp.* in all sampling locations. Regarding the efficiency of the Marine Protected Area, the study revealed the existence of very perturbed zones. Consequently, there is the need of coral restoration in MPLS.

Coral restoration methodologies and techniques

In its early days, coral restoration mostly concentrated on the design and execution of complex and structural engineering projects and solutions to recover damaged reefs, mainly impacted by physical disturbances such as ship groundings (Precht and Robbart, 2006). Nowadays, a considerable amount of strategies has been established (Lirman and Schopmeyer, 2016) and among coral restoration, the most performed and popular techniques are addressed for tropical scleractinian corals (Clark and Edwards, 1995; Epstein et al., 2001; Yap et al., 1998), although some efforts have been conducted for temperate species (e.g. *Paramuricea clavata* in the Mediterranean Sea; (Linares et al., 2008a)).

Coral transplantation

During the last decades, coral transplantation was considered as the prime tool for coral restoration (Epstein et al., 2003). The fundamental principle behind this practice is to restore damaged coral areas by replacing dead coral colonies with new healthy ones collected from a stable donor site to speed up natural recovery (Epstein et al., 2003). Moreover, transplantation should benefit recruitment, accelerate recovery and as well improve the visual perspective (Jaap, 2000) and enhance the state of diminished resilience of the ecosystem (Dobson et al., 1997). Several transplantation techniques are widely used including throwing bits and fragments to the damage areas and securing individual pieces or whole organisms to the reef platform with cement, epoxy glue, cables, plastic tubes, among other methods (Jaap, 2000; Linares et al., 2008a; Weinberg, 1979).

Numerous studies have discussed different aspects and parameters intrinsic to coral transplantation: (a) choice of coral species, (b) transplantation efficacy, (c) colony pattern formation, (d) survivorship of transplanted corals, (e) growth rates of transplanted colonies as compared to naturally recruited colonies and (f) type of substrate, to name a few (Epstein et al., 2001; Epstein et al., 2003; Yap, 2000; Yap, 2004). Therefore, coral transplantation is best employed when (a) natural recruitment is improbable or limited and also there is the evidence of unconsolidated and unstable substrate (Lindahl, 2003); and (b) when there are enough healthy donor colonies available (Bowden-Kerby, 2001; Gleason et al., 2001; Ortiz-Prosper et al., 2001). However, there are a significant amount of challenges associated to this restoration measure and its protocols (Edwards and Clark, 1999) particularly high labour and financial costs (Harriott and Fisk, 1988) and the introduction of alien material (i.e. pathogens and diseases) (Clark and Edwards, 1995) and reduction of genetic diversity (Jahnke et al., 2015). Another adverse aspect associated to this approach is that the harvesting of corals for transplantation disturbs healthy and undamaged reef, inflecting stress on donor colonies (Epstein et al., 2003).

Since the potential of restoration of temperate gorgonian populations is scarcely explored, especially in Portugal, the main goal of this study is to test transplant techniques for gorgonians in open ocean to contribute to the elaboration of an effective ecological restoration strategy for gorgonian populations in Portugal to restore degraded areas. Therefore, it will focus on (1) the development of transplantation techniques involving low labour effort and low economic cost and (2) on the evaluation of the outplants potential in terms of growth, survival and adaptability to the environmental conditions of the transplant site; (3) based on this work, to compile a set of guidelines of "good practices" for temperate gorgonians restoration in Portugal and (4) focus on repopulation of the gorgonian populations in Marine Park Luíz Saldanha and monitoring their adaptation and success and ultimately answer the following questions:
Will the transplant method chosen succeed, meaning will the gorgonian outplants survive and grow in the environmental conditions of the Marine Park Luíz Saldanha?

Based on other similar studies with temperate gorgonian species we establish the following hypothesis: (1) Regarding the transplant procedures, we expect a certain level of success respecting the chosen transplant technique and also growth of the outplants to the new location conditions. However, it is expected the disappearance of some colonies due to natural events and not because the ineffectiveness of the transplant procedures;

MATERIALS AND METHODS

Study area: Arrábida Natural Park – Marine Park Luíz Saldanha

The Marine Park Luiz Saldanha (MPLS) was the first marine park established in continental Portugal in 1998 and it is included in the list of Natura 2000 proposed sites since 2003 (Cunha et al., 2014). This site extends through a 53 km^2 area along 38 km of coastline from the Setúbal peninsula, more specifically from the mouth of Sado estuary at Figuerinha beach, to the rocky biotopes located just north of Cape Espichel limited by Foz beach (Stratoudakis et al., 2015). The mountain chain of Arrábida protects this area from the north and northwest winds while most of the prevailing area faces south (Stratoudakis et al., 2015); this kind of protection from north currents is rare through the Iberian Peninsula coastline and enables the development of structured ecological communities (Stratoudakis et al., 2015). This area is recognized for its high and unique biodiversity and also its ecological importance, harboring more than a thousand recorded marine species (Cunha et al., 2014).

The aim of establishing the marine park is mainly to protect coastal ecosystems and safeguard its nursing areas, thus promoting the sustainability of fisheries resources (Cunha et al., 2014). The usage of the Marine Park Luiz Saldanha was only regulated in August 2005 by official legislation (Ministers Council Resolution No. 141/2005, 23rd August 2005) dividing the region in three different protection areas: Complementary, partial and total, differing in level of restrictions imposed (Figure 3.1) (Sousa, 2011). This zoning was established to reconcile the socioeconomical activities that take place in the park with the ecological and biodiversity importance (Cunha et al., 2014; Sousa, 2011). Concerning the restrictions imposed to the different zones, when it comes to total protection, human activities are not allowed; if it is partial it is only allowed a certain gear of fishing and in case of complementary protection, fishing is allowed but restricted to vessels under 7m length and licensed to operate within the marine park (Carneiro, 2011; Cunha et al., 2014; Sousa, 2011). Organized recreational activities, such as SCUBA diving and boating pending on the granting of specific licenses (Carneiro, 2011).

Six species of gorgonians have been identified in MPLS, namely *Leptogorgia lusitanica*, *Leptogorgia sarmentosa*, *Eunicella verrucosa*, *Eunicella gazella*, *Eunicella labiata* and *Paramuricea clavata* (Rodrigues, 2008). Regarding gorgonian species diversity, the number of species found in MPLS is similar to certain areas in the Mediterranean, such as the coast of Catalunya (Gili et al., 1989). Additionally, these species are also found along the coast of the Bay of Biscay (Altuna Prados, 1991). The presence of the Setúbal submarine cannon is an influential factor for the presence of these species, since the thermohaline characteristics of the water in this region indicate the presence of Mediterranean waters (Rodrigues, 2008). In the study area, the species from the genera *Eunicella* and *Leptogorgia* are found along the total area of MPLS and coexist at same depth, between 4 and 24 m (Rodrigues, 2008). There is a particularity regarding very shallow minimum depth limits, since gorgonians were found at only 4.2 m for *L. sarmentosa* and *E. gazella* and 4.6 m for the remaining species, except *P. clavata*. Note that the minimum depth recorded for *L. sarmentosa* in the Mediterranean is 10 m (Ribes et al., 2003), 6 m for *E. verrucosa* in South-west England (Hall-Spencer et al., 2007) and 5 m for *E. labiata* in Malaga coast (Grasshoff, 1992). This fact could be explained by the characteristics of the study area such as the low turbulence of the water and the turbidity of the water, which protect from high solar radiation (Rodrigues, 2008). Concerning species frequency, there was evidence of the existence of geographical zoning, since there were differences in species frequency between the different regions of the marine park, which could be explained by the diversity of the habitats in each region (Rodrigues, 2008). *L. sarmentosa* and *L. lusitanica* are more frequent in east of MPLS, on the other hand, the distribution of *P. clavata* is limited to the Cabo Espichel region, albeit being observed in Arcanzil and Pedra Milú, *E. verrucosa* and *E. labiata* are more frequent in west regions of the marine park (e.g. Arcanzil and Pedra Milú), while *E. gazella and L. sarmentosa* are less represented (Rodrigues, 2008). As matter of fact, *L. sarmentosa* recorded densities in MPLS are very similar to those found in the Mediterranean (Rossi et al., 2004).

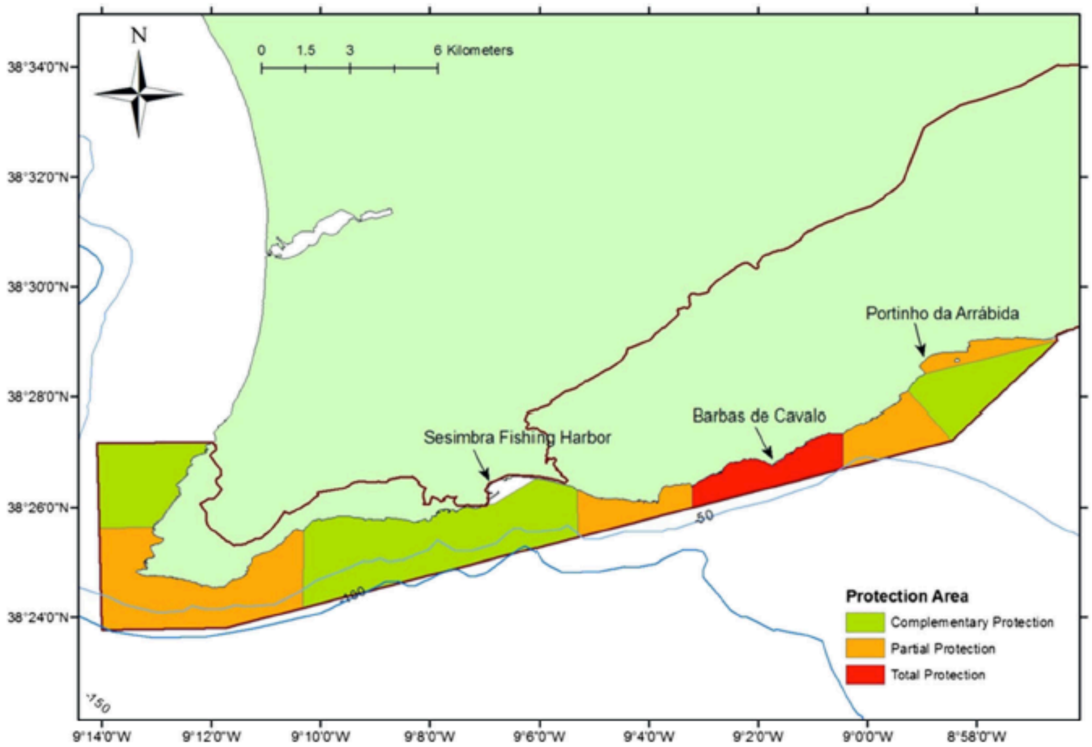

Figure 3.1. The Arrábida Marine Park with its three different types of protection areas, CPA (Complementary Protection Area), PPA (Partial Protection Area), TPA (Total Protection Area) (Cunha et al., 2014).

Fragments transplantation

On 30th and 31st August 2017, 67 healthy fragments of gorgonians from Porto da Baleeira, Sagres, south Portugal, previously subjected to thermotolerance experiments in a controlled set aquarium system (14 *Paramuricea clavata*, 13 *Leptogorgia sarmentosa*, 21 *Eunicella verrucosa* and 19 *Eunicella labiata*), were transplanted by the CCMAR scientific diving team to Marine Park Luíz Saldanha. The fragments were transported within the discs inside cooler boxes with aeration to oxygenate the water and maintain the fragments alive during the transportation.

After the arrival to the transplantation site, the fragments were detached from the discs and placed inside individual small sampling zip lock bags previously labelled and posteriorly assembled together by a rope for transplantation purposes. The fragments were glued individually using an underwater two-part epoxy putty glue (Z-Spar Splash Zone A-788 Kit**),** that was previously mixed: two equal parts of each component (yellow and black) were mixed until reach an homogeneous mixture with colour grey (this mixture process was previously done outside water); then the glue was moulded underwater to glue each fragment individually after scrapping the bottom to remove algae and epibiont vestigial, thus providing more adherence, and then each fragment was placed in the middle following a structured scheme design (see the experimental protocol in Annexes section). The transplantation procedures were executed on two different sites (two sets: rock1, 2 and 3) (- 9.01838597, 38.45213502 decimal coordinates) and the first segment of each set was tagged for guidance. After some tests with some fragments, we choose the "Raw technique" of transplantation from Linares et al., (2008), which consists in gluing the fragments directly to the rock as previously described.

Monitoring

The monitoring of the transplants includes regular systematic visual census surveys to measure the progress of the project, in particular to collect data concerning growth, survival and mortality rates and also to identify potential problems and maintenance (e.g. remove overgrowth). The first monitoring survey was realized on December 21st 2017 by CCMAR Scientific Diving Team in the MPLS transplantation site, where all the locations of transplanted fragments were surveyed and growth measurements (only the principal axis) were performed for each fragment using a scale device that consists of a set of two rectangular plates with a written scale. The outplant locations were also photographed with a Canon PowerShot G16. The second monitoring campaign was realized on 7th June 2018.

RESULTS

Four months after the transplantation procedures, there was evidence of disappearance of 23 fragments. The remaining fragments did not show any signs of coenenchyme necrosis, suggesting good health of the colonies. The fragments with 7 cm and 8 cm length were the ones that had 100% transplant success, followed by the 5 cm length fragments with 90% success. In contrast, the lowest transplantation success was for 3 cm length fragments with only 17% success (Figure 3.2). There were no significant differences between the length of the fragments since the day of transplantation and after the first monitoring campaign, suggesting practically no growth.

In June 2018, ten months after the outplanting, there was no evidence of any fragments in the transplant locations, suggesting a 0% of success. The outplant rocks were colonized by seaweeds of the genera Cystoseira and Asparagopsis, that formed a massive canopy over the rock.

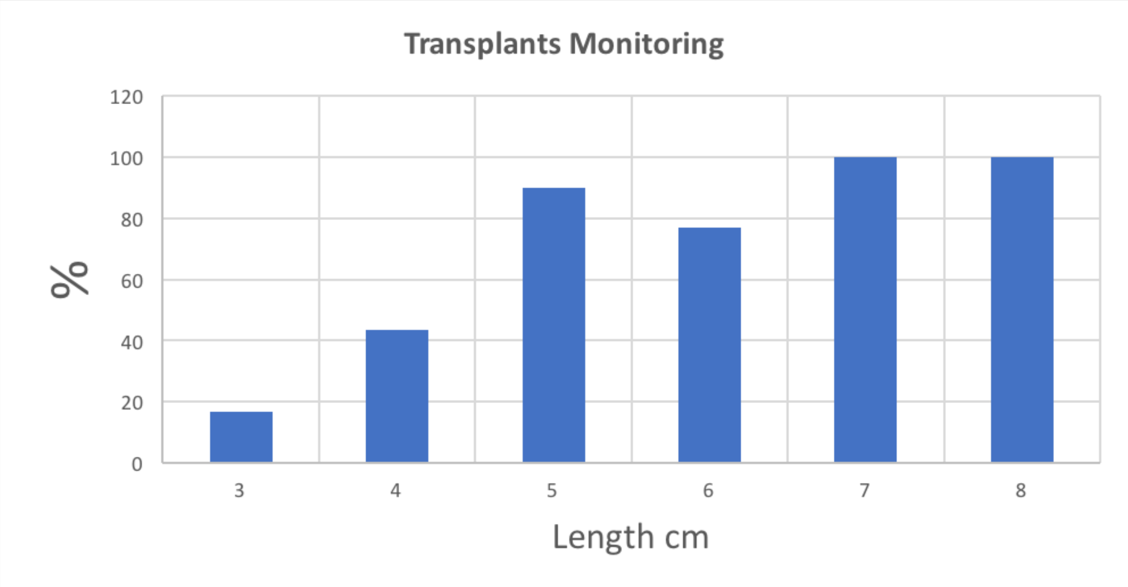

Figure 3.2. Mean relative success of transplantation by fragment length (cm) of temperate gorgonians (species) after 11 weeks of transplantation.

DISCUSSION

Our results show that the four species tested are suitable for transplantation purposes, since they survived in the new location site in the first months next to transplantation. During the first four months, there was a high rate of survival success of the transplants, between 90% and 100% for the longer fragments (7-8 cm length). The loss of 34% of fragments was probably due to natural events such as strong currents or animals that dislodged the glued fragments, because they were simply not found there, whereas the ones found on the site were in good health. This indicates that the chosen transplantation technique is suitable although with some rate of fragment loss, and that the technique allows successful establishment in the new site of transplantation for the fragments that remained glued. However, in terms of growth, there were no significant differences after four months of transplantation, which is not unexpected given the very slow growth rates of gorgonians. Unfortunately, after 10 months of transplantation, all the remaining fragments (n=44) disappeared due to natural events of comptetion by macroalgae, as in 2018 there was a very high rate of growth of *Cystoseira sp*. and *Asparagopsis armata* that occupied the transplantation site. Consequently, in the long run there was no success of transplantation and no data about fragments growth. To avoid this outcome, in future efforts there is the need for constant monitoring during the first year after the transplantation: such as every month in the first months and later every six months monitoring to clean the area and control the state of the fragment colonies. A better evaluation of the transplanting site is advisable, but it could not predicted that an area without seaweed in August (a time when *Cystoseira* canopies are maximal) would have a major cover in the following year. The best way to avoid major macroalgal competition is to avoid very shallow areas with high light.

In conclusion, this study demonstrated that the transplantation method tested worked successfully in ensuring good survival for some months of the fragments that remained attached. However, a small proportion of the fragments did not remain attached and this suggests that it would be beneficial to develop an improvement on the attachment method to reduce such losses of transplanted fragments. In the long run, the habitat selected for the transplants became unsuitable due to novel macroalgal recruitment that could not have been predicted in the previous year at the transplanting time, indicating that in future transplant efforts it is better to divide the risk among many habitats by spreading the transplants across different areas, so that such stochastic effects will not affect all the transplants. A program of long-term growth surveys of the new transplanted populations would be important for evaluating the success of these conservation strategies. This cannot be achieved in short-term studies due to the very slow growth rates of gorgonian colonies.

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ANNEXES

Gorgonian Transplantation Protocol

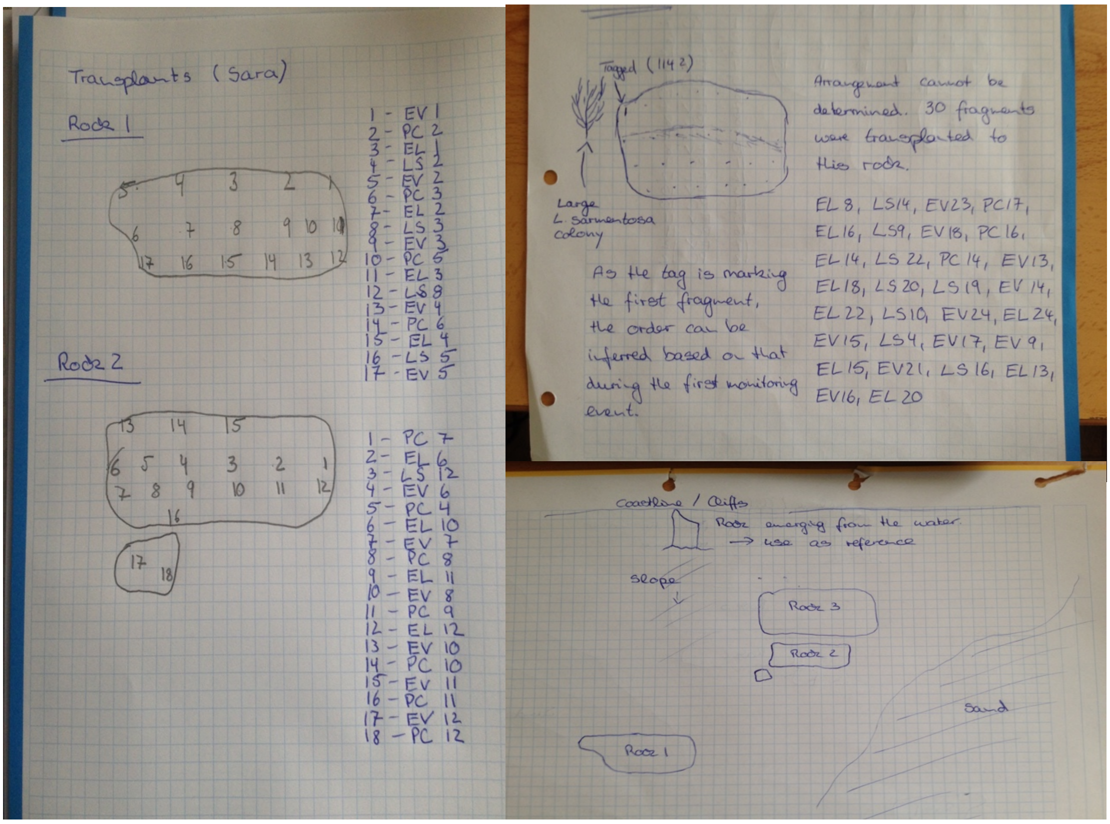
1) Tagging

On 30th and 31st August 67 fragments of gorgonians were transplanted (14 *Paramuricea clavata*, 13 *Leptogorgia sarmentosa*, 21 *Eunicella verrucosa* and 19 *Eunicella labiata*) on Arrábida

Coordinates (rocks 1,2 and 3): - 9.01838597, 38.45213502

The fragments were glued individually using a two-component epoxy (Z-Spar) glue that is previously mixed: two equal parts of each component (yellow and black) are mixed until homogeneous mixture is reached, with color grey (this mixture process is done outside water just before the transplants, as it solidifies quickly after);

The glue is moulded underwater to glue each fragment individually after scrapping the bottom and the fragment is placed in the middle. The design in Fig. XX indicates the distribution of the fragments.



The tags will be placed next to each fragment using the glue previously mixture.

2) Measurements

The measurements will be performed using a ruler and a contrasting background and each fragment will be measured and photographed. First, the tag is photographed and then the fragment with the background and ruler is photographed.

3) Monitoring

The aim of the monitoring is to assess growth and survival in the first four months where they will be monitored through growth measurements and photography of each individual, mortality and fragments disappear will also be recorded through photography following the described on point 2) and also recording the number of missing fragments.

Dates:

25 to 30 September/October/November 2017 and June 2018