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Octocoral restoration - improving methods for transplantation success

UNIVERSIDADE DO ALGARVE

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho:

Eu, Nuno Miguel Machado Padrão, declaro de ser o autor deste trabalho de tese de mestrado: '**Octocoral restoration - improving methods for transplantation success**', que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Abstract

Octocorals are among the main habitat-engineering species, generating complex three-dimensional ecosystems of unquestioned importance. Despite their ecological value, gorgonian habitats have dramatically declined in the last decades. Consequently, gorgonian gardens were internationally recognized as Vulnerable Marine Ecosystems, highlighting the importance to act and protect these valuable ecosystems. To reverse the actual scenario, it is important to not only develop policies and management directives but also methodologies to recover impacted, but still existing, endangered habitats. In this 2 years' study we tested and compared the outcome of the "Stick" transplant method and the Direct Substrate Attachment (DSA), a novel transplantation method here developed. In the transplants performed using the octocoral *Paramuricea clavata*, size of the transplant, attachment, survival, holdfast formation, growth and ecological state were monitored and compared to assess the success of the transplant. The results obtained using the "Stick" methodology were not comparable with those previously published. Several issues resulting from the capacity to retain the gorgonians emerged, which together with a high mortality, culminated in a premature end of these treatments. Contrastingly, the DSA methodology produced positive results, with 70% survival and annual positive growth. Moreover, by the end of the study, the transplants were healthy and reattached to the natural substratum. The transplant done with the DSA method are to date the first successful gorgonian transplant in temperate seas with proven long-term success. The results achieved are especially important in a moment where ecological degradation and mitigation efforts are a hot topic among decision makers. Using the DSA method it has been proved that transplantation is possible and should be considered in conservation and restoration efforts of impacted areas. This study is an encouraging step, laying a base foundation for future restoration efforts using octocorals in temperate seas.

Keywords: restoration, transplantation success, methods, gorgonian, octocoral growth

Sumário Executivo

Espécies com a capacidade de modificar significativamente fatores bióticos ou abióticos, mantendo, modificando ou destruindo dessa forma o habitat circundante são conhecidos como engenheiros de ecossistema ou espécies estruturantes. Estes organismos têm a capacidade de criar estruturas tridimensionais complexas através de processos endógenos, em que a modificação da sua própria estrutura modifica o habitat, ou através de processos alogénicos, em que o organismo modifica o espaço que o rodeia. Agregações de organismos autogénicos são extremamente importantes em zonas costeiras, e ainda mais no oceano profundo, onde a falta de relevo ambiental faz das estruturas criadas por estas espécies autênticos oásis. Várias espécies usam os espaços criados no seio destas agregações como refúgio para desovar, ou para os seus juvenis se desenvolverem ao abrigo de predadores naturais. Octocorais, também conhecidos como gorgónias, fazem parte deste grupo importante de espécies estruturantes. As gorgónias são organismos coloniais sésseis, que capturam o seu alimento da coluna de água por via dos seus pólipos. A sua importância é inquestionável, não só pelo intricado habitat que criam com a sua forma arbórea e ereta, mas também por contribuírem para as trocas energéticas que se verificam entre a coluna de água e as comunidades bentónicas. Vários estudos apontam para espécies estruturantes como as gorgónias, as ervas marinhas e diversas algas como sendo responsáveis pela abundante biodiversidade encontrada em diversos ecossistemas, nomeadamente em zonas costeiras. No entanto, as zonas mais impactadas nas últimas décadas são exatamente as plataformas continentais e zonas costeiras. O incremento da pesca com artes que têm como alvo o fundo marinho é uma das atividades antropogénicas com um impacto significativo nas comunidades bentónicas. Deste tipo de atividade pesqueira resulta a captura de um incalculável número de espécies que não são espécies alvo (*bycatch*). Os corais, em particular as gorgónias, encontram-se entre as espécies frequentemente impactadas, devido à sua morfologia. Outro fator com impacto crescente é a poluição das águas costeiras, resultante da massificação urbana e industrial no litoral. O turismo marinho (mergulho com escafandro e náuticas desportivas) e fenómenos associados às alterações climáticas (*blooms* de algas e infeções virais), são outros exemplos de impactos que têm sido reportados nas últimas décadas. Devido ao contínuo e crescente impacto sobre estes ecossistemas, associado ao declínio verificado nas populações ou agregações de gorgónias - tipicamente conhecidos como “Jardins de Gorgónias” -, estes foram classificados como Ecossistemas Marinhos Vulneráveis em 2010, pela Comissão OSPAR. Sem dúvida, este passo legitimou os pedidos de muitos

investigadores para que se aumentem os esforços para a proteção e restauro destes habitats tão importantes. A criação de Áreas Marinhas Protegidas tem sido de extrema importância, porém, ecossistemas que foram impactados durante décadas podem não ter capacidade de se regenerar sem uma intervenção externa. Nesse sentido a restauração de habitat é uma disciplina que tem ganho relevância e obtido resultados positivos. A reintrodução de biomassa, recorrendo a transplantes, não só tem um impacto direto e imediato no aumento da estrutura do habitat, como visa aumentar a capacidade de autorregeneração da população alvo. Esta capacidade de autorregeneração resulta do incremento de organismos reprodutores na área transplantada e por consequência, o aumento de recrutamento na área. Para atingir os resultados desejados, muitas foram as tentativas de desenvolver métodos de transplante de corais, embora historicamente, o foco tenha sido nos corais rochosos. Só recentemente as gorgónias foram alvo de mais atenção no que toca a estudos de restauração de habitat. Apesar de alocações de recursos desiguais, foram desenvolvidos esforços para transplantar gorgónias desde os anos 70. No entanto, as dificuldades encontradas foram muitas e os estudos, na sua generalidade, de curta duração. Historicamente, nos estudos publicados, ocorreram acima de tudo problemas na fixação dos corais no novo local, o que limita a sua sobrevivência. Estudos mais recentes reportam resultados mais promissores no que toca à sobrevivência, porém são estudos de curta duração e sem monitorização do crescimento das colónias transplantadas. Estes resultados podem ser tendenciosos e induzir a comunidade científica em erro, uma vez que a curta duração dos estudos não engloba as várias estações do ano e as associadas alterações climatéricas, ou seja, a resiliência dos transplantes não é comprovada. Por outro lado, o não seguimento do crescimento dos corais transplantados não permite entender se a população transplantada está a crescer ou a perder tamanho, o que poderia inclusive levar à morte da colónia no espaço temporal já fora do qual decorreu o estudo. O seguimento do crescimento de corais sempre levantou questões e dificuldades. Seja pela complexidade da estrutura do coral ou pelo seu crescimento ser pouco previsível e influenciado por fatores ambientais (outros organismos e relevo do substrato). No entanto, há vários protocolos de monitorização de crescimento de corais desenvolvidos e testados, que podem ser aplicados. Em particular na última década, com o recurso a novas tecnologias, tais como câmaras fotográficas subaquáticas e programas para processamento de imagem, algumas destas metodologias geram a capacidade de seguir o crescimento destes organismos mesmo com monitorizações pouco espaçadas no tempo. De todas as metodologias disponíveis, tendo em consideração a sua precisão, provavelmente as que são mais adequadas para o seguimento de transplantes de gorgónias são: o método que segue o crescimento de todos os ramos da colónia "*Total Branch Lenght*" e o método que acompanha a área ocupada pelos ramos da colónia. Ainda assim, o método mais utilizado na literatura é a medição da altura da colónia, devido à facilidade logística e pouco tempo de processamento dos dados.

Neste estudo, o objetivo foi comparar a longo prazo (2 anos) os resultados obtidos com a metodologia "*Stick*" (Linares et al., 2008) com uma metodologia desenvolvida recentemente no âmbito desta tese, a "*Direct Substrate Attachment*" (DSA). A metodologia "*Stick*" é descrita como fácil e rápida, para que possa ser implementada em projetos com voluntários. O processo é simples e envolve a remoção (raspagem) do material biológico do substrato para onde vai ser transplantada a gorgónia, a colocação de uma base de epóxi, e for fim a fixação da gorgónia no epóxi. A gorgónia deve ser preparada de antemão, pondo um pequeno tubo de PVC preso à mesma para lhe dar suporte quando colocada no epóxi. A metodologia DSA envolve a realização de um furo de pequenas dimensões no substrato, a inserção de epóxi no furo realizado, e a colocação da gorgónia de forma que o tecido da mesma fique em contacto com o substrato. Para a realização da experiência foi utilizada *Paramuricea clavata*, a espécie de gorgónia também utilizada no estudo realizado por Linares et al., 2008, permitindo assim comparar resultados entre estudos. Foram seguidos fatores como a fixação artificial (resultante da metodologia), a sobrevivência, a criação de uma fixação natural (*Holdfast*), o crescimento, e o estado ecológico (necroses, perda de cor, perda de tecido). Para além destes fatores, foi também testado o efeito do transplante no tamanho da colónia transplantada. Foram para isso reproduzidos os tratamentos acima referidos em 2 classes de tamanho: <10cm e 10-20cm. Este estudo testou 4 tratamentos distintos, considerando os dois métodos de transplante e 2 classes de tamanho.

Os resultados obtidos com a metodologia "*Stick*" foram aquém do esperado, contrariando os resultados do estudo publicado por Linares et al., 2008. Problemas associados à fixação dos transplantes da classe <10cm tiveram um papel decisivo nos resultados obtidos. A maioria dos transplantes não ficou na base de epóxi, acabando por cair. Dos restantes transplantes, observou-se uma mortalidade que foi atribuída a eventos naturais. Com a classe 10-20cm, tal como descrito no estudo original, não foi possível transplantar. O tamanho e peso dos transplantes, aliado à normal movimentação da água, não permitiu manter os transplantes perpendiculares ao substrato. Devido à falta de sucesso na fase de transplantação, não foi possível a análise dos restantes fatores para fragmentos de 10-

20cm. Com a metodologia DSA o sucesso na fixação dos transplantes foi de 100% tanto no dia do transplante como 24h depois, estabilizando em 95% de sucesso ao fim de 3 meses. No final do primeiro ano de estudo, todas as colónias transplantadas tinham desenvolvido uma fixação natural com o substrato, sendo que as gorgónias de classe de tamanho 10-20cm desenvolveram esta fixação mais rapidamente. A sobrevivência dos transplantes ao fim de 2 anos foi de 70% e 68% na classe de tamanho <10cm e 10-20cm, respetivamente. Um resultado acima da média de sobrevivência reportada na literatura, em estudos que, na sua maioria, têm durações inferiores a 1 ano. O crescimento, também demonstrou resultados encorajadores. As gorgónias de ambas as classes de tamanho tiveram um crescimento positivo anual, sendo o crescimento significativo no fim da experiência. O estado ecológico dos transplantes também revelou uma boa adaptação ao processo de transplantação com este método, não havendo um número significativo de colónias com necroses, descoloração, ou inibição dos pólipos (ausência de alimentação). Neste estudo podem destacar resultados contrários aos reportados na literatura ao implementar a metodologia "*Stick*". Apesar do objetivo da metodologia ser a aplicação em projetos de ciência cidadã, a sua implementação por parte de uma equipa de biólogos com experiencia na área, revelou maus resultados. Na classe de tamanho <10cm, foram observadas colisões entre peixes e as colónias transplantadas enquanto o epóxi ainda estava a solidificar. Os peixes atraídos pelo sedimento resultante do processo de raspar o substrato acabariam por derrubar várias colónias. A maioria das colónias transplantadas viria a ser perdida nas 24 h seguintes ao transplante, e supõe-se que este tenha sido o principal fator. Das colónias que não foram perdidas nas 24 h após o transplante por falha na sua fixação, todas foram encontradas sem tecido vido e com o esqueleto coberto por algas. A perda das colónias transplantadas e a incapacidade de transplantar tamanhos acima dos 10cm limita as conclusões que se podem tirar desta experiência. Estes resultados expõem, porém, dificuldades inerentes ao processo de transplantação, que aparentam não ter sido ultrapassadas com a metodologia "*Stick*". Este facto põe em questão a sua aplicação com voluntários sem experiência na área, e se o método é exequível em águas com fortes correntes, visto que nas 2 a 3h em que o epóxi solidifica os transplantes podem ser removidos da sua base de epóxi. A metodologia DSA, apresentou resultados positivos, particularmente tendo em conta a duração e fatores não referenciados na literatura tal como o crescimento após a transplantação. O estudo foi conduzido durante 2 anos (longo prazo), houve crescimento positivo das colónias e uma sobrevivência de 69% no fim do estudo (acima da média na literatura). A adaptação das

gorgónias após a transplantação foi boa, todas as colónias exibiam pólipos abertos 24h depois do transplante, um indicador de que não estavam sobre stress e se estavam a alimentar. Ambas as classes de tamanho obtiveram resultados semelhantes no final do estudo, tanto a nível de sobrevivência como de crescimento e estado ecológico. Este facto permite a escolha do tamanho dos fragmentos a ser utilizados em projetos de restauração, tendo em conta as necessidades e objetivos definidos no desenho experimental e não por limitações inerentes ao método. Não havendo escassez de biomassa para a realização dos transplantes, deverão ser transplantadas colónias entre os 10 e 20cm. Transplantar fragmentos maiores, permitirá transplantar colónias com tamanho reprodutivo (dependendo da espécie), ou que poderão atingir a maturidade reprodutiva mais depressa. No caso de existir falta de biomassa, podem utilizar-se colónias mais pequenas, ou secções de colónias. Tendo em conta que as taxas de crescimento entre as duas classes de tamanho não foram significativamente diferentes, irá demorar mais tempo até que as colónias atinjam um tamanho que lhe permita alocar recursos na sua reprodução. Apesar deste facto, tendo em conta objetivos a longo termo seccionar colónias obtendo mais fragmentos para transplantar permite aumentar o número de transplantes no local.

Este estudo é o primeiro a realizar transplantes de gorgónias com sucesso na costa portuguesa e provavelmente o primeiro em todo o mundo em águas temperadas. Projetos de restauração de habitat são essenciais na recuperação de áreas impactadas. A restauração ativa aliada à restauração passiva é defendida por muitos autores como a mais eficiente. Depois da remoção dos fatores de impacto, tais como a pesca ou poluição, poder-se-ia esperar a recuperação do habitat por si mesmo (restauração passiva). Porém habitats fragilizados, demoram mais tempo a recuperar sem uma ação de restauração ativa (tal como a transplantação), ficando à mercê de eventos naturais que podem levar à sua destruição. Ao adicionar biomassa as populações afetadas ficam imediatamente mais resilientes e podem recuperar mais rapidamente. É também relevante que a restauração ativa, nomeadamente por via de transplantes é na sua na generalidade um processo dispendioso e demorado. Devido a este facto é essencial que as metodologias de transplantação tenham resultados credíveis e robustos. Os resultados descritos neste estudo podem ajudar na tomada de decisão pelas entidades competentes, permitindo a formulação de projetos de restauração de habitats de gorgónias exequíveis, nomeadamente em áreas protegidas.

Palavras-chave: restauração, sucesso na transplantação, metodologias, gorgónias, crescimento

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List of Abbreviations

BFSA	Branch Fan Surface Area
CP	Complementary Protection
DSA	Direct Substrate Attachment
FP	Full Protection
GLM	Generalized Linear Model
ICNF	Instituto Conservação da Natureza e das Florestas
MPA	Marine Protected Area
PP1	Partially Protected Level 1
PP2	Partially Protected Level 2
POC	Particulate Organic Carbon
POM	Particulate Organic Matter
PNSACV	Parque Natural do Sudoeste Alentejano e Costa Vicentina
PVC	Polyvinyl chloride
RFSA	Rectangularized Fan Surface Area
TBL	Total Branch Length
2-D	Two Dimensions
3-D	Three Dimensions

1 Introduction

1.1 Ecosystem engineers

Ecosystem engineers are autogenic or allogenic organisms that have the ability to significantly modify abiotic and biotic factors, creating, maintaining or destroying or destroying a habitat (Jones et al., 1997). Autogenic engineers transform the environment via endogenous processes that alter the structure of the organism itself (e.g., seagrass or coral growth), and the engineer remains as part of the engineered environment. Allogenic engineers, on the contrary, shape the environment by altering living or non-living materials that will in turn transform the surrounding landscape (Jones et al., 1994). Aggregations of marine autogenic engineering species create intricate three-dimensional environments and are extremely important in shallow waters, and even more in the deep ocean where the lack of relief and hard substrate are common habitat features (Carvalho et al., 2014; Gutiérrez et al., 2011; Buhl-Mortensen et al., 2010; Coma et al., 1998; Jones et al. 1994). The influence ecosystem engineers have in the habitat reflects directly on the species richness and habitat landscape heterogeneity of an area (Carvalho et al., 2014; Cerrano et al., 2010; Wright, 2002), and in turn, the health and delicate balance of the ecosystem depends on these species' population stability (Ponti et al., 2016).

1.2 Gorgonians

Gorgonians (Octocorallia: Alcyonacea), commonly known as soft corals or sea fans, are long-lived ecosystem engineering species, and can live up to centuries of age (Martinez et al., 2016; Cerrano et al., 2010). These sessile colonial organisms have a simple but unique morphophysiological composition. The coenenchyme (skeleton) is flexible, unlike scleratinian corals, and is composed of an organic substance called gorgonina (Pérez Zaballos et al., 2009; Barnes, 1980). Over its skeleton, grows a complex matrix called mesogleal tissue, composed of living tissue and small calcareous spicules. The tissue is perforated by numerous tiny channels that interconnect the gastrovascular cavities of the polyps. These pathways allow water and nutrients to flow freely between all the members of the colony. Embedded in the coenenchyma and the mesogleal are numerous polyps (Pérez Zaballos et al., 2009; Barnes, 1980). Only a small part of the polyp projects out of this tissue matrix (Fabricius & Alderslade, 2001). The exposed area is where the tentacles and mouth are. The mouth has a siphonoglyph, which helps controlling the water flow. It opens into a tubular pharynx that continues towards the gastrovascular cavity. The gonads are located right under this complex structure allowing embryos to be released through it to the outside of the organism (Pérez Zaballos et al., 2009; Brazeau & Lasker, 1990; Barnes, 1980). Gorgonians attach to rocky reefs by the form of a holdfast (part of the coenenchyma) and demonstrate an erect branching growth form that can vary in size, shape, and orientation of branching (Linares et al., 2007; Coma et al., 1998). Shape can be influenced or restricted by the heterogeneity of the surrounding environment, substrate relief or presence of other organisms, while growth orientation is predominantly perpendicular to the general water current in the area (Grigg, 1972). They can be found sparsely distributed or in dense populations composed of one or more species, which are referred to as gorgonian gardens or forests (Cúrdia et al., 2013; Coma et al., 1998). Such clusters can be explained by their reproductive strategies and larval stages (Mokhtar-Jamai et al., 2011). Gorgonian corals are iteroparous and can reproduce both sexually and asexually, being generally gonochoric. Fertilization is achieved through internal (in some cases external) larvae brooding or broadcast spawning (Kinzie, 1970; Grigg, 1977; Brazeau & Lasker, 1989). Successful fertilization and recruitment are guaranteed by seasonal gamete production followed by short synchronous episodes where spawning occurs. Brooders' fertilized eggs are retained by the colonies, allowing larval formation to occur internally or on its surface. The short life span and lack of swimming ability of some species' larvae (ie *P. clavata*) increases the likelihood of settlement and recruitment in the area of the parent population (Mokhtar-Jamai et al., 2011; Coma et al., 1995). Other species exhibit the capacity to swim (mostly vertically), extending their time in the water column and allowing bigger dispersion (Guizien, et al, 2020). These strategies are paramount for population stability, growth and survival as it increases the success of sexual reproduction and genetic diversity. Moreover such diverse larvae swimming capacity affect distribution dynamics and consequently, directly alter and shape the surrounding environment (Mokhtar-Jamai et al., 2011; Tsounis et al., 2006; Coma et al., 1995; Ribes et al., 2007). Sexual maturity is size dependent and species specific, thus, many factors that affect growth and size, such as predation, water flow, fragmentation, or lack of food, will dictate when a colony will be able to reproduce. For example, *Paramuricea clavata* (Risso, 1826) will achieve sexual maturity when the colony is 11 cm (height) or bigger (Coma et al., 1995) while *Leptogorgia sarmentosa* (Esper, 1789) will mature at 21 cm (Rossi, S. & Gili, J.M., 2009) and *Plexaura* A will require 20 cm height (Brazeu & Lasker, 1989). These results show how different sexual maturity size can be among different species. Gorgonians also have a protection mechanisms to avoid predation, and prolonged contact with other organisms. The polyps contain sensorial cellules and cnidocytes. Cnidocytes are explosive cell-like capsules containing one giant secretory organelle or cnida. Its content is extremely toxic (Holstein et al., 1984), and when the sensorial structures detect contact, the cnidocyte is activated (Nüchter et al., 2006; Fabricius & Alderslade, 2001), forcing the attacking organism to die or to move away (Fabricius & Alderslade, 2001).

Gorgonians exhibit an interesting morphophysiological complexity, diverse reproduction and dispersion strategies and efficient protection mechanisms, allowing these sessile organisms to thrive (Lasker et al., 2003; Done, 1987, 1988).

1.3 Ecological Relevance

Gorgonian forests are of great ecological relevance. The three-dimensional structures of these organisms can reduce water flow, particularly in proximity to the substrate, creating Hydrologic storage areas (Nepf et al., 2007). Hydrologic storage areas are areas under the canopy in which water flow cannot penetrate due to energy dispersion, an effect also known as skimming flow (Guizien & Ghisalberti, 2016). The ability to disperse energy and reduce water flow has a direct impact on sedimentation rates and larvae retention. As a result, sessile species recruitment is increased under the canopy of these corals (Nepf et al., 2007; Cerrano et al., 2010; Buhl-Mortensen & Mortensen, 2005; Coma et al., 1998). The shelter created by gorgonian gardens also provides habitat and refugia for juvenile's growth and reproduction of several species (Geist & Hawkins, 2016; Nagelkerken et al., 2000; Goh et al., 1999; Sanchez et al., 1998). Furthermore, other than shelter and refugia, gorgonians provide other ecosystem services. They produce secondary metabolites such as julieannafuran, that other organisms (ie nudibranchs) are able to sequester for protection (Cronin et al. 1995) and provide food for polychaetes (*Hermodice carunculata*), gastropods (*Cyphome sp.*) and fish (Gerhart, 1989; Vreeland and Lasker, 1989; Botero, 1990; Ruesink and Harvell, 1990). Gorgonians are also able to extract and process energy from the water column. The ability to sequester particles from the water column plays a fundamental role in organic matter transfer between planktonic communities and benthos (Coppari et al., 2019; Mistri & Ceccherelli, 1994). When suspended Particulate Organic Matter (POM) and Particulate Organic Carbon (POC) are within reach of their polyps, the particles are captured and processed (Gili & Coma, 1998). Gorgonian gardens can be important carbon recyclers, *P. clavata* can remove up to 170 mg C m^{-2} day^{-1} (Coma et al. 1994; Ribes et al. 1999) while a gorgonian garden (octocoral community) can sequester up to 2.5 g C m^{-2} day^{-1} or 897 ± 394 g m^{-2} yr^{-1} (Fabricius & Dommisse, 2000). Worldwide seagrass meadows' carbon retention is estimated to be 138 ± 38 g C m^{-2} yr^{-1} (Greiner et al., 2013; Mcleod et al., 2011), 167 g C m^{-2} yr^{-1} for mangroves (Alongi, 2012) and 150 g C m^{-2} yr^{-1} for saltmarshes (Alongi, 2012; Laffoley et al., 2009). Estimations of carbon sequestration by different habitats have great fluctuations, from study to study. Despite that, these results position gorgonian gardens as one important carbon sinker (Rossi et al., 2017). Sequestered carbon is then reintroduced back in the habitat through secondary production (growth and reproduction) (Rossi et al. 2004). Ecosystem engineers are known to have important carbon sequestration rates. Gorgonians are no exception, being key species for a stable and diverse ecosystem and important ecological players in their habitats, contributing positively to the species that thrive in their vicinity (Cerrano et al., 2010; Coma et al., 1998).

1.4 Natural and Anthropogenic Impacts

Gorgonians are long-lived species exhibiting slow growth rates, low recruitment and fecundity, characteristics that explain their slow population dynamics (Coma et al., 1995b, Santangelo et al, 1993). These characteristics make them highly vulnerable to rapid and long-lasting biological and anthropogenic disturbances (Garrabou et al. 2009; Cerrano et al. 2005; Cúrdia et al., 2013). In the last decades climate anomalies became more frequent leading to widespread mass mortality episodes of benthic species. In addition, there was also increasing anthropogenic pressure in coastal areas (Montseny et al., 2019). This left gorgonian gardens under extreme pressure and partially explain the recent population decline reported by several studies (Fava et al., 2010; Mokhtar-Jamai et al., 2011). Disturbances such as pollution (Chan et al., 2012), fishing (Betti, F. et al., 2020; Mytilineou et al., 2014; Sardá et al., 2012), anchorage of boats or structures and leisure activities (e.g. SCUBA diving) are good examples of anthropogenic activities that have a direct impact in benthic assemblages (Waters, 2015). Direct physical impact can damage gorgonians resulting in broken sections or dislodgement of entire colonies. In some cases the elasticity of the gorgonians' skeleton avoids breakage, but when abraded by friction with a foreign object it can remove the tissue and expose the skeleton. Such situations seem to favour epibiosis and given time, the whole colony may be completely overrun and destroyed (Bavestrello et al., 1997). Other mortality episodes are related with algae blooms and viral infections that affect wider areas and are also generally linked to climate anomalies and pollution. In the Mediterranean Sea the loss of 50-60% of gorgonian branch length and mass mortality events have been recently associated to biological agents such as filamentous algae aggregations, also known as mucilage (Bally & Garrabou, 2007; Mistri & Ceccherelli, 1996). The proliferation of the *Vibrio coralliilyticus* bacterium also had a big impact in gorgonian populations, being considered one of the most likely agents linked with the diseases affecting *P.* clavata (Vezzulli et al., 2010). In SW England, *Vibrio splendidus* was identified in *Eunicella verrucosa* and linked to impacts in the local populations (Hall-Spencer et al. 2007). These events occur in a wide-spread geographical area demonstrating these occurrences are not confined to a particular region. The stressors are often similar, and the result of these mass events produces deep changes on the entire benthic community and consequently, in the ecosystem balance (Garrabou et al. 2009; Cerrano et al. 2005).

1.5 Conservation Efforts

Ecosystem engineer's importance in marine biodiversity has attracted attention from management and policy makers. Gorgonian gardens are no exception to this and taking in consideration their high vulnerability to disturbances, gorgonian gardens were internationally recognized as Vulnerable Marine Ecosystems (Fabri et al., 2014; OSPAR Commission, 2010). Despite this important step towards gorgonian protection, more research and conservation efforts are needed to protect and recover these habitats (Linares et al., 2008).

The creation of marine protected areas (MPA's) was one of the steps taken to achieve this goal, establishing concrete management and protection policies and strategies (Edgar et al., 2014; OSPAR Commission, 2010). However, to fully recover these habitats, considering that many have already been impacted, ecological restoration efforts are often a necessity to ensure habitat recovery (Goreau, 2010). One of the strategies that have been devised to recover gorgonian populations is through transplants of living fragments. Increasing the number of colonies through transplantation aims to enhance recruitment to levels that are sufficient for population persistence and recovery (Montseny et al., 2019; Linares et al., 2008; Kumagai et al., 2004). Moreover, the immediate increase of engineered structures through the addition of living colonies will help stabilize the existing population (Possingham et al., 2015). Based on the stable states' theory (Lewontin, 1969; Sutherland, 1974; May, 1977), ecosystems are considered stable when persisting for long periods of time under a specific set of physical, chemical, and biological factors. The ability for an ecosystem to remain unaltered demonstrates that enough resilience has been achieved to ensure stability. To encourage a shift from current state, a disturbance needs to have an impact of enough magnitude to overcome the current resilience threshold (Lewontin, 1969; Sutherland, 1974; May, 1977). Such has been accomplished through transplantation with other engineering species such as seagrasses (i.e. seagrass meadow was reinstated in a barren area where once a meadow existed (Paulo et al., 2019)). The introduction of biomass through transplantation in fragile or impacted ecosystems may help stabilize the current population, while recovery of completely lost ecosystems may prove difficult (Possingham et al., 2015; Linares et al., 2008). When targeting big geographical areas that were diminished by mass mortality events, transplantation of gorgonians as a restoration measure alone is not known to be sufficient. On smaller scales though, in areas where the impacts and disturbances affecting ecosystem engineering populations were extinguished or controlled, transplantation has been considered a feasible tool (Paulo et al., 2019; Linares et al., 2008). This is the case within well managed marine protected areas (MPAs) (Coma et al., 2004; Coppari et al., 2019). Defaunation and addition of epifauna can be difficult processes, especially when conducted *in situ*. As such, established transplantation methods that are effective, efficient or allow a great number of gorgonian colonies to be transplanted are required. Studies with the goal of developing new methodologies will allow to collect reliable ecological data, and how species react to manipulation. Such knowledge will support further research and provide essential information in the recovery of impacted coral populations and habitats (Linares et al., 2008; Kumagai et al., 2004).

1.6 Transplantation Challenges

The quest to find an effective coral restoration strategy resulted in numerous experimental trials, from which transplantation showed promise, but also significant challenges (Rinkevich, 2014). Despite the challenges faced, the potential and apparent success of transplantation methodologies (Goreau, 2010), has led for resources to be invested in researching and developing new transplantation methodologies (Montseny et al., 2020;

Montero-Serra et al., 2018). For many years these efforts were mainly directed towards scleractinian corals (Rinkevich, 2014), and not only until recently did temperate water corals restoration become a focus of research (Montseny et al., 2020). The challenges faced in transplantation of scleractinian corals are similar to those faced when transplanting octocorals, with the exception of the delicate nature of gorgonians. Substrate hardness, attachment difficulties, intrusiveness of the methodology, toxicity and abrasion of materials, inherent stress of manipulation and logistic feasibility are just some factors that need to be taken into account when transplanting corals (scleractinian or octocorals) (Edwards & Clark, 1999; Weinberg, 1979; Brinkhuis, 2008). In order to overcome such challenges, several forms of transplantation have been tried so far, with two main strategies being followed: (1) the use of mobile or temporarily fixed artificial anchor points or (2) to fix the transplants directly and permanently to natural substrate. On the use of temporary fixed anchorage points, the proposed methodologies consisted of using PVC clamps with nails (Weinberg, 1979; Gunnill, 1982), stainless steel eyebolts used to secure macroalgae (Taylor, 1998), nylon to attach coral branches to nails (Okubo & Omori, 2001) and climbing hooks were used in gorgonian transplantation (Velimirov, 1973). Plastic mesh was used for attachment of gorgonian fragments and had a short term success (Ponti et al., 2016; Kumagai et al. 2004). Unfortunately, in the long term, the plastic tends to deteriorate and comes loose, eventually propagating microplastics in the habitat and throughout the trophic chain (Nadal et al., 2016). Moreover, the length of the studies was short, at times conducted only for 8 weeks, which is insufficient time to assess the real success of the transplantation. Other similar procedures have been attempted but with mobile anchorage points; Kim & Lasker (1997) fastened colonies of the gorgonian *Plexaura homomalla* (Esper) to PVC posts set on a small cement flat base (24 cm - 18 cm ~5 cm), but the base proved to be too small and would drift or fall, being inefficient in stabilizing itself. Attaching concrete blocks with erect structures to the substrate or clothes pegs set in PVC racks was also attempted (Weinberg, S., 1979), though mortality was high and the procedure cumbersome. In the same trial, another method used by Weinberg (1979) consisted of transplanting big stone blocks that bear small gorgonian colonies. The stones needed to be heavy enough not to be dislodged by water current and then tied to the substrate with either nylon rope or a metal rack. This method reported some success, although it is highly invasive and logistically complicated technique that requires moving big blocks of substrate and removing full growing colonies from donor areas. These constrains make the method possible, but to be avoided due to the impact in the donor populations and the difficult logistics involved. Most of the aforementioned methods are invasive, present low efficiency, and hold samples too rigidly to be applicable with fragile organisms like gorgonians, while others are logistically extremely challenging. This fact likely explains why other different approaches were attempted, in the pursue of a higher success percentage. Fixing the transplants directly to the substrate partially accomplished that goal. Using epoxy resin adhesive, Sakai et al. (1989) transplanted coral branches permanently to artificial substrata. In Florida, epoxy and cement were also successfully used to transplant *Pseudopterogorgia acerosa* and *Plexaura flexuosa*. The procedure involved using a hydraulic drill and doing a wide, 10 cm deep hole in the horizontal substrate (soft substrate, calcareous reef), that would later be filled with epoxy or cement securing the colony inside. This procedure obtained 70% to 90% of survival after 1 year but reported no growth rates. Nonetheless despite the small sampling size (20 individuals per treatment) it demonstrates that in these conditions this procedure is possible and attains a high survival rate in short term trials (Brinkhuis, 2008). Also using epoxy glue for attachment, three different procedures were tested in the Mediterranean Sea (Linares et al., 2008). The most successful method, the “Stick” procedure, consisted of transplanting the gorgonian fragment by gluing it to the substrate with epoxy, and then adding a small PVC tube for support. With this procedure, considering successfully attached colonies alone, a 70% survival rate was reported. After 3 years though, only 16% of the colonies transplanted were still alive (Linares et al., 2008). This shows that this apparent successful technique, is not appropriate in long-term restoration efforts in hard substrate. Moreover, the lack of data regarding ecological state and growth, does not allow to understand how the transplants adapted to the new environment. Despite its shortcomings, this study places in evidence the need to report long term results of transplant trials. Only then can the real success be gauged.

1.7 Monitoring

Most published research on transplant restoration only present short to mid-term results (up to 1 year), and focus on survival (Montero-Serra et al., 2018; Edwards & Gomez, 2007). Although survival is a key factor, keeping in mind the main purpose of a transplantation effort, without monitoring growth and ecological state, it is hard to assess the true success attained with the transplant (Montero-Serra et al., 2018). The ultimate restoration goal is to positively affect the local populations or to reinstate a lost population and habitat. In order to achieve this, growth and reproduction of the transplanted colonies should be attained (McDonald et al., 2016; Edwards & Clark, 1999). As such, colonies need to settle and grow healthy, recovering from any stress resulting from manipulation. Once the colony is stable, it will then be able to achieve the size in which energy is allocated towards reproduction (Okubo et al., 2007). In this context, growth monitoring becomes an important part of assessing the true success of a coral transplant. One of the explanations for the lack of monitoring of these aspects are the difficulties in assessing growth of corals (Mistri, 1995). Growth does not follow a specific direction or trend and varies regarding colony size (multiple branches and thickness of skeleton), water currents and available environmental space (Matsumoto et al., 2004; Mistri et al., 1994). Environmental space is mainly constringed by other organisms or the heterogeneity of the substrate. These factors force corals, in particular gorgonians, to assume a variety of shapes. Colonies can split at the base and create parallel fans growing in undetermined directions (Santavy et al., 2013), creating difficulties in following its growth. Moreover, negative growth resulting from fragmentation can influence colony size, and as a consequence its reproductive capacity without affecting its total height. The assumption derives from the fact that reproductive height referred in published studies assumes a ratio between height and branching size, or in other words a minimum total branch length (Brazeu & Lasker, 1990). Indeed, the tri-dimensional structure of these organisms poses real challenges when it comes to accurately following its? development. To overcome these challenges, several studies using different protocols and parameters have attempted to minimize error in order to acquire accurate data (Mistri, 1995; Santavy et al., 2013).

Height of the colony and average growth was correlated with actual colony growth and branch growth, providing an estimate of growth based on one parameter alone (Coma et al., 1994). A similar approach was done using distance from base to the most distant end branch (Mistri, 1995). Although simple, this method introduces error when a smaller scale is necessary due to the growth dynamics of these organisms. A slightly more precise combination of height and width has also been used, and although the associated error is acceptable in large scale monitoring, it is not in fine scale with short sampling episodes (Goh and Chou 1995; Mistri 1995; Goffredo & Lasker 2006). Skeleton growth rings analyses have also been used, and this method provides accurate growth rates but requires the colony to be removed and processed. For this reason, this method is not often used in restoration efforts (Grigg 1974; Mitchell & Dardeau et al. 1993; Mistri 1995; Goffredo & Lasker 2006). Rectangularized Fan Surface Area (RFSA), is a method that conceptually is similar to that of using height and width to create and approximate area of the fan. This is a more accurate method, with a smaller associated error regarding the aforementioned technique (height and width) (Mistri 1995; Weinbauer & Velimirov 1995). By measuring the total length and branch length increments, the monitoring of gorgonian colonies, and in particular, those of small growth increments is possible. This is a strategy that even though time consuming, produces accurate results (Lasker et al. 2003). In order to expedite this kind of refined analyses, methodologies combining pictures plus software as the "tabletop-PVC-matrix" (Vago et al. 1994) have been tried with some success. Also using software to process photographs, measures of area cover have been made. This methodology much like measuring total length and branch increments, can produce accurate measures of small growth increments. The method aims to calculate the total branch fan surface area (BFSA); the ''area'' occupied by the organism on photographs in 2-D projection. It requires a scaled picture taken *in situ* and has the added benefit to avoid damaging the colonies. The associated error is also smaller than the previously mentioned methodologies, but it is a time consuming process (Matsumoto, A.K., 2004; Coz et al., 2012). The most recent method published makes use of recent, 3-D model technology. It was used to establish a relation between size and biomass. The measurements resulting from this method might be appropriate for comparing population structures at large scales (Palma et al., 2018). Yet it cannot reflect the age of a colony precisely, nor small growth increments such as those recorded in frequent monitoring events on small transplants (Linares et al. 2007). From all monitoring methods published, height still seems to be the most used method overall (Johnson et al., 2020). Despite that, the methods that seem to be more adequate to follow small transplanted colonies' growth, with relatively short growth increments, are the BFSA, the branch total length and branch increment methods. These are the methods with higher resolution and likely to allow to record the slow growth of these organisms.

1.8 Aims of this study

Additional efforts are still required to test appropriate gorgonian transplantation and monitoring techniques on natural hard substrata (Linares et al., 2008; Fava et al., 2010). In fact, restoration studies must test the viability of transplantation techniques with several gorgonian species and consider mid to long term monitoring. It is crucial to obtain knowledge regarding the adaptation of the transplanted colonies to the conditions of the new local environment after manipulation to assess its viability. Many factors can influence the adaptation and success of a colony. Often, stress, injured tissues, environment, competition or genetic adaptations play a negative role on survival and health of transplants (Mokhtar-Jamaï et al., 2011). The success of the transplantation technique is a key contributor towards the restoration of ecosystem engineer species and is a subject still requiring attention. As new technology becomes available, new opportunities arise to create more accurate and successful methodologies. Experimental trials that do not achieve efficiency and success should still be seen as examples to learn from (Geist & Hawk, 2016).

This study aims to test and compare two different transplantation methodologies and the effect of size in the transplant success. The methodologies used are the "Stick" method (Linares et al., 2008) and the Direct Substrate Attachment (DSA) on *Paramuricea clavata*, one of the gorgonian species found in Portuguese coastal waters. The first size class will use gorgonian fragments up to 10 cm of height (class size - < 10cms). Method success in attaching the fragments will be examined in all size classes and methodologies ("Stick" vs DSA). Gorgonians were considered successfully attached by the epoxy if found in place and firmly secured after 3 months. At an initial stage, this will allow to assess the losses due to method application and substrate influence in attachment. Once the fragments are secured, growth, ecological state, holdfast and survival will be compared in order to assess the outcome of the transplants using both techniques. Since colony size defines reproductive maturity, this study also aims to replicate the previous trial with a second size class (transplant size of 10 to 20cm). Results from both trials will be compared to assess transplant size influence in transplantation success. Finally, donor colonies will be examined to determine if fragment collection has negative impacts on donor colonies, and if the colony recovers after being sampled. The results of this study should help define the feasibility and most appropriate transplantation methodology to be used with *P. clavata*.

It is paramount for MPA's management bodies and decision makers to have reliable data concerning transplantation success using different techniques (Edwards & Clark, 1999). Only then efficient experimental design and resources can be allocated in accordance, enabling all factors involved to achieve the desired restoration success.

2 Material and Methods

2.1 Study Site

This study took place from December 2017 to December 2019, in the marine reserve of "*Parque Natural do Sudoeste Alentejano e Costa Vicentina"* (PNSACV), more specifically in Sagres village. The PNSACV was created in 1995 *(Decreto Lei n. º 26/95)* and is located in the southwest coast of Portugal. The PNSACV is constituted by land and marine reserves, extending 2 km into the sea. It covers a narrow strip of coastline from São Torpes (37.91915 N, -8.80873 O) to Burgau (37.07719 N, -8.77530 O), with a total extension of 110 km, a coastline of 134 km (Figure 1), and an area of approximately 1310 km^2 (ICNF; Jesus, 2004). The extension and coastline orientation, allied to typical swell direction, allow to divide this marine park in two main areas. The occidental and the meridional (southern) coast. The occidental coast is exposed to the typical NW Atlantic swell while the southern coastline is mostly sheltered from the high energy waves that hit the occidental side (Dias, 1998; Pena & Cabral, 1997). Along this region, cliffs slowly decrease in height (W-E). This is an area of geological and landscape transition. Within the marine reserve the continental shelf sinks slowly up to 60-80 m depth, in the park's outer limits.

The marine reserve has three different protection levels that are applied within its limits: full protection (FP), partially protected level 1 (PP1), partially protected level 2 (PP2) and a complementary protection (CP) (*Resolução do Conselho de Ministros n. º 11-B/2011*). In FP areas, no human activity whatsoever is permitted, unless for scientific purposes and with particular licenses issued by ICNF (Instituto Conservação da Natureza e das Florestas). In PP1 areas, only gooseneck barnacles (*Pollicipes pollicipes*) can be collected on the land-sea interface, no other fishing methods are allowed. Within these areas leisure activities are allowed with some exceptions, such as spearfishing. As such PP1 is a marine reserve or no-take area for fish and invertebrates with the aforementioned exceptions. In the PP2 areas estuaries and small water reservoirs are subject to local regulation. In the buffer zone legal fishing methods are not restricted for registered fishing vessels with a valid license for PNSACV, although temporary restrictions can at times be issued. Leisure activities are also not restricted if in accordance with local safety regulations, which can change according to season.

The area of interest for this study is within the limits of the fishing harbor Porto da Baleeira in Sagres, a small fishing village in the southern coastline. This infrastructure was built in 1978, and its protective breakwaters were built with a mix of concrete blocks and natural calcareous rock. The process of pilling up the puzzle like concrete pieces and the natural rock, created an intricated maze beneath the rocks. Here the habitat is highly heterogenous, with small passages and sciaphilous areas, some big enough for a human to enter. The breakwaters wall has a slope of about 45º, which allows divers to reach the bottom by merely moving 10-15m away from it. Around the breakwaters the bottom is flat and composed by thin sediment and sand. In high tide maximum depth is of 18m (tide variation is around 2.5-3m). After 40 years from its construction the breakwaters became an artificial reef colonized by several species. A gorgonian garden (Appendix A) can be found at the very end of the breakwaters, where 5 species can be observed: *Eunicella verrucosa*, *Eunicella labiata*, *Eunicella gazella*, *Leptogorgia sarmentosa* and *Paramuricea clavata*. This area is closed to both leisure and fishing activities, eliminating anthropogenic impacts as a factor in the success of the transplantation.

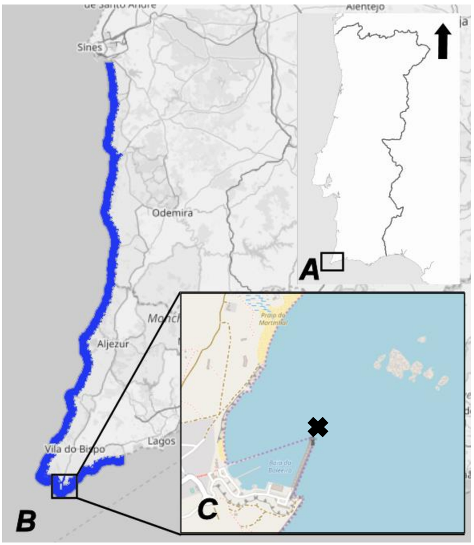

Figure 1: A) Location of the PNSACV regarding the Portuguese continental territory. B) The map of PNSACV, with the MPA marked in blue. C) Map of Sagres' Baleeira harbor, where the breakwaters can be seen. The study site is marked with a "X".

The easy access and inherent protection of the location makes this a suitable area to conduct experimental trials. Direct anthropogenic impacts are unlikely to be an issue, effectively allowing to isolate environmental factors affecting this study. Furthermore, the existence of a gorgonian forest was a deciding factor in selecting this area as a potential site to test methodologies.

2.2 Sample Collection and Preparation

In the breakwaters of Sagres' harbor (Figure 1), at 16 m of depth, where higher gorgonian density was found (Appendix A), 30 individual donor colonies of *P. clavata* were chosen via visual identification (one fragment per individual collected). The selection criteria used was being healthy and big enough that a collected fragment would have minimal impact in the overall donor colony. With these criteria, the objective was to avoid a negative impact on unhealthy and on small colonies. The sampled fragments would have at least one apical branch of around 8 cm. Sampling was performed using cutting plyers, while the divers had special care to hold the samples by their base (cut side), as it would be scraped of living tissue latter. The fragments were stored in plastic zip-lock bags while transported underwater, with no more than 5 fragments per bag. This number aimed to minimize direct friction between the fragments that could lead to necrosis. Outside of the water the zip-lock bags were opened and placed in a cooler filled with fresh sea water and cooling pellets to allow temperature stability before being placed in aquariums.

The fragments were further split and trimmed to match the two size classes: <10cm (up to 10 cm) and 10-20cm (from 10 to 20 cm) as described by Linares et al., (2008). The size was measured not considering 2cm at the base. These would be scrapped latter. In the day of the transplant the fragments were prepared before being transplanted, the first 2 cm of the base were scrapped and cleaned of side branches. Living tissue was removed and the skeleton exposed (Figure 2). The average width of the bare skeletons was also measured, and found to be of 2 mm.

Figure 2: *P. clavata* colony fragment being prepared for the transplant. The first 2cm of the base was scrapped out of the living tissue exposing the skeleton.

2.3 Transplantation

All transplants were performed taking in consideration the preferred habitat characteristics of the species being transplanted, such as: depth, orientation (preference for vertical walls) and light incidence (area not directly exposed to light)

.

Some specific materials and equipment were common to both methodologies:

- Two component epoxy glue (Z-spar Splash Zone A788), was prepared mixing the two components 30-40 minutes before being used underwater. This epoxy glue was used in all the experiments, either to secure the gorgonian fragments to the substrate directly or inside the holes drilled for the transplant.

- Nemo Divers Hammer Drill underwater electric driller, with underwater exchangeable batteries, was used whenever it was necessary to open a hole in the substrate, either to fix a monitoring station or transplant fragments (DSA methodology only).

2.3.1 DSA methodology

The Direct Substrate Attachment (DSA) methodology has the primary goal of attaching the gorgonian transplant directly to the substrate. The methodology aims to create conditions for the gorgonian to overgrow the material (epoxy glue, or other fixating material) used to secure it, reaching the substrate in which it was transplanted. With the formation of a holdfast that secures the colony directly to the substrate, we aim to create conditions for a healthy growth, minimizing chances of skeletal breakage, or glue detachment. These are common reasons for transplant loss in other transplant methodologies (Linares et al., 2008,; Brinkhuis, 2008). In the present study successful attachment to the natural substrate was considered to have been achieved once coenenchyme (gorgoninan's skeleton) was found to be part of the observable holdfast, and big enough to overgrow the thin layer (roughly 1-2 mm) of epoxy between the rocky substrate and the skeleton, effectively securing the colony to the underlying rock.

The process proposed was implemented as follows: two metallic rod were fixed in the substrate 30m apart, establishing a baseline. To identify the direction of transplantation and monitoring, tie wraps were attached to the metal rods indicating their number (i.e. Station 1 had one tie wrap and Station 2 had two tie wraps). After having the two stations in place a transect of 30m was deployed at 16m of depth in an heading of 110º. Once the stations and transect were set, gorgonian transplants took place. In order to transplant the gorgonian fragments, using the underwater driller, a hole 2 cm deep was opened, with a 4 mm diameter drill bit. After removing the

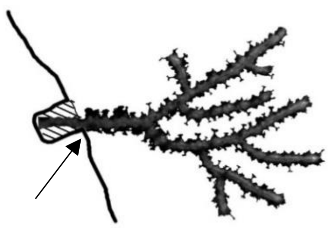

Figure 3: Sketch of a gorgonian transplant done with DSA methodology. The solid line represents the substrate, and the stripped area is the glue material. The arrow places in evidence that the living tissue is touching the substrate.

resulting particles from inside the hole, a small amount of epoxy glue was inserted in the hole and pushed strongly inwards with an extra drilling bit. The epoxy was verified to be compact and the gorgonian fragment was carefully inserted, making sure the living tissue was left touching the substrate (Figure 3; Figure 4). Any epoxy glue excess was removed, leaving the natural substrate clean and as little evidence of epoxy as possible. After the process was finished, the transplanted fragment was surveyed: distance along the transect, offset distance, species and size of fragment were recorded. The fragments were all transplanted in a row, at the same depth, and in intervals of about 30-50 cm, when possible, to facilitate monitoring. Size classes were alternated to decrease possible environment heterogeneity bias.

Despite the initial plan only contemplating 2 size classes, due to an unexpected event, the study regarding attachment success was expanded to include a third size class. During the first months of this study several storms hit Algarve's coastline and as a result many gorgonian colonies of a variety of species washed ashore. Among them, gorgonian colonies of great proportions (up to 50 cm height) were retrieved from the beach of Armação de Pera. The colonies were selected; unhealthy colonies were discarded, and healthy colonies were kept in tanks at CCMAR's station "Ramalhete". The tanks were covered to protect the colonies from direct light exposure and had running saltwater at a temperature of 18º approximately. The available biomass allied with the apparent success of the transplants already done at the time (<10cm and 10-20cm size classes), was an opportunity to test the methodology further. To accomplish this, a third size class (20-40 cm) was tested using *L. sarmentosa*. This new treatment had the sole objective to test the methodology capacity to hold bigger fragments.

In total 70 gorgonian fragments were transplanted, 58 of *P. clavata* (30 of <10cm and 28 of 10-20cm) and 12 of *L. sarmentosa*. The fragments were divided by three size classes, in a total of 3 different treatments.

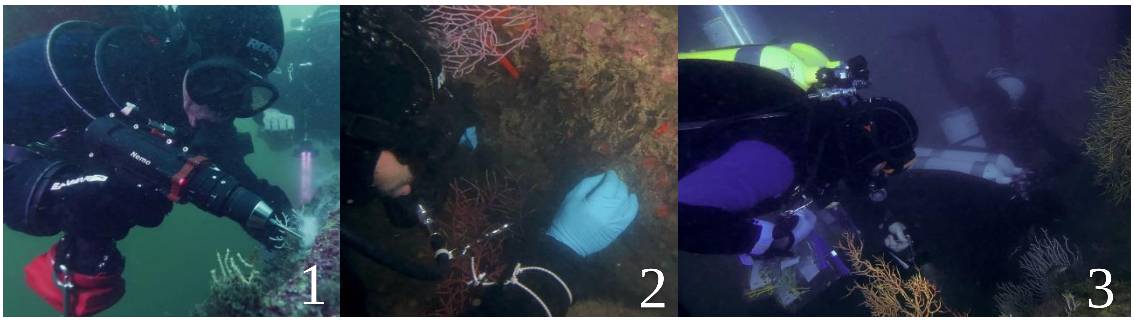

Figure 4: DSA Methodology sequence: 1) A hole in the substrate is opened using an underwater driller. 2) the gorgonian fragment is inserted in the hole already filled with epoxy. 3) the team can be seen working in the transplantation process at 18m of depth.

2.3.2 Stick Method (Linares et al., 2008)

The "Stick" method was implemented as described in the literature (Linares et al., 2008). Since this method requires a flat surface, a location close to where the DSA was performed, was selected. To maintain the same conditions, depth of the transplant was also 16m. The proximity of big rocky boulders provided some shade. To implement the methodology the gorgonian fragments were previously attached to 5 cm long PVC sticks by means of a plastic bridle (Figure 5). This process took place underwater, and needs to be done with care not to cut the gorgoninan tissue when securing the bridle. Once all fragments were ready, two metalic rods were placed to establish a baseline. The substrate at the location where the transplant would be performed was scraped of any organic material using a metal brush, exposing the substrate surface. The removal of organic material increases epoxi attachment to the substrate. From the previously mixed epoxi, an epoxi putty was placed directly over the substrate that was scrapped, and firmly pressed to increase adherence.It is important to verify the consistency of the epoxi, too soft will not hold the transplant and too hard will not adhere to the transplant. Once the consistency is right (identified by touch) the transplant can be performed. A fragment attached to the PVC stick is placed in the epoxi putty and the base is hard-pressed to ensure adherence to the epoxi. For each epoxi putty, one fragment was transplanted (Figure 6). Using the "Stick" methodology, 30 fragments were transplanted in the size class <10cm. Transplantation with bigger sizes was not possible, since the transplants fell off right after being introduced in the epoxy putty.

Figure 5: PVC sticks ready to be attached to gorgonian fragments with small tie wraps.

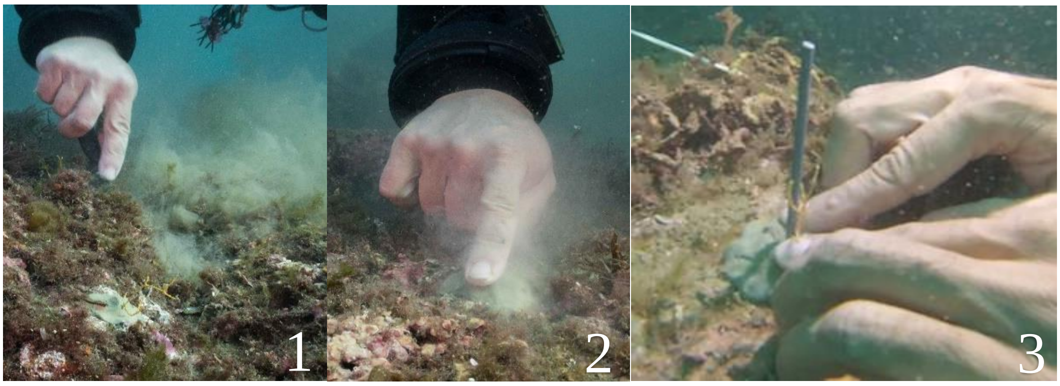

Figure 6: "Stick" (Linares et al., 2008) method sequence: 1) The substrate is scrapped using a metal brush. 2) The epoxy putty is placed in the area where substrate was scrapped. 3) The gorgonian fragment already attached to the PVC stick is inserted in the epoxy putty, and the base is pressed to force the epoxy to adhere to the base of the transplant.

2.4 Monitoring

2.4.1 Attachment, Size and Ecological State

Figure 7: *P. clavata* transplant being monitored. The scaled plate behind the gorgonian provides a perfect contrast which will facilitate processing of images latter on.

Attachment success was assessed by recording the presence or absence of the transplanted colonies and if they were firmly fixed. Size was recorded using scaled photography. Ecological state was defined as the health condition of the gorgonian. The gorgonian was considered healthy when no necrosis, bare skeleton, discoloration, or epiphytes were present. The monitoring procedure consisted of placing a scaled slate of contrasting color behind the colony and taking a photo on a perpendicular angle, avoiding distortion and parallax effect (Figure 7). Photographs were taken with a Canon G16 inside a waterproof housing. Holdfast monitoring was performed in a non-intrusive manner. To avoid removal of the transplants to verify holdfast formation, parts of the base living tissue were slightly scrapped to observe coenenchyme existence. Only in this case was organic attachment considered. To facilitate monitoring, both the transect tape and a line were extended parallel to each other between monitoring stations. The position of all transplanted gorgonian fragments was marked underwater with duct tape wrapped around the line. This strategy increased the monitoring efficiency and facilitated the sampler's identification of the transplanted gorgonian fragments.

The monitoring schedule was defined to be more frequent in early stages and more spaced later. The first monitoring events took place in the first two days, one right after transplantation and another in the following day. The goal was to assess immediate attachment success of the method and potential technique failure, survival, and ecological state of the colonies. In the second day, size was also recorded. This monitoring aimed to establish a baseline for growth and ecological state. Moreover, immediate adaptation of the colonies was verified by recording if the polyps were open or closed to assess if the colony was feeding or stressed and unable to feed in the new habitat (Fava et al., 2010). Following the first 2 monitoring's, there were three monthly monitoring events (until March), a time period deemed sufficient to verify if attachment issues not obvious in the first days (e.g. epoxy lack of adherence) had impact in the treatments. In these monthly monitoring events, attachment, survival, holdfast presence and ecological state were recorded. Subsequent monitoring events were held every six months and, adding to the aforementioned factors, growth was also monitored.

2.4.2 Photographic analyses

Photographs resulting from monitoring events were used to assess the total branch lenght (TBL) and the height of the transplanted gorgonians. The TBL process aims to measure the length of all gorgonian branches of the transplant, while height was measured from the holdfast (substrate level) to the highest point of the colony. Digital processing occurred using Adobe Corel PaintshopPro and ImageJ. With the Adobe Corel PaintshopPro (Jasc Software, Inc.) photographs were pre-processed: when found, distortion caused by the camera lenses was corrected, and the background of the photo deleted leaving only the gorgonian and the scale. The photo would then be converted to a binary color frame (black and white) (Figure 8). Finally, using ImageJ image processor, the scale in the photograph was used as a reference for the TBL calculation (Fava et al., 2010). This data allowed to estimate colony size (in cm) and growth in between monitoring events using both methods.

Figure 8: Gorgonian transplant photographs of the same *P. clavata* colony after being processed using PaintshopPro software. The background was removed and the gorgonian was colored in black. In the image from left to right, can be observed the colony in the transplant day, in the middle 1 year monitoring, and on the right 2 years monitoring.

2.5 Data Processing and Statistical Analyses

After the pictures' processing using ImageJ software, from which size of the transplants was recorded, net growth was calculated by subtracting the initial size (day after transplant day) from the size of the gorgonian fragment in monitoring events post-transplant. The growth rate was determined by subtracting from the most recent monitoring event the TBL of the previous monitoring, effectively being the relative size increment or decrement between monitoring events. Monthly growth rate was found dividing the growth increments in successive monitoring events by the numbers of months between the monitoring events. To assess ecological state, epiphytes, discoloration, polyp release and necrosis percentage was recorded regarding the total area of the colony. The results were then fitted into a class: 0%, 20%, 40%, 60%, 80% or 100% of the colony impacted (Francour, 1998).

Statistical analyses were performed using open source R software version 3.4.3 (R Core Team, 2017). The effects of size factors (Size class <10cm and 10-20cm) on response variables of attachment, survival, size, growth, holdfast formation and ecological state along the time (monitoring event) were tested. Analyses were done using a generalized linear model (GLM) package "gml2" in accordance with the binomial nature of the data. Data subsets normality was tested using a Shapiro–Wilk test. Since some subsets were not normally distributed a fit-Dist function (R package propagate) was ran to verify the subset distribution, allowing to run the GLM with the correct distribution.

3 Results

3.1 Attachment Success

Attachment was monitored in the first 3 months after the transplant. Successful attachment was considered after 3 months (March 2018) through the presence or absence of the gorgonian transplant. This allowed to verify proper attachment once the epoxy was dry, and enough time had passed for a loose fragment to detach.

3.1.1 DSA Methodology

Using the DSA methodology attachment was 100% successful in all treatments (size classes) on the day of the transplant. After 3 months attachment success dropped to 97% on small fragments (size <10cm), and 93% on medium sized fragments (size 10-20cm). There were no significant differences of attachment success between size classes (GLM, p=1.000), nor between the transplant event and the monitoring on March 2018 (GLM, p=0.993). Overall attachment success was of 95% after 3 months (Table 1).

The third class, tested with bigger fragments (20-40cm), had 100% attachment success after one month, and of 75% three months after the transplant.

3.1.2 Stick Methodology

Using the smaller sized branches (<10cm size), attachment success was of 100% on the day of the transplant. With the bigger fragments (10-20 cm), the success was of 0%, where the fragments were observed to fall after a few minutes of being transplanted. After 3 months, 20% of the smaller size class fragments remained.

The third size class (20-40cm) was not tested with this method due to the lack of success with the size class 10-20cm.

3.2 Survival

3.2.1 DSA Methodology

The survival of transplanted gorgonians was influenced by transplant technique failure (methodology attachment success), natural mortality and anthropogenic impacts. Over the course of the study, size had no influence in the survival of the transplants (GLM, p=0.100) whereas time significantly influenced survival. After 2 years, small transplants (size <10cm) had a survival of 70% while medium-sized transplants (size 10-20cm) had 68% survival. Despite that, there are differences concerning survival between the first and the second year of the study. In the first year, when comparing the transplant moment and the monitoring 1 year latter survival changed significantly (GLM p=0.010), whereas on the second year, when comparing the monitoring of December 2018 and the monitoring 1 year latter (Dec 2019) it did not (GLM p=0.741).

Even though the third size class (20-40cm), was only intended to test attachment, survival kept being monitored throughout the study. After 1 year survival was 67% and after 2 years remained the same, a similar result when comparing with the other to the other size classes.

Table 1: Survival (%) of *P. Clavata* transplants done using the DSA method for the different monitoring events for the duration of the trial. Survival was monitored monthly in the first 3 months and then on every 6 months, by verifying the presence of the colony and if it was alive. Most lost transplants happened in the first year. After 2 years 69% of the transplants were alive.

	Size <10cm	Size 10-20cm	Overall
Transplant	100%	100%	100%
Day after	100%	100%	100%
1 Month	97%	93%	95%
2Months	97%	93%	95%
3 Months	97%	93%	95%
1 Year	80%	78%	79%
2 Years	70%	68%	69%

3.2.2 Stick Methodology

Using the “Stick” methodology, survival was 0%. There was high mortality in the first months and after 3 months all transplants were either detached or dead.

3.3 Mortality Fate

3.3.1 *DSA* Methodology

In total, from 58 colonies transplanted, 18 colony fragments were lost. In the medium size class (10-20cm) 9 fragments were lost, from which 33.3% were associated to natural events, 22.2% lost due to technique failure and 45.5% due to observable anthropogenic impacts (fishing nets, lines litter and others). In the smaller sized transplants (<10cm) 9 fragments were lost, with 77.7% associated to natural events, 11.1% lost to technique failure and 11.1% lost due to anthropogenic impacts. Overall, 50% of the mortality was associated with natural events. Anthropogenic impacts leading to the death of the transplanted colony were observed in 5 transplants. From those, 4 were in the 10-20cm size class and 1 in the <10cm size class which corresponds to 8.6% of the total transplanted colonies (Figure 9).

In the 20-40cm size class, on the last monitoring event (December 2019), 1 gorgonian was missing due to natural causes. Despite that, survival was still of 67%.

3.3.2 Stick Methodology

In the monitoring event performed 3 months after the transplant event the 20% remaining transplants were dead. The transplanted gorgonians had the skeleton completely overrun by epiphytes and no living tissue was observed. Due to the absence of evidence of anthropogenic impacts, mortality was attributed to natural causes.

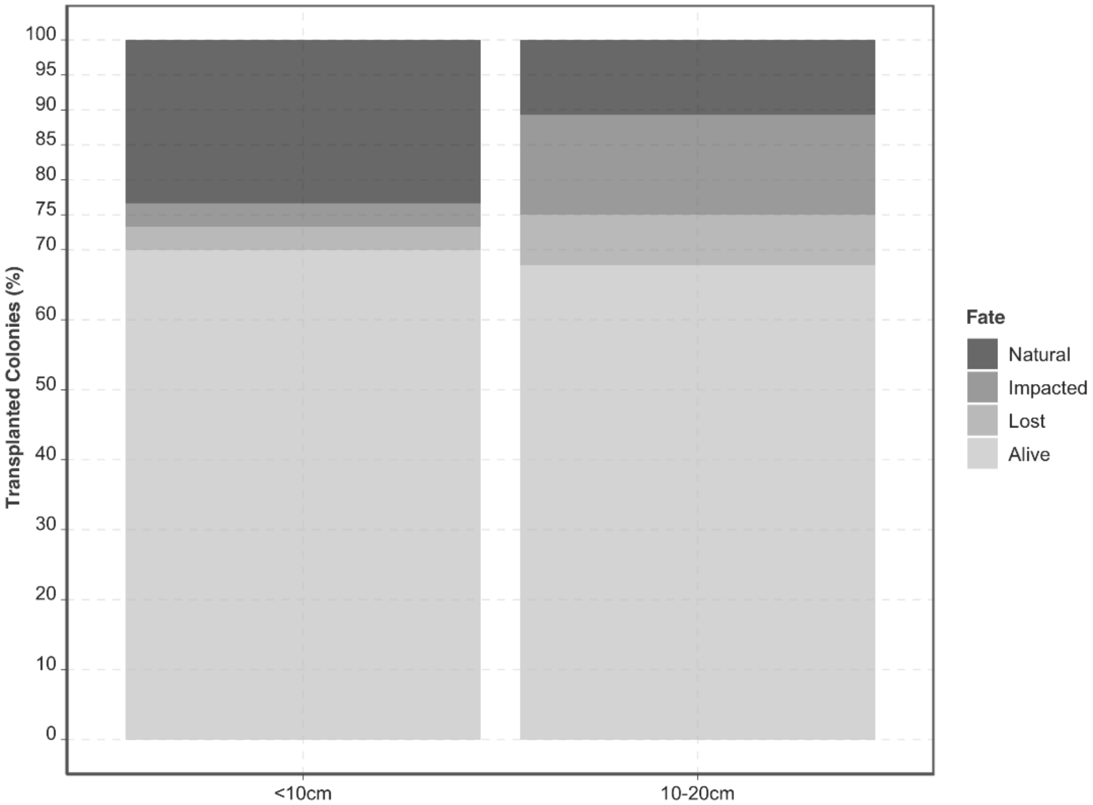

Figure 9: Mortality fate of the *P. clavata* colonies transplanted with the DSA methodology, for the two size classes (<10cm and 10-20cm). Fate was identified as natural mortality (marked as "Natural"), "Impacted" when found to have been lost due to observable anthropogenic impacts, "Lost" when the gorgonian fell from the epoxy (method failure) and Alive when the colony was in place and alive.

3.4 Holdfast

3.4.1 DSA Methodology

Holdfast formation was observed on all transplanted fragments remaining at the end of this experiment. There were differences between size classes when it concerns holdfast formation (GLM $p=0.009$). The first occurrences of holdfast formation were observed after 3 months. After 5 months, the 10-20cm size class, had 58.3% colonies with a developed holdfast. From April'18 to May'18 a significant growth holdfast occurred (GLM $p<0.001$), where the <10 cm class size treatment changed from 34.5 to 96.3 % colonies with holdfast, and the 10-20cm size class from 58.4% to 95.6% (Figure 10). After one year all transplants independent of their size class had created a holdfast.

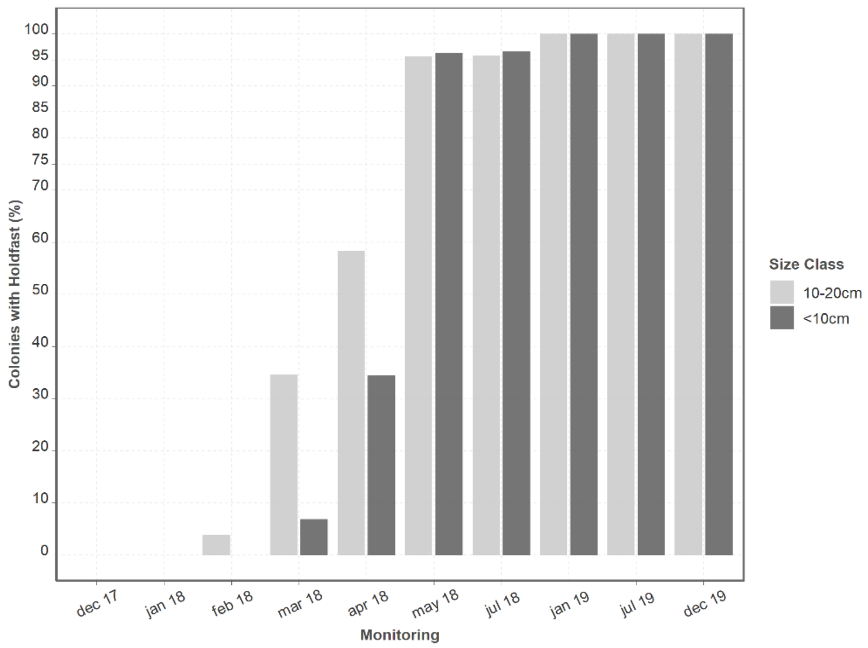

Figure 10: Percentage of transplanted colonies of *P. clavata* that have developed a natural holdfast during the study period. Both size classes are represented for each monitoring event. After 5 months (May' 18) over 90% of the colonies had an organic holdfast.

3.4.2 Stick Methodology

Due to the high mortality and lack of live transplants, holdfast formation was not observed.

3.5 Size

3.5.1 DSA Methodology

3.5.1.1 Height

The height of the transplants had no significative differences when comparing the start with the end of the experimental trial. Overall height suffered no significative changes on both size classes. Despite that the <10cm size class had a slight increase in height, while the 10-20cm size class had a decrease (Table 2).

Table 2: *P. clavata*'s transplants size (height) for both treatments (<10cm and 10-20cm) in all monitoring events performed in the 2 years experimental trial. Size is presented with its standard error and a 95% confidence interval.

	Size <10cm	**Size 10-20cm**
Transplant	4.17±0.29se	9.43±0.61se
6 Months	4.1±0.42se	8.68±0.65se
1 Year	4.81±0.41se	8.01±0.69se
1.5 Years	4.72±0.37se	7.95±0.71se
2 Years	5.15±0.41se	7.93±0.62se

3.5.1.2 Total Branch Length

Both size classes treatments after 2 years had a significant increase in total branch length (GLM p<0.001). The <10cm size class had a significative increase (GLM *p*=0.044) from 9.28±1.25se cm of total branch length in the transplant moment to 25.38±3.67se cm after 2 years. The 10-20cm size class also had an increase, growing from 33.98±2.23se cm to 45.66±5.23se cm after 2 years. Despite this there were found no significative differences between the initial and final total length size concerning the 10-20cm size class (*p*=0.499). Both treatments evidence an annual total branch length increase, but it is always in the second semester of each year that this increase becomes more relevant, particularly in the <10cm size class (Table 3).

Table 3: *P. clavata*'s transplants size (total branch length) for both treatments (<10cm and 10-20cm), in the all monitoring events of the 2 years experimental trial. Size is presented with its standard error and a 95% confidence interval.

	Size <10cm	**Size 10-20cm**
Transplant	9.28±1.25se	33.98±2.23se
6 Months	10.64±2.45se	31.92±3.62se
1 Year	19.47±3.55se	38.74±4.77se
1.5 Years	19.38±2.92se	36.33±4.35se
2 Years	25.38±3.67se	45.66±5.23se

There is an apparent inversed coupling between total branch length and water temperature changes. As soon as water temperature decreases size increased. By January 2019 and December 2019 during the winter and when the water was colder (16º and 18º respectively), was when size increased the most (Figure 11). Contrary to that in 2018 and 2019 summer there was loss or stabilization in size. This trend is more noticeable in the second year, when in July and August water temperatures start to peak, and a loss of gorgonian size is observed in the 10-20cm treatment, while the <10cm treatment stops growing (Figure 11).

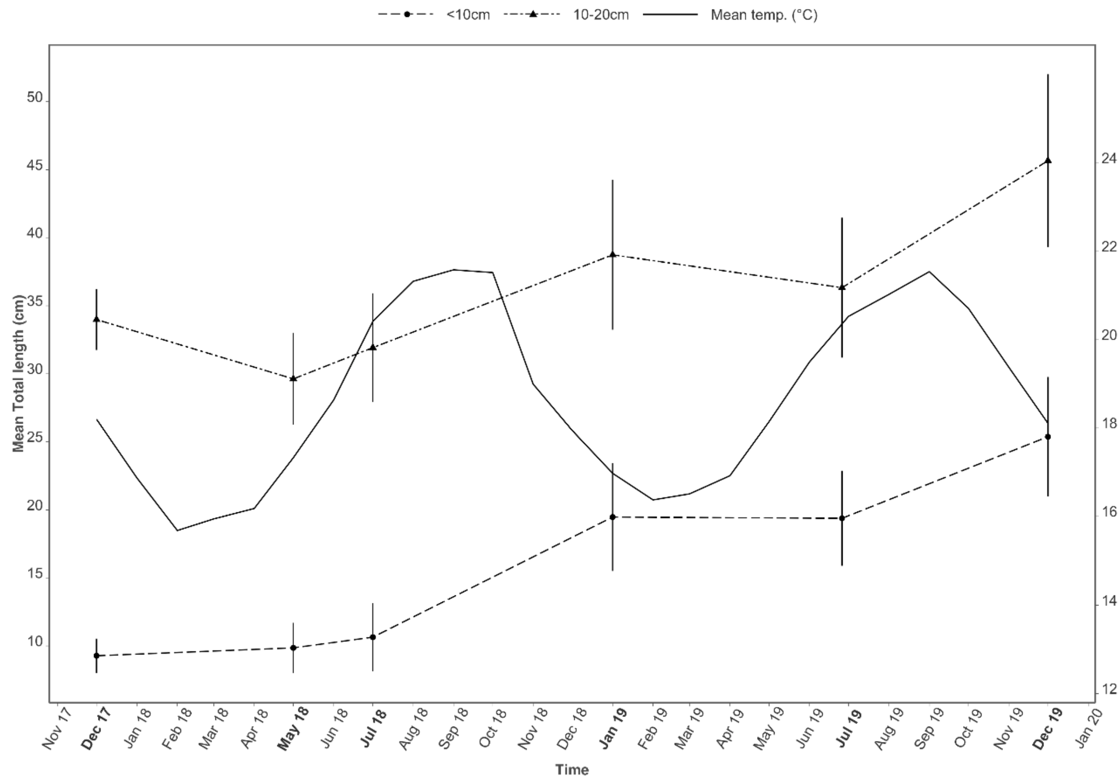

Figure 11: *P. clavata* treatments' (<10cm and 10-20cm) total branch length size evolution through the duration of the experiment. Monitoring events are marked in bold letters. The error bars have a 95% confidence interval. The size evolution is plotted with sea surface water temperature variation (NOAA), represented by the continuous line.

3.5.2 Stick Methodology

Due to the high mortality and lack of live transplants, growth was not able to be assessed.

3.6 Net Growth

3.6.1 DSA Methodology

3.6.1.1 Height (Net Growth)

The growth of the transplants (height) had no significant differences between the monitoring events of the experimental trial. Despite that, considering the heterogeneity effects of the data on the mean, both size classes show a positive growth trend (Figure 12).

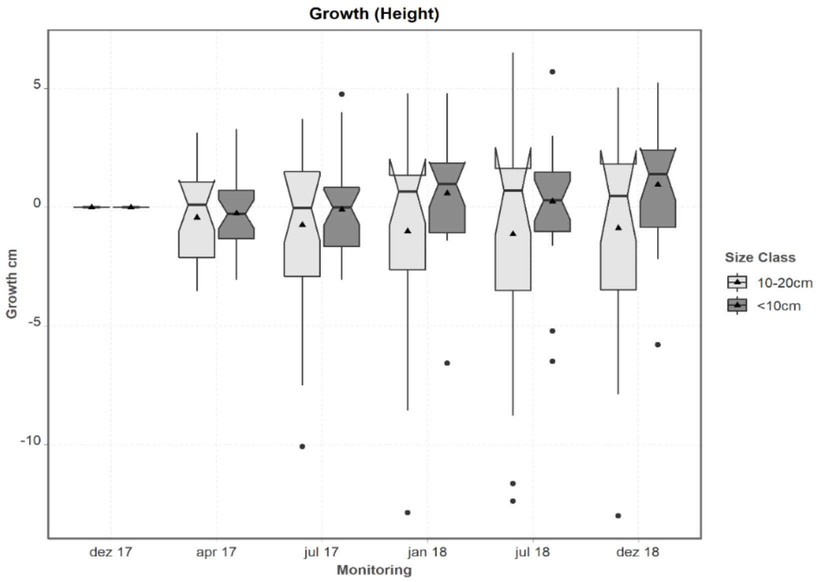

Figure 12: Boxplot (Tukey style) of *P. clavata* transplants' height net growth, for both size classes used in the experimental trial (<10cm and 10-20cm). Boxes are paired by size class and displayed regarding its monitoring event. The triangular mark represents mean and middle bar the median. Notches (limited by the top and lower hinges) are displayed with 95% confidence interval and outliers with a dot. No significant growth is observable.

3.6.1.2 Total Length (Net Growth)

Both size classes grew during the 2 year trial, demonstrating a positive growth trend. The <10cm class had a net growth of 16.51cm±3.66se, while the 10-20cm class had a net growth of 12.02cm±6.3se. The net growth was not significantly different between size classes. For < 10cm size class, net growth was significantly different (GLM p=0.029), between the transplant monitoring and the end of the trial. Overall, differences in net growth are significant between the transplantation event (December 2017) and the last monitoring event (December 2019) (GLM p<0.001).

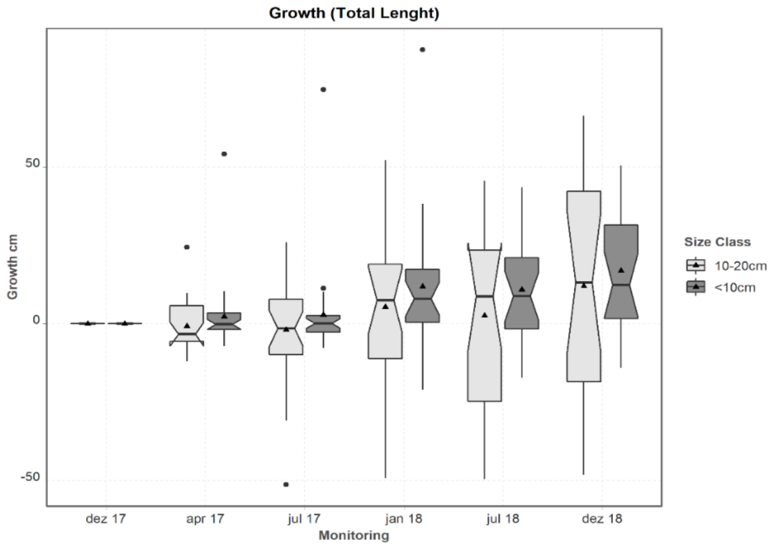

Figure 13: Boxplot (Tukey style) of *P. clavata* transplants' total branch length net growth, for both size classes used in the experimental trial (<10cm and 10-20cm). Boxes are paired by size class and displayed regarding its monitoring event. The triangular mark represents mean and middle bar the median. Notches (limited by the top and lower hinges) are displayed with 95% confidence interval and outliers with a dot. Overall a positive growth trend is observable in both size classes, being significantly different between the start and the end of the trial for the <10cm class.

3.6.2 Stick Methodology

Due to the high mortality and lack of live transplants, net growth could not be studied this treatment.

3.7 Growth Rate

3.7.1 DSA Methodology

3.7.1.1 Height (Growth Rate)

There were no significant differences in monthly height growth rate in any of the treatments. Overall height growth was non-existent and did not change significantly in what concerns height (Table 4). In between the transplant moment and the last monitoring 2 years later, there were also no significant differences (<10cm: GLM p=0.72; 10-20cm: GML p=0.87).

Table 4: Monthly height's growth rate in cm, of both *P. clavata* transplant treatments (<10cm and 10-20cm), in intervals of 6 months, through the experiment duration. Growth rates are presented with its standard error (95% confidence interval).

	Size <10cm	**Size 10-20cm**
Transplant	0.09±0.04se	-0.09±0.06se
6 Months	0.09±0.07se	0±0.16se
1 Year	0.09±0.07se	0±0.11se
1.5 Years	-0.05±0.06se	-0.05±0.07se
2 Years	0.07±0.03se	-0.07±0.04se

3.7.1.2 Total Length (Growth Rate)

The monthly growth rate concerning total branch length had no significant differences (GML p=0.66) and was positive in both treatments. It evidences two clear distinct moments which occur during the 6months monitoring and 1.5 year monitoring (both in the summer). The monitoring after 6 months shows a growth rate of 0,39±0,3se cm in the <10cm treatment, similar to that of the 10-20cm treatment that had 0.48±0.64se, in clear contrast with the monitoring after 1 year where both treatments had a higher growth rate with 1.11±0.27se cm and 1.39±0.64se for the <10cm and 10-20cm treatments (Table 5). The same trend was observed in the second year of monitoring, when growth rate decreased in the 1.5 years monitoring, being negative in the 10-20cm treatment. Despite this it increased again at the 2 years monitoring, to more than 1cm per month in both treatments (Table 5). Annually growth rate in the <10cm treatment was of 8.34±1.7se cm in the first and second year, while at the 10-20cm treatment it was of 6.22±3.4se cm and 5.1±2.7se cm for the first and second years respectively.

Table 5: Monthly total branch length's growth rate in cm, of both *P. clavata* transplant treatments (<10cm and 10-20cm). Growth rates calculated every 6 months, from the transplant monitoring until the end of the experiment. Growth rates are presented with its standard error (95% confidence interval).

	Size <10cm	**Size 10-20cm**
Transplant	0.09±0,16se	-0.16±0.26se
6 Months	0.39±0,3se	0.48±0.64se
1 Year	1.11±0,27se	1.39±0.64se
1.5 Years	0.41±0,14se	-0.64±0.33se
2 Years	1±0,23se	1.17±0.3se

There is in both treatments, like observed on total size branch length, a coupling of total branch length growth with water temperature. Both the 6 months and 1.5 years monitoring occurred during the summer when the water temperature increased. Growth rate had an inversed response to water temperature rising. When temperature went up, growth decreased reaching negative values in the 10-20cm treatment. And when water temperature decreased growth rate increased again (Figure 11).

3.7.2 Stick Methodology

Due to the high mortality and lack of live transplants, growth rates could not be studied in this treatment.

3.8 Ecological State

3.8.1 DSA Methodology

The ecological state of the gorgonian transplants did not change significantly in both size classes from the transplantation event to the end of the experimental trial (GLM p=0,999) and (GLM p=0,983) for the <10m and 10-20cm respectively. Moreover, all transplanted fragments had open polyps and were feeding in the first two monitoring events. There were significant differences between size classes (GLM p=0,021). By the end of the study 90% of the small fragments and 84% of the medium size transplants had no signs of disease, necrosis nor had epiphytes and had an overall healthy condition (Figure 3). The colonies found to have been impacted only showed small epiphytes in extremities or exposed branch tips. In all cases recorded to be impacted, colonies had less than 5% of the total colony affected.

3.8.2 Stick Methodology

Due to the high mortality and lack of live transplants, ecological state was not able to be assessed.

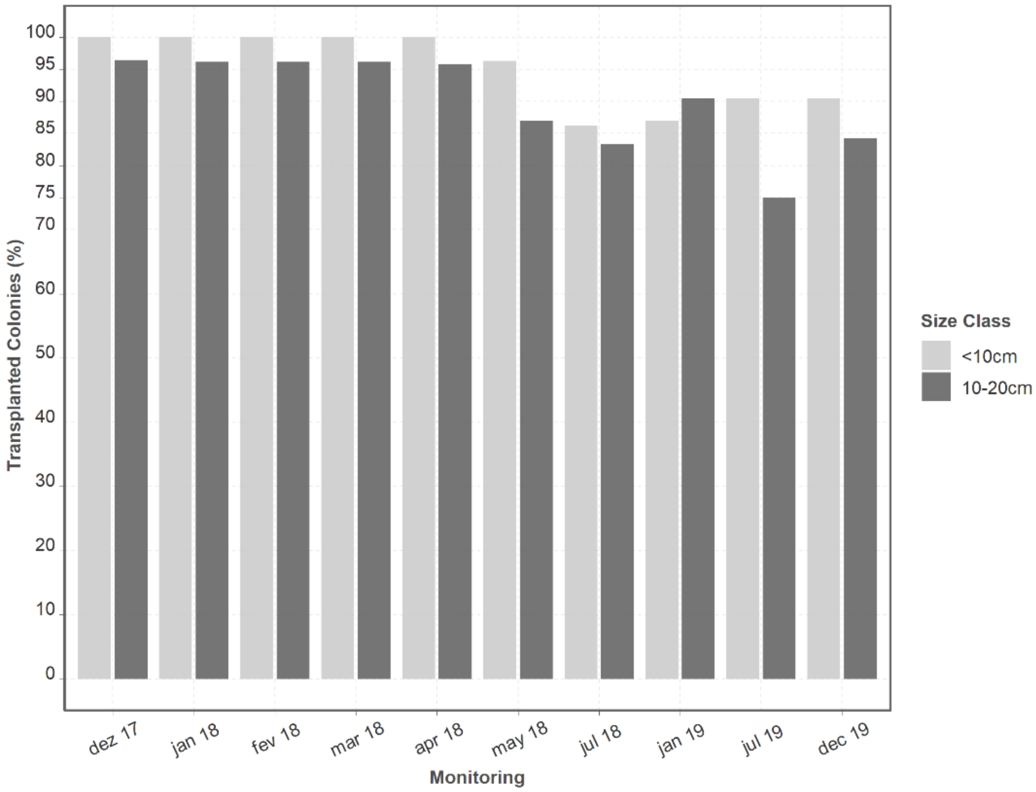

Figure 14: Transplanted *P. clavata* colonies treatments' (<10cm and 10-20cm) ecological state. Percentage of transplants that had no visible impacts (disease, discoloration, polyp release, exposed skeleton or epiphytes), through the duration of the experiment. No significant differences were found between the start and end of the trial (95% confidence interval).

4 Discussion

In the present study we demonstrate that the novel DSA transplantation methodology is a successful and viable restoration tool when used with *P. clavata*. It is a success not only considering the short-term results but also the long-term scope, necessary in modern restoration attempts (Montero et al., 2017).

4.1 Attachment

Two different methodologies have been attempted in this study, using epoxy to secure the transplants: the "Stick" methodology (Linares et al., 2008) and the DSA methodology developed in this study. With the DSA attachment success was not only higher (95% after 3 months), but proved to be long lasting, allowing the gorgonian transplants to develop a holdfast in the natural substrate. This was one of the goals of the DSA methodology, and a key accomplishment in order to achieve long term success. With the "Stick" methodology, attachment proved inefficient, and most transplants were lost. A distinct characteristic when comparing these two methodologies is labor and logistics. The "Stick" methodology is described as a low labor effort and low economic cost, easy technique. When implementing this transplant procedure our team of two divers was able to transplant around 60 colonies in 80 minutes. The DSA methodology in which cost was similar to that of the "Stick" method, was a slower procedure and only 35 transplants were able to be accomplished in the same amount of time. The differences between both methodologies are related with the technique itself where scraping the substrate turned out to be a faster procedure, while drilling the rocky substrate took significantly more time. In the literature, the "Stick" methodology reported over 40% failure in attachment due to technique failure. The main reasons were breakage of the epoxy or loss of the supporting rod used in fixating the fragment. In this study, using the "Stick" methodology, the attachment success attained with fragments of <10cm was of 20% after 3 months. Other than epoxy detachment and loss of the support rod, it was also observed that the interaction of local fauna can be a critical factor in achieving success in the initial stages using this technique (personal observation while executing the procedure). The epoxy putty needs to be soft enough to allow the insertion of the transplant and to be manipulated and pressed against the base of the transplant. During the time necessary for the epoxy to cure and become rigid, small fish, attracted by the suspended particles resulting from scraping the substrate, were observed colliding with the transplants. These small impacts loosened several transplants from the epoxy putty, and some were observed to fall from the epoxy base. This situation might explain the higher percentage failure observed in this study when compared to the reported in the literature (60% success). Contrastingly, the success obtained with the DSA methodology using <10cm fragments was of 97%, demonstrating that it has a high efficiency in securing the transplanted colonies despite being a more time-consuming process. Unlike what was observed with the “Stick” method, small impacts from fish, or even water currents seemed less likely to have an impact in the transplanted fragments. This resilience to displacement results from the fact that the hole drilled in the substrate is only marginally larger than the gorgonian skeleton. When subject to a force, the fragment is pushed against the hard substrate and is more likely to stay immobile while the epoxy becomes rigid. More importantly, the DSA methodology showed great plasticity regarding size, with 95% success on fragments of 10-20cm and 75% success on fragments of 20-40cm. While the “Stick” methodology proved inefficient in retaining any fragments of 10-20cm, in accordance with what was reported in the original study.

Despite the importance of the attachment step in any transplant efforts, there is a somewhat vague definition of what is a successful attachment. Attachment literal definition is fixating, coupling, or connecting an object or organism, but in a coral transplant framework it is lacking context. In an ecological context, considering the long-term goals that should be pondered in coral restoration methodologies, without the development of a natural holdfast it is likely that the transplant will break or loosen up and fall given enough time (Linares et al., 2008; Brinkhuis, 2008; Edwards & Gomez., 2007). This fact derives from the stress placed in the first segment of the transplant’s skeleton and artificial base and will likely increase as the transplanted coral grows larger and interacts with water currents and local fauna. It is then safe to assume that without a continuous enlargement, strengthening and organic recovery of the attachment point, such as that observed in natural colonies, the transplants will be lost. Due to this fact, the present study supports that on long-term restoration, attachment success needs to be coupled with the ability to stimulate a natural adherence with the substrate, creating what one could describe as organic attachment.

Attachment success is paramount to any transplantation efforts (Montseny et al., 2019). Only with a strong attachment, that does not inhibit growth or damages the transplanted gorgonian fragment, can the colony grow and allow long-term restoration success (Edwards & Gomez., 2007). Cost and efficiency are also factors that need to be taken in consideration, and a concern in restoration studies (Edwards & Clark., 1999). Indeed, restoration efforts are still limited by the allocated funding and pressed to achieve the best results with the available resources. It is then imperative for decision makers to take in consideration cost-efficient procedures such as the DSA, that while slower, proved to be more effective and with long term success.

4.2 Holdfast Formation

In this trial, holdfast formation was met in all treatments of the DSA methodology. Over 95% of transplanted colonies in both <10cm and 10-20cm treatments had observable organic coupling after six months, reaching 100% after one year. In the 10-20cm size treatment, living tissue was observed to cover and extend over the natural substrata as soon as one month after the transplant event. At this time, none was observed in the <10cm treatment. Even though no gorgonin was observed in this monitoring event on any treatment, it gives an indication that the 10-20cm transplants were allocating resources to reattachment earlier when compared to the smaller <10cm treatment. Moreover, after 5 months, the 10-20cm treatment had 58,4% of holdfast formation, while in the <10cm treatment only 34,5% of the transplanted colonies had a holdfast. These results seem to indicate that bigger sized transplants have a higher and faster capacity to reattach to the substrate. Yet, if given time, smaller transplants (<10cm) can recover, with six months to one year being a good time frame in which holdfast is expected to be observed. Unfortunately, due to the lack of survival obtained in the treatments using the “Stick” methodology, no consideration can be made regarding the formation of a holdfast when using this technique. Despite this, in previous studies the formation of holdfast was also observed on coral transplanted with epoxy (Jaap., 2000). The success in growing over the base material used to hold the transplants when using epoxy contrasted with the poor results when using cement (Brinkhuis, 2008). This indicates that holdfast formation using the “Stick” methodology combined with the use of epoxy glue should be possible, given enough time; should the issues directly related with physical attachment and breakage be resolved. Holdfast formation, or the capacity for the transplanted gorgonians to develop an organic attachment to the natural substrate was one of the proposed goals of the DSA methodology. It was also a hypothesis to be tested with the “Stick” methodology, since there were no reports of holdfast existence in the original study. The ability to reattach the gorgonian skeleton to the natural substrate might be a key factor to ensure the long-term survival of the transplanted colonies (Montseny et al., 2019; Edwards & Gomez., 2007). Considering previous studies, mortality is largely related directly or indirectly with attachment issues. Loss of transplants is often reported due to detachment of the base, inability for the living tissue to form over the material or breakage of the material or of the skeleton close to the base material due to abrasion or friction (Linares et al., 2008; Brinkhuis, 2008). Concerning substrate differences and despite not being a goal of the study, it was also observed that holdfast grew in both natural and artificial blocks of the breakwaters in which the transplant was performed. A clear indication that artificial material might also be suitable for restoration efforts, and an important factor to consider in future projects.

4.3 Survival

In the present study, size had no direct impact in survival, allowing other factors to be prioritized when defining restoration projects using *P. clavata*. After one year, the DSA methodology had a high survival rate (79%) using this species (all treatments considered). An auspicious outcome, taking into account the average success of published results where the mean annual observed survival was 48% for several gorgonian species (Montseny et al., 2020); specifically, 35–45% survival for *Eunicella singularis*, 30% for *Eunicella verrucosa* and 35–50% for *P. clavata*, one year after the transplants (Montero-Serra et al., 2018; Fava et al., 2010; Linares et al., 2008). For the “Stick” methodology, after a year, survival was of 0%, mainly due to technique failure (attachment) which was responsible for around 80% direct mortality. During the first three months, the subsequent cause of death for the few remaining transplants was unknown. However, as the remaining 20% of transplants were found overrun by epiphytes, but still in place, it was most probably due to result of manipulation and natural causes.

Despite the aforementioned results, one important factor that requires particular attention and is often neglected in the literature is the timescale of published studies. There is a general lack of results of long-term studies (over one year duration), with most published studies reporting results obtained in small-time frames (Montero-Serra et al., 2018). This fact might create a bias towards positive results as suggested by Bayraktarov et al., 2016. The short duration of the studies results in a lack of exposure to full year seasonal changes, which can be a determining factor towards positive results. In this study, two years after transplant, the DSA still had a high survival rate (69%, both treatments considered). These results are particularly encouraging, considering that during the study multiple devastating storms hit the Portuguese coastline with waves of 5-6m (IPMA; personal observation), and despite such rough maritime conditions the transplants resisted. Another aspect to consider is that 8,6% (potentially more) of the transplanted colonies were lost due to illegal sport fishing in the location of the transplant. Fishing gear and lines were observed to be entangled around some of the transplanted colonies, in some cases causing branches to break, in other cases removing tissue through friction or cutting the whole gorgonian colony close to the substrate. Survival of gorgonian transplants has been object of study for decades and is suggested in the literature as a primal proxy for the success of a methodology to be applied in future restoration efforts (Montseny et al., 2019; Bayraktarov et al., 2016; Fava et al., 2010; Linares et al., 2008). Yet, survival alone can be a misguiding indicator to validate or rule out a methodology. Among the many acting factors, different life history strategies might play a key role in survival, which places in evidence that some species might be more suitable to manipulation than others (Montero-Serra et al., 2018). In fact, different species might react to a methodology differently (Fava et al., 2010), stressing the necessity of validating methodologies for the specific gorgonian species to be used in the restoration efforts. Another factor evidenced as potentially relevant is transplant size (Linares et al., 2008). In general, published studies focus in only one size class – transplants up to 10cm length. Linares et al., 2008, attempted to expand on this knowledge, however, was unsuccessful in transplanting larger sizes (>10cm). This clearly reveals a void of knowledge in the subject matter. The results obtained in this study demonstrate that using the DSA methodology with *P. clavata*, survival of the transplants is above the average of published outcomes, in particular on longer timescales.

4.4 Growth

In this study it is demonstrated that *P. clavata*, transplanted using the DSA methodology, attains positive net growth after 2 years (long-term). Positive growth was observed in both treatments (<10cm and 10-20cm) by the end of this study. The <10cm treatment had greater growth than the 10-20cm treatment, which was an expected result since colonial organisms, in particular gorgonians, tend to decrease growth speed with increasing size (Lasker et al., 2003). As observed in net growth results, annual growth rates were also negligeable when considering height, but positive when considering total branch length monitoring results. It was only by monitoring branch length that it was possible to determine both treatments attained an obvious growth. In fact, the height of both treatments showed no differences between the transplant moment and the last monitoring 2 years later. The lack of height growth attests that despite height measuring being one the most used metrics (Palma et al., 2018), measuring transplant height can lead to misleading interpretations of the experimental trial outcomes. It is of extreme importance to consider gorgonian growth dynamics (Montero-Serra et al., 2018), and that the transplanted fragments are removed from the extremities of other gorgonians and then trimmed closer to the base, to be inserted in the epoxy glue. This unnatural shape of the transplanted gorgonian fragment might lead to an allocation of resources towards growth in different sections of the colony (Matsumoto et al., 2004). Therefore, typical growth dynamics are altered in the transplanted colony, affecting the increase in height, while other branches are formed and grow. Nonetheless, growth rates were not stable throughout the year. There is an inversed coupling between growth and water temperature, which might result from temperature stress. In this study, both treatments had moments of higher growth rates (above 1cm month) during wintertime, while during the summer, growth rates dropped to less than half, including an observed negative growth in the 10-20cm treatment. Suffering phases have been previously reported and are directly linked to the conditions experienced during the warmer periods of the year (Fava et al., 2010; Matsumoto et al., 2004). Temperature stress reactions have also been reported by Rakka et al., 2019, where gorgonians subject to increased temperature were reported to release polyps and exhibit negative growth. This is a consideration of utter importance when selecting the moment of the year in which to perform the transplant. Transplanting the gorgonian colonies during the summertime, when the environment naturally leads to a suffering phase, coupled with the natural stress caused by manipulation, might lead to unsuccessful results.

4.5 Ecological State

The transplanted colonies, in both treatments (<10cm and 10-20cm), had overall healthy conditions by the end of the study. The smaller fragments (<10cm) had a higher percentage of healthy colonies without necrosis, polyp release or epiphytes. Nonetheless, both treatments had a high percentage of healthy transplants, with 90% in the <10cm and 84% in the 10-20cm treatments not exhibiting any signs of disease, necrosis or epiphytes.

These results fall in line with the expected, since a small number of impacted natural colonies were observed in the surrounding area (personal observation). Moreover, the impacted colonies all had less than 5% of their total area impacted, in some cases a small epiphyte in a branch extremity was recorded. Small impacts are expected to be observed in healthy gorgonian gardens, be it due to predation, or other natural stress responses such as temperature shifts. Due to this in cases where the impacted colony percentage is less than 5%, it has been suggested that colonies should be considered healthy (Francour, 1998), which allows to consider the transplant healthy as a whole. Despite that fact, there was no significant degradation of the gorgonian ecological state directly after the transplantation, and the day after the colonies had open active polyps and were feeding. These results demonstrate that *P. clavata* is a species suitable to be manipulated and resilient to the inherent stress caused by the transplantation procedure. It also puts in evidence that there was no negative interaction between the epoxy material and the living tissue since no degradation was observed in the base branches or holdfast area.

5 Final Considerations

Coral restoration studies have focused in scleractinian corals for decades, while octocorals have only recently drawn more attention from researchers. In this study, we demonstrate that the DSA methodology, when performed using the octocoral species *P. clavata*, is the first successful long term (2 years) transplantation procedure with published results. The colonies transplanted in both treatments exhibit a healthy condition and developed a natural holdfast in the first year of the experiment. The holdfast, now coupled with the substrate, will ensure the capacity to support increased stress as the colony grows and interacts with the environment (water currents, fish impacts and others). Moreover, growth was positive, which demonstrated that given time, the small corals transplants are expected to reach reproductive maturity and a natural repopulation of the area will ensue. The procedure described in this trial works equally well with small (<10cm) and mid-sized fragments (10-20cm). It also shows potential to hold bigger colonies as demonstrated by the attachment success achieved with 20-40cm transplants. This fact leaves fragment size choice an open criterium for decision makers. Size should be chosen depending on the objectives and limitations of the project to be implemented. Transplantation of bigger fragments will ensure earlier reproductive maturity but will require more biomass or less colonies transplanted; transplanting smaller fragments will bypass a potential lack of available colonies to be transplanted but require a longer time to reach reproductive maturity size (Coma et al., 1995). The resources and research efforts concerning octocoral growth after transplantation are lacking when compared to scleractinian. There are very few published studies reporting growth after transplantation and even less so presenting long term results. It is urgent to increase research efforts in this restoration area and have a clear idea of the fate of transplants on long term scales. Without a true understanding of the fate of the transplanted colonies, claiming positive transplantation outcomes may be biased towards success.

The results obtained in this experimental trial support a proven methodology with potential to be used in large scale restoration projects. Nonetheless, the fact that it was tested with only one species should make this study a baseline for future work. The different physiological and morphological characteristics of other gorgonians species may prove an obstacle and require testing. It is then imperative to test this methodology with other species before implementation in restoration projects. Another important aspect to consider in future studies is the monitoring of recruitment and reproductive output of transplanted colonies (Lloret and Planes 2003). In this case, genetic analyses could provide insights on the success achieved by sampling recruits and transplanted colonies to verify kinship through loci examination (Ledoux et al., 2015). Such data would allow to assess if repopulation is occurring as a result of the transplantation efforts. In the future, given the success achieved in the present study using the DSA method, we aim to develop citizen science protocols. Easy to implement citizen science protocols not only allow to involve local partners (dive centers and general population) but also increase human resources available to the project. The increased capacity resulting from local involvement effectively make restoration efforts more affordable and enable large scale efforts. Moreover, the involvement of local populations allows to leverage opportunities to educate and raise awareness to the protection of these structuring species and its habitats. Despite the success achieved in this study, to achieve this goal, we should overcome the difficulties met with substrate drilling. We believe that bypassing the necessity to drill holes underwater, which is time consuming, would make the whole process easier and faster. Such would be possible through the deployment of artificial or natural structures with pre-made holes (done outside of the water) in areas requiring restoration. After having the structures in place, local divers (after briefed) can simply fill up the small cavities with epoxy and introduce the already prepared gorgonian fragments. With the help of local volunteers, a great number of corals can be transplanted in a small timeframe, and in larger areas. When possible, citizen science effectively provides a solution to some of the biggest obstacles found in restoration methodologies: area coverage and cost-efficient efforts. With the implementation of pre-drilled structures, the DSA methodology would then have the potential to be used in large scale citizen science restoration projects expanding its scope and versatility.

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7 Appendix

Supplement A1

Characterization of Sagres harbor breakwaters' Gorgonian Population

Methods

A preliminary study was made in order to characterize the gorgonian population in the breakwaters of Sagres harbor, located inside the *Parque Natural do Sudoeste Alentejano e Costa Vicentina* marine park (PNSACV). In December, 2017, the population identified at the end-tip of Sagres harbor's breakwaters (Sagres' population here onwards) was object of study. The study consisted of identifying the geomorphology of the habitat where the gorgonian garden occurred, gorgonian populations species, distribution, density, height and ecological state. Geomorphology was assessed according four main aspects: substrate, vertical profile and if the habitat is continuous or heterogenous.

Vertical distribution was verified through the deployment of a vertical transects (three replicates). After identification of the vertical limits of the populations' distribution, a 50m band transect was deployed per depth (extension of the habitat allowing), from deepest to shallowest, in intervals of 3 m. The adjacent area, up to 0.5 m (1m in total) from the transect was sampled (Lloret et al., 2006; Coz et al., 2012). When density of gorgonians was high (visual assessment, 10 colonies per m^2), quadrats of 1 m^2 were randomly sampled along the transect, instead of doing a continuous band transect (Coma et al., 2006; Coz et al., 2012). Considering the aims of the study, sampling took place in the 60m long area facing north-northwest. In this area the highest density of gorgonians (visual assessment) was found. Outside of this area, despite the same substratum, colonies could be observed but in lower density and in patches. All identified gorgonians were sampled considering several parameters: species, height, necrosis and epiphytes presence and if the colony was in flat area or in a wall/overhang area. The percentage of necrosis and epibionts was documented and used as a sign of colony health. The coverage assessment was done visually using 5 distinct classes: 1 to 5 (1=0-10%; 2=10-25%; 3=26-50%; 4=51-75% and 5=76-100%), following a modification of Fancour and Koukouras (2000) methodology, where up to 10% coverage is considered a healthy gorgonian.

Distribution, Density and Demographic Structure

Gorgonian populations were characterized regarding its distribution, density and demographic structure. After assessing the site, density was calculated for each band transect, as the number of organisms per area sampled. This choice resulted from the fact the gorgonian population occupies a 60m wide area. For every depth, density was calculated for the whole population and per species. Final densities were presented as the mean of the densities. The demographic structure was determined considering population size (height). Distribution was assessed using the standardized Morisita Index of Dispersion (I_{Mr}). The standardized Morisita index, was applied where: -1 = no similarity; 1 = complete similarity and a setting where -0.5 and 0.5 were set as confidence limits around random distribution with rescaled value 0 (Smith-Gill., 1975; Krebs., 1999).

Results

Geomorphology

The site where the gorgonian garden was identified is located at the tip of the breakwaters of Sagres' harbour facing north-northwest. The breakwaters were built using a mix of natural calcareous boulders and tetrapod and H-interlocking concrete structures. The way these structures were piled and settled to form the breakwaters, created a continuous habitat. The breakwaters exhibit a continuous slope of 40º from the top up to the sandy bottom of the bay. Maximum depth at the bottom ranges from 18 to 16m depth depending on the tide. Despite being continuous the habitat itself is highly heterogenous. When diving around the construction, several crevices and holes can be observed in between the many concrete and calcareous structures. Some of these hollow areas are big enough for a human to enter, creating multiple sciaphilous areas. The random disposition of the many structures and boulders that compose these breakwaters also lead to available rocky substrate in all angles, ranging from flat, to diagonal facing upwards, vertical walls, and overhangs.

Species

The gorgonian species recorded in this area were: *Eunicella labiata*, *Eunicella verrucosa*, *Leptogrogia sarmentosa*, *Eunicella gazella* and *Paramuricea clavata*.

During characterization of the area, positive identification of *Eunicella verrucosa*, *Eunicella labiata* was hard underwater. The morphology of these two species is so similar, that through observation the risk of a wrongful identification was too high. Taking this into account both *E. verrucosa* and *E. labiata*, were referred to as *Eunicella sp* and were treated as one for statistical analyses.

Distribution

Gorgonian population vertical distribution was found to be comprised between 7m and 16m (maximum depth of the site). Despite this only at 13 and 16m were all species observed. Above 13m only *Eunicella sp. and L. sarmentosa* were recorded (**Erro! A origem da referência não foi encontrada.**). Horizontal distribution was found to be continuous at 16m and 13m of depth for all species, with the exception of *E. gazella* population at 13m of depth that exhibits a patchy distribution (**Erro! A origem da referência não foi encontrada.**). Shallower than 13m all species were found to have a non-continuous distribution with exception of *Eunicella sp.* at 10m of depth (I_{Mr}=0,52).

Table 6: Morisita's standardized index of dispersion for all species identified in the location where the study took place. The distribution is divided by the depths sampled, with intervals of 3m starting from the deepest point possible.

Depth (m)	Species	Morisita's Index (I_{Mr})
7	*Eunicella sp.*	0
	E. gazella	-
	L. Sarmentosa	-
	P. clavata	-
10	*Eunicella sp.*	0,52
	E. gazella	-
	L. Sarmentosa	-0,12
	P. clavata	-
13	*Eunicella sp.*	0,34
	E. gazella	0,08
	L. Sarmentosa	0,52
	P. clavata	0,33
16	*Eunicella sp.*	0,5
	E. gazella	0,55
	L. Sarmentosa	0,5
	P. clavata	0,33

Gorgonian Substratum Orientation

Gorgonians' orientation preference varied depending on species. While *P. clavata* was found to have 100% of the sampled colonies in walls/overhangs, *L. sarmentosa and Eunicella sp.* both showed plasticity regarding substratum orientation, growing in both flat areas and walls. Despite this fact there still seems to be preference for walls and overhangs (**Erro! A origem da referência não foi encontrada.**). The species *E. gazella* seems to favor walls and overhang areas with 82,6% of the sampled colonies having been found in such areas.

Table 7: Percentage of colonies per species sampled regarding substratum orientation. Two main orientation were considered, wall/overhand and flat areas. Results are presented per species.

	P. clavata	*Eunicella sp.*	*E. gazella*	*L. sarmentosa*
Wall/Overhang	100%	72,6%	82,6%	60,5%
Flat	0%	27,4%	17,4%	39,5%

Density

The highest gorgonian density at the breakwaters was found at 16m of depth where population's density was 12,3±0,91 col./m^2 (Figure 14). At this depth, *Eunicella sp.* is the dominant species with a density of 5,5±1,05 col./m^2, followed by *L. sarmentosa* and *L. Clavata* with 4,93±0,89 col./m^2 and 1±0,32 col./m^2 respectively (Figure 14). At 13m of depth gorgonian density was of 5,8±0,53 col./m^2 where the species with higher density is *L. sarmentosa* with 2,47±0,74 col./m^2. At 7m of depth, the upper limit of the gorgonian population at this site, *Eunicella sp* was the only species recorded with a density of 0,4±0,16 col./m^2 (Figure 14).

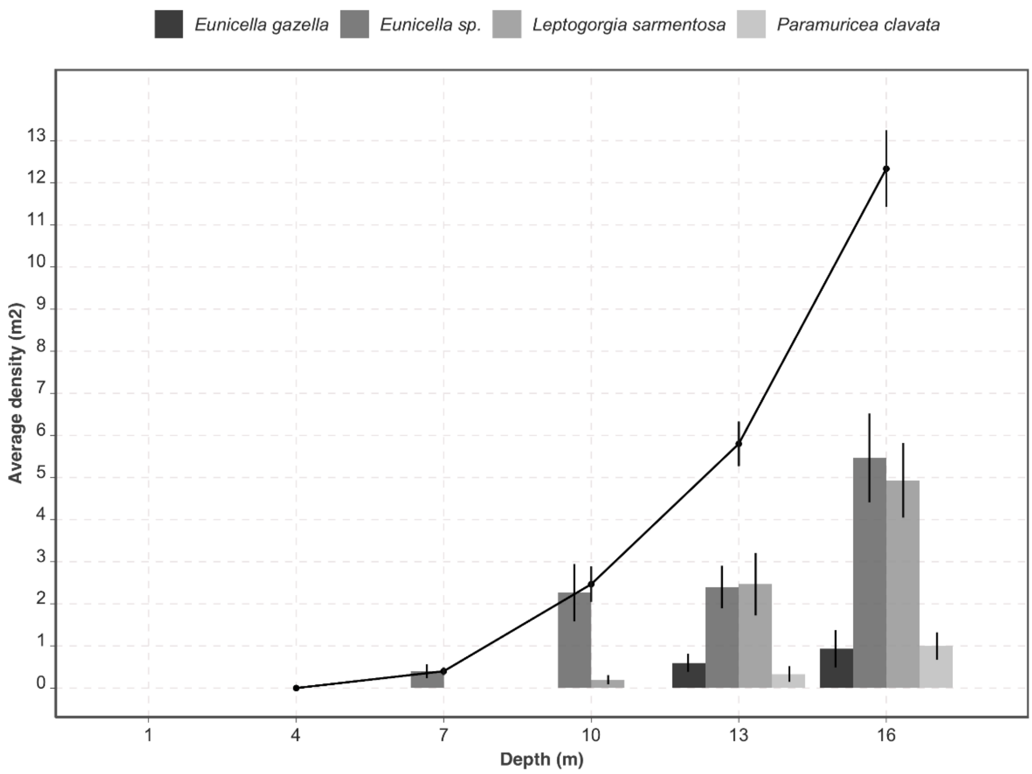

Figure 14: Density of gorgonian species per depth at the end tip of Sagres' harbor breakwaters depicted by the bar plots, while overall gorgonian population's density is represented by the line plot.

Demography

Populations' height was higher at the 13m of depth with an average height of 18,3±1,3 cm. Despite the fact that populations height was higher at 13m, for *P.clavata* the biggest colonies were found at 16m with an average height of 13,4±1,78 cm, while at 13m on average colonies had 11,8±1,15 cm height. For *L. sarmentosa*, *Eunicella sp.* and *Eunicella gazella*, on other hand, height was found to be higher at 13m of depth (Figure 15).

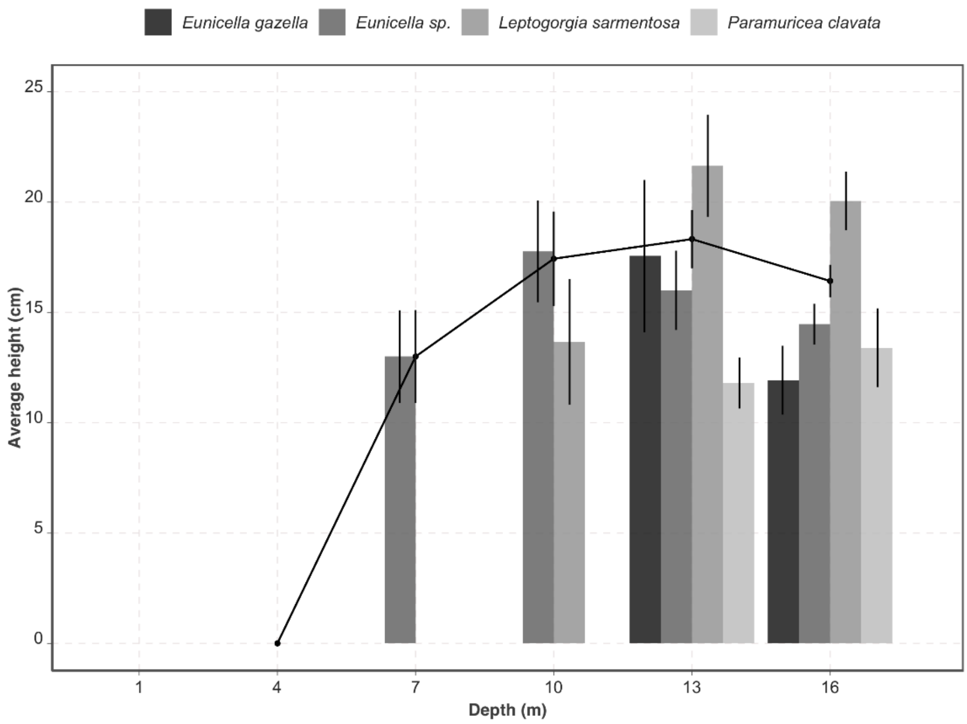

Figure 15: Height of gorgonian species per depth at the end tip of Sagres' harbor breakwaters depicted by the bar plots, while overall gorgonian population's height is represented by the line plot.

Ecological State

The ecological state of the gorgonian garden was overall considered to be healthy, with 70,7% of all sampled colonies showing either negligible or no signs of necrosis and epiphytes, and 19,7% had less then 25% of the colony affected. The species with the least number of impacted colonies was *P. clavata*, where 95% of the sampled colonies showed negligible necrosis or epiphytes coverage (**Erro! A origem da referência não foi encontrada.**).

Table 8: Ecological state percentage of the gorgonian species found at Sagres' breakwaters. The classes reflect the percentage of gorgonian found to be affected by necroses or epiphytes (ie <10% refers to a colony where less than 10% was found to be impacted by necrosis or epiphites).

	<10%	**10 - 25%**	**26 – 50%**	**51-75%**	**76-100%**
E. gazella	60,8	26,1	8,7	4,4	0
Eunicella sp.	64,3	24,8	5,1	3,8	1,9
L. sarmentosa	77,2	14,0	5,3	2,6	0,9
P. clavata	95	5	0	0	0
Overall	70,7	19,7	5,1	3,2	1,3