



D4.3. Effectiveness of tools/techniques for restoration in the deep-sea

Marine Ecosystem Restoration in Changing European Seas MERCES

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Executive Summary

Deep-sea environments host one of the most extensive ecosystems on Earth, playing a key role in the functioning of our planet and providing essential goods and services for human well-being. Among deep-sea ecosystems, cold-water corals (CWC) are among the main habitat-forming species generating complex three-dimensional ecosystems that create hotspots of biodiversity over large areas. Unfortunately, they are increasingly affected by several stressors, mainly fishing but also oil and gas extraction, and are exposed to potential impacts from deep-sea mining that could ultimately be the largest scale human activity impacting deep-sea ecosystems in the near future. As a consequence, CWC communities have been recently internationally recognized as Vulnerable Marine Ecosystems (VMEs), stressing the urgent need for their sustainable management and conservation.

Ecological restoration practices are receiving worldwide attention as they offer the opportunity to assist the recovery of damaged ecosystems. While marine restoration practices are increasingly common in shallow tropical environments, assisted regeneration initiatives focusing on degraded deeper benthic ecosystems are still extremely uncommon. As such, the main goal of this Deliverable 4.3 was to assess the effectiveness of tools and techniques developed for assisted (active) restoration in the deep sea (focusing mainly on cold-water corals habitats) and to synthesize the restoration actions developed and performed within the MERCES project through the presentation of the three case studies within WP4. Finally, we also discuss the success of these restoration actions, the pros and cons of the tools and techniques developed until now and we suggest some ways forward (risks, challenges, uncertainties) and how to scale up these actions at spatial and temporal scales.

The aim of the first two study cases was to restore cold-water coral gardens impacted by deep-water fishing using assisted regeneration through coral transplantation techniques in the NW Mediterranean (Cap de Creus) and the Atlantic Sea (Azores). The third study case assesses the feasibility of relocation of a marine benthic fish from areas within island slopes impacted by bottom long-line fisheries to a nearby seamount with fishing closure in Azores as means to restore fish stocks.

The three study cases showed successful results demonstrating the feasibility of assisted regeneration actions in deep sea, mainly in CWC habitats. However, given the life history traits of corals and fishes, with high longevity, slow growth and late reproduction, only a long-term monitoring (i.e. beyond the lifetime of the MERCES project) will reveal fully restored habitats.

Ecological restoration of deep-sea habitats involves considerable constraints due to the difficult access to deep environments, requiring for the use of advanced underwater technology entailing high economic costs. Future availability of accessible cost-effective underwater technology (such as relatively low-cost AUV) will be paramount for the wide application and up-scaling of coral and gorgonian restoration actions at depths below conventional or technical scuba diving limits. Regarding to fish translocation scaling up in spatial sense can only be considered after the animal wellbeing is secured and more experiments should be done on small scale in order to improve methods, prove concepts and fill knowledge gaps. Finally, the involvement of professional fishers in restoration actions like those implemented in MERCES project can help to increase the awareness of local society about the need for the protection of cold-water coral gardens and could facilitate in combination with low-cost technology as commented before the application to larger scales.

1. General Introduction

1.1 Deep-sea ecosystems and cold-water coral communities: current and emerging threats for their conservation

The deep-sea is the largest biome of Earth comprising diverse ecosystems, with a large portion of biodiversity that plays a key role in the functioning of our planet and provides essential goods and services for human well-being (Danovaro et al., 2010, 2017; Thurber et al., 2014). Deep-water ecosystems have received an increasing attention during the last decades as a result of the continued advancements in deep-sea exploration technology, which have contributed to expand knowledge about benthic communities dwelling on continental shelves and deep-sea bottoms, evidencing the negative effects of fishing activities (Freiwald et al., 2004; Althaus et al., 2009). The continuously increasing exploitation of deep-sea resources is currently recognized as a major concern (Morato et al., 2006; Ramirez-LLodra et al., 2011) where the potential expansion of mineral exploitation and bottom-contact fisheries threat the conservation of deep-sea ecosystems at long-term (Barbier et al., 2014; Mengerink et al., 2014).

Among deep-sea environments, cold-water corals (CWC) are among the main habitat-forming species generating complex three-dimensional ecosystems that create hotspots of biodiversity over large areas (Hovland, 2008; Roberts et al., 2009). They provide suitable habitat, acting as feeding, reproductive, nursery and refuge areas for a wide variety of associated species, many of which are of commercial interest (Krieger & Wing, 2002; Henry & Roberts, 2007; Cartes et al., 2013). At the same time, these organisms also play a paramount role in the benthic-pelagic transfer of energy and matter (Graf, 1989; Gili & Coma, 1998) as well as in most of the biogeochemical cycles in the deep-sea (Van Oevelen et al., 2009; Wild et al., 2009; Cathalot et al., 2015). Unsustainable and destructive fishing activities have been identified as one of the most pervasive threats to CWC communities occurring on seamounts as well as on the continental shelf and slope (~60–1000 m depth), as these areas endures the bulk of commercial fishing activity (Watling & Norse, 1998; Hall-Spencer et al., 2002). Since the last century, seamounts, continental shelves and upper slopes have been heavily impacted by fishing activities (Jones, 1992; Koslow et al., 2000; Althaus et al.

2009; Oberle et al., 2017). Bottom-contact fishing gears, and especially bottom trawling, represent one of the main threats to benthic ecosystems (Hall-Spencer et al., 2002; Clark et al., 2016), resulting in a severe oversimplification of benthic communities (Walting & Norse, 1998; Thrush & Dayton, 2002; Reed et al., 2007; Rossi, 2013). A large amount of the deep-sea fishing by-catch (the untargeted catch occurring unintentionally in a fishery) of sessile fauna comprises corals, gorgonians, and sponges species, as they are easily entangled in trawled and trammel nets, as well as longlines, due to their branching morphology and erect structure (Wareham & Edinger, 2007; Althaus et al., 2009; Durán Muñoz et al., 2011; Sampaio et al., 2012; Bo & Bava et al., 2014). Additionally, these benthic species are also highly exposed to partial mechanical damage (i.e. breakage and tissue abrasion) from the direct impact of fishing activities (Althaus et al., 2009; Sampaio et al., 2012; Mytilineou et al., 2014). Oil and gas extraction can also result in direct damage to CWC communities, and potential impacts from deep-sea mining could ultimately be the largest scale human activity impacting deep-sea ecosystems in the near future (Ramirez-Llodra et al., 2011; Cordes et al., 2016). Furthermore, all these activities have indirect impacts through increased suspended sediment concentration in the water and sediment deposition rates in benthic ecosystems adjacent to the directly impacted areas (Martin et al., 2014). Smothering by sediments results in damage and decreased growth rates of CWCs (Larsson & Purser, 2011; Larsson, 2013; Grant et al., 2018). The loss of these benthic habitat-forming species can result in overall loss of the associated biodiversity and is comparable to the impact of forest clear-cutting on terrestrial ecosystems (Watling & Norse, 1998). Consequently, the vast majority of benthic communities inhabiting these depths have been degraded for decades (Hall, 2002).

As a consequence, CWC communities have been recently international recognized as Vulnerable Marine Ecosystems (VMEs) (OSPAR, 2010), stressing the urgent need for their sustainable management and conservation (Davies et al., 2007; FAO, 2009; Aguilar et al., 2013). The conservation and recovery of benthic engineering species such as CWC will also preserve all their associated fauna, maintaining the ecosystem functioning and the ecosystem services that they provide (Byers et al., 2006; Geist & Hawkins, 2016). It is thus highly desirable to actively initiate or improve the slow natural recovery of impacted cold-water coral assemblages and associated fish fauna by means of reducing impacts and active ecological restoration actions (Van Dover et al.,

2014; Possingham et al., 2015; Da Ros et al., 2019). Natural recovery of these communities may take centuries, if it is possible at all (Dayton, 2003). In order to enhance their recovery, active intervention to aid the regeneration of these communities is highly desirable (Rinkevich, 2005).

1.2 Ecological restoration in deep-sea: active restoration in CWC habitats

Ecological restoration practices are receiving worldwide attention as they offer the opportunity to redirect the environmental damage caused by anthropogenic impacts and mismanagement of natural resources, by assisting the recovery of a natural range of ecosystem composition, structure, and dynamics (Falk, 1990; Allen et al., 2002; Palmer et al., 2005; Falk et al., 2006). Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed by human activities, bringing it back as close as possible to its undisturbed state (SER, 2004). While marine restoration practices are widespread, mainly in shallow tropical environments (Rinkevich, 2005; Precht & Robbart, 2006; Young et al., 2012), active restoration initiatives focusing on degraded deeper benthic ecosystems are still extremely uncommon (Brooke et al., 2006; Dahl, 2013; Da Ros et al., 2019).

There are only a few restoration initiatives carried out to date targeting the recovery of CWC populations. First attempts to restore CWC using assisted regeneration techniques began in the early 21st century and focused on the CWC reef forming species *Oculina variciosa* (Brooke et al., 2006) and *Lophelia pertusa* (Dahl, 2013; Jonsson et al., 2015). These actions consisted of using coral transplantation techniques where coral fragments from a healthy donor reef were collected, using Remotely Operated Vehicles (ROVs), and transplanted on degraded reefs. Transplants were attached to artificial structures such as concrete modules or racks and deployed at a depth range from 70 m to 100 m in the impaired areas. After more than two years, the restoration actions demonstrated high survival of coral transplants (>76%), coral growth, re-establishment of associated coral reefs fauna and few larval settlement and juvenile recruitment (Brooke et al., 2006; Dahl, 2013; Jonsson et al., 2015). Recently two restoration actions have been developed within the MERCES project simultaneously in the Western Mediterranean Sea and North Atlantic Ocean, focused on the restoration of CWC gardens composed by gorgonian species (see two first study cases in the section below). These actions consisted mainly in testing the

feasibility of using coral transplantation techniques developed for tropical (Bowden-Kerby, 2001) and temperate gorgonians (Bavestrello et al., 1999; Linares et al., 2008; Fava et al., 2010) to restore cold-water gorgonian populations. In the Mediterranean Sea, a pilot action focusing on populations of the gorgonian *Eunicella cavolini* on the Mediterranean continental shelf (NW Mediterranean, SPAIN) was performed at ~90 m depth, (Montseny et al., 2019 and first study case, section 2.1). Following this small-scale pilot study, the same authors have carried out a large-scale restoration action in close collaboration with artisanal fishers, where by-catch colonies of *Eunicella cavolini* were recovered from trammel nets, transplanted to supporting cobbles and returned to their natural habitat using a pioneering technique ("badminton method"). At the same time in the Atlantic, restoration actions in the Azores have been performed in order to restore cold-water coral gardens and associated commercial fish species impacted by bottom-longline fishing (second and third study case, section 2.2 and 2.3). Outside the MERCES project, Boch and collaborators (2019) have carried out a translocation study with seven different cold-water coral species, in the Sur Ridge seamount (Monterey, USA) (>800 m depth). Coral branches were collected with a ROV, fragmented at the surface and attached to "coral pots" using two different methods (zip ties and cement). Then the fragments were placed back in the same habitat, where their survivorship was assessed. After the first year the mean survival detected was around 52% with the higher mortality occurring in the first 3 months. Although results indicated differences in sensitivities to transplanting methods among coral species, they suggested repopulation efforts may accelerate the recovery of disturbed coral communities (Boch et al., 2019). On the other hand, relocation of fish species in the deep-sea seems to be first of this kind (del Mar Gil et al., 2015). There are some initiatives in the shallow waters, but they are mainly focused on release of captive bred fish in the wild as a form of reinstatement of fish stocks and/or biodiversity. Another interesting aspect of this study is that the targeted fish species is often associated to CWC that are a focus of the second case study (Gomes-Pereira et al., 2014).

Since CWCs are highly vulnerable to human pressure, restoration actions should act in concert with protection measures that remove as much pressures as possible from the area to be restored (e.g. closures to fishing activities), until a certain threshold of

size/biomass of coral colonies or area covered by coral colonies is attained. An important feature of CWC is the presence, diversity and biomass of associated fauna which should also be in focus of restoration activities, either as an indicator or as a target for restoration activities. Moreover, because of the patchy or fragmented nature of deep-sea coral gardens, a combination of restoration approaches will likely be necessary, with natural spontaneous regeneration (through fisheries closures, MPAs) at large scales, and assisted regeneration and reconstruction at smaller scales

2. Case study content: Implementing active restoration activities in the deep-sea

2.1. Feasibility of coral transplantation techniques and the deployment of artificial substrates for the active restoration of CWC gardens on the continental shelf (CSIC/UB)

2.2. Feasibility of coral transplantation techniques and the deployment of artificial substrates for the active restoration of deep-sea CWC gardens on seamounts (IMAR)

2.3. Feasibility of fish transplantation for restoration of fish stocks in areas impacted by fishing (IMAR)

The goals and objectives of these ecological restoration experiments are presented in Tables 1, 4 and 8. The overall goal is an overarching and time bounded statement that describes a state of the ecosystem expected at a specific time (McDonald et al., 2016). The objectives are used to evaluate the goal in smaller time periods with a specific indicator, which allows for timely interventions if needed.

2.1. Feasibility of coral transplantation techniques and the deployment of artificial substrates for the active restoration of CWC gardens on the continental shelf

2.1.1 Introduction

The main objective of this study case was to use gorgonians recovered from the bycatch of artisanal fishery, to restore cold-water coral gardens on the continental shelf.

Environmental setting of the ecosystem

The marine area of Cap de Creus is located in the southernmost part of the Gulf of Lions in the Western Mediterranean Sea, and includes an extensive continental shelf together with the submarine canyon of Cap de Creus (Figure 1). The area is influenced by the Liguro-Provençal current running close to the shelf edge, and by the winter formation of cascading through the submarine canyon toward the deep basin of dense cold shallow waters generated by the predominant north and northwesterly winds on the area (Tramuntana and Mistral). At the Cap de Creus continental shelf (60–120 meters depth) the prevailing strong bottom currents generate areas of low sediment deposition where coarse-grained deposits with a significant gravel fraction are dominant.

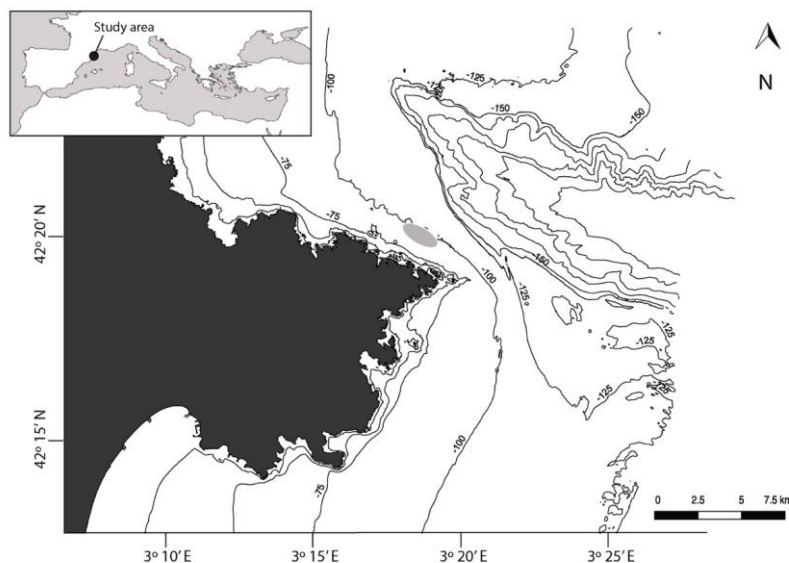


Figure 1 · Location of the study area. The grey ellipse corresponds to the area where the artificial structures were deployed.

There, the combination of strong bottom currents, outcropping rocks, and coarse-grained sediments with nutrient-rich waters derived from river runoff in the gulf, supports the offshore rocky community, mainly composed by gorgonian-dominated assemblages (Gili et al., 2011; Lo Iacono et al., 2012). Both temperature and salinity are stable around 13°C and 38, respectively.

Species composition and diversity

Over the continental shelf, cold-water coral gardens on offshore rocky bottoms at 80–110 m depth are mainly composed by dense populations of the gorgonian *Eunicella cavolini*, together with several sponges (e.g. *Suberites syringella*). Gorgonian populations are found in well-developed patches dominated by medium to large sized colonies and reaching densities of the order of 25 colonies m⁻². Populations of *E. cavolini* on the Cap de Creus continental shelf are closely associated to high diversity of associated fauna (e.g. sponges *Suberites syringella*, *Stelligera stuposa*, *Raspailia viminalis*, *Haliclona elegans* and *Dysidea avara*, soft corals *Parazoanthus axinellae* and *Paralcyonium spinulosum*, and an extent list of bryozoans, hydrozoans and polychaets) (Dominguez-Carrió, 2014, 2017). Soft bottoms around rocky patches are dominated by the soft coral *Alcyonium palmatum* and the seapen *Pteroeides spinosum* (Gili et al., 2011; Dominguez-Carrió, 2018).

Main life-history and other characteristics

There is currently little information on the life-history traits of deep populations of the gorgonian *E. cavolini* (Koch, 1887), which is one of the most common Mediterranean gorgonian species (Carpine & Grasshoff, 1975; Weinberg, 1976), showing a wide bathymetric distribution (<10–220 m depth) (Carpine, 1963; Russo, 1985; Sini et al., 2015) (Figure 2). Seasonal sampling and laboratory incubations showed that this is a dioecious internal brooder species, reproducing in summer (July), with a slow growth a few mm per year (linear extension of apical branches) (Dominguez-Carrió et al., 2017). This gorgonian presents a fan-like morphology with a variable ramification pattern, depending on the main hydrodynamic conditions.

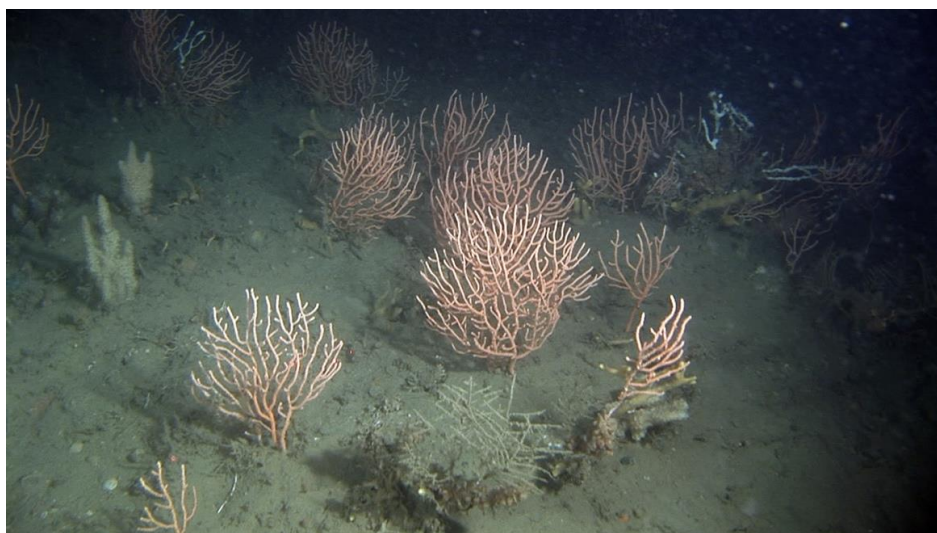


Figure 2 · Cold-water coral garden on the Mediterranean continental shelf.

Structural complexity (habitat forming)

Coral gardens are among the main complex three-dimensional communities on the Mediterranean continental shelf, generating spatial heterogeneity and providing suitable habitat for hundreds of associated species (Bo et al., 2012; Grinyó et al., 2016; Gori et al., 2017, and references therein).

Vulnerability and fragility / recovery capacity

Taking into account the life history traits of *E. cavolini* known to date (i.e. slow growth, high longevity, low reproductive potential), their population are considered as sensitive communities and vulnerable marine ecosystems (VME), mainly affected by fishing activities.

Main ecosystem services

Sub-outcropping rocky bottoms with *E. cavolini* populations on the continental shelf (mainly 70–100 m depth) are the main fishing ground for local artisanal fishers in Cap de Creus, targeting at lobster (*Palinurus elephas*) during the fishing season from March to August.

Activities, pressures and impacts

Artisanal fishing activities, mainly trammel nets, are largely extended in the Cap de Creus marine area (Dominguez-Carrió et al., 2014). Corals, gorgonians and sponges

are among the most frequent bycatch species (accidental capture) as they remain easily entangled in nets and longlines due to their branching morphology and erect position. The gorgonian colonies that remain on the seafloor are also mechanically damaged (submitted to breakage, displacement and partial mortality) (Althaus et al., 2009; Sampaio et al., 2012). This fishing impact, in turn, can reduce the habitat for associated species, resulting in overall loss of biodiversity and the ecosystem services that cold-water coral gardens provide.

Management landscape

Marine coastal areas (between 0.2–1.3 miles offshore) are included since 1998 in the Natural Park of Cap de Creus, where professional fishing activities, recreational navigation and other touristic activities are allowed except within the small no-take area where all activities are prohibited. The deeper area including most of the continental shelf and the submarine canyon has been recently declared Site of Community Interest within Natura 2000 network. However, a management plan has still to be defined. Due the rough topography of their bottom, most of the CWC gardens in the area are preserved by the impacts of bottom trawling fishing. However, as previously explained, they are largely exploited by artisanal fishermen using trammel nets.

Existing restoration actions and potential future techniques

Restoration protocols being tested in Cap de Creus are based on techniques developed for tropical coral reefs and Mediterranean shallow gorgonian populations (Rinkevich, 1995; Linares et al., 2008), as well as on fishing practices from artisanal fishermen in the Menorca Channel (Grinyó et al., 2016).

2.1.2 Material and Methods

Gorgonian collection and maintenance

Colonies of the gorgonian *E. cavolini* (Koch, 1887) were obtained from artisanal fishers's bycatch from Cap de Creus (north-western Mediterranean Sea, 42°19.12'N; 03°19.34'E), at a depth range of 70 to 100 m, during three fishing sorties in June and one in August. Fishers picked up gorgonians entangled in trammel nets and kept them in containers filled with surface seawater (~20–23°C). Once back on land (1–2 hr after collection), gorgonians were transported to the experimental aquarium facilities of the

Institute of Marine Sciences (ICM–CSIC) in Barcelona (within 3–4 hr after the initial pick up), while seawater temperature was kept at $14 \pm 1.0^{\circ}\text{C}$ at all times. A total of 120 gorgonians were held in 100 L tanks, simulating Cap de Creus continental shelf's natural conditions, between a few days and a maximum of 2 months.

Transplant on artificial structures and deployment on the sea bottom

In June, 80 gorgonians were transplanted onto two stainless steel structures (40 gorgonians onto each; outer diameter: 2 m; inner diameter: 1.5 m), with a base grid ($10 \times 10 \text{ cm}^2$) surrounded by four concrete plates and a central 1 m vertical axis holding an acoustic reflector (30 cm in diameter) supported by four stainless steel bars (12 mm in diameter) (Figure 3). Forty conical supports for the gorgonians (80 mm high, 20 mm diameter) were placed on the grid. The inside of the supports was filled by polyester fibreglass resin and, once dry, 8 mm boreholes were made in order to attach the gorgonians colonies with epoxy putty (Corafix SuperFast, GROTECH®). Each structure weighed 137 kg in the air. Initially, the structures were deployed at 6 m depth north of the marine protected area of Cap de Creus, where gorgonians (entire colonies) were attached to the supports by scuba divers. Each structure was then raised up to below the water surface by means of a buoy and transported by boat at a slow and constant speed ($\sim 0.5 \text{ kn}$) towards the continental shelf, where they were deployed at 85 m depth (structure 1: $42^{\circ}20.06'\text{N}$; $03^{\circ}18.67'\text{E}$; structure 2: $42^{\circ}20.05'\text{N}$; $03^{\circ}18.67'\text{E}$). Since an additional 40 gorgonian colonies were collected as bycatch in fishing events in August, they were transplanted later on a third structure on October, and deployed nearby the first two structures (structure 3: $42^{\circ}20.05'\text{N}$; $03^{\circ}18.64'\text{E}$) following exactly the same procedure. The density value of colonies transplanted onto each structure corresponds to $\sim 15 \text{ colonies m}^{-2}$, and was selected based on data about Mediterranean gorgonian assemblages dwelling at 40–300 m depth ($10\text{--}20 \text{ colonies m}^{-2}$; Bo et al., 2009; Grinyó et al., 2016).

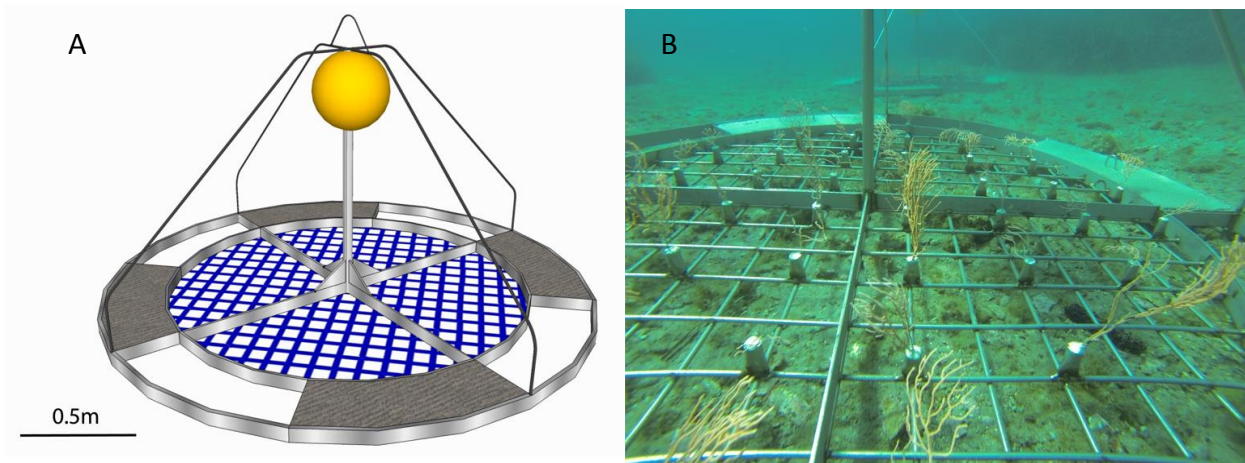


Figure 3 · (A) Schematic representation of the stainless-steel structures used in this study.
(B) Transplanted gorgonians on the structures at 6 m depth.

Monitoring of transplanted colonies

The structures were monitored through three consecutive surveys using the Girona 500 autonomous underwater vehicle, equipped with the Bumblebee stereo camera, working as a hybrid ROV (Carreras et al., 2016). Surveys were conducted on July (21 days after deployment for structures 1 and 2), December (6 months after deployment for structures 1 and 2; 47 days for structure 3), and September (14 months after deployment for structures 1 and 2; 10 months for structure 3). During each survey, the hybrid ROV used sonar to locate the acoustic reflector and approach each structure. The images, with a resolution of 1,024 x 768 px, were subsequently collected by encircling each of the structures, while maintaining the gorgonians in the centre of the view. The robot maintained an approximately constant distance of 2 m between the camera and the centre of the structure, enabling observations of the gorgonians from various directions with sufficient image quality to allow successful assessment of their survivorship. Gorgonian survival was assessed by individually observing if each transplanted colony was still in place and alive (with no evidence of necrotic tissue). Moreover, a three-dimensional (3D) reconstructions of the three structures deployed on the continental shelf with transplanted gorgonians (Figure 4) were made using an optical 3D reconstruction procedure (Hernández et al., 2016).

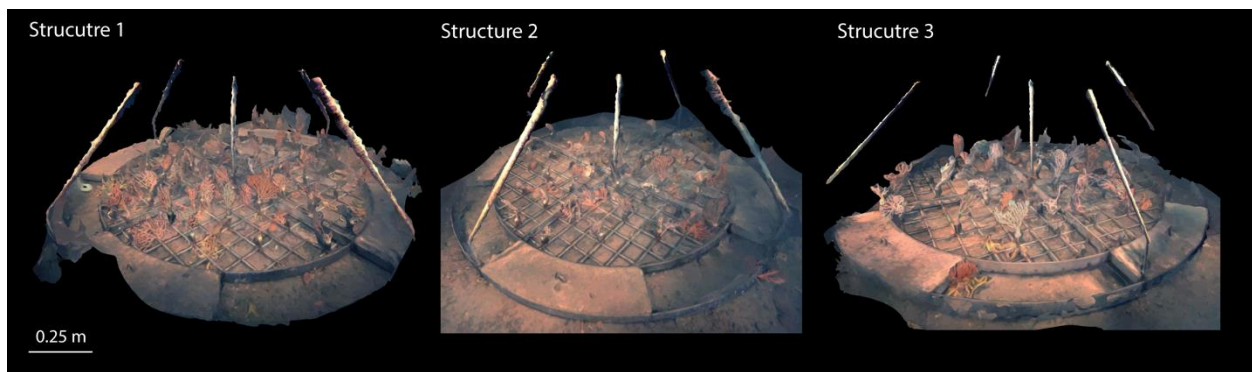


Figure 4 · Three-dimensional (3D) reconstruction of the three structures deployed on the continental shelf with transplanted gorgonians.

The main goal of this study is to eventually re-establish CWC coral gardens in the marine protected of Cap de Creus National Park area, which is measured by survival and increase in size of transplanted gorgonian (Table 1).

Table 1 · Proposed goals and objectives for the restoration for active restoration of CWC gardens on the continental shelf study.

Target	Goals categories	Goals	Objectives for 1 year period	Indicators
reference ecosystem	Ecological	re-establishment of CWC coral gardens in 15 years	survival of transplanted gorgonians	survival >50%
			increase in size of transplanted gorgonians	increase in size >10%

2.1.3 Results

Several of the gorgonians collected from fishermen showed partial breakage and a little evidence of tissue abrasion. Even so, they all recovered and survived while being maintained in aquaria at ICM–CSIC prior to redeployment at sea.

On structure 3, 85% of the transplanted gorgonians were still in place at the time of the second survey (47 days after deployment). Finally, approximately 1 year after deployment (14 months for structures 1 and 2; 10 months for structure 3) $87.5 \pm 9.0\%$ (mean \pm SD) of the gorgonians were in place and alive, with no necrosis, on the three structures (Figure 5).

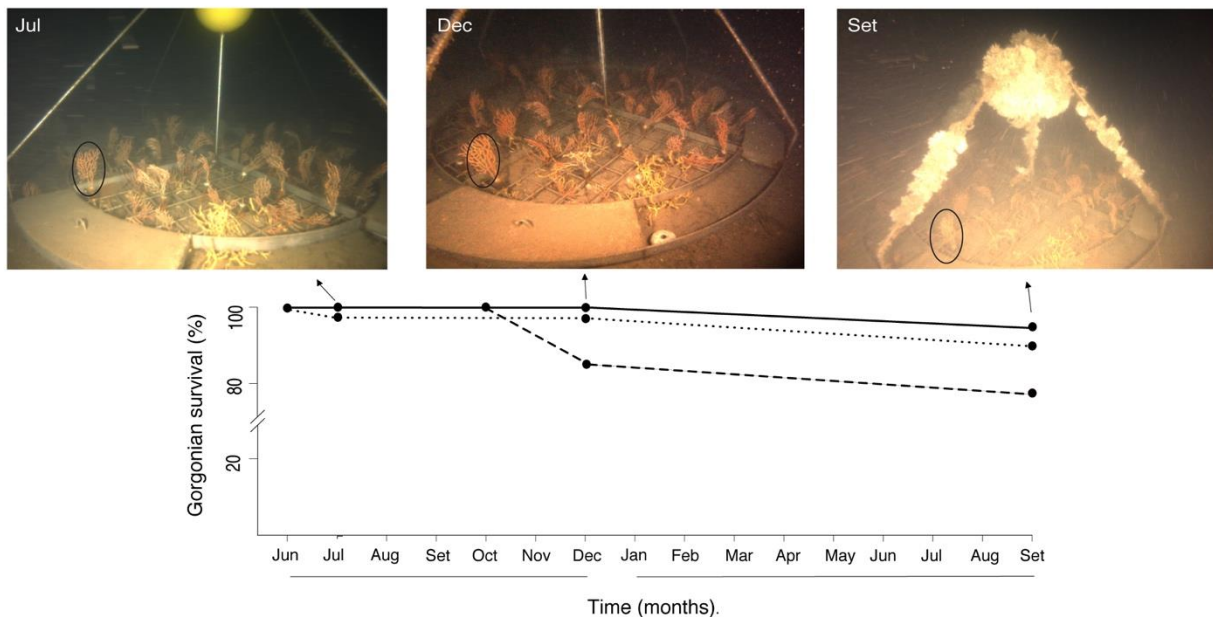


Figure 5 · Survival rate of transplanted gorgonians for each structure during the study period. Solid line corresponds to structure 1, dashed line to Structure 2, and dotted line to structure 3.

The first objective regarding the survival of the transplanted colonies over a 1-year period was successfully achieved, while the objective regarding the increase in the size of the transplanted colonies could not be assessed due to limitations in methodology (Table 2). From the images acquired by the HROV, we could not assess eventually growth in the transplanted colonies. The visibility was limited, there were many particles in the water column that makes difficult detecting the increase in size.

Table 2 · Objectives, results and evaluation 1 year after coral transplantation in the Cap de Creus National Park.

Case study	Objectives for 1 year period	Results	Evaluation
reference ecosystem	survival of transplanted gorgonians	average survival is 87.5%	objective was achieved
	increase in size of transplanted gorgonians	NA	objective was not evaluated due to limitations in methodology

2.1.4 Discussion

This pilot study has assessed, for the first time, the feasibility of successfully returning by-catch gorgonians recovered from artisanal fishery to their natural environment on the Mediterranean continental shelf. Initial results showed that, in spite of some *E. cavolini* colonies suffering partial breakage, tissue abrasion, or both, all colonies survived while being maintained in aquaria. This survival may be attributable to the species' high healing rate (0.085 mm of tissue recovery·d⁻¹) (Fava et al., 2010). In contrast, other Mediterranean common gorgonians, such as the red gorgonian *Paramuricea clavata* (which is also frequently collected by artisanal fishermen in Cap de Creus), shows low survival rates when recovered from by-catch and maintained in aquaria, with a rapid degradation of living tissues and high colony mortality (Montseny, pers. observation). These observations highlight the importance of understanding the biological and ecological characteristics of each species before engaging in any restoration initiative (Montero-Serra et al., 2018), and points at *E. cavolini* as a suitable gorgonian species for restoration projects in the Mediterranean continental shelf.

Monitoring of structures shortly after their deployment (21 days or 47 days, depending on the structure) suggested that initial loss of gorgonians was mainly due to colony detachment during the structure deployment on the continental shelf (Figure 3). Although we cannot strictly exclude natural mortality causes, the high survival following initial losses (Figure 3), that is in accordance with previous gorgonian transplantations in Mediterranean shallower habitats (Linares et al., 2008; Fava et al.,

2010), suggests that initial securement of a right colony attachment to the substrate is critical to their long-term survival with rather small effect of stress due to transplantation (Linares et al., 2008). Gorgonian transplants in the present study shown high survival (almost 85%) approximately one year after deployment, inline with the high survival observed for *Corallium rubrum* after 4 years from transplantation (about 99.1%) (Montero-Serra et al., 2018), and much higher compared to transplanted *Eunicella singularis* (35–45% survival after 1 year), *Eunicella verrucosa* (30% survival after 1 year) and *Paramuricea clavata* (35–50% survival after 1 year) (Linares et al., 2008; Fava et al., 2010; Montero-Serra et al., 2018). In comparison with the few examples of ecological restorations in deeper habitats, transplanted gorgonians in this study also showed similar or even higher survivability rates compared with *Oculina varicosa* (50-60% survival after 1 year) (Brooke et al., 2006) and *Lophelia pertusa*, (76%, after more than 3 years) (Dahl, 2013; Jonsson et al., 2015). Long-term survival of *E. cavolini* transplants on the continental shelf may thus be partially explained by the higher stability of environmental factors in deeper habitats (below ~40 m depth) (Garrabou et al., 2002; Grinyó et al., 2018). Indeed, outcomes from shallow restoration studies in tropical ecosystems under high environmental stability, are in accordance with the high gorgonian survivorship detected in the present study (Guzman, 1991; Edwards & Gomez, 2007). However, tropical corals encompass species with contrasting life history traits, including fast to slow growing species (Darling et al., 2012), which make tropical transplant survival rates highly variable (43% to 95% during the first year) (Yap et al., 1992; Lindahl, 2003; Young et al., 2012). Therefore, the high survival rate detected in this study is consistent with the notion that slow-growing species require little initial transplantation effort, since they show high survival rates after transplantation in comparison with fast-growing species, but the period required to fully reestablish habitat complexity will tend to be far longer (Montero-Serra et al., 2018).

In contrast to most restoration practices using coral transplants obtained from fragmentation of donor colonies (Brooke et al., 2006; Dahl, 2013), restoration based on by-catch gorgonians, such as one developed in the present study, would minimize damage to other colonies or populations. Moreover, directly involving professional fishers in restoration actions, will also increase the awareness of local society about the need for the protection of cold-water coral gardens and would facilitate the

application of this methodology in an extensive manner, which is crucial for the restoration success (Gobster & Hull, 2000; Yap, 2000). Restoring with artificial structures with transplanted corals, such as those used in the present study, have advantages and constraints. As an advantage this artificial substrate can also represent an opportunity for the settlement of natural corals (Bramanti et al., 2005) that could accelerate the recovery of the community. However, their spatial scale of application is very limited, while the main stressors that affect most of the ecosystems of the continental shelf and deep waters are widespread (Halpern et al., 2008). Other limitation of this study was the large number of suspended particles present in the study habitat that make it difficult to acquire high resolution images for a proper subsequent analysis. Regarding this aspect, it has not been possible to analyze the potential growth of the transplanted gorgonians and it is necessary to continue improving in the development of better underwater technology.

The ultimate goal of restoration initiatives should be to achieve the recovery of the structure and ecological functioning of affected ecosystems (SER, 2004; McDonald et al., 2016). For coral gardens, restoration of sessile engineering species can drastically alter the abiotic system state triggering a consequent response in the biotic state (Byers et al., 2006), such as that transplanted gorgonians not only provide habitat structure, but also enhance the recovery of its associated biodiversity and positively influence ecosystem functioning (Geist & Hawkins, 2016). Overall, restoration is often a long-term investment and its potential results are still highly uncertain (Suding, 2011; Van Dover et al., 2014). To overcome those uncertainties restoration actions has to be complemented by a rigorous and long-term monitoring to ensure a long-term success (McDonald et al. 2016). Furthermore, to be effective, these restoration actions should be accompanied by a reduction of fishing impacts in the restored areas, by partial closures or by improving fishing techniques.

As above-mentioned, upscaling ecological restoration actions it is a foremost challenge (Aronson & Alexander, 2013; Perring et al., 2018), since the main stressors that affect most of the deep sea ecosystems are widespread (Halpern et al., 2008). The present study is a first essential step, but now new methodologies target at large-scale should have to be proved, like the one developed by Montseny and collaborators (in press). This methodology is named “badminton method” and consist in gently throwing

from the sea surface, gorgonians colonies attached to cobble supports, returning them back to the sea floor. Performing multiple throws could be an option to reach large areas with no high cost associated (Montseny et al., in press).

In this case study the deployment of artificial structures and subsequent HROV, involve the use of an underwater technology that raises the cost, limiting the application of the restoration action. Deep-sea restoration cost per hectare has been estimated at two to three orders of magnitude higher than for shallow marine ecosystems (Van Dover et al., 2014). That is another argument that supports the idea of continuously improving underwater technology to better know and access to deep-sea habitats.

2.2 Feasibility of coral transplantation techniques and the deployment of artificial substrates for the active restoration of deep-sea CWC gardens on seamounts

2.2.1 Introduction

The aim of this case study was to restore cold-water coral gardens impacted by deep-water using assisted restoration of with coral transplantation techniques. Two pilot studies were conducted: (1) one study testing the feasibility of transplanting the gorgonian *Dentomuricea* aff. *meteor*, a common species in coral gardens in the Azores, after the simulated impacts of deep-sea mining and fishing; (2) a second study testing the feasibility of transplanting multiple species of cold-water corals accidentally caught during hook-and-line fisheries operations. The success of the transplantation studies was measured in terms of survival, growth rates, and physiological condition using antioxidant biomarkers in coral tissues. In addition, the capacity to attract sessile and mobile fauna was assessed by characterizing the diversity and density of fauna found in the recovered corals and images from landers.

Environmental setting of the ecosystem

The Condor Seamount is an elongated volcanic ridge, rising from 1700 m to a flat summit at ca. 200 m depth located 17 km southwest of Faial Island (Figure 6). The summit of Condor is characterized by hard substrate, mainly rocky outcrops and boulders, mixed with areas of soft sediments, while the slopes constitute of mostly soft sediments such as gravel, sand and mud. The oceanographic conditions over Condor are different from the surrounding environment, mainly characterized as enclosed circulation around the seamount, pronounced mixing most probably due to semidiurnal tidal effects. The temperature ranges between 12–16 °C throughout the year, whereas salinity is stable at 36. Such environmental setting supports the existence of rich biological communities found in Condor.

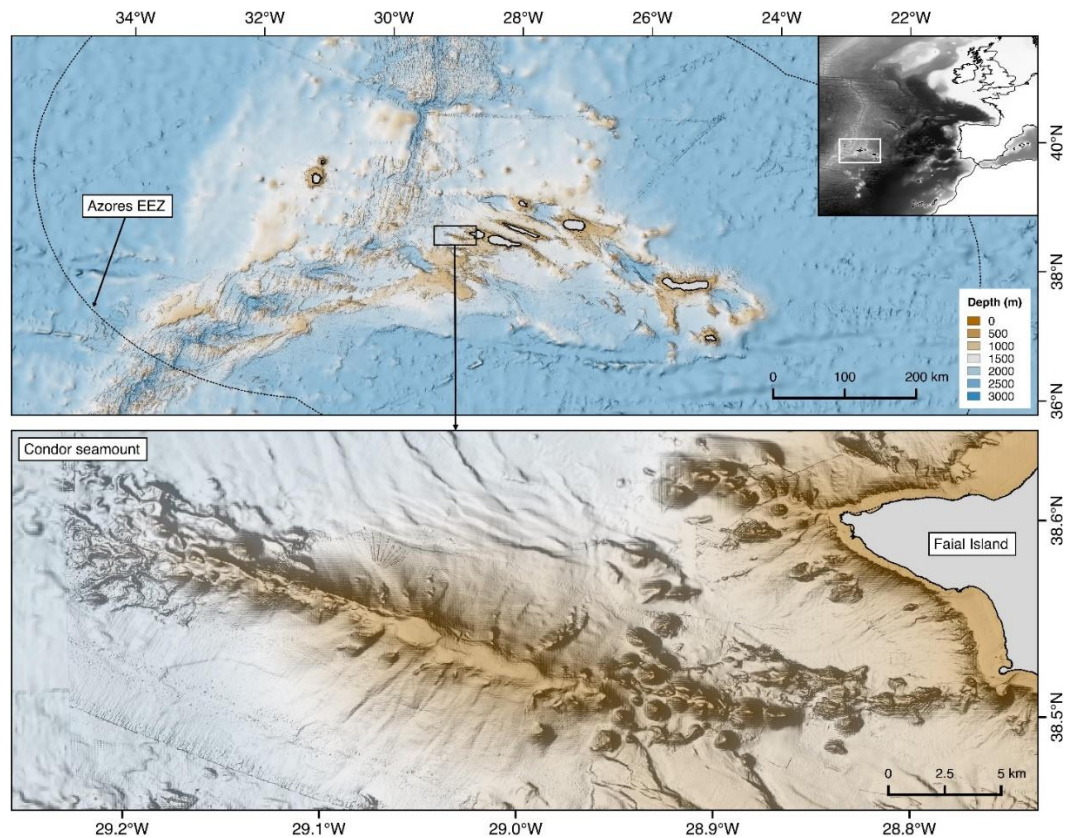


Figure 6 · Location of the Condor Seamount within the Azores Archipelago.

Species composition and diversity

Condor seamount shows a very clear zonation pattern strongly linked to depth, with the composition of the assemblages located on the summit displaying significant differences from those found on the seamount flanks. Coral gardens formed by the octocorals *Viminella flagellum* (Figure 7A), *Dentomuricea* aff. *meteor* (Figure 7B) and large colonies *Callogorgia verticillata* (up to 2 m in height and 1 m in width, Figure 7C), together with the hydrozoan cf. *Lytocarpia myriophyllum* dominate the summit of Condor. Large colonies of octocoral *Paracalyptrophora josephinae* (Figure 7D) and more rarely the black coral *Leiopathes* sp. are also present, as well as small-sized corals, such as the octocoral *Bebryce mollis* and the soft coral *Schizophytum echinatum* (endemic to the Azores). Coral gardens in Condor are found in small and fragmented patches (3.8 ± 3.2 colonies m^{-2}), largely reflecting substrate type and oceanographic conditions (hard substrates where the current flow is accelerated and food input is potentially high).

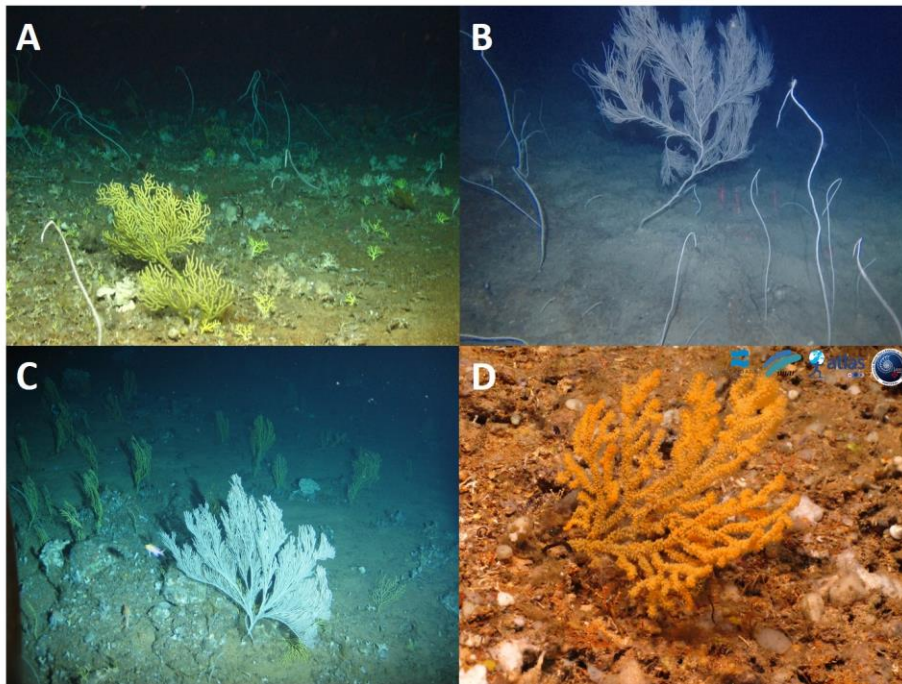


Figure 7 · Coral gardens in the Condor seamount. (A) Coral garden formed by the octocorals *Viminella flagellum* and *Dentomuricea* aff. *Meteor*; (B) large colonies of the octocoral *Callogorgia verticillata* and (c) *Paracalyptrophora josephinae*; (D) small *Acanthogorgia* sp.

The flanks of the seamount are dominated by sponge aggregations dominated lithistid sponges and aggregations of the hexactinellid sponge *Pheronema carpenteri*. Coral gardens formed by of the octocoral *Candidella imbricata* can be observed in the deepest parts of the seamount. Until now, 61 coral taxa have been described for Condor seamount, the highest biomass is found on the summit, between 165 to 262 m water depth (Tempera et al., 2012; Braga-Henriques et al., 2015).

Main life-history and other characteristics

There is currently no information on growth and age of the dominant gorgonian species in Condor. However, studies on deep-sea gorgonians elsewhere show slow growth rates of 0.44-2.32 mm/year, with ages spanning from 30 to more than 400 years (reviewed by Watling et al., 2011). Deep-sea black corals are generally at the end of the spectrum of slow growing organisms with rates of 0.002-0.066 mm/year and estimated ages in the range of nearly hundreds to thousands of years in the Azores and other regions (82-4000 years: Sherwood & Edinger, 2009; Roark et al., 2009;

Carreiro-Silva et al., 2013). Knowledge on the reproductive biology of these organisms is also still very limited. Studies on the reproductive biology of black corals and gorgonians in the Azores show that gorgonians have low fecundity (5-10 oocytes per coral polyp) and larvae with potentially low dispersal capabilities (Rakka et al., 2016; Rakka & Carreiro-Silva, unpublished data). Genetic connectivity of coral populations in the Azores has not been studied yet.

Structural complexity (habitat forming)

Coral gardens, especially if built by tall and arborescent gorgonian and black coral colonies, form tri-dimensional complex habitats and add functional capacity to the surrounding deep-sea environment. A high number of associated sessile (e.g. zoantharians, anemones, hydroids) and vagile (e.g. polychaetes, echinoderms, crustaceans, fish) species use coral gardens as refuge, source of food, spawning and nursery areas (Braga-Henriques et al., 2015; Pham et al., 2015). Several commercial fish species inhabit the seamount, including *Helicolenus dactylopterus*, *Polyprion americanus*, *Pagellus bogaraveo*, etc. The food web in Condor is complex with mesopelagic organisms having an important role in the transfer of energy between the epipelagic environment and the benthic and benthopelagic organisms (Colaço et al., 2013).

Vulnerability and fragility / recovery capacity

Because of cold-water corals life history characteristics (i.e. slow growth, high longevity, low reproductive potential) and fragmented habitat, cold-water corals are perceived as very vulnerable to damage by fisheries or other human activities, with recovery of individual coral colonies and communities requiring decades to centuries. These characteristics have resulted in coral gardens' being listed as vulnerable marine ecosystems (VME) (UNGA, 2007; OSPAR, 2010).

Main ecosystem services

In addition to bioengineering role, coral gardens provide important provisioning services such as fisheries resources and pharmaceutical compounds, regulation services as carbon storage and nutrient remineralization, cultural services for aesthetical, educational and scientific purposes (Matsumoto, 2010; Thurber et al., 2014).

Activities, pressures and impacts

Since the 1990's Condor seamount has been largely targeted by local demersal fisheries. Fishing activities mainly comprised bottom longline and handline fishing down to depths of ca. 600 m. Longline fisheries can impact coral gardens through the accidental capture (bycatch) of corals during fishing activities or by mechanically damaging corals that remain on the seafloor (e.g. breakage, displacement, tissue abrasion) (Sampaio et al 2012; Pham et al. 2014). Moreover, longline fishing impacts mostly organisms with complex morphology, which may eventually threaten their population health since growth and recruitment may be outbalanced by the amount removed and population recovery is highly unlikely. This in turn will reduce the habitat for associated species, resulting in overall loss of biodiversity and the ecosystem services they provide. Because of the "selective" impact of fisheries on larger coral colonies, information on the maximum size that corals can attain is uncertain. Therefore, it may be necessary to use historical records of coral maximum size from early century oceanographic campaigns in the Azores, such as Prince Albert I of Monaco expeditions.

Management landscape

The Condor Seamount is located southwest of Faial Island within the Azores EEZ. An area of 242 km² surrounding the seamount had been closed to fisheries due to research purposes since 2010, and was included in the Azores Marine Park since 2016.

Existing restoration actions and potential future techniques

No restoration techniques have yet been validated for deep-sea coral gardens. Restoration actions and techniques are currently being tested for deep-sea coral gardens in European Seas within MERCES and in the Pacific Ocean by the Monterey Bay Aquarium Research Institute (MBARI). Knowledge gaps, restoration techniques and management issue are being evaluated for cold water coral communities impacted by the Deep-Water Horizon blowout in the Gulf of Mexico.

Restoration protocols being tested in the Azores are based on techniques developed for tropical coral reefs and Mediterranean red coral populations, whereby transplants

of small to medium size coral fragments from adult donor specimens are transplanted to impacted areas (Rinkevich, 1995; Linares et al., 2008).

2.2.2 Materials and Methods

Study location

The coral transplantation studies were conducted in the Condor seamount because it has been close to fishing since 2010 and thus coral landers were not at risk of being accidental removed by fishing or other activities.

Pilot study 1: Coral transplantation

Nine adult colonies of the octocoral *Dentomuricea meteor* were collected at the summit of the Condor seamount at depths of 185-210 m using the IMAR-UAz's ROV SP-300 in March 2016. Corals were transferred to the DeepSeaLab facilities (Orejas et al., 2019), fragmented into 108 coral nubbins (5-7 cm in length). The recovery capacity of transplanted corals after the simulated impacts of deep-sea mining, fishing and both were assessed by deploying landers with *D. meteor* under three different conditions/experimental treatments: i) intoxicated with cooper (the main trace metal present in seafloor massive sulfide sediment plumes), ii) injured with superficial scratches (to mimic fisheries impact), or iii) with both impacts. Intoxication was achieved by placing coral nubbins in a seawater solution with a copper concentration of 150µg/L following Martins et al (2018), a concentration that is known to cause damage without killing the corals. Coral injury consisted of removing a small portion of tissue 2x20 mm of the surface of the coral colony.

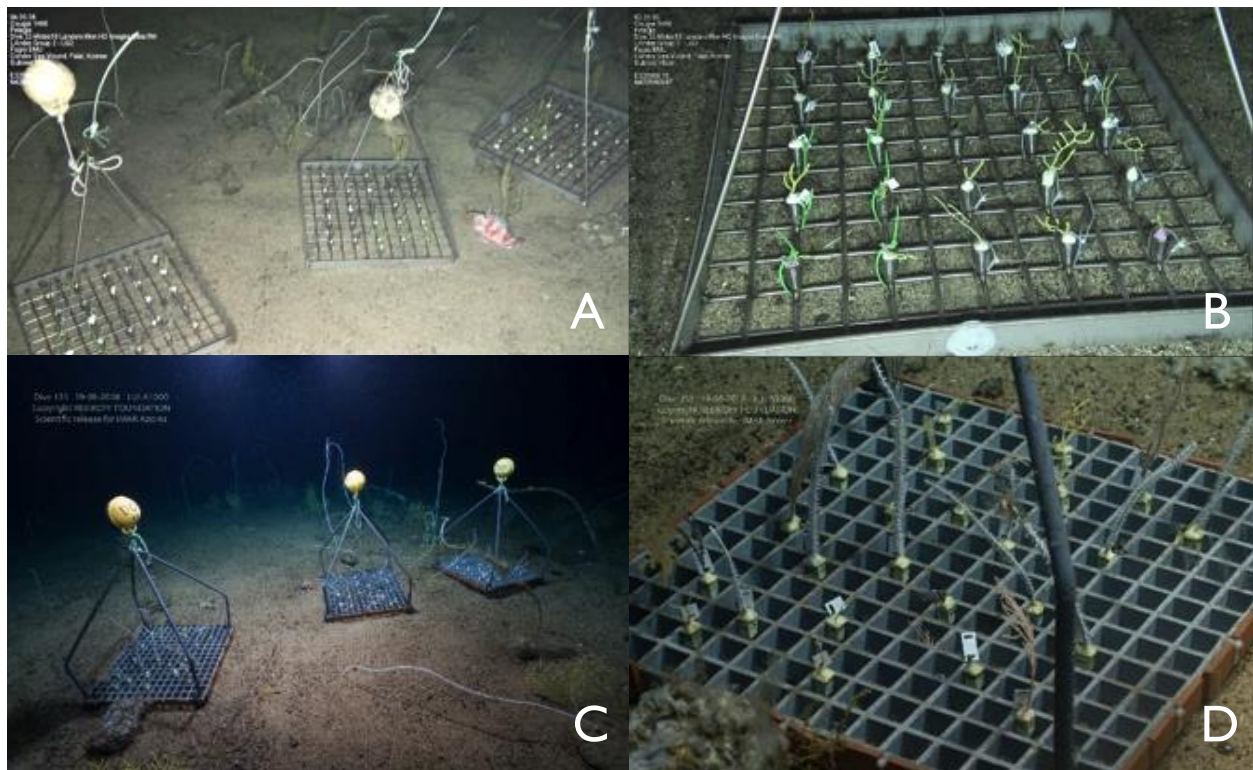


Figure 8 • Landers used in the octocoral transplantation studies; (A) Metal landers used in pilot study 1; (B) close-up of coral nubbins used in the landers; (C) Plastic landers used in pilot study 2; (D) close-up of coral nubbins used in the landers.

Coral nubbins were transferred back to the Condor seamount using 1.08 m² stainless steel landers (Figure 8) on July 25th 2016 during a field cruise onboard the RV Pelagia to the Condor Seamount, funded by the FP7 MIDAS project (Managing Impacts of Deep-sea reSource exploitation). Fauna landers were deployed with the light work class ROV Seaeye Cougar XT, and positioned in three different areas of coral density (low, medium and high) on the summit of Condor seamount at 230 m depth (Figure 9). These areas were chosen with the objective to determine the effect of the surrounding density of natural coral populations and oceanographic conditions on the transplanted corals' survival. Each lander had 6 fragments from every experimental treatment and 3 landers were placed in each coral density site.

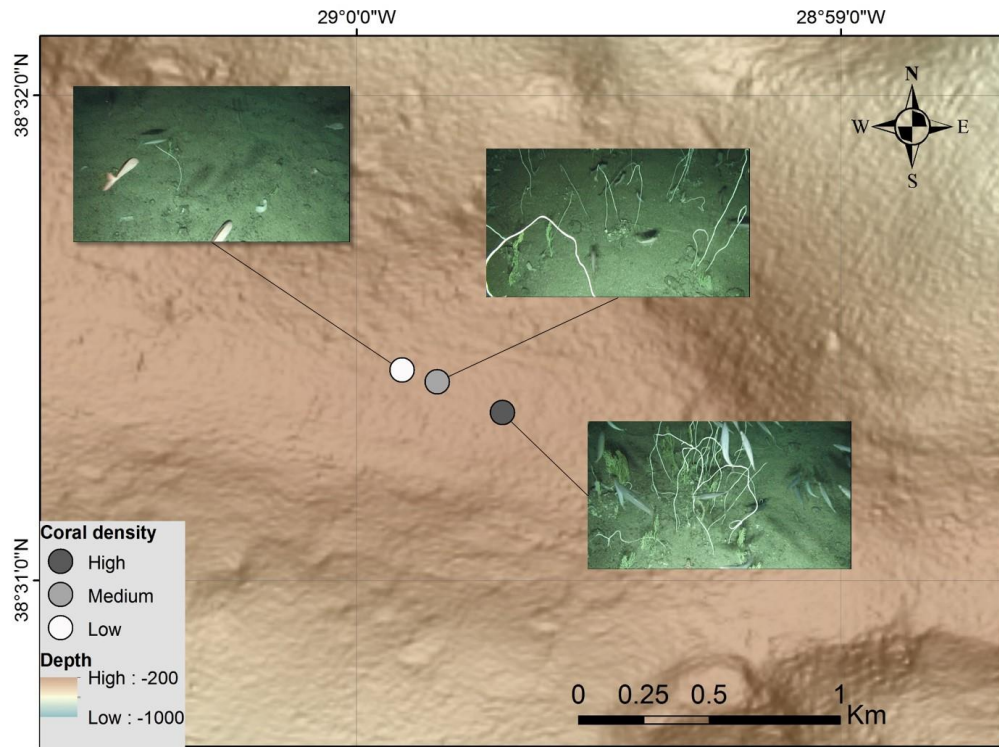


Figure 9 · Locations of coral landers deployed in 2016 at the summit of Condor Seamount, in three sites with varying coral densities (low, medium and high) at ~230m depth.

The recovery of the landers was initially planned to take place after 12 and 24 months of deployment, however due to the advanced corrosive damage of the landers, these were collected in March and August 2017 (8 months and 13 months after deployment). We were not able to retrieve one lander in the high density and long exposure (13 months) group. This lander was accidentally found and recovered in 2019. Immediately upon recovery, living coral fragments were photographed for growth rate assessment and subsequently stored in liquid nitrogen for enzymatic cellular biomarker analysis.

Pilot study 2: Coral transplantation

During February and June 2017, IMAR-UAz recovered coral specimens that were accidentally caught during longline and hand-line fisheries by the local fishermen and fisheries observers in the Azores EEZ from a depth range of 180 to 700 m depth (Figure 5, reported in Milestone 13). All the corals were maintained onboard in a cooler box with chilled seawater. Upon arrival to shore they were transferred to the Deep-Sea Lab where they are maintained in aquaria. The exception was for the octocoral

D. aff. meteor, for which fragments from colonies collected with an ROV were used for transplantation because of the low number of bycaught corals of this species.

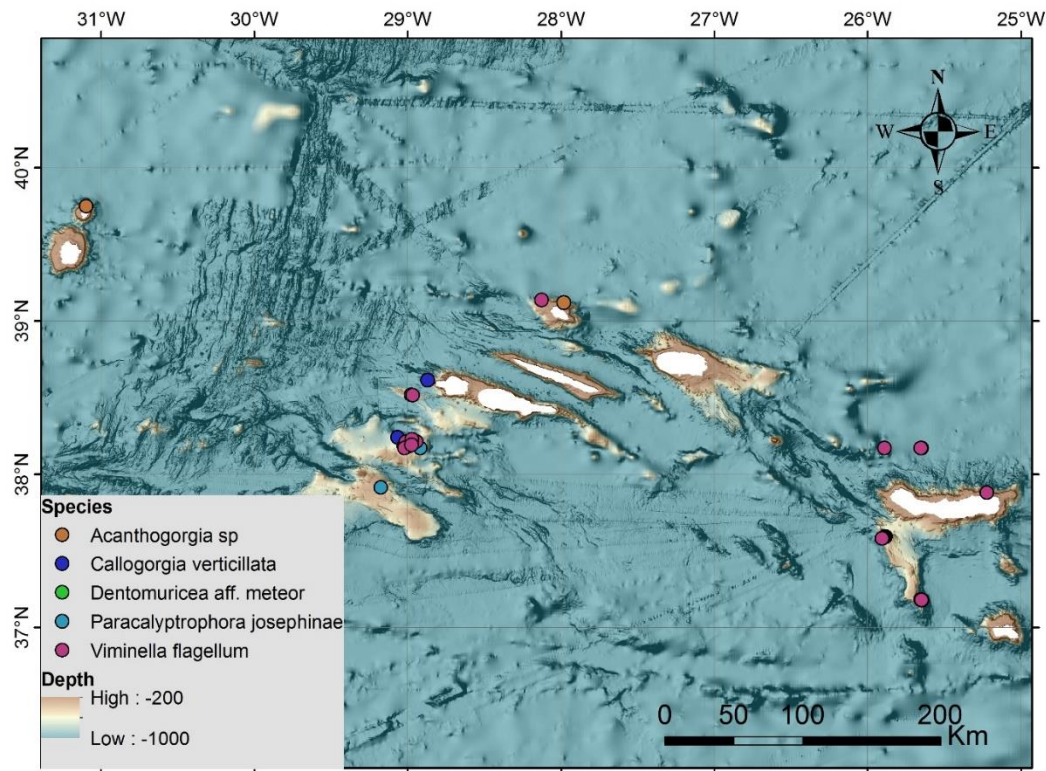


Figure 10 · Location of corals collected as bycatch during longline and hand-line fisheries by the local fishermen and fisheries observers.

The corals used in this transplantation study were chosen based upon the native coral species that can be found on Condor seamount and survivability of corals in aquaria. The selected species were the octocoral species - *Acanthogorgia armata*, *Callogorgia verticillata*, *Dentomuricea aff. meteor*, *Paracalyptophora josephinae* and *Viminella flagellum* (Figure 7). Collected colonies were fragmented in the DeepSeaLab yielding a total of 250 coral fragments from 66 colonies (Table 3).

Table 3 · Coral species maintained in the Deep-Sea Lab and total number of fragments used in pilot study 2.

Coral species	Number of coral colonies maintained in Deep-Sea Lab	Total number of fragments used in the transplantation action
<i>Acanthogorgia armata</i>	4	12
<i>Callogorgia verticillata</i>	7	72
<i>Dentomuricea meteor</i>	9	168
<i>Paracalyptrophora josephinae</i>	2	24
<i>Viminella flagellum</i>	44	96

Coral nubbins were deployed in October 2017 on the High Density site selected for pilot study 1 (see Figure 9), using 11 landers constructed with plastic to avoid corrosion, (Figure 10). The landers were deployed by hand from a vessel using a 3m long metal bar where we attached 3 structures at a time (Figure 11). The bar was connected to an acoustic releaser that would liberate the structures at the bottom, a camera system to document the deployment and a pinger that was transmitting the position during the deployment. In addition, 13 settlement structures carrying 25 basalt tiles (10 x 10 cm) each were deployed in the same sites as the landers. Landers were visited in August 2018 using the Lula submersible (Rebikoff-Niggeler Foundation) to assess the condition of the corals and take more precise coordinates of the lander positioning.

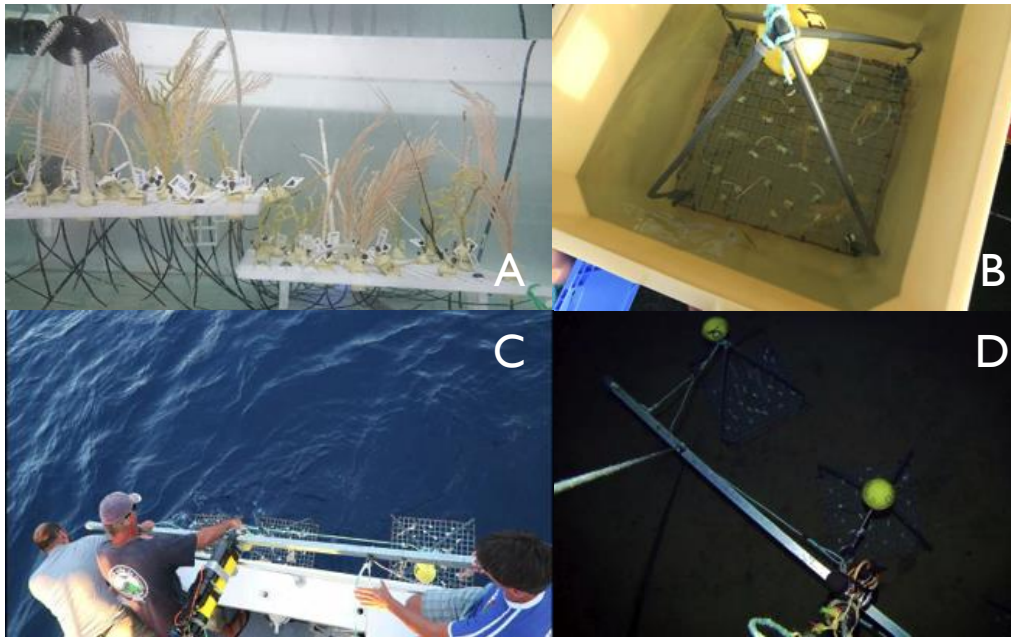


Figure 11 · (A) Fragments of different octocoral species after attaching to the epoxy base; (B) lander prepared for deployment; (C) and (D) Deployment structure including 3 coral landers, a metal bar and an acoustic releaser.

Seven landers were subsequently collected with the submersible in July 2019, while four landers could not be found. Coral survival was visually inspected from the photographs taken in 2018, while the survival in 2019 was directly counted from the recovered landers. Three settlement structures were collected together with the landers. Immediately upon recovery, living coral fragments were moved to chilled aquaria and subsequently transferred to DeepSeaLab facilities to obtain 3D photographs of the fragments. Small fragments of *D. meteor* were stored in liquid nitrogen for enzymatic bioassays and in 10% buffered formalin for reproduction studies.

Coral growth rate measurements

To estimate growth rates, each branch was firstly manually annotated (e.g. primary, secondary branch: Brazeau & Lasker 1988) and subsequently measured using ImageJ software (<https://imagej.nih.gov/>). The classification is done from the periphery of the colony towards the interior, ultimately reaching the base of the coral that is of a higher rank. The primary (P) branches are the peripheral branches which are classified first. Only when two branches of the same rank meet, the rank of the next branch changes (e.g. two primary make one secondary (S), two secondary make one tertiary

(T), two tertiary make one quarterly (Q)). Growth rates were then calculated by subtracting pre- from post-deployment linear measurements of coral branches based on photographs of the fragments taken before and after coral deployment. Some examples of the assessment of growth or absence of coral branches and recovery from injury based on photographs is presented in Figure 12. Growth was calculated using average branch length (no matter the classification) per fragment per year.

IMAR-UAz is also developing a new way of acquiring growth information from images, using 3D reconstruction. The objective is to assess architectural complexity, rugosity, volume, and other structural characteristics of cold-water octocorals, that play a significant role in habitat provision and ecosystem processes, but cannot be accurately measured from traditional 2D models (photographs). This method consisted in placing the coral nubbin in a rotation plate inside an aquarium. The rotation of the plate was triggered through a series of magnets below the aquarium.

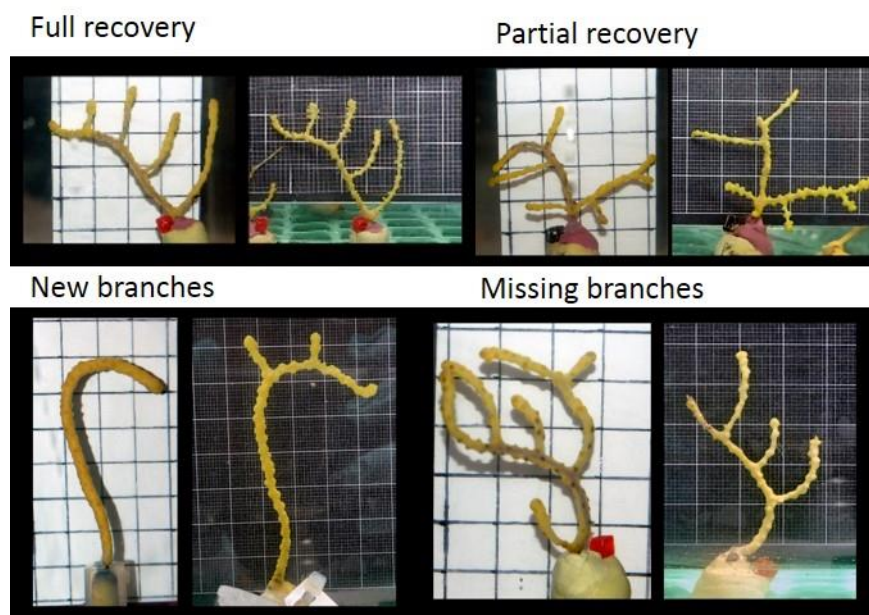


Figure 12 · Assessment of coral growth and recovery from injury in pilot study 1.



Figure 13 · Set-up for 3D image acquisition of transplanted coral nubbins.

A camera outside the aquaria took approximately 100 images of the coral nubbin in 20 sec, which was enough time for a full round of photographs of the coral (Figure 13). However, the analyses are still underway and thus results reported here were based on 2D images of the coral nubbin in the exact same position in the aquaria before and after deployment. Fragments of the octocoral *Acanthogorgia* sp. (n=3) were not included in the growth analysis since it was difficult to define branches from the photographs.

Preparation of tissue extracts for antioxidant biomarker essays

Frozen coral samples were homogenized and prepared for antioxidant biomarker essays (Martins et al., 2017). The homogenate was centrifuged at 16000g for 30 min at 4 °C and enzyme activities were measured in the supernatant fraction. All enzyme assays were tested with commercial enzymes obtained from Sigma® and each sample was run in triplicate (technical replicates). Enzymatic activity of glutathione peroxidase, superoxide dismutase, and lipid peroxidation were used to evaluate coral physiological condition and capacity to recover from the stress of transplantation after deployment in natural conditions.

Processing of settlement tiles

Upon recovery of the settlement structures, basalt tiles were removed and individually placed in the containers with 30 % ethanol. For each tile, we removed all invertebrate specimens manually, enumerated them, assigned them to Phyla level based on descriptions in Hayward & Ryland (1990) and preserved in 95% ethanol for further investigations.

Statistical analysis

For all statistical analyses, we followed the procedure described by (Crawley, 2007). Data exploration of each dataset was done according to (Zuur et al., 2010). To study the effect of the independent variables on the dependent variables in question, first were added progressively in the respective model and the Akaike Information Criterion (AIC) along with maximum likelihood ratio (MLR) tests were used to select the most appropriate model. Model diagnostics were inspected to ensure that there were no violations of the model assumptions. Afterwards model summaries were inspected to reveal statistical differences among levels of the significant treatments. In order to analyze coral survival the survival of each coral fragment was taken into account as a binomial variable (1-survival, 0-no survival) and Generalized Linear Models (GLMs) were used to model it. Coral growth was also modelled with GLMs. More specifically, we used generalized linear models to test for differences in the survival of *D. meteor* in different coral density sites, treatments and time after deployment in Pilot Study 1; and to test for differences in survival and growth rates for transplanted octocoral species and time after deployment in pilot study 2. Sites, treatments and time were treated as fixed effects in the statistical models for pilot study 1. Species and time were treated as fixed effects in the statistical models for pilot study 2. These were added progressively to the respective models and the Akaike Information Criterion (AIC) was used to select the most appropriate model. For growth rates measured in Pilot study 1, we separated the data by time and analyzed each time in separate to increase degrees of freedom for a small dataset. For the lander collected in September 2019 we solely compared response variables between different treatments. Statistical analyses were performed in the R statistical computing environment version 3.5.0 (R Development Core Team, 2008).

A permutation multivariate analysis of variance (PERMANOVA; Anderson et al. 2008) was used to test for the influence of the different treatments and coral density sites on the concentrations of the antioxidant mechanisms of *D. meteor*. The analyses were conducted using the software PRIMER 6 & PERMANOVA using a resemblance matrix based on Euclidean distance (Anderson et al., 2008). The PERMANOVA was run using 9999 permutations to produce p values using PERMANOVA to produce p values using the Monte Carlo method. When the main test produced a significant result ($p < 0.05$), a pairwise test was conducted to identify the individual differences between treatments.

Evaluation of the success of the restoration studies

The success of the restoration study was evaluated using the goals, objectives and indicators described in Table 4.

Table 4 · Ecological goals, objectives and indicators for evaluation of assisted restoration of coral gardens in Condor seamount 1-2 years after coral transplantation

Target	Ecological Goals	Objectives	Indicator
reference ecosystem	re-establishment of dominant octocoral populations in 15 y	transplanted CWC survive – at least 85% survival after 1-2 years	survival rates of transplanted octocoral species
		increase in size of transplanted octocorals – linear growth more than 1 mm	length of octocoral colonies
	re-establishment of genetic diversity of dominant octocoral species after 10 years after transplantation	presence of reproductively active colonies after 1-2 years	presence of gametes, evidence of spawning

Target	Ecological Goals	Objectives	Indicator
		presence of coral recruits in artificial substrates after 2 years;	number of coral recruits
	reinstatement of diversity and biomass of fauna associated with dominant gorgonian species within 10 years after transplantation	increase in the number and diversity of fauna associated with transplanted octocoral species; presence of associated fauna after 1-2 years	number and diversity of epibenthic macrofauna species and vagile fauna (e.g. fish, crustaceans)

2.2.3 Results

Pilot study 1

Survival rates

Survival rates differed significantly among treatments and coral density sites, but not between times of deployment (8 and 13 months) (Table 4, Figure 14). Survival rates were significantly higher in the “High” and “Medium” coral density in comparison with “Low” coral density for all experimental conditions (GLM, $p=0.002$). Maximal survival rates (85%) were recorded for non-injured coral nubbins (Control and Cu-intoxicated treatments, Figure 14) for 8 and 13 months deployment times in the “High” density coral site. Coral survival in the injured and in the combined injury and Cu-intoxication treatment in these sites were 1.5 to 2-times lower than in other treatments (30-50% survival) (GLM $3 \times 10^{-5} < p < 0.04$). Survival rates in the “Low” coral density site were below 50% for Control and injured treatments after 13 months exposure and no coral survived under the Cu-intoxicated treatment after 8 months deployment and in combined injury and Cu-intoxication treatment after 13 months of coral transplantation. After 36 months deployment, coral survival in the “High” coral density site was overall lower than for other times of deployment, particularly for the control treatment where

coral survival was almost half of 8-13 months deployment (50% compared with 80% survival).

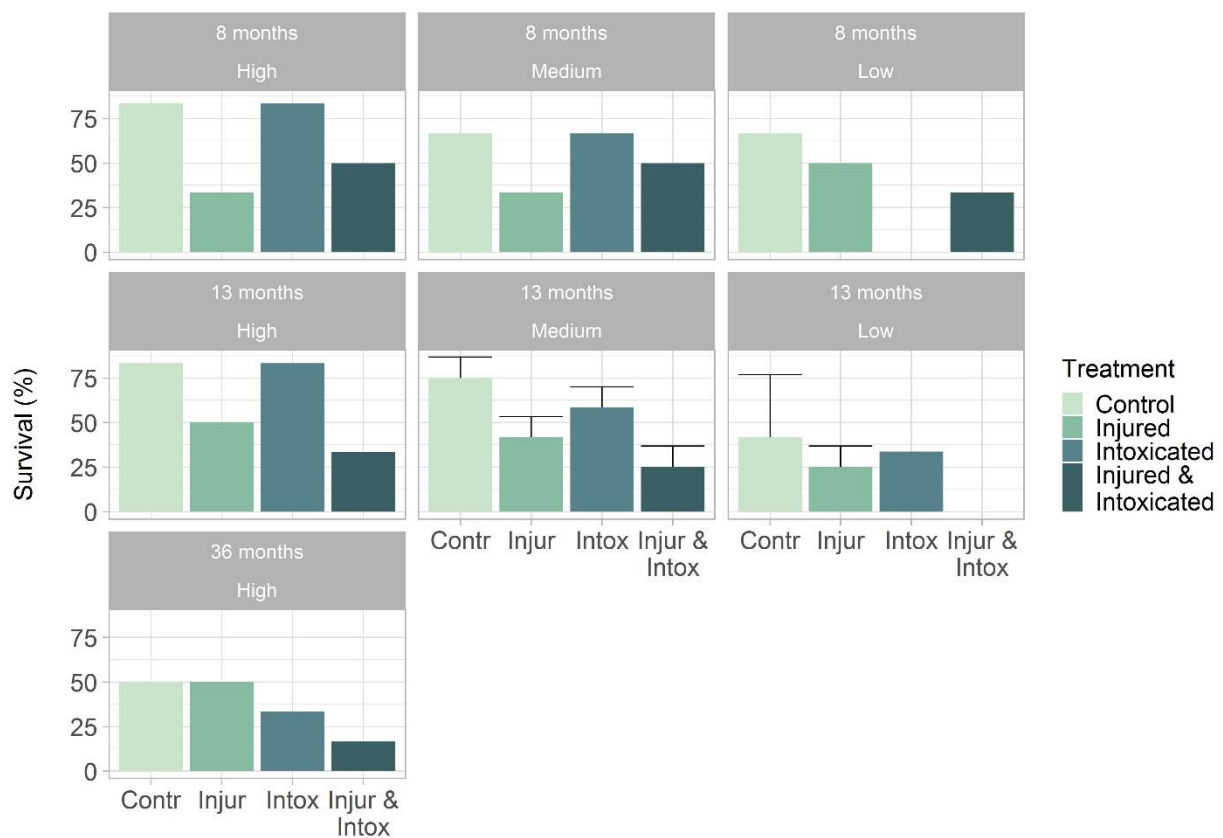


Figure 14 · Survival rates of the octocoral *Dentomuricea aff. meteor* for different coral density sites (High, Medium, Low), treatments, and times of deployment (8, 13 and 36 months during Pilot study 1 in Condor Seamount).

Growth rates

Growth rates of corals recovered after 8 months deployment differed significantly between treatments and coral density sites (Table 4, Figure 15). Coral fragments in the “High” and “Medium” coral density sites generally increased in length, with the exception of corals in the “High” coral density site under the Intoxicated treatment which mainly lost coral branches (GLM, $p=0.01$, depicted as negative growth in Figure 15). Coral growth rates differed significantly in the “Low” coral density site in comparison to other sites (GLM, $p=4.07 \cdot 10^{-6}$), with corals showing signs of tissue paling or necrosis and loss of branches in all treatments, with complete absence of coral fragments in the Intoxicated treatment. After 13 months deployment, coral growth rates differed

between sites but not between treatments (Table 4, Figure 15), with a general increase in coral linear extensions in the “High” and “Medium” coral density sites and loss of coral branches in the “Low” coral density site. No corals survived in the combine Injured and Intoxicated treatment. There were no significant differences in rates of coral growth between treatments in the “High” coral density site after 36 months deployment (Table 4, Figure 15). Maximal coral growth rates ($60 \pm 39 \text{ mm} \cdot \text{year}^{-1}$) were recorded for corals in the Control treatment in the “High” coral density site.

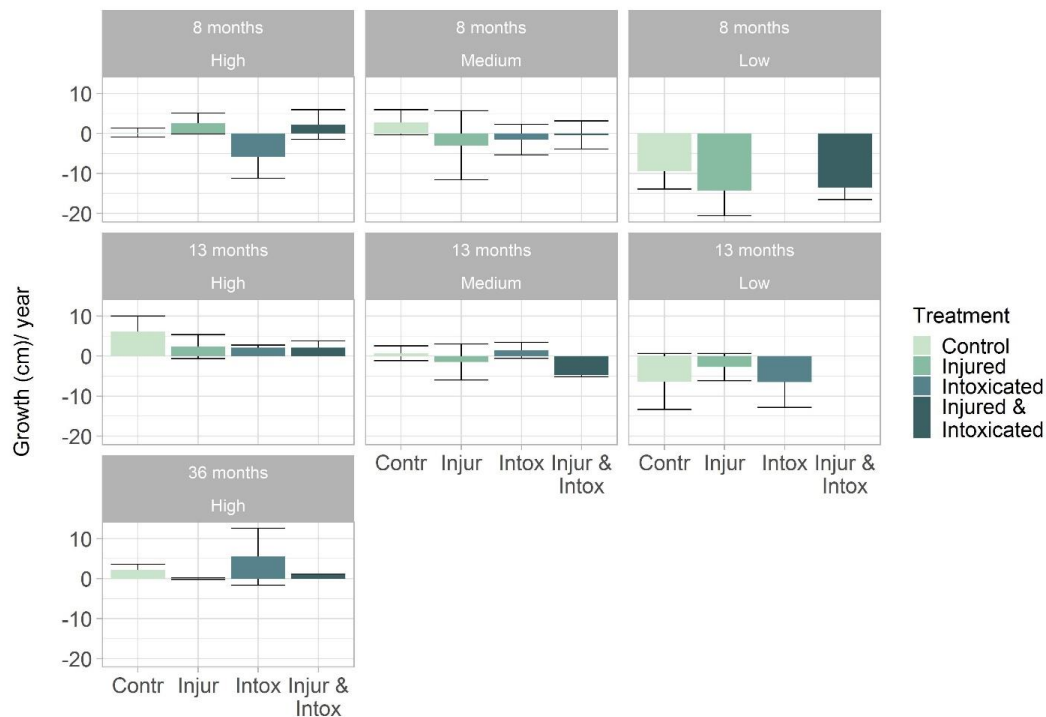


Figure 15 · Growth rates of *Dentomuricea .aff. meteor* for different coral density sites (High, Medium, Low), treatments, and times of deployment (8, 13 and 36 months during Pilot study 1 in Condor Seamount).

Physiological condition

Antioxidant biomarkers in *D. meteor* tissues were used to evaluate the degree of cellular stress induced by Cu exposure and physical damage and the coral capacity to recover from such induced stress after deployment in natural conditions. Results of antioxidant biomarkers essays (Figure 16) indicated that *D. meteor* responded to the induced stress by eliciting their antioxidant defense system (Figure 16A) and increasing the activity of the enzymes SuperOxidase Dismutase (SOD, Figure 16B) and Glutathione S-transferase (Figure 11A-C) involved in the antioxidant defense response against Reactive Oxygen Species (e.g. metal ions; lipid peroxidation). This

response was detected immediately after coral collection and acclimation to aquaria conditions (comparison between “wild” corals and T0 corals, PERMANOVA, $p < 0.05$). This response was reduced after 13 months deployment, particularly in the injury and Cu-intoxication treatments (PERMANOVA, $p < 0.05$). Nevertheless, high levels of Malondialdehyde (MDA, Figure 16D), an indicator of cellular oxidative damage, in the treatment with combined injury and Cu intoxication (Inj-Intox) were recorded after 13 months in natural conditions.

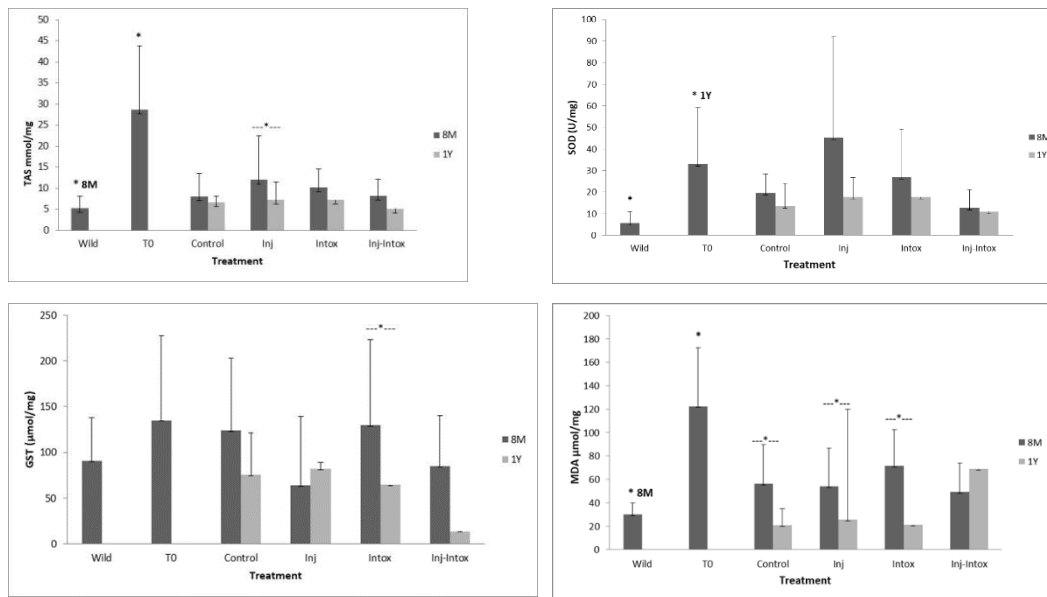


Figure 16 • Antioxidant biomarkers in the coral *Dentomuricea Meteor* tissues under different experimental treatments and after eight months (black bars) and one year (grey bars) deployment at Condor seamount. A. Total Antioxidant Status (TAS, mmol/mg; wet weight); B. SuperOxidase Dismutase enzyme (SOD, mmol/mg; wet weight); C. Glutathione S-transferase (GST, mmol/mg; wet weight); D. Malondialdehyde (MDA, mmol/mg; wet weight). Wild: fragments collected at Condor seamount; T0: fragments acclimatized at the lab and sampled before deployment; Control: deployed fragments with no treatment; Inj: deployed fragments with injury; Intox: deployed fragments after 48h exposure to 150 $\mu\text{g/L}$ Cu concentration; Inj-Intox: Injured fragments deployed after 48h exposure to 150 $\mu\text{g/L}$ Cu concentration. Symbol (*) indicates significant statistical difference among treatments (Permanova, pairwise test $p < 0.05$).

Pilot study 2

Survival rates

Survival rates of transplanted coral nubbins differed significantly among species and time of deployment (Figure 17). Survival rate decreased significantly between 10 and 21 months of deployment (2018 and 2019) (GLM, $p=0.004$) for all species, except *Acanthogorgia* sp., which had a survival rate of 100% from both time periods, although this was based on only three transplanted coral fragments. *Viminella flagellum* had the highest survival for both deployment times (90 and 75% after 10 and 21 months, respectively) and in comparison the other species (GLM $p<2\cdot10^{-16}$). *Dentomuricea* aff. *meteor* presented the second highest survival rates (52 and 45% after 10 and 21 months, respectively) and it was significantly different from all other species (GLM $p<0.0003$). Fragments of the large structuring octocorals *Callogorgia verticillata* and *Paracalyptrophora josephinae* presented less than half lower survival rates than other species but did not differ between them (30 and 15% after 10 and 21 months, respectively).

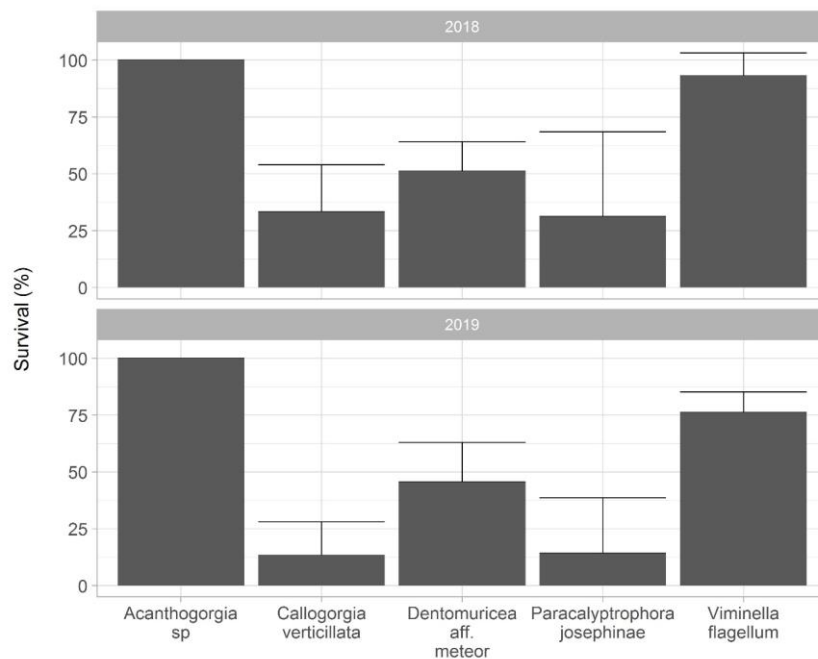


Figure 17 · Survival rates of coral nubbins of different octocoral species used in the transplantation study 2. Values are mean and standard deviation.

Coral growth rates and structural complexity

Coral growth was evaluated in terms of linear growth rates of coral branches and changes in structural complexity of the transplanted fragments. *Dentomuricea* aff. *meteor* showed an increase in structural complexity, based on the increase of all branching orders before deployment and after 21 months deployment (Table 5). In contrast, both *C. verticillata* and *P. josephinae* showed a decrease in structural complexity related to loss of branches.

Table 5 · Branch ratio for the different species used in pilot study 2, before and 2 years after deployment.

Species	Branch ratio 2017	Branch ration 2019
<i>Paracalytrophora josephinae</i>	16P : 5S : 1.5T : 0.5Q	13.5P : 4S : 1T
<i>Callogorgia verticillata</i>	67.2P : 7.8S : 2T : 0.6Q	37.2P : 4.6S : 1.6T : 0.2Q
<i>Dentomuricea</i> aff. <i>meteor</i>	11P : 4S : 0.7T : 0.02Q	10.5P : 5.3S : 1.1T : 0.02Q

Growth rates varied significantly among species (Table 6, Figure 18). *Viminella flagellum* presented the highest growth rates ($11 \pm 20 \text{ mm} \cdot \text{year}^{-1}$) of all transplanted species (GLM, $p=0.04$). *Dentomuricea* aff. *meteor* did not present measurable growth, resulting from a higher loss of coral branches than growth of coral branches. Fragments of both *C. verticillata* and *P. josephinae* showed only the loss of branches with no addition of new branches or growth of existing branches, represented in Figure 18 as negative growth.

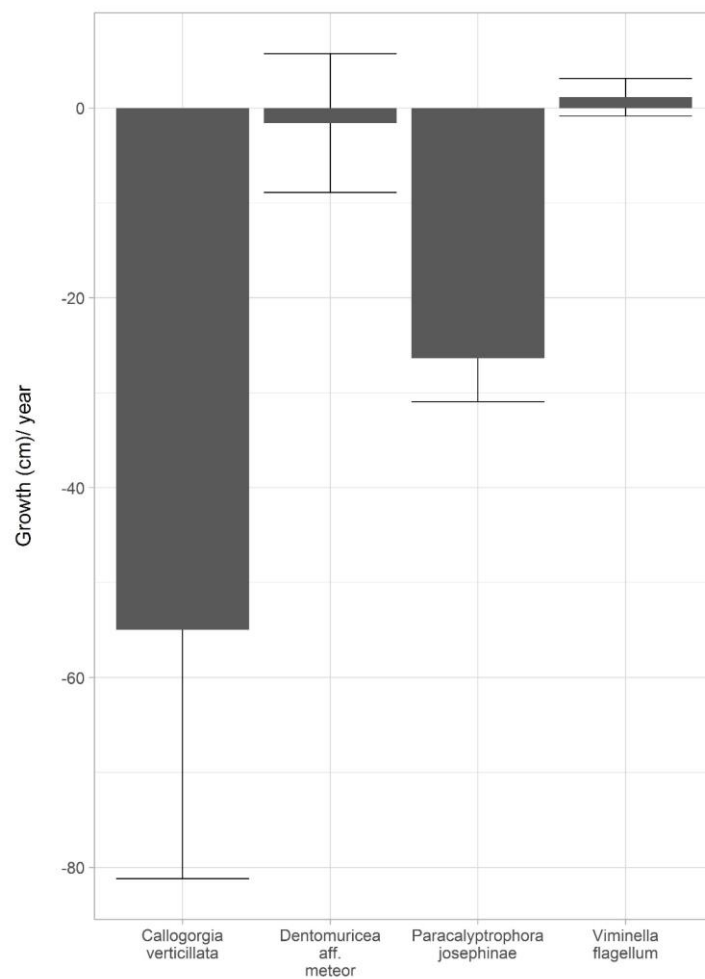


Figure 18 · Growth rates of coral fragments for the different species of octocorals transplanted in pilot study 2. Values are mean \pm standard deviation.

Associated fauna to transplanted corals

The capacity for transplanted octocorals to attract mobile and sessile fauna was assessed through in situ photographs of the landers and examination of corals in the laboratory. Associated fauna included small crustaceans, including anemones, hermit crabs and spider crabs (*Anamathia* sp.), nudibranch tritoniid gastropods, ovoidae gastropods, polychaetes and some fish, such as *Helicolenus dactylopterus* (Figure 19). In some cases, fauna was found on the lander after recovery, not directly on the corals making it difficult to evaluate if fauna is attracted by the corals or the lander structures.

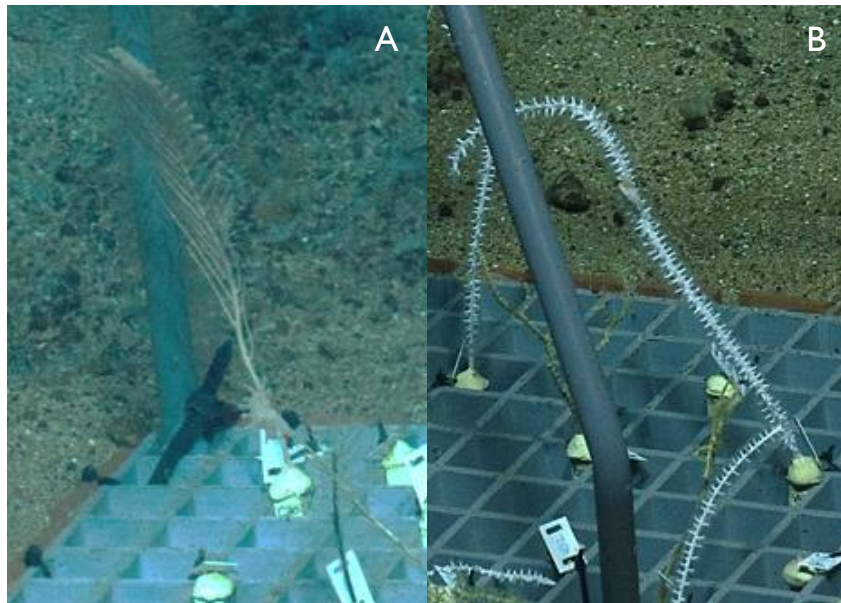


Figure 19 · Examples of mobile fauna associated with transplanted corals. (A) Nudibranch tritoniid gastropod; (B) ovolidae gastropod; (C) spider crab; (D) fish.

Deployment of artificial substrates

Settlement plates made of basalt recovered after 21 months deployment presented very little fauna colonization and none of the targeted coral species (Figure 20). Taxonomic analysis of the fauna is underway.



Figure 20 · Fauna found on the settlement plates after 21 months deployment. (1) Hydrozoa sp.1 (2) Hydrozoa sp.2 (3) Crustacea sp.1 (4) Gastropoda sp.1 (5) Cnidaria sp.1 (6) Polycheta sp. 1 (7) Bivalvia sp.1 (8) Hydrozoa sp.3 (9) Hydrozoa sp.4 (10) Polycheta sp.2 (12) Gastropoda sp.2.

Evaluation of the success of the restoration action

The results obtained in this study are evaluated against the initial objectives that were set to be possible after 2 years of coral transplantation in Table 6. The 50% threshold set for rates of survival of transplanted coral fragments were only achieved for the small to medium sized and/or less structurally complex coral species, *D. meteor*, *Acanthogorgia* sp and *V. flagellum* but not the larger more complex species *C. verticillata* and *P. josephinae*. Linear growth rates were measurable only for *D. meteor* in pilot study 1 and *V. flagellum* in pilot study 2. Some of the set objectives were however not achieved. Transplanted *D. meteor* fragments showed no gametes after 13 months deployment in pilot study 1, while these are still under evaluation for other species transplanted in pilot study 2. Furthermore, no coral recruits were observed on basalt settlement plates after nearly 2 years of deployment. The capacity for transplanted corals to attract associated mobile fauna was also difficult to assess because it is difficult to know if fauna is attracted by the corals or the lander structures.

Table 6 · Evaluation of the success in accomplishing the objectives set for a 1-2 year period after coral transplantation.

Case study	Objectives for 6 year period	Results	Evaluation
Condor seamount	transplanted octocorals survive – at least 50% survival	<i>Dentomuricea meteor</i> 85% survival after 13 months; 50% survival after 36 months deployment in pilot study 1; <i>Acanthogorgia</i> sp. 100% survival; <i>Viminella flagellum</i> 75% survival after 21 months deployment in pilot study 2	<i>Acanthogorgia</i> sp. results were based on the transplantation of only 3 coral fragments. Failure in accomplishing this target for the other 3 species transplanted. Success of transplantation depends on the species
	increase in size of transplanted octocorals – more than 1mm after 2 years	<i>D. meteor</i> 0.02±0.2 -60±39 mm/year increase in size after 13 months in pilot study 1; <i>V. flagellum</i> 11±20 mm/year increase in size	objective achieved for 2 species, but not for the other 3 species studied. Large variability in growth rates between fragments

Case study	Objectives for 6 year period	Results	Evaluation
		after 21 months deployment in pilot study 2	
	presence of reproductively active colonies	no gametes present for <i>D. meteor</i> in pilot study 1; still under evaluation for pilot study 2	not achieved for pilot study 1; under evaluation for pilot study 2
	presence of coral recruits in artificial substrates after 2 years	no coral recruits observed	objective not achieved
	increase in the number and diversity of fauna associated with transplanted octocoral species	some fauna present	objective achieved but difficult to know if coral or lander effect

2.2.4 Discussion

Restoration protocols tested in the Azores were based on techniques developed for tropical coral reefs and Mediterranean red coral populations, whereby transplants of small to medium size coral fragments from adult donor specimens are transplanted to impacted areas (Rinkevich, 1995; Linares et al., 2008). Results from both of our pilot studies indicate that coral transplantation is a viable technique to restore coral gardens impacted by fishing or other anthropogenic impacts. However, the survival of transplanted corals depends on the octocoral species, the condition of coral colonies (intact vs. injured or damaged colonies) and the location of the restoration action. Corals were better able to recover from copper intoxication and injuries in areas with high to medium coral densities than in low coral density areas. This suggests that our low coral density area probably had less favorable conditions for coral survival and growth, e.g. current speed, food availability, than the other sites with higher coral cover. Thus, knowledge of the oceanographic conditions and abundance of natural

food are likely important for the selection of the site where the restoration action will take place.

In our study, transplants of large, more structurally complex colonies of the octocorals *Callogorgia verticillata* and *Paracalyptophora josephinae* had low survival rates than other smaller or less complex coral species. Variation in mortality among coral taxa was also reported for transplantation studies conducted with deep-sea corals in the Pacific (Boch et al., 2019). There, transplants of larger more arborescent coral species (e.g. *Paragorgia arborea*, *Sibogagorgia cauliflora*) presented lower survival rates than fragments of smaller or less ramified coral species. These differences in survival rates among species were suggested to be related to high sensitivity of species at the point of attachment with tissue sloughing followed by breakage. This could have been also likely the case for *C. verticillata* and *P. josephinae*, which presented more rigid main axis making them more susceptible to breakage during the lander deployment or due to natural disturbances such as fish movement. Loss of transplants during deployment due to detachment and breakage has been evoked as a major cause of initial loss of transplants in the other studies in the Mediterranean (e.g. Linares et al., 2008; Montseny et al., 2019).

Transplantation studies at shallow and mesophotic depths in the Mediterranean have also reported differing transplant survival among coral taxa. Transplants of *Viminella flagellum* *Acanthogorgia* sp. in the present study showed comparable survival rates (75-100% after 2 years) to *Eunicella cavolini* transplants approximately 1 year after deployment depth Cap de Creus (Montseny et al., 2019) and *Corallium rubrum* 4 years after transplantation (about 99.1%) (Montero-Serra et al., 2018). Survival rates of *C. verticillata*, *P. josephinae* (15-30% after nearly 2 years) and *D. meteor* (45-50% after 2-3 years) were more comparable to survival rates of transplanted *Eunicella singularis* (35–45% survival after 1 year), *Eunicella verrucosa* (30% survival after 1 year) and *Paramuricea clavata* (35–50% survival after 1 year) (Linares et al., 2008; Fava et al., 2010; Montero-Serra et al., 2018).

In addition to mortality or loss transplants during deployment, differing mortality rates of transplants reported above could be related to the intrinsic physiological sensitivities of the differing taxa and the ability to cope with environmental variations. Our cellular

biomarkers essays in *D. meteor* show that corals elicit a stress response when removed from their natural environment and are placed under aquaria conditions before deployment back in the field. Results suggest that *D. meteor* transplants were able to return to baseline levels of the stress response after 1 year. However, the physiological responses may vary between coral taxa and likely not all taxa can overcome stress caused by fragmentation and transplantation.

Variations in the survival rates of *D. meteor* between pilot study 1 (85-50% survival after 13 to 36 months, respectively) and pilot study 2 (52-45% survival after 10 to 21 months) suggest that other factors may also influence the success of the transplantation. Coral fragments in pilot study 2 were kept in aquaria for a longer period of time, which may have influenced their physiological condition at the time of the transplantation. The deployment method was also different between the two pilot studies, with deployment with an ROV in pilot study 1 likely being less stressful on corals than deployment of landers from the vessel by hand. Therefore, it is recommended that corals are kept out of their natural conditions for the shorter possible time with transplantation of bycaught corals requiring a higher number of transplants to account for mortality due to deployment caused stress.

Artificial structures made of basalt deployed as a method of enhancing coral recruitment in impacted areas were very poorly colonized with no presence of coral recruits after almost 2 years of deployment. These results are in line with other colonization studies using artificial substrates which showed low colonization after 4 years of substrate deployment at 600-800 m depth in the Northeast Channel Coral Conservation area, off Canada (Lacharité & Metaxas, 2013; Girard et al., 2016). These studies showed very low recruitment by corals, with varying success among different taxa likely related to their life history traits (Lacharité & Metaxas, 2013). The octocoral *Primnoa resedaeformis* presented higher number of recruits in comparison with *Paragorgia arborea* which was suggested to be related to divergent reproductive modes, i.e. *P. resedaeformis* is a broadcast spawner with wider larvae dispersal and *P. arborea* as a brooder and lower larval dispersal capacity. Moreover, studies with *Oculina variciosa* have shown very little coral recruitment or even no recruitment at all, after 5 years from the deployment of the concrete modules (Brooke & Young, 2003).

This points out to the need of better knowledge of life history stages of coral taxa that need to be restored.

The differing recovery capabilities of the coral taxa used in the transplantation studies together with the lack of recruitment by coral larvae in artificial substrates points out to the need of using a combination of assisted and natural restoration approaches. Assisted restoration approaches are best used at local scales for the species that show good high survival and growth rates, while natural restoration approaches (e.g. fisheries closures, marine protected areas) at the large scale will ensure removal of as much pressure as possible from the area while ensuring the long term recovery of individual native species that cannot be transplanted. An option to be considered would be to have several small local restoration sites with transplanted corals that would be connected by oceanographic patterns (currents) and would ensure natural seeding of the coral populations.

2.3 Feasibility of fish transplantation for restoration of deep-sea fish stocks in areas impacted by fishing

2.3.1. Introduction

Reintroduction or translocation is an established approach to manage endangered species (Seddon et al., 2007). Fish translocation, in particular, has been widely used to re-establish populations of threatened species or re-stocking exploited species in freshwater and shallow coastal environments (e.g. Ebner et al., 2009; Sard et al., 2016). Yet, these actions have not been previously conducted on deep-sea fishes, and the potential of this tool to support management and conservation of deep-sea fish biodiversity and fisheries remains virtually untested.

The blackbelly rosefish, *Helicolenus dactylopterus*, is a widely distributed Atlantic and Mediterranean deep-water rockfish. It is a sit-and-wait bottom predator feeding on benthic crustaceans and fishes (Neves et al., 2012). In the Azores, it is abundant on island slopes and seamounts from 200 to 1000 m depth (Abecasis et al., 2006), often associating to Vulnerable Marine Ecosystems (VMEs) of coral gardens and sponge grounds, especially the juveniles (Pham et al., 2015). As many other deep-sea fishes, blackbelly rosefish is a long-living, slow-growing, low-productivity species. In the Azores it reaches 49 cm total length and at least 32 years (Abecasis et al., 2006) but other studies indicated a 49 years longevity (Allain Lorange, 2000). Sexual maturity is only reached after five to seven years at an average size of 21 cm for males and 26 cm for females (Isidro, 1987). The reproductive strategy of this species, zygoparous oviparity, is also peculiar and complex: after mating, supposedly with internal insemination, the sperm is stored inside the female's ovaries until fertilization, up to seven months later (Mendonça et al., 2006), with egg deposition at an intermediate embryo developmental stage. Therefore, the spawning season can last for almost a year. In the Azores, this corresponds to a mating period of June to December, as indicated by the male maturity stages, and a (female) spawning season from December to March (Mendonça et al., 2006; Sequeira et al., 2012).

Blackbelly rosefish has a substantial role in providing ecosystem services across its range and, specifically, in the Azores region. First, it ranks second in importance for the main Azorean fishery, the multispecific deep-sea demersal fishery with hooks-and-

lines. Second, as this is one of the most abundant benthic predators in Azorean demersal ecosystems (e.g. Menezes, 2003), it is believed to substantially contribute to maintain the ecosystem health via top-down control of the food-web, as well as by supporting the higher trophic levels in these habitats (e.g. sharks, wreckfish, eels). The Azorean population is relatively isolated from other populations in the Atlantic, including those from Cabo Verde, Madeira, and the European or NW Atlantic continental shelf slopes, due to the isolation-by-distance and hydrographic conditions that limit larvae mixing between these regions (Aboim et al., 2015). The reduced chances of mixing may also be reinforced the reduced planktonic larval duration (due to the oviparity) and the putative sedentary behaviour of this species.

These characteristics render the blackbelly rosefish particularly vulnerable to overfishing. Indeed, the intensive fishing supported by this populations in the last three decades in the Azores has led to concerns regarding the sustainability of the fisheries and the integrity and resilience of the rosefish population and its habitats. There are also wide evidences that populations intensively exploited for longer, such as the Mediterranean populations, have already suffered population-wide effects, including a substantial reduction in maximum size. In the Azores, this species is also frequently discarded at sea, especially because of the legal Minimum Size Limit (MSL). Thus, it is an excellent candidate to evaluate the potential of translocation as a tool to help conserving the local population as well as promoting a sustainable fishery via the net benefits of spatial protection where healthier, reproductively productive sub-populations may contribute to the overall fishery productivity.

This work aimed to test, for the first time, the use of fish restoration approaches to deep-sea fishes and previously fished seamounts, such as many Azorean seamounts. Specifically, we tested the applicability of translocating fished blackbelly rosefish from fished areas into a protected area, the Condor Seamount MPA. We used an experimental approach with acoustic telemetry to follow the fate of the tagged and released fish along time. This is also the first study ever to characterize the movements and residency of this species using electronic tagging.

2.3.2 Material and Methods

We conducted this experiment at the Condor seamount marine reserve. The Condor Seamount is located 17 km off Faial island with depth ranging 200 m to 1700 m (Figure 22). It is a long and narrow flat summit oriented East-West, characterized by hard substrate mixed with soft sediments, while slopes are mainly unconsolidated sediments. The bottom temperature varies between 12-16 °C (see detailed description in section 2.2). Being very close to shore, the seamount was intensively targeted by the local demersal fleet since the 1980's, using both bottom longline and handline down 600 m depth. Blackbelly rosefish was one of the main catches alongside blackspot seabream (*Pagellus bogaraveo*). A no-take area (except tuna fishing) of 242 km² surrounding the seamount was established in 2010 for scientific study purposes, and later incorporated in the Azores Marine Park in 2016. At present, it is still the only Azorean seamount, in addition to the Formigas shallow, closed to fishing.

Experimental design, fish capture and tagging

We tested the feasibility of translocating rosefish by comparing the survival rates and habitat use/residency of translocated versus non-translocated individuals. The hypothesis was that translocated fishes would not differ in both metrics, thus allowing the future translocation (under certain conditions) by fishermen of unwanted catch (e.g. undersized or over-quota) to closed areas as a possible restoration tool. We used long-term acoustic telemetry to verify the fate and the movements of fishes caught at a fished site – the experimental group – versus that of fishes caught at the Condor seamount – the control group - after releasing them on the seamount. The fish were tagged with long-lasting acoustic transmitters and their presence monitored over a year (14 months) using a network of acoustic receivers moored at the summit and flanks of the seamount. This (sub) network is part of a larger network that also includes other neighbouring seamounts as well as the flanks of Faial and Pico island. The site of release and placement of additional acoustic stations (see below) was first informed by preliminary analyses of video transects, where preferred habitat conditions for rosefish were evaluated.

Fish were caught using bottom fishing. We targeted larger individuals because 1) laboratory trials with dead specimens showed that >30 cm TL individual were needed to guarantee enough space in the abdominal cavity necessary to accommodate the

smaller commercially available transmitters and respect a maximum 2% of total weight rule, 2) larger individuals are generally recommended for translocation studies, as they are believed to be more resilient to environmental stress and predation mortality, and 3) by using sexually mature fish we could increase the potential for larval subsidy to other areas and genetic diversity on the local population (Leber, 1995, del Mar Gil et al., 2015), ultimately reaching the objectives of the restoration quicker.

The experimental group was collected at 325-515 m depth on the north slope of Faial island on 07.06.2019 by bottom longlining onboard the RV Arquipélago as a part of the annual demersal fisheries cruise. The fish were unhooked upon capture and immediately transferred onto a 700L tank filled with surface seawater onboard a fast RHIB equipped with a (Figure 22). All fish were vigorous and seemed to be in a good condition. After capture, we moved to Condor seamount (30 km), the journey lasting 2 h with occasional stops to partially replace the tank's seawater. The control group was collected on 04 to 05.07.2019 on the eastern part of Condor seamount summit. Initially, we deployed a small bottom longline (200 hooks) from a small commercial boat (Figure 22). However, this proven to be unproductive (only one rosefish vs. 54 bycatch fish) and we therefore changed to handlining (3 hooks) in order to maximize the effort and reduce the bycatch, especially given the restrictions of fishing in the protected area. During next two days we collected 26 rosefish with almost no bycatch. After capture, the control fish were maintained in the same tank as experimental fish for the amount of time.

We tagged 20 fish in each group. Each fish was first checked for vitality signs before tagging, measured and tagged by surgically inserting an acoustic tag (Vemco V9-2H, 9 mm diameter) in the abdominal cavity. During surgery, fish were kept in tonic immobility in a V-shaped cradle with constant flow of seawater running through the gills. We didn't use anaesthesia as we wanted to minimize surface time and predation upon release. Incision was closed using a catgut absorbable suture. The surgery was performed by experienced scientists following well tested methodologies (e.g. Afonso et al. 2009). After a short (max 20 min) period of observation on a smaller tank (200 L) to check for recovery signs (Figure 22), fish were released at the control site by lowering them to a depth of 150 m (about 50-80 m over the summit) using a barbless hook and heavier weight on the fishing (Figure 22). This way we minimized the

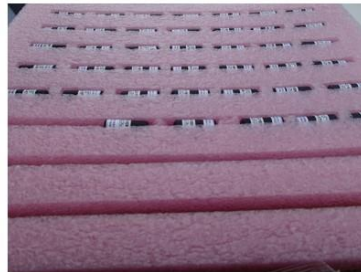
probability of mortality due to predation while increasing the chances of the fish landing on the finer-scale detection array and we made sure the fish were released in the middle of the receiver (see below). Time between capture and release of individuals varied between 2 and 5 hours for both groups.

To monitor the presence of the tagged fish, we used Vemco VR2 acoustic receivers which are part of the Azores Acoustic Tracking Network. The receivers continuously listen to the presence of the tags detecting any fish within a radius of ca. 500 m (Afonso et al. 2012) and log the tags' identity and time stamp, which can later be retrieved by physically downloading the receivers memory. The receivers were moored ca. 3 to 5 m above the seafloor on a nylon rope mooring with floats ca. 1.5 m above it. We used either VR2AR receivers with integrated acoustic releasers or VR2W receivers with Edgetech ORE releasers. The monitoring was two-phased: on phase 1, during the first three months (June-August 2019), we monitored the fine-scale habitat use of tagged fish around the release area using five closely spaced receivers on a 'fine array' together with monitoring eventual broader movements with another four receivers along the seamount summit (the 'broad array'). Additional manual monitoring was conducted monthly around the mooring array with a manual receiver and hydrophone (VR100) to detect fish that could be outside of the array listening range. On phase 2, from November 2019 to July 2020 (another 8 months), we monitored the fishes' residency and broad movements by using only the five receivers along the summit (the 'broad array') complemented with another two receivers on the western and eastern sections of the southern slope. Final retrieval of the receivers and phase 2 data was done on 10.07.2020, 13 months after transplanting the experimental group.

Fishing



Tagging



Releasing



Figure 22 · Acoustic telemetry translocation experiment – Experimental (translocated) and control (native) blackbelly rosefish were caught on RV Arquipelago and a commercial hand line boat, surgically tagged with a long-lasting acoustic tag inserted in the abdominal cavity, and drop-down released on the Condor seamount summit after a short recovery period.

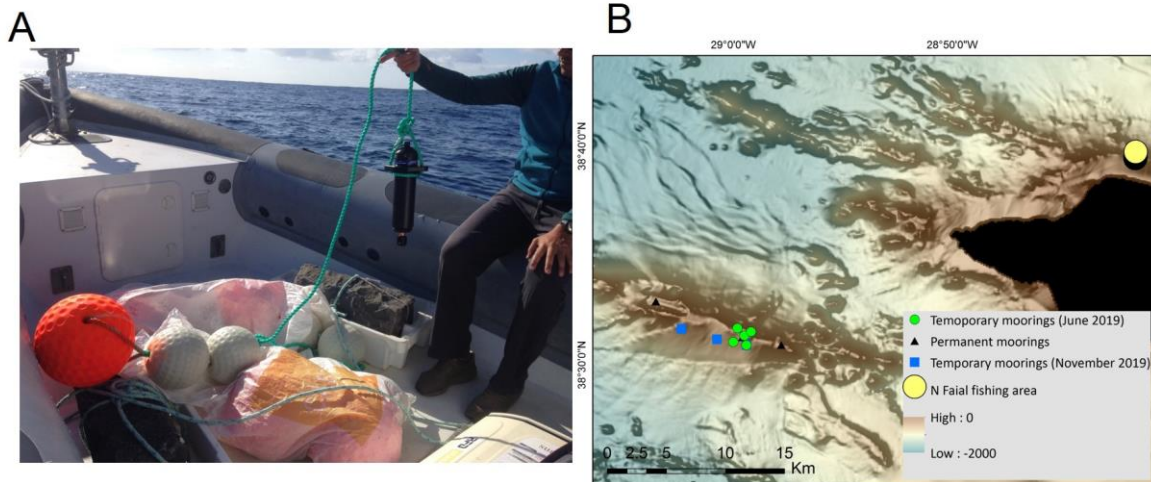


Figure 22 · (A) A line mooring with VR2AR receiver (with an integrated releaser) and buoys, ready to be deployed on Condor seamount. (B) Map of the fishing area in N Faial, permanent receiver array and receiver array deployed 2019.

Table 7 · Details of acoustic receivers on Condor seamount to monitor tagged rosefish during phases 1 and 2 of the translocation experiment.

station	area	depth (m)	scale of study	phase 1 Jun–Aug19	phase 2 Nov19-Jul20
202	slope	276	fine	X	
203	slope	303	fine	X	
204	slope	296	fine	X	
205	slope	299	fine	X	
25	summit	217	fine/broad	X	X
56	summit	280	broad	X	X
57	summit	300	broad	X	X
58	summit	237	broad	X	X
24	summit	186	broad		X
206	slope	320	broad		X
207	slope	306	broad		X

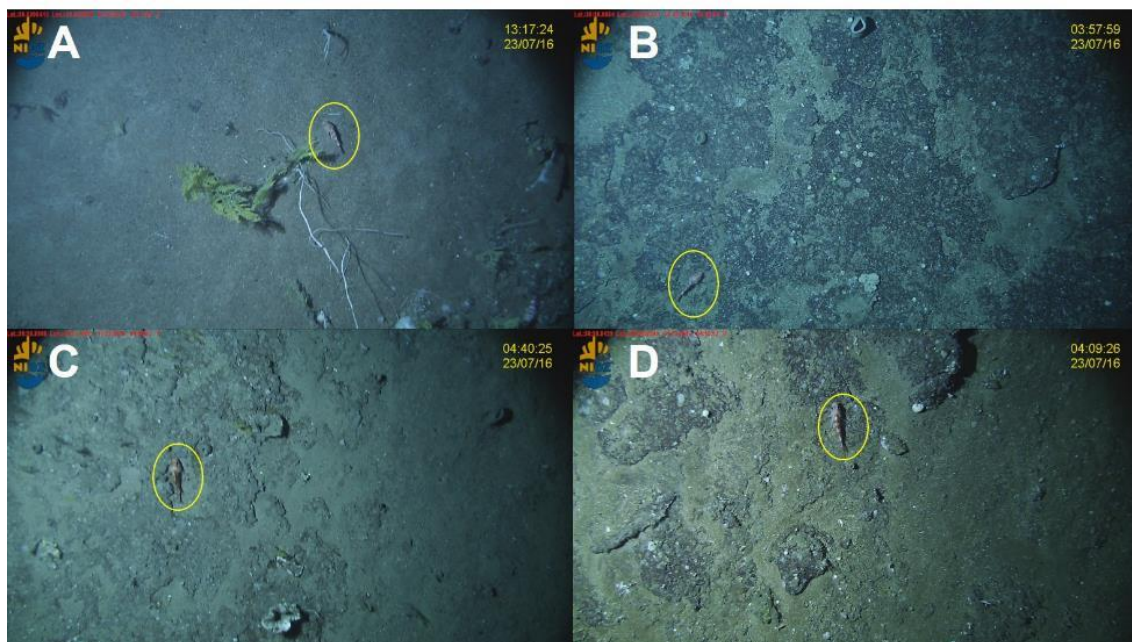


Figure 22 · Examples of substrate and different types of habitat building where blackbelly rosefish (yellow circles) can found on the Condor seamount seabed: A) coral on soft substrate B) sponge on hard substrate C) mix on hard substrate and D) None on hard substrate.

Data analysis

To select the best area of translocation and subsequent placement of the acoustic stations within the 17 km long seamount, in particular the fine-scale array, we analysed a bottom-video transect conducted in 2016 using a towed camera equipped with lasers. We extracted an image for every 5 m of the transect using the OFOP software. The images were analysed and scored for 1) dominant substrate type (hard or soft) and 2) presence of habitat building organisms (coral, sponge, mix or none) (Figure 22). We measured every fish based on the lasers using the ImageJ software.

The acoustic tagging data collected from phase 1 was used to calculate 1) the immediate and medium-term survival, and 2) the medium-term residency and habitat use, while data from phase 2 was used to evaluate long-term residency and potential emigration. A fish was defined as short-term survivor if it was detected in the array after the first 24 hours upon release, and as a medium-term survivor if detected after the first eight days. Medium-term habitat use patterns (In or Out of the fine-scale array) were analysed using the fine-scale monitoring data. If the fish were found in the square array during the period of the study, they were considered “In”, all the rest were considered “Out”. A Residency Index (R_i) was then calculated by dividing the number of days a fish was detected in the fine array (DD) with total study period (TP) (Afonso et al., 2008), ranging from 0 (not resident) to 1 (resident). TPs were corrected for differences in experimental groups (the control group was released one month after the experimental group). Finally, data from the fine array was used to estimate home range areas calculating Minimum Convex Polygons (MCP) (Kernohan et al., 2001) in the *adehabitatHR* package in R using centres of activity (COA) for 180 minutes periods (Simpfendorfer et al., 2002). The minimum number of unique relocations for the calculation of MCP is five, so all fish with less than five COAs were removed from this analysis. Survival, RI and Home range were then tested for significant differences based on experimental treatments, size and moving patterns. The statistical analysis followed the same protocol as described in the second case study (Crawley, 2007; Zuur et al., 2010) and was performed in R 3.5.0 (R Core Team, 2018). Finally, we used the survival and residency estimates to assess the potential to achieve the conservation goals and objectives in this study (Table 8).

Table 8 · Proposed goals, objectives and indicators for the fish transplantation study.

Target	Goals categories	Goals	Objectives for 3 months period	Indicators
reference ecosystem	ecological	re-establishment of fishing stock and genetic diversity of commercial deep-sea fish <i>Helicolenus dactylopterus</i> in 20 years	survival of the relocated fish over 50 %	survival >50%
			residency of relocated fish established	residency index >0.5
				home range area of relocated fish similar to the control

2.3.3 Results

Transect Image analysis

We analysed 2783 images along a 13 km transect on the summit of Condor seamount. We counted 505 rosefish in 381 images and measured 442 fish (Figure 24A). The highest abundance on an image frame was 10 rosefish, while an average 0.18. We can roughly identify two dominant size classes, one ranging 15-20 cm and another larger size class ranging 23-28 cm (Figure 4A), which indicates a prevalence of immature fishes on the shallower summit habitat. Rosefish occurred mostly over soft sediment (73%, $p < 0.001$) and associated to habitat building organisms (56%), especially coral gardens ($p < 0.001$) (Figure 24B). As two peaks of fish abundance were found along the transect (Figure 24C), we decided to place the fine array on the eastern peak and one of the broad stations in the western peak.

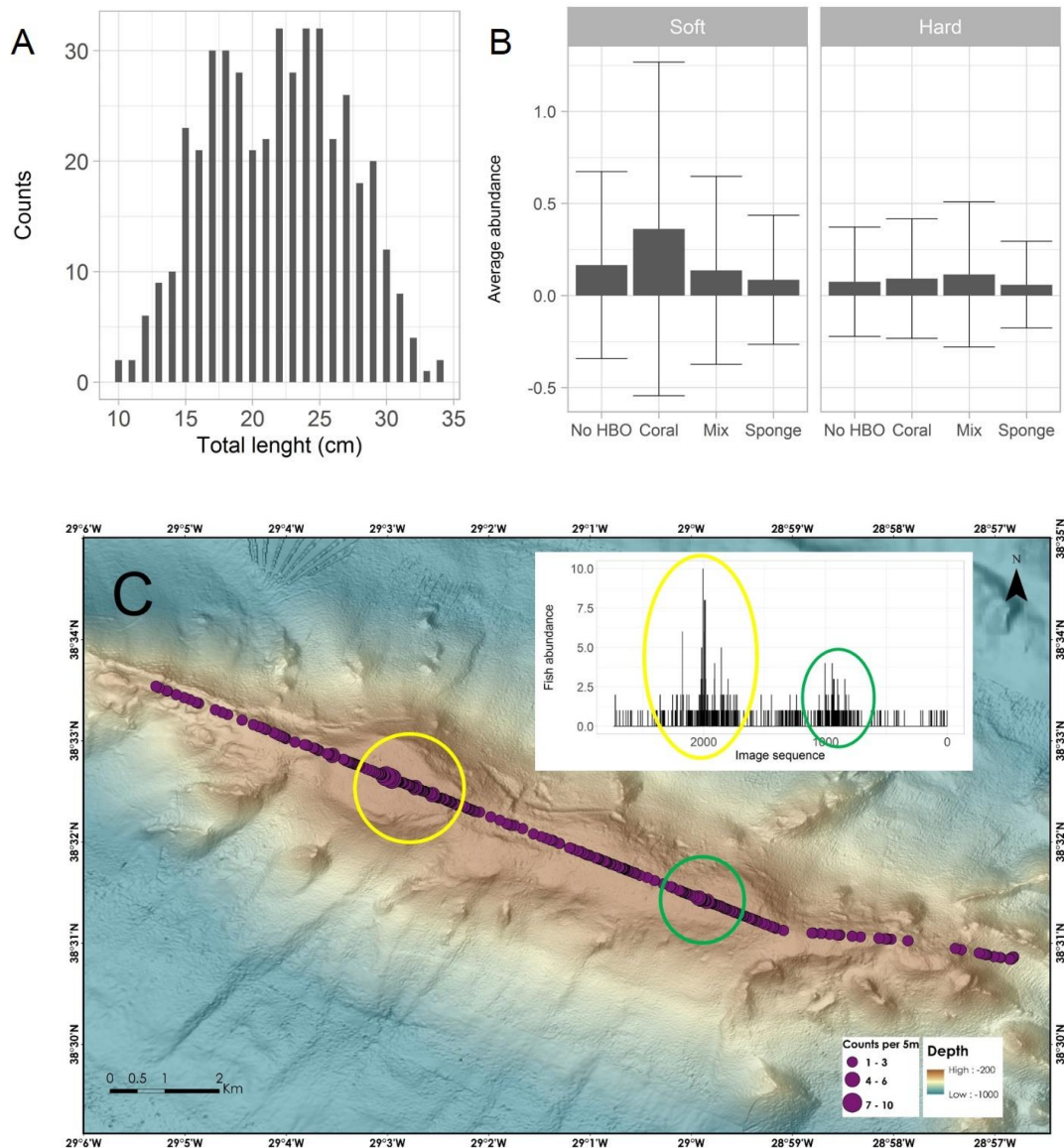


Figure 24 · Characteristics of blackbelly rosefish detected in the video transect: (A) size distribution of rosefish; (B) average abundance of rosefish in respect to different substrates and habitat building organisms (HBO); (C) abundance of rosefish along the transect with two peaks noted with circles.

Acoustic telemetry

During the whole study, we detected a total 18 out of 20 experimental fish and 14 out of 20 control fish in the Condor network (table 9). There was a substantial decay in number of fishes detected per month after the first month of experiment for each group, especially on the control group (7 vs 2). Only 3 and 2 fish were still being detected at the end of the study, respectively. There was no difference between the average presence of tagged fish per month (2.92 vs. 2.88).

Table 9 · Summary of detections per Control (top) and Experiment (bottom) fish/month in the Condor network during phases 1 and 2.

Control fish																		
fish	size	Jun	Jul	Aug	---	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	months	total		
21646	35.5		184													1	184	
21647	33.5		1395	3741												2	5136	
21648	32.5		3								8	342	167	96		5	616	
21649	29															0		
21650	31.7															0		
21651	28															0		
21652	32.5		125													1	125	
21653	28.5		123													1	123	
21654	28		2299	198		1766	1899	2413	2699	1161	381	491	1341	999		11	15647	
21655	25.5								39	44	33	10	22	8		6	156	
21656	37															0		
21657	33		60													1	60	
21658	36.5		2													1	2	
21659	31.3															0		
21661	39															0		
21662	34.8		16													1	16	
21663	37.5		17			9	8	2		3	2	7	8			8	56	
21664	33		62													1	62	
21665	33.5		95													1	95	
21666	35.5		97													1	97	
no. control fish			13	2		2	2	2	2	3	4	4	4	3				

Experiment fish

fish	size	Jun	Jul	Aug	---	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	months	total
21660	41															0
21667	26	207														1 207
21668	38	258														1 258
21669	44		4			9	2		28	4	7	44	42	59		9 199
21670	28	40	6													2 46
21671	46			3												1 3
21672	40	338														1 338
21673	37	35														1 35
21674	31	31														1 31
21675	37	18														1 18
21676	36	12	339	431												3 782
21677	42	39														1 39
21678	34	419	8	14												3 441
21679	45															0
21680	40	709	232													2 941
21681	31	161														1 161
21682	43	741	1083	2193		62	198	101	105	165	237	15				10 4900
21683	45	1189														1 1189
21684	43	6	204	2016		54	128	52	63	14	19	156	284	65		12 3061
21685	41.5	3														1 3
no. exper fish		16	7	5		3	3	2	3	3	3	3	2	2		

Eight fish (6 control, 2 experimental) were never detected. All fish (6 control, 3 experimental) detected at station 56 (far west of the seamount) soon after release were assumed predated, as a visual inspection of the data showed it would imply an improbable behaviour of crossing 4 km in 3 hours. All remaining fish were considered alive. Average medium-term survival (after 8 days) of tagged fish was 40% for the control group and 85% for the experimental group (Figure 26A).

During phase 1, the receiver located right at the center of the fine array (24) had by far the highest number of detections (57653), followed by those located nearby on the south flank (204: 6079 and 205: 3897), then on the north flank (202: 1704 and 203: 245), and on the farthest west (56: 158). The far-east station 57 did not have any detections. The manual monitoring around the fine array identified 14, 8 and 20 tagged fish on 19 June, 30 July and 27 August, respectively. Fish were significantly smaller in control than experimental groups (32.5 vs. 38.5 cm, $p < 0.002$) (Figure 25).

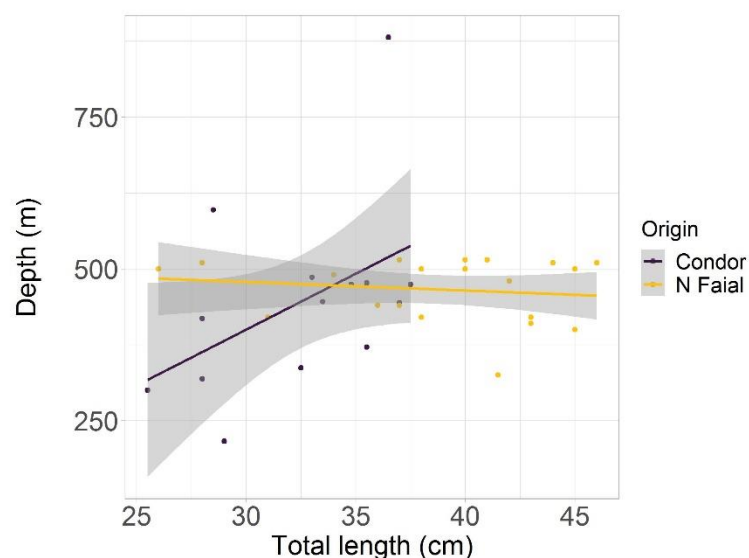


Figure 25 · Relationship between total length and depth of capture of tagged fish in respect to the sites of origin. The confidence interval is 0.95.

The residency index (RI) during phase 1 was very similar between groups (0.52 for control vs. 0.43 for experimental groups, n.s.) during phase 1, even if the experimental group had a somewhat lower RI due to more animals being detected outside of the array (Figure 26B). During phase 2 we only detected 16 fish (10 control and 6

experimental), not only with a lower RI, but also a much higher RI in the control than experimental group (0.32 vs. 0.05).

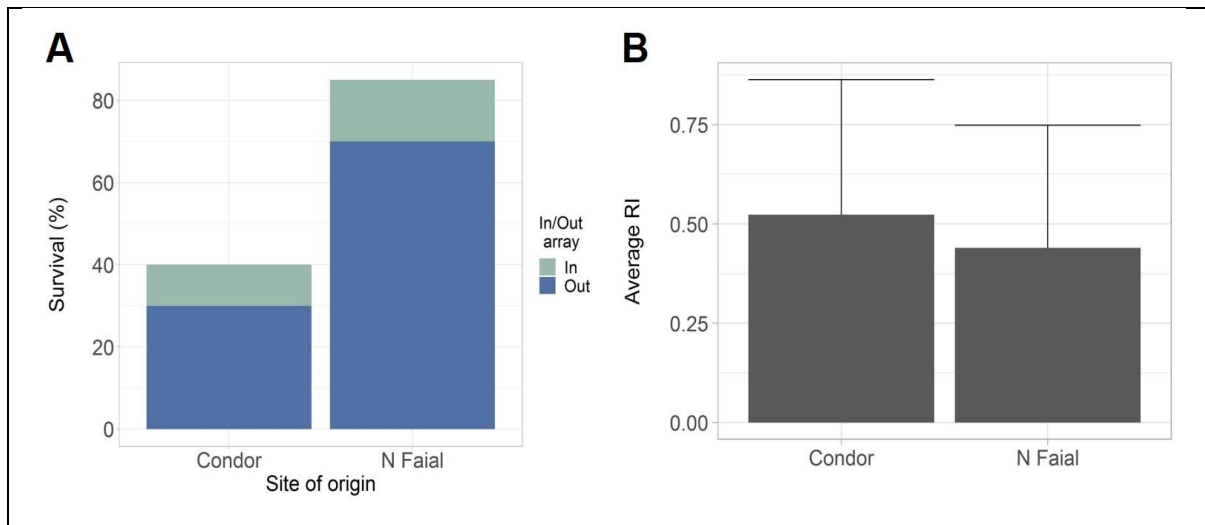


Figure 26 · (A) Fish survival per site of origin and residency type; (B) Residency index (RI) per site of origin.

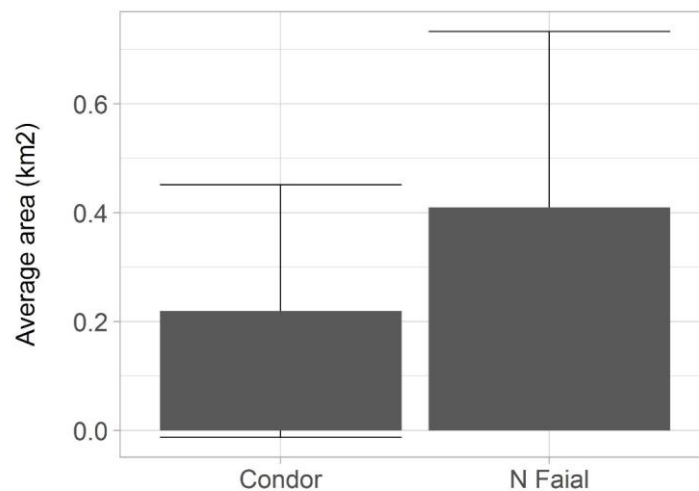


Figure 27 · Average home range area calculated by MCP

Although most fish stayed in the central part of the fine array, some localized emigration occurred towards the south flank and west of the fine array. Home range MCP areas ranged from for and for the experimental group. The average home range was higher in the experimental group (0.02 to 0.9 km²) than the control group (0.05 to 0.38 km²) due to a larger number of emigrating control fish, but this difference was not significant (Figure 27).

2.3.4 Discussion

Fish translocations/relocations are one of the tools used to replenish depleted stocks or increasing local biodiversity, but it has essentially used in freshwater ecosystems, especially using captive breed individuals and releasing them into the wild (Seddon et al., 2007). Wild marine fish translocations have seldom been conducted, and virtually never in the case of deep-sea fishes and habitats. To our knowledge, this is the first study to explicitly address this possibility using an experimental setup and hypothesis testing, and indeed our results show that this tool may have great potential for restoration of deep-sea fish populations,

The overall performance objectives set for the 3-month phase 1 monitoring period were generally met (Table 9): estimated survival of translocated animals was high (85%) and even larger than anticipated, while residency was slightly under the 0.5 target (0.43). Furthermore, patterns of habitat use as determined by home ranges didn't differ from the control group. Thus, in spite of the limitations in this study, we conclude that there is potential in applying translocation as means of replenishing the native fishing stocks in deep-sea species of commercial interest.

Table 9 · *Evaluation of the success in accomplishing the objectives set for the a 3 months period of phase 1 after fish relocation.*

Case study	Objectives for 3 months period	Results	Evaluation
reference ecosystem	survival of the relocated fish over 50 %	average survival is 85%	objective was achieved
	residency of relocated fish established	average residency index is 0.43	objective not achieved, but it is very close to what was expected
		home range area of relocated fish similar to the control	objective achieved, MCP areas are not different between control and relocated fish

However, our study also pinpoints a number of weaknesses and technical and methodological challenges to address in the future.

The first is the potential for a size related influence in the results, as the experimental group consisted of larger individuals on average than the control group. One of our goals was to speed up the recovery of native species by using larger, sexually mature individuals which also have a better chance of surviving the stressful procedure of fish translocation. The Blackbelly rosefish is native to the Condor seamount, and the local population has been apparently slowly recovering since the fishing closure in 2010. We encountered two smaller size classes (~ 15 cm and ~ 22 cm) dominating the transect over the summit, which indicates that individuals in the summit is either typically immature or that the seamount hasn't yet recovered to support a population of larger individuals. The fact that there is a typical size segregation with depth in this species as shown in experimental monitoring campaigns (Macpherson & Duarte, 1991; Santos et al 2020), including in the Condor seamount (Eva Giacomello, pers. comm.), seems to support the first hypothesis. Additionally, both the need to reduce potential physiological stress and to operate the acoustic telemetry gear in shallower depths led us to target the summit and shallower parts of the flanks when fishing there, especially after the first attempt using longline proved unsuccessful. It is possible that this size difference partially explains the difference in survival rates, as the control group had lower survival in comparison to the experimental group and smaller individuals may be more susceptible to predation and stress. However, it seems unreasonable to expect that a putative lower survival of translocated animals would be substantially outcome by such a size effect.

An alternative explanation is that the experimental fish were initially more stressed than the control fish, as they were caught using longline and thus stayed hooked for a longer period. This could might have affected their mobility in the short term and reduce the chance of premature (8-day) emigration that can be misinterpreted as non-survivals. Indeed, the control group had three times more early non-survivors (undetected individuals) than the experimental group but, as in many other acoustic telemetry studies, it is impossible to ascertain what the fate of these animals was. Nevertheless, the habitat use patterns were very similar between the two groups,

showing that individuals from both groups were equally prone to moving away from the array, and that the home range area was also similar between the groups.

Home range was only calculated for animals that showed movement between receivers in the array, therefore some very resident fish which were mostly detected on one receiver were left out from this part of the analysis. For these individuals the home range would be very small ($\sim 0.2 \text{ km}^2$) further showing that this species seems to use a small vital area in the short-to medium term. This conclusion agrees with previous findings in the Azores using standard tag-release (Santos et al 2020) and supports the established notion that rockfishes are sit-and-wait predators that move little and use small areas (Matthews 1990a,b, Tolimieri et al., 2009).

Previous studies of site fidelity and movement of shallower rockfishes (Sebastidae) also showed that, in suitable rocky habitat, the animals have smaller home ranges than in plain substrates (soft substrates) (Matthews 1990a,b, Tolimieri et al., 2009). From the image analysis we have seen that the summit of Condor seamount is dominated by soft sediments with patchy islands of rocky boulders with habitat building organisms. This could also be another factor promoting of emigration from the summit after some time, apart from the size-related preference for deeper environments., There was a substantial decrease in the number of detected fishes during phase 2. Again, it is impossible to know whether this was the result of mortality or emigration. Although we had two receivers located on the flanks during this period, there is a substantial probability that many fish went undetected towards the unmonitored flanks of the seamount and that rockfish moves more than previously thought in order to forage or find shelter.

For future studies of similar purpose, it would be optimal to use the same fishing technique and individual sizes. The new release system we developed for this project proved useful as it drastically minimized the spatial error of the release point even if it would be hard to expect fishermen to use such a system. Since the Condor seamount is a vast area, a denser array of acoustic receivers would be useful in detecting movements outside of the receiver array especially on the flanks.

One major problem in this type of studies is fish well-being, which needs to be carefully evaluated and maximized in order to secure high survival rates, including appropriate fish capture and handling techniques, maintenance of good water quality in transport tanks (including temperature and oxygen), and reduction of time at surface. More experiments should be done on small scale in order to improve methods, prove concepts and fill knowledge gaps. Other main barriers are the time needed for significant results that will be valued by local communities and governments, and knowledge gaps about the life histories of deep-sea fishes which directly influences potential management benefits, including the larval export and emigration effects. Scaling up the number of individuals and receivers also has cost implications, especially given the relatively high cost of deep-sea actions and acoustic telemetry equipment. Thus, it is crucial that similar ecological restoration studies are conducted in protected areas where the main pressures and impacts are controlled.

3. General Discussion

Regarding to the restoration actions focusing on CWC, both pilot studies showed the survival potential of by-catch cold-water gorgonians once returned to their habitats, and suggests the potential success of future scaled-up restoration actions. The third case study was the first one its kind, providing insight into the possibilities of restoring commercial deep-sea fish populations. This should encourage future initiatives aimed at recovering, preserving, and sustainably managing VMEs. However assisted regeneration techniques like transplantation of coral fragments or individuals may not be easily applicable for all CWC and fish species. Collecting and maintaining cold-water corals in aquarium facilities before returning them to their natural habitat can be complicated. The stress suffered by corals during the collection process (pressure and thermal changes) and the complexity of replicating their natural environment in the laboratory, can compromise the survival of corals in captivity (Orejas et al., 2019). One restoration action with no success took place in the CWC coral garden community of Sur Ridge seamount, within the Monterey Bay National Marine Sanctuary (US), where none of the transplanted corals survived after one year from their reintroduction. However, years after, the scientific team improved the technique, by avoiding long-term coral maintenance in aquaria and improving the attachment of corals to transplantation structures, overcome the problems and set up a new restoration experiment with successful results (Boch et al., 2019). Using adult fish individuals has shown a promising technique, having in mind the fishing techniques used and post-fishing-pre-release protocols that need to ensure good physiological status of the fish in order to survive release.

The advantage of using transplants of adult coral colonies (usually with branching forms) instead of early life stages (e.g. fertilized eggs, larvae), is the short-term recovery of the three-dimensional structure of coral populations, facilitating the recovery of habitat-forming functions such as structural habitat for large number of associated species (Horoszowski-Fridman et al. 2015; Geist & Hawkins, 2016). Conversely, the main disadvantage is that coral fragments are normally obtained from healthy coral donor areas, fragmented and then transplanted to the impaired ones altering thereby the preserved areas. In addition, by using larger transplants, which

suffer less natural mortality after transplantation (Brooke et al., 2006), more material needs to be taken from the donor site. A potential solution to overcome this drawback could be collaborative initiatives with fishers using bycaught corals as in the Mediterranean and Azores case studies. By using coral bycatch material, the impact on natural coral populations is minimized, while it also overcomes the need for expensive technology for coral collection, reducing the overall cost of the restoration action. Moreover, since fishing operations cover a much wider spatial scale that could be covered with technological means (e.g. using ROVs), the genetic diversity of the parent donor coral colonies is also potentially increased.

The fish relocation case study has indicated that the mortality due to predation and stress may be reduced when using larger individuals (del Mar Gil et al., 2015). This was an important element in the case study because of the use of commercially important fish species. The larger individuals can contribute to the greater reproductive output of the population as they are sexually matured. This is an advantage for short term studies, where the recovery of essential parts of the ecosystem structure and functioning is accelerated (McDonald et al., 2015). The study has shown that the relocated target fish species remained on Condor seamount at least on the medium term, meaning that they may becoming integrated to the local population. The setback of this study is that we cannot be sure how many individuals will remain alive during a longer period of time due to technological restrictions (battery life of acoustic tags, coverage of the receivers). Since this study has shown promising preliminary results, more studies should be done on other, more mobile fish species (e.g. Afonso et al., 2012) to see if residency can be achieved by relocation to a marine protected seamount.

One of the techniques that still needs to be considered and tested for deep-sea, especially for CWC is culturing and rearing coral larvae, which is a widely applied techniques in coral reef restoration actions (Rinkevich, 1995; Shafir et al. 2006; Mbije et al., 2010). The use of larval rearing techniques has also the advantage of reducing the damage to existing donor reefs and also in producing higher genetic diversity, which provides more potential for habitat shelf-support. However, the little knowledge about CWC larvae (small-scale recruitment patterns, dispersal abilities and settlement cues) (Brooke & Young 2003, 2009; Strömberg, 2016; Strömberg & Larsson, 2017)

together with the slow-growing pattern of deep-sea coral species (Andrews et al., 2002; Risk et al., 2002; Prouty et al., 2014) hinders the application of rearing techniques to CWC habitats. Up to this date, no studies have yet explored the viability of producing larvae neither “in situ” using coral nurseries nor in aquaria. An alternative approach, which does not require larval rearing, is to deploy artificial or natural substrates where corals can naturally settle (Bramanti et al., 2005). Finding the best larval settlement surface is a first key step. Complex substrates have been shown to promote higher colonization by benthic deep-sea invertebrates than simple substrates (Girard et al., 2016). However, in situ larval settlement experiments targeting CWCs have had mixed results. While a study found high recruitment rates for *Primnoa resedaeformis* on artificial substrate deployed for four years in the Northeast Channel Coral Conservation area, off Canada, very few *Paragorgia arborea* recruits were recovered on these same substrates (Lacharité & Metaxas, 2013). Differences in the number of recruits could be due to differences in the reproductive strategies (broadcast spawning vs brooding) of these two deep-sea coral species. Moreover, studies with *Oculina variciosa* have shown very little coral recruitment or even no recruitment at all, after 5 years from the deployment of the concrete modules (Brooke & Young, 2003). Deploying substrates in areas receiving high recruit densities during coral mass spawning, with subsequent transfer of the settlement substrata with settled corals to the degraded areas could be an approach to improve the settlement success (Guest et al., 2010). Likewise, introducing large amounts of coral larvae directly to degraded coral gardens or reef areas may also increase the chances of recolonization such as in shallow-water coral examples (Heyward et al., 2002), but this method has not been tested for cold-water coral species yet. One applied example of this approach is the worldwide increasing implementation of “rigs-to-reefs” (RTR) program, whereby obsolete rigs are decommissioned and converted into artificial reefs (Larcom et al., 2014 and Kaiser & Pulsipher, 2005 RTR program in Gulf of Mexico; Bergmark & Jørgensen, 2014 and Dannheim et al., 2018 in the North Sea). Obsolete industry platforms become de facto a no-trawling zones, thus providing a suitable habitat for colonization of corals and other epifaunal species (Larcom et al., 2014; Macreadie et al., 2011; Bergmark & Jørgensen, 2014). With more than 6500 oil and gas rigs dismantled by 2025, “rigs-to-reef” programs are expected to provide the opportunity to create complex deep-sea artificial reefs on unprecedentedly large scales (Macreadie et al., 2011). Colonization of those artificial reefs is determined by the

arrival of species larvae and propagules and a subsequent local survival (Dannheim et al., 2018). The *Lophelia pertusa* planulae capability to survive for one year and disperse over long distances confirms the potential of this wide-spread cold-water coral to colonize artificial structures (Strömberg & Larsson, 2017). The type of structure used, age and depth of artificial reefs influence colony density and growth as observed in *Lophelia pertusa* on 10 artificial structures in the Northern Gulf of Mexico (Larcom et al., 2014).

A long-term monitoring and evaluation should be included in any restoration project to ensure viability and the accomplishment of goals and objectives set for a specific time period (McDonald et al., 2016). Monitoring expenses in deep-sea can be very high due to the difficult access, so it is desirable to choose methods that are relatively cheap and easy to maintain. Thus, the availability and continuous development of specialized underwater tools such as low-cost ROVs, drop-down and towed camera systems and Autonomous Underwater Vehicle (AUV) to monitor deep ecosystems, provides the possibility to perform an accurate monitoring of the restoration actions and improve their evaluation. Non-destructive methods based on imagery can provide a valuable tool to assess the success of a restoration project. Image-based monitoring, where high-resolution images of the same individual coral colonies are taken every year (or less frequently if annual monitoring is not feasible), allows the detection of small changes in the health of coral colonies as well as the measurement of in situ growth rates (Hsing et al., 2013; Girard & Fisher, 2018; Girard et al., 2019). Moreover, 3D model reconstructions can provide information about in situ growth dynamics (Bennecke et al., 2016; Montseny et al., 2019). Those monitoring methods could be employed to monitor the health and growth of coral transplants. Additionally, staining techniques for assessing in situ growth rates of cold-water corals have been developed, offering another methodological basis to monitor the transplants growth (Brooke & Young, 2009; Lartaud et al., 2013). The development of better imaging techniques can provide more information on the recovery of associated fauna, such as commercial fish species and cryptic small invertebrates.

Since CWC's are extremely vulnerable species to anthropogenic activities (long-lived, slow-growing and fragile) (Frieiwald et al., 2004; Clark et al., 2015) there is a need to combine assisted and natural spontaneous regeneration strategies. Avoidance of the

main stressors (e.g. closure to fishing activities) seem to be the more sustainable and economical approach for mitigating impacts and protect these vulnerable ecosystems as demonstrated by the implementation of deep-sea marine protected areas (MPAs) around the world (Van Dover et al., 2014; Van Dover, 2014). International agreements and directives supporting the protection of cold-water coral's ecosystems are nowadays also increasing worldwide (Armstrong et al., 2014). To date, a number of countries have created MPAs for the conservation of cold-water coral ecosystems: The Darwin Mounds (UK), Oculina Bank marine protected area (US), Aleutian gorgonian garden ecosystems (US), Corner Rise Seamount (US), Northeast Channel Coral Conservation Area (US) (Harter et al., 2009; Huvenne et al., 2016; Bennecke & Metaxas 2017). Harter and collaborators (2009), was the first study that reveals several positive effects of a deep sea MPA (Oculina Bank marine protected area). High coral abundance and the presence of large colonies and recruits point to signs of recovery after 12 years since the closure of fishing in the Northeast Channel Coral Conservation Area (Bennecke & Metaxas, 2017). However, in some cases, protection is not always successful, for instance in the Eastern Darwin Mounds, very little regrowth and no coral recolonization were detected, evidencing the low resilience and slow potential of deep-sea ecosystems (Huvenne et al., 2016). Furthermore, to protect a desirable area and to avoid all the coral degradation drivers is not always possible. For instance, in MPAs of Cap de Creus and Azores artisanal fisheries are allowed despite their well-known detrimental effects on gorgonian populations, particularly the species that are larger and have a more complex morphology (e.g. Sampaio et al., 2012). Nevertheless, in order to suitably manage these areas and minimize fishing impacts to coral habitats, scientists in the Cap de Creus and Azores are working together with local fishers to change and improve fishing techniques that are less impacting on coral communities. In the Mediterranean, it has been tested the possibility for replacing trammel nets for other gears that have lower impact on the benthic communities, such as baited traps and modified trammel nets (e.g. enlarged mesh size and reduced height) to reduce the amount of bycatch. In the Azores, effort has been placed to replace bottom longline fishing with by hand-lines, which have been shown to be much less impacting to benthic communities. Similar applies for fish species, as there is growing concern about the reduced deep-sea fish stocks (Morato et al., 2006). Techniques to reduce the catch of undersized individuals and

uncommercial fish species (e.g. deep-sea sharks) are underway (Fauconnet et al., 2019).

Ecological restoration of mesophotic and deep-sea habitats involves considerable constraints due to the difficult access, requiring for the use of advanced underwater technology entailing high economic cost. Deep-sea restoration cost per hectare has been estimated at two to three orders of magnitude higher than for shallow marine ecosystems (Van Dover et al., 2014). Future availability of accessible cost-effective underwater technology (such as relatively low-cost AUV) will be paramount for the wide application and up-scaling of coral and gorgonian restoration actions at depths below conventional or technical scuba diving limits.

To date, the few examples of cold-water coral restoration included the study cases developed within MERCES project are based on coral fragments transplantation on artificial structures that greatly limit the restoration area that can be reached (Brooke et al., 2006; Dahl, 2013; Montseny et al., 2019). Thus, in order to achieve large-scale restoration actions, many large artificial structures would be needed, further increasing the economic cost and the technical constraints. In this sense, and given that the main threats impacting natural habitats occur on large scales (Halpern et al., 2008), it is a foremost challenge to develop effective methods for upscaling ecological restoration actions (Aronson & Alexander, 2013; Perring et al., 2018). Indeed, a mismatch between the scale at which ecological restoration can currently be done and the scale at which major impacts act has been highlighted for tropical shallow coral reefs (Edwards & Gomez, 2007; Maya et al., 2016; Pollock et al., 2017). Further investigation is needed to evaluate the cost effectiveness of restoration of fish stocks in the deep-sea.

Overall, technical and logistical difficulties of working below the limit of scuba diving commonly imply the use of high cost underwater technology (Van Dover et al., 2014). Future availability of accessible cost-effective underwater technology (such as relatively low-cost AUV for monitoring) will be paramount for the wide application and up-scale of restoration at depths below conventional or technical scuba diving limits.

4. General Conclusions and Ways Forward

Overall, outcomes from the few assisted regeneration actions performed in conjunction with parallel studies provide evidence confirming the feasibility to restore cold-water coral reefs and coral gardens by transplanting fragments of adult coral colonies on artificial structures. Similarly, the fish transplantation case study showed that the relocation of deep-sea fishes from island slope to a nearby seamount is feasible and the translocated fishes showed similar patterns of movement in comparison to the control group in order to restore fish stocks in deep environments.

Differing recovery capabilities of the coral taxa used in the transplantation studies together with the lack of recruitment by coral larvae in artificial substrates points out to the need of using a combination of assisted and natural restoration approaches. In the same line, fishes were translocated within a fishery closure to speed-up the recovery of seamount species. Therefore, any restoration actions should act in concert with protection measures that remove as much pressures as possible from the area to be restored (e.g. closure to fishing activities), until a certain threshold of size/biomass of coral colonies or area covered by coral colonies is attained. Moreover, assisted regeneration (such as transplantation) may be used for some species, while natural regeneration (through fisheries closures, marine protected areas) at large scales may be used to assist individual native species that cannot be transplanted and may take longer to recover.

The cost of all of these restoration actions is high, highlighting the need to search for new low-cost technologies and the collaboration of fishermen could help to reduce these costs in a near future.

Finally, given the life history traits of corals and fishes, short-term monitoring (i.e. within the lifetime of the MERCES project) cannot be expected to reveal fully restored habitats. Therefore, management measures should be taken to ensure the long-term monitoring.

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