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Growth rate and survival of transplanted corals: *Paramuricea clavata, Corallium rubrum* and *Cladocora caespitosa* case studies

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ABSTRACT

Le foreste animali marine sono complesse strutture viventi composte da specie strutturanti che garantiscono, con la loro tridimensionalità, la formazione e il mantenimento di un'elevata biodiversità e di svariati servizi ecosistemici.

A causa delle minacce passate e recenti, sia di origine naturale che umana, queste comunità si stanno rapidamente impoverendo, passando da strutture complesse altamente resilienti a strutture più semplificate. Quando la resilienza degli ecosistemi naturali è compromessa, le azioni di restauro potrebbero essere significative per il recupero delle popolazioni e delle comunità danneggiate. Questo studio analizza il tasso di crescita e sopravvivenza legato al trapianto di tre specie di coralli presenti nel Mediterraneo: *Paramuricea clavata, Corallium rubrum* e *Cladocora caespitosa.* I parametri sono stati studiati in laboratorio per *C. caespitosa*, e mediante attività di campo per le altre due specie, con trapianto da un sito donatore (Monte Argentario, Mar Tirreno settentrionale) a uno ricevente (Isola Gallinara, Mar Ligure).

In laboratorio, 40 frammenti di *C. caespitosa* sono stati divisi in trattamento luce (12h:12h luce:buio) e trattamento buio (24h buio). I frammenti sono stati divisi in 4 vasche sperimentali in base al numero di coralliti iniziali (1, 2, 3 o 4) e studiati per due mesi. Tutti i frammenti in condizioni di oscurità

sono sbiancati, al contrario, di quelli mantenuti con il trattamento con la luce. La variazione dell'area dei frammenti è risultata quasi nulla per entrambi i trattamenti, ad eccezione dei frammenti con 3 e 4 coralliti esposti alla luce, che hanno mostrato differenze significative, grazie alla produzione di nuovi coralliti durante i due mesi di esperimento.

Le altre due specie sono state trapiantate a una profondità di 35 e 40 m rispettivamente per *C. rubrum* e *P. clavata*. Frammenti di entrambe le specie sono stati attaccati al substrato roccioso utilizzando una resina bicomponente, e per il corallo rosso sono state studiate tre tecniche di trapianto (ovvero resina epossidica, griglia e polistirolo) testate nel progetto MERCES. Dalla prima attività di trapianto, avvenuta nel 2017, sono stati trapiantati complessivamente 87 frammenti, di cui 46 sopravvissuti. Considerando l'efficacia della griglia (34 colonie su 57 ancora in sede), è stata indagata la variazione nel numero di apici delle sole colonie trapiantate con questa tecnica. Tutte le colonie hanno mostrato una diminuzione media mensile di -0,106 ± 0,130 apici/mese, con un solo trapianto che ha mostrato 1 nuovo apice.

Delle 25 colonie di *P. clavata* trapiantate, 16 sono ancora in sede dopo 2 mesi e appaiono sane. La maggior parte delle colonie ha mostrato un aumento almeno in altezza, con la produzione di nuovi rami, mentre altre hanno subito la perdita di numerosi rami. Inoltre, una delle colonie che mostrava inizialmente danni tissutali, ha mostrato, dopo 2 mesi, un pieno recupero.

Un monitoraggio a lungo termine aiuterà a comprendere meglio il tasso di crescita delle popolazioni trapiantate e a confermare l'efficacia dei trapianti e la sua possibile applicazione su scala più ampia.

CONTENTS

1. Introduction

1.1. Marine Animal Forests: ecological role and threats

Marine animal forests (MAFs) are living three-dimensional structure composed mostly of sponges, cnidarians, bryozoans, ascidians and other sessile benthic organisms (Rossi et al., 2017). MAFs are dominated by ecosystem engineering species, organisms that create and maintain these habitats, while other may take advantage of these structures to create their own niche (Rossi et al., 2013). These represents nursery areas where many species spend part of or all their life cycle: the more complex and mature the MAF structures, the higher the nursery effect, since more complex structures provides more places to hide and feed (Marliave et al., 2009; Baillon et al., 2012, Miller et al., 2012). Mature forests mainly consist of slow-growing long-living organisms (Gili and Coma, 1998; Baillon et al., 2012), and the species composing MAFs may depend on the type of substrate and on the productivity of the entire system (Rossi et al., 2017) (Figure 1).

MAFs generally develop on rocky substrates, which represent at the same time the most common environments in coastal areas worldwide, as well as the most threatened ones (Rossi, 2011; Coutinho et al., 2016). Apart from their role of nursery areas, MAFs provides several ecosystem services, providing for food and pharmaceutical compounds, supporting the primary productivity, and nutrient cycling, regulating the carbon sequestration and storage, preventing the coastal erosion and, lastly, providing recreational, esthetic, and touristic benefits. However, the limited information on the composition and structure of the assemblages composing these crucial environments prevent the possibility to properly protect them (Sardà et al., 2005, 2012).

Figure 1: A technical diver enjoying the beauty of a Marine Animal Forest, here represented by the red gorgonian *Paramuricea clavata* in a Mediterranean coralligenous assemblages (Photo credits: Claudio Provenzani).

Due to past and recent threats, including both natural or human (e.g., direct anthropogenic activities and climate change), benthic communities are rapidly changing, shifting from highly resilient complex structures to more simplified communities (Rossi et al., 2008, 2013; Rife, 2018), leading to a reduction in biodiversity and to a shift in the ecosystem functioning of marine ecosystems.

The mechanical impact of fishing activities has dramatically reduced the coverage of habitat-forming species, with consequences on the diversity and abundance of associated organisms (Blanchard et al., 2004; Althaus et al., 2009). Negative effects of fishing have been known for a long time and represent a world-wide concern (Kaiser et al., 2001; Gage et al., 2005; Morato et al., 2006), both for the direct action of fishing gears (e.g., gillnets, trammel nets, long lines and traps) on the benthic organisms and the sea floor integrity, and the deleterious effect of sediment resuspension caused by trawling activities (Chiappone et al., 2005; Martín et al., 2014; Paradis et al., 2017). However, fishing gears produce an impact not only when in use, but also when they are lost in the environment. As a point of fact, being made of non-biodegradable synthetic fibers, once lost, they persist in the environment for hundreds of years (Carr, 1987; Thompson et al., 2004; Moore, 2008; Barnes et al., 2009). Additionally, lost fishing gears (LFGs) can still catch a large spectrum of organisms (Carr, 1987; Matsuoka et al., 2005; Brown & Macfadyen, 2007), and can damage others abrading and entangling while free to drift on the bottom (Laist, 1995; Brown & Macfadyen, 2007), representing one of the major concerns for the marine environments (Brown & Macfadyen, 2007) (Figure 2).

Figure 2: A lost fishing net on the Mediterranean red gorgonian *Paramuricea clavata* at Punta Manara (Ligurian Sea, Italy) (Photo credits: Torcuato Pulido Mantas).

Climate change (CC) represents another crucial threat to marine benthic ecosystems (Hughes et al., 2003). Three are the main effects of CC on MAFs: ocean warming, ocean acidification and sea level rising; the synergistic activity of these impacts are negatively influencing the ability of many organisms to adapt to stressful conditions (Rossi et al., 2013).

The Mediterranean Sea have previously been identified as major contributors to the degradation of coralligenous communities as it is considered the most investigated sea globally (Costello et al., 2012), however the knowledge of marine and coastal biodiversity is still insufficient and inhomogeneous, with data still not available for many species, habitats, and communities (UNEP-MAP RAC/SPA, 2010). The lack of historical long-term data is one of the major problems in understanding fluctuations of marine communities (McClenachan et al., 2012; Fortibuoni et al., 2017), especially when related to changes induced by anthropogenic pressures. LFGs are omnipresent in the Mediterranean Sea, and they have previously been identified as major contributors to the degradation, especially in coralligenous communities (Angiolillo & Fortibuoni, 2020). The most severally damaged organisms are the once characterized by three-dimensional erect morphologies, such as, among others, octocorals (e.g., *Corallium rubrum, Eunicella singularis, E. cavolini, E. verrucosa, Leptogorgia sarmentosa, Paramuricea clavata*) and sponges (e.g., *Aplysina* spp., *Axinella* spp.) (Betti et al., 2020).

Owing to its relatively reduced area (2.3 x 10^6 km^2) and being an almost enclosed basin, the Mediterranean Sea is suffering the rising temperature more than any other seas or oceans. The water temperature anomalies recorded in the last decades (Garrabou et al., 2022) as well as the acute warming events occurred in 1999, 2003, and 2006, caused catastrophic mass mortalities of up to 100% of octocorals, mucilaginous proliferation and triggered the arrival of alien species with warm water affinity (Bavestrello et al., 1994; Cerrano et al., 2000; Perez et al., 2000; Garrabou et al., 2001, 2009; Bianchi et al., 2001; Bianchi & Morri, 2004; Schiaparelli et al., 2007;

Cupido et al., 2008; Puce et al., 2009; Roghi et al., 2010; Bianchi et al., 2012; Parravicini et al., 2013, 2015; Gatti et al., 2015). Anthozoans mass mortality episodes mainly involved shallow water communities (up to 40 m depth), where seasonal temperature fluctuations are more acute. Effects of this phenomena are visible in necrotic portions of the branches, lesions, increase of epibiosis, and in the denudation of the axial skeleton (Garrabou et al., 2001, 2009, 2019, 2022). Consequences of these catastrophic heatwaves are now well documented, and recovery of populations appeared to be slow for many species, and, in some cases, only partial (Linares et al., 2005; Cerrano & Bavestrello, 2009; Lima & Wethey, 2012).

Several Mediterranean coastal communities host gorgonians, sponges, and bryozoans, which can form dense MAFs (Linares et al., 2008), able to support highly structured and diverse associated fauna (Gibson et al., 2006; Gori et al., 2011).

1.2. Restoration: a promising tool for marine habitat recovery

When the resilience of natural ecosystems is severely compromised, active restoration actions may be necessary as a complementary tool to recover damaged populations and communities (Possingham et al., 2015). 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 pristine condition (SER, 2004). Recently, ecological restoration is receiving increasing attention worldwide trying to reverse the environmental damages caused in the last decades by anthropogenic activities (Falk et al., 2006).

As habitat-forming species, corals and seagrasses have been the primary targets of marine restoration activities (Edwards & Clark, 1998). Until now, restoration actions were mainly conducted on shallow tropical habitats (i.e., Rinkevich, 2005; Precht & Robbart, 2006; Young et al., 2012), applying different techniques, mainly depending on the species (e.g., Shaish et al., 2008; Williams et al., 2010; Johnson et al., 2011; Fujiwara et al., 2016) and mostly focusing on survival rates of transplanted individuals over relatively short monitoring periods (usually less than 2 years), while its effectiveness needs to be evaluated on long term, being comparable to rates similar to natural ones (i.e., investigating structural complexity and ecosystem services), thus providing more reliable data (Bayraktarov et al., 2016; Boström-Einarsson et al., 2020). On the other hand, considering the Mediterranean Sea, only a few attempts have been carried out so far (Dahl, 2013; Boch et al., 2019; Montseny et al., 2019).

Restoration can be passive or active: passive restoration “relies on increases in individuals, without the human intervention (e.g., direct planting or seeding), after the removal of causal factors alone”, while active restoration relies on reintroductions or augmentations. The type of restoration corresponds to the level of degradation sustained by the environment: passive restoration can be applied to sites less damaged, while active restoration is necessary where natural recovery is not enough (Boström-Einarsson et al., 2020). Regarding corals, approaches to active restoration include (Boström-Einarsson et al., 2020; Hein et al., 2021):

1. Direct transplantation: direct transplantation of coral fragments, from a donor to a recipient site, without an intermediate nursery phase;
2. Coral gardening: transplanting fragments with an intermediate nursery phase, which can be *in situ* (ocean) or *ex situ* (aquaria);
3. Larval propagation: coral larvae are released at a restoration site, after collection and holding phase (in the ocean or in aquaria);
4. Artificial reefs positioning: positioning of artificial structures as a substrate for coral recruitment and/or transplanting;
5. Micro-fragmentation: technique that involve the use of a diamond blade band to cut small fragments (1cm^2), a methodology particularly suitable for massive corals, which are less prone to fragmentation;
6. Coralclip®: stainless-steel spring clip that clamps the fragment to the reef for long enough to begin growing onto the substrate securely (Suggett et al., 2020).

Evidences of restoration are present in the Mediterranean Sea, for *P. clavata*, *E. singularis, E. verrucosa, E. cavolini* (Fava et al., 2010; Montseny et al., 2019, 2020), and *C. rubrum* (Montero-Serra et al., 2018). These corals have been obtained from artisanal fishermen's bycatch, collected from healthy adult colonies, or intercepted from illegal harvesting and subsequently transplanted. Adopted technique includes the use of aquarium facilities and later transplantation on rocky substrate using a two-component epoxy putty or stainless-steel structures (Montero-Serra et al., 2018; Montseny et al., 2019, 2020) as well as glued on 1.5 ml plastic tubes (Fava et al., 2010). Additionally, high survival rates of Mediterranean cold-water corals transplants were found in several studies (Davies et al., 2007; FAO, 2009; Aguilar & Marín, 2013), highlighting the feasibility of active restoration even for these environments despite the considerable limitations associated with the difficulties of working at intermediate and deep depths (Clauss & Hoog, 2002). Another project that is worth to mention is the MERCES project (Marine Ecosystem Restoration in Changing European Seas - http://www.merces-project.eu/?q=content/about-project), focused on the restoration of shallow and mesophotic degraded habitats. MERCES tried to put together marine biology and ecology, economy, policy and governance, as well as knowledge dissemination and communication, thanks to the creation of a multi-disciplinary consortium. Among all considered species, MERCES focused on habitat forming species of the upper mesophotic such as *P. clavata*, *C. rubrum* and *Eunicella* spp. combining different restoration techniques. Spread all over the Mediterranean Sea, studies on thermotolerance, facilitation, and restoration effectiveness (tested with different methods, e.g., different epoxy application method, artificial panels for larvae settlement and landers), as well as citizen science approaches and divers cooperation have been carried out trying to enhance habitat recovery (http://www.merces-project.eu/?q=content/list-deliverables).

1.3. Target species

1.3.1. Corallium rubrum (Linnaeus, 1758)

Endemic of the Mediterranean Sea and Atlantic Ocean, with remarkable abundance in the western Mediterranean (Cerrano et al., 2000; Giannini et al., 2003; Zibrowius, 1979), the precious red coral *C. rubrum* (Figure 3) is a colonial octocoral (Cnidaria, Anthozoa, Octocorallia) of the Coralliidae family currently listed among the Habitat Directive and Barcelona Convention due to its ecological and economical value, and also considered Endangered in the International Union for Conservation of Nature (IUCN) Red List (Bramanti et al., 2011; https://www.iucnredlist.org/species/50013405/110609252).

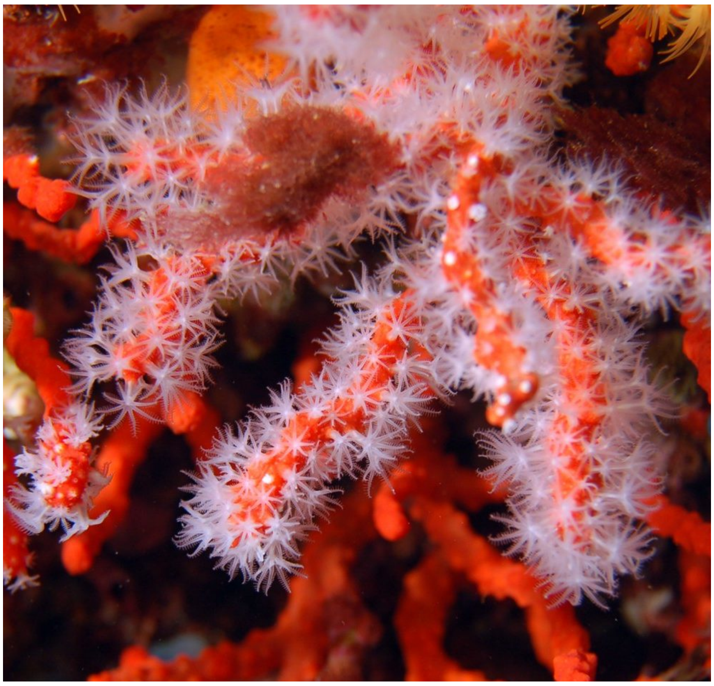

Figure 3: A colony of *Corallium rubrum* thriving on a typical coralligenous overhang (https://museialghero.it/museo-del-corallo/biologia/)

The species is a slow-growing ecosystem engineer of Mediterranean benthic communities (Rossi et al., 2008), presenting a wide bathymetric range up to 1000 m depth (Knittweis et al., 2016), even though it mainly occurs at mesophotic depths, generally between 20 and 200 m (Zibrowius et al., 1984). The species is characterized by an arborescent morphology and, under the optimal environmental condition, can reach up to 50 cm in height and more than 2 kg weight. However, due to intense harvesting and the frequent mass mortality events occurred in the past, colonies taller than 20 cm and thicker than 2 cm are, today, very rarely found (Garrabou & Harmellin, 2002; Roveta et al., 2023). The mineralized skeleton exhibits a bright red color, attributed to the presence of canthaxanthin, a chemical compound belonging to the group of carotenoids (Cvejic et al., 2007). This aspect is strictly related to the historical exploitation of the coral: the red colored calcium carbonate skeleton was used for the manufacture of precious objects for magical, ornamental and religious application, and traded worldwide (Ascione, 1993; Weinberg, 1993). The coral was intensively fished by using boats ("coralline") hosting fishing devices called "Croci di Sant'Andrea" consisting of two wooden planks fixed together in a cross, weighted by metal balls or stones, to which groups of nets were fixed to collect the coral. Subsequently, during the middle of the 20th century, this tool was replaced by the "Sbarra italiana", a structure of a single wooden plank to which groups of nets were connected (Marongiu, 1996; Cattaneo-Vietti et al., 2017). Several centuries of intense commercial harvesting led to a decline of the species at basin scale, before more stringent management rules were applied in the '80s (Tsounis et al., 2010). Additionally, in the last decades, repeated mass mortalities mainly linked to marine heat waves were identified as the major threat for the shallow populations of the red coral (Garrabou et al., 2019, 2022). In particular, those of 1999 and 2003 caused mortality rates of up to 100% (Cerrano et al., 2000; Perez et al. 2000; Garrabou & Harmelin, 2002; Garrabou et al., 2009). The red coral is a gonochoric brooder species with internal fertilization. Sexual maturity is reached at 7/10 years, when the organism is almost 6 cm high. Reproduction occurs once a year within a limited time interval from the end of July to early August (Santangelo et al., 2003; Bramanti et al., 2005) and the spawned larvae (planulae) exhibited negative buoyancy and active swimming behavior for most of the time, conferring high dispersive potential (Martínez et al., 2015). Reproduction can also take place asexually through fragmentation, by breaking one portion of the colony, with subsequent potential adhesion on a hard substrate and the development of a new colony. Using this type of reproduction, the species generates clones, a strategy typically adopted in conditions of stress and disturbance (Russo, 1995; Russo et al., 1997), and useful for restoration methodologies (Highsmith, 1982; Gallmetzer et al., 2010).

1.3.2. Paramuricea clavata (Risso, 1827)

The red gorgonian *P. clavata* (Cnidaria, Anthozoa, Octocorallia) (Figure 4), belonging to the family Paramuriceidae, is one of the typical ecosystem engineers of Mediterranean coralligenous assemblages, playing a crucial role in the maintenance of biomass and structural complexity of this bioconstruction (True, 1970; Jones et al., 1994; Ballesteros, 2006).

Despite its importance as habitat forming species, this gorgonian is not listed in any conservation or protection directive. The species can be found in rocky substrates from 15 to 200 m depth, with a wide geographical distribution (Linares et al., 2007; Gori et al. 2017).

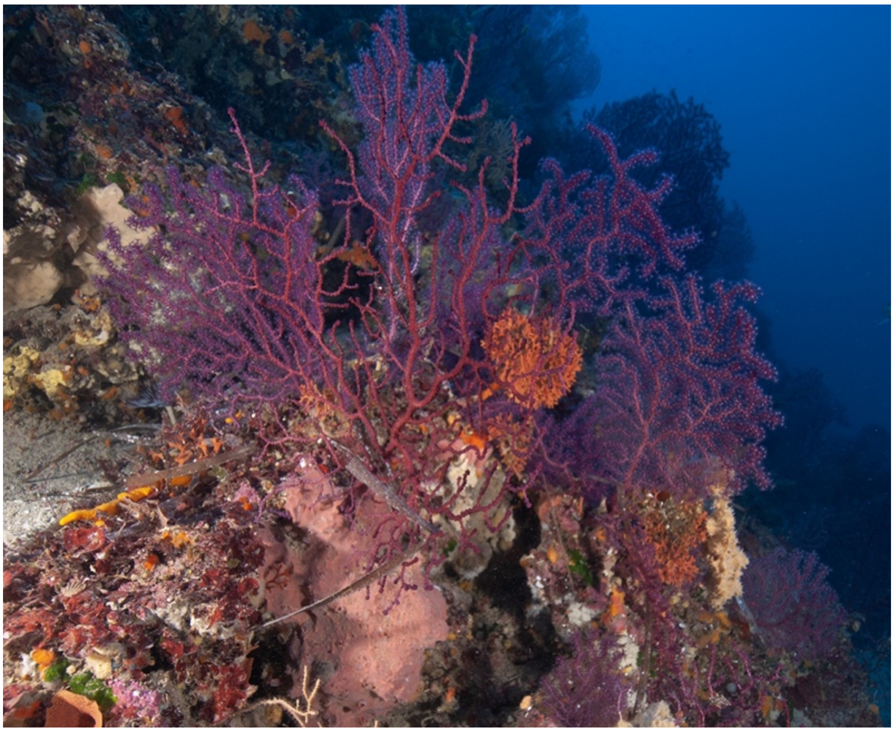

Figure 4: A colony of the red gorgonian *Paramuricea clavata* (Photo credits: Claudio Provenzani).

It is typically abundant in sciaphilic rocky walls, but it also grows on horizontal or sub-horizontal surfaces if light levels are low. Being a suspension feeder, the species prefers areas of high hydrodynamic activity with presence of strong currents (Palma et al., 2018).

P. clavata is an azooxanthellate, therefore biomass accumulation is only due to heterotrophic nutrition, consisting in zooplankton, POM (Particulate Organic Matter) and DOM (Dissolved Organic Matter) (Grigg, 1972; Coma et al., 1994). The shape of the fan, its growth and orientation adapt to local water current regimes, with the maximal filtration surface always opposing the water flow to maximize feeding efficiency (Grigg et al., 1972; Palma et al., 2018). The growth rate of *P. clavata* varies depending on the area but is estimated to be of few cm per year (Mistri & Ceccherelli, 1994). It is a gonochoric species, with a sex ratio generally not significantly different from 1:1, and low fecundity (Linares et al., 2008). Spawning is synchronous and occurs every year generally in May-June, coinciding with the increasing of the water temperature and correlated with the lunar cycle (from 3 to 6 days after the full moon or the new moon). Fertilization is external and embryogenesis takes place on the surface of the colony. The mature larvae (planulae) have limited mobility, and they rapidly settle on the surrounding substrate. Asexual reproduction by fragmentation is possible, but not responsible for the maintenance of the population that is based on the sexual reproduction (Coma et al., 1995). As cold-affinity species, *P. clavata* is highly sensitive to thermal stress, and is therefore considered an indicator species for climate effects on benthic assemblages (Linares et al., 2008; Previati et al., 2010; Vezzulli et al., 2013). Considering this, first documented mass mortality event in the Ligurian Sea occurred in the 1980s: the red coral *C. rubrum* and the gorgonian *P. clavata* have been heavy impacted (Bavestrello & Boero, 1986; Bavestrello et al., 1994). Also, fishing is a threat for this species: long lines, for example, can be considered one of the main causes of mortality due to mechanical injuries. The lines cause severe lesions to the gorgonian coenenchyme, favoring the development of epibionts that increase the friction of the colonies, eventually causing the rupture of the branches (Betti et al., 2020).

1.3.3. Cladocora caespitosa (Linnaeus, 1758)

C. caespitosa (Figure 5) is a colonial scleractinian coral of the Faviidae family, present in all the Mediterranean Sea and in the Ibero-Maroccan Gulf (Kružić et al., 2003) and included in the Annex II of the Protocol SPA/BD and in the Annex B of the EU Regulation Trade wild fauna and flora species (Relini & Tunesi, 2009; Otero et al., 2017). Additionally, it is considered of Least Concern by the IUCN Red List (https://www.iucnredlist.org/species/133142/165739749).

Figure 5: A colony of the Mediterranean cushion coral *Cladocora caespitosa* (Photo credits: Fabrizio Torsani).

It is commonly distributed from 5 to 20 m depth, although it can be found deeper, up to 40/50 m, where light is still sufficient for the species (Morri et al., 1994; Kružić et al., 2002).

Light availability is in fact crucial for this coral, since it hosts a symbiosis with zooxanthellae, being the only hermatypic Mediterranean coral able to build reef-like structures (Kružić & Benković, 2008). *C. caespitosa* colonizes both hard and soft bottoms, modifying its shape depending on the hydrodynamic environmental conditions of the area: encrusting with short corallite where there is a high hydrodynamism, ramified with longer corallites where lower hydrodynamic conditions are present (Abel, 1959). Massive and subspherical colonies are the most common, formed by cylindrical corallites developed by extra-tentacular (i.e., on the lateral side of the old polyp), rarely intra-tentacular (i.e., longitudinal fission of the disc of the original polyp) budding. When abundant, colonies may form beds (i.e., dense populations of numerous small globose colonies) or banks (i.e., large frameworks of colonies, more than 1m high and covering several square meters). However, this species can be also found in mixed distributions, often associated with rhodolits (Kružić et al., 2002, 2017). Asexual reproduction occurs by budding or fragmentation (Kružic, 2005). Regarding the sexual reproduction, one gametogenic cycle happens per year, usually in late spring and early summer, according to seasonal changes in temperature and lunar cycles (Harrison et al., 1984; Baird et al., 2001; Wilson & Harrison, 2003): oocytes and spermaries (digonic; i.e., male and female gametes produced in separate gonads in the same individual) are produced few days before the full moon, by the same hermaphroditic polyp (Kružić, 2005; Kružić et al., 2008). *C. caespitosa* mortality seems to be related to climate variation in the Mediterranean Sea (Perez et al., 2000; Rodolfo-Metalpa et al., 2005; Garrabou et al., 2009), as well as to the presence of alien species and eutrophication (Kružić & Benkovic, 2008; Kružić et al., 2008; Kružić et al., 2007).

1.4. Aim of the study

This study aims to understand the possibility to restore and recover the anthozoans *Paramuricea clavata*, *Corallium rubrum* and *Cladocora caespitosa*, with both laboratory and field studies. Considering the growing impact that society and climate change are having on marine ecosystems, it is urgent to find a way to reverse this trend. Concretely, this research has been conducted questioning the feasibility of transplanting these three Mediterranean coral species. For *C. caespitosa*, the study was performed in the laboratory, while for the other two on the field. *C. caespitosa* growth in controlled conditions was tested and evaluated as a possibility to use aquaria as potential *ex situ* nurseries for restoration projects. For the octocorals *C.*

rubrum and *P. clavata*, the objective was to evaluate their survivor and growth once translocated from a donor site (Punta Finestra, Monte Argentario, Northern Tyrrhenian Sea) to a recipient site (Punta Sciusciaù, Gallinara Island, Ligurian Sea).

2. Materials and methods

2.1. *Cladocora caespitosa* laboratory experiment

2.1.1. Treatment and experimental setup

A colony of *Cladocora caespitosa* collected in Alassio (Ligurian Sea) was divided in 40 fragments: 10 fragments with 1, 2, 3, 4 corallites, respectively. Fragments were left to acclimate for two weeks, before the start of the experiment. Eight 30 L aquaria (25 cm x 38 cm x 50 cm) were set up and equipped with a pump (NEWA® Maxi, 375 L/H) and a biological filter. All aquaria were positioned inside a bigger tank of around 400 L (150 cm x 95 cm x 40 cm) to guarantee a continuous recirculation of the water (Figure 6). Temperature was maintained at 18°C with a heat exchanger (Askoll THERM XL 200W) and salinity was maintained constant at 35 PSU.

Five fragments, with the same number of corallites, were placed in each aquaria, and were subjected to two different light treatments: four aquaria received a 12h:12h light:dark photoperiod using a POWER-GLO lighting system (1750 lumen, 40 W), while four were maintained in complete darkness. The darkness treatment was guaranteed by a black plastic panel between and above the aquaria preventing light penetration (Figure 6).

The experiment lasted from 2 months from December 19th 2022 to February 20th 2023.

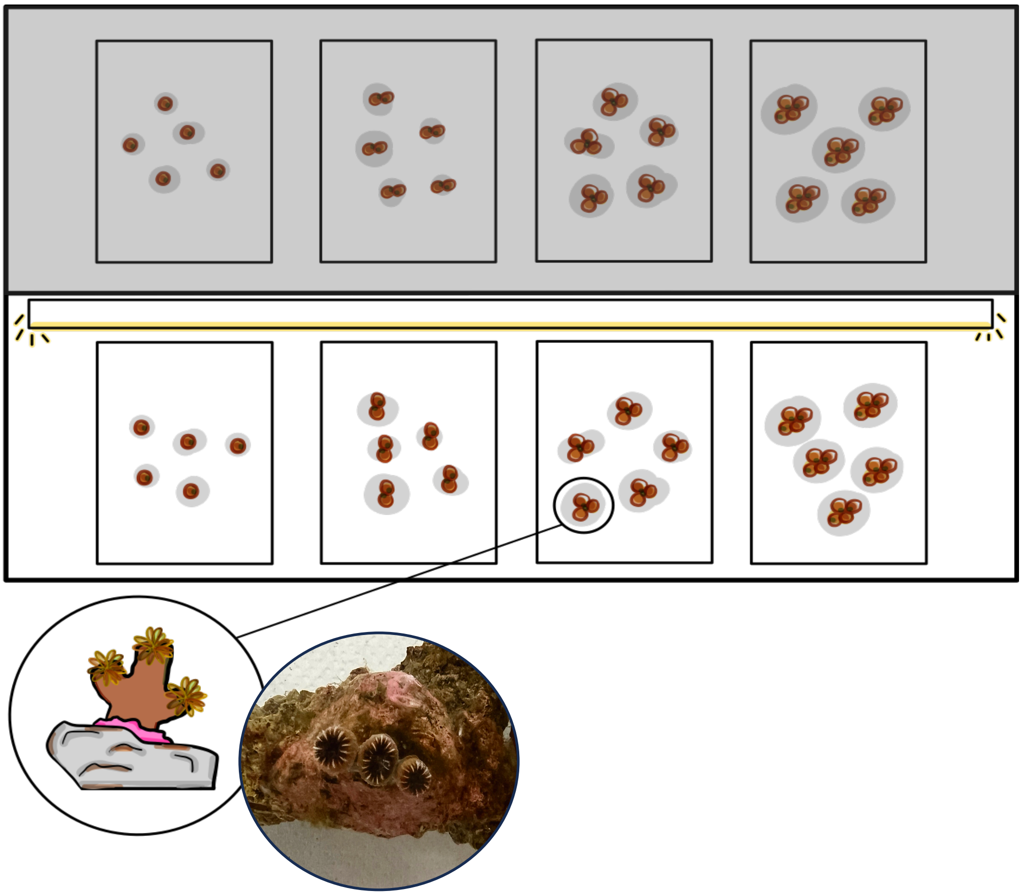

Figure 6: Schematic representation of the experimental setup. Eight aquaria were divided, within a single big tank, in two treatments: light (below in the representation), and dark (on top). Each aquaria contained 5 fragments, based on the number of corallites. The highlighted nubbin comes from the light treatment aquaria, from the group of 3 corallites (c3).

2.1.2. *Artemia salina* preparation and feeding methodology

During feeding periods, the continuous recirculation was stopped for 1 hour to avoid changes in the water parameters, while nubbins in each aquaria were covered with a transparent plastic bowl of 1.4L to maintain a high food concentration and optimize the possibility of polyps' to feed. Small holes were present in each plastic bowl to inject *Artemia salina* nauplii using a pipette.

A. salina was prepared 2/3 days before the administration: cists were added into a 500 ml beaker with filtered seawater and placed on a heated plate with an aerator to facilitate the hatch. The quantity of food distributed per aquaria was calculated following Leal et al. (2014) and a specific concentration of *A. salina* nauplii was given to each group of fragments proportionally to the number of corallites: fragments with 1 corallite received a 1 ml solution of *A. salina* nauplii, 2 corallites 2 ml, 3 corallites 3 ml, and 4 corallites 4 ml. After one hour, bowls were removed and the system was reactivated. Fragments were fed twice a week (generally Monday and Thursday), in the morning, after testing water temperature, nitrates (using a Salifert Nitrate Profi Test) and salinity (with a refractometer). Every Thursday, after the feeding procedure was completed, 60 L of water were changed.

2.1.3. Data collection and analysis

During the 2 months-experiment, photo surveys of the fragments were conducted to investigate corallites status and growth. Data were collected on the 12/12/2022 (T1), 09/01/2023 (T2), 19/01/2023 (T3), 26/01/2023 (T4), 09/02/2023 (T5) and on the 20/02/2023 (T6). Photos were taken from above and sideways in order to get the best perspective of the corallites, including in the frame both the fragments and a metric reference.

Photos were analyzed using the free software ImageJ (Rasband, 2012), and the surface area (mm^2) of fragments and of new corallites was measured. The bleaching status of the fragments was assessed using the 'Coral health chart' (Figure 7), a simple table produced by Australian CoralWatch program (https://coralwatch.org).

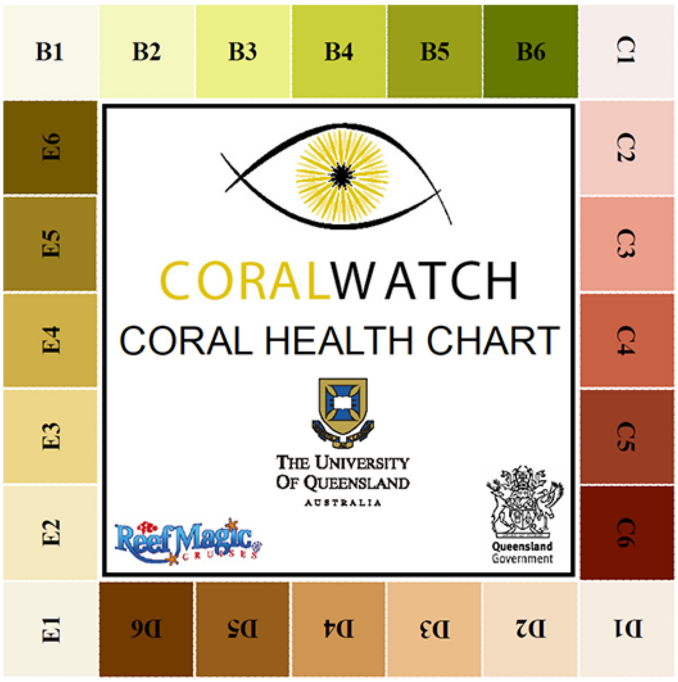

Figure 7: The Australian CoralWatch Coral health chart.

The measures of the areas were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene's tests. Then, a repeated measure analysis of variance (ANOVA) was used to test changes of the fragments' area through time. The Tukey's post-hoc comparison was used when significant differences were found. Statistical analyses were performed with the free software PAST (PAleontological STatistics), version 4.05 (Hammer et al., 2001).

2.2. *Paramuricea clavata* and *Corallium rubrum* transplantation activities

2.2.1. Donor and recipient sites

Fragments were collected in Punta Finestra (Northern Tyrrhenian Sea) and transplanted in Punta Sciusciaù at the Gallinara island (Ligurian Sea).

Corals’ collection took place in the diving site Punta Finestra (42°21'45.4"N, 11°10'56.8"E), close to Porto Ercole, in the eastern area of Monte Argentario, a broad promontory up to 635m high and 60km^2, central of the Tyrrhenian Sea (Figure 8A).

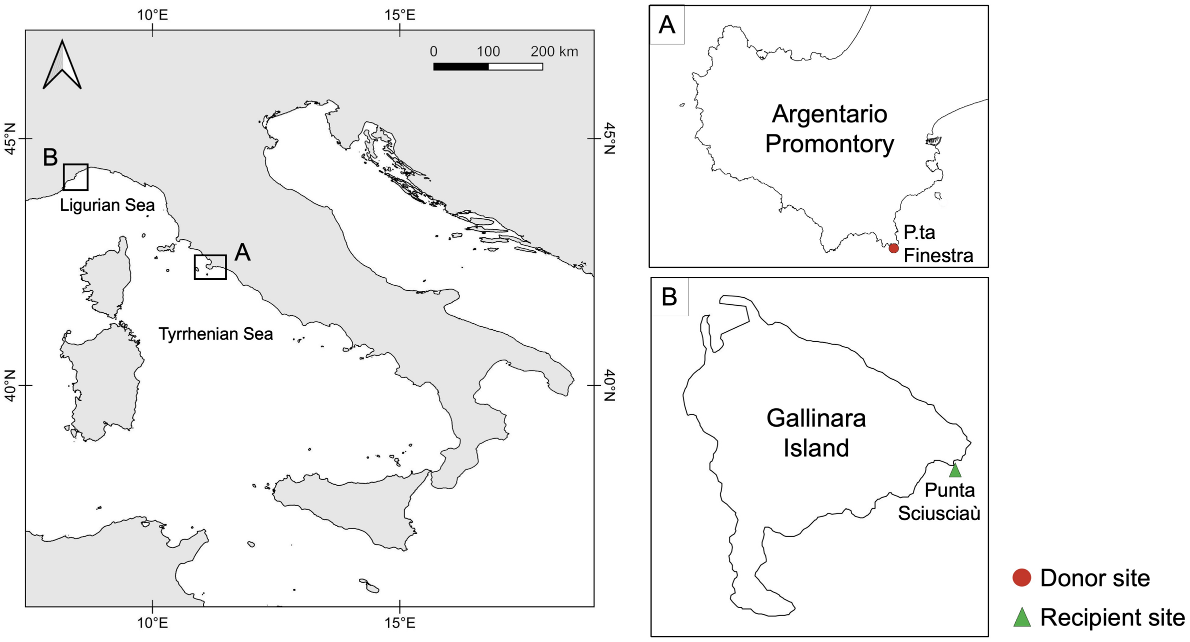

Figure 8: Map of the location of the (A) recipient and (B) donor sites, respectively.

The name of the diving spot comes from a hole situated on a cliff, right above the sea water. Underwater, from the first meters, the site is characterized by small tunnels and caves, where eels and shrimps can be found; the area is also subjected to the transit of pelagic fish and presence of nudibranchs. From 15 m depth, several individuals of *Eunicella singularis* and *E. cavolini* can be found, while around 27 m depth, the red gorgonian *Paramuricea clavata*, together with *Corallium rubrum, Parazoanthus axinellae* and *Leptosammia pruvoti* thriving in small crevices, characterize the area (https://www.tuttomaremma.com/subacquea puntafinestra.htm).

Transplantation activities were carried out in the Ligurian Sea, at Punta Sciusciaù (44°01’30.0” N, 8°13’45.9” E), situated in the Gallinara Island, a small island with an extent of 0.11 km^2 and a maximum elevation of 87 m above sea level, which became Regional Natural Park in 1989 (Guida et al., 2019) (Figure 8B).

The site is characterized by the typical Mediterranean rocky bottom assemblages. The submerged profile presents a large sub-horizontal terrace between 6 and 12 m, while deeper, a sciaphilous algal population can be found, together with an animal component consisting of sponges, bryozoans and tunicates. Below 25 m, coralligenous biocenosis constitute the main habitat, with cnidarians, bryozoans and numerous large specimens of porifera, including several individuals of *Axinella polypoides* (Abbiati et al., 2011).

2.2.2. Collection and transplantation activities

In February 2023, at the donor site Punta Finestra, 25 fragments of *P. clavata* and 29 of *C. rubrum* were collected at around 20 m depth using scissors and a wire cutter for the red gorgonian and the red coral, respectively (Figure 9). Collected fragments were placed in plastic bags and brought to the surface, where they were moved into a tank filled with seawater. Corals were then taken to the recipient site by car. During the transport, two aerators were used to oxygenate the water in the tank, and ice packs were used to maintain the water temperature of the collection site (15℃). Once at destination, fragments of *C. rubrum* were fixed on previously cut plastic grids. Five fragments were attached to 5 different grids (for a total of 25 fragments) using a two-component epoxy (Subcoat S, Veneziani Yachting; https://venezianiyachting.com/). During this phase, fragments were never exposed to air. The grids with *C. rubrum* fragments were then placed again in the tank, kept oxygenated and at optimal temperature. The day after, fragments of both octocorals were transplanted at the recipient site (Punta Sciusciaù, Gallinara Island) by six scuba divers, divided in two teams. *P. clavata* was placed around 40 m depth on a rocky terrace, previously cleaned from epibionts with a metal brush, using the two-component epoxy. *C. rubrum* fragments were instead transplanted on a vertical wall at around 35 m depth, using two techniques: the grids were attached with stakes nails in crevices on the vertical wall, while the remaining free fragments were individually placed using the two-component epoxy (Figure 9).

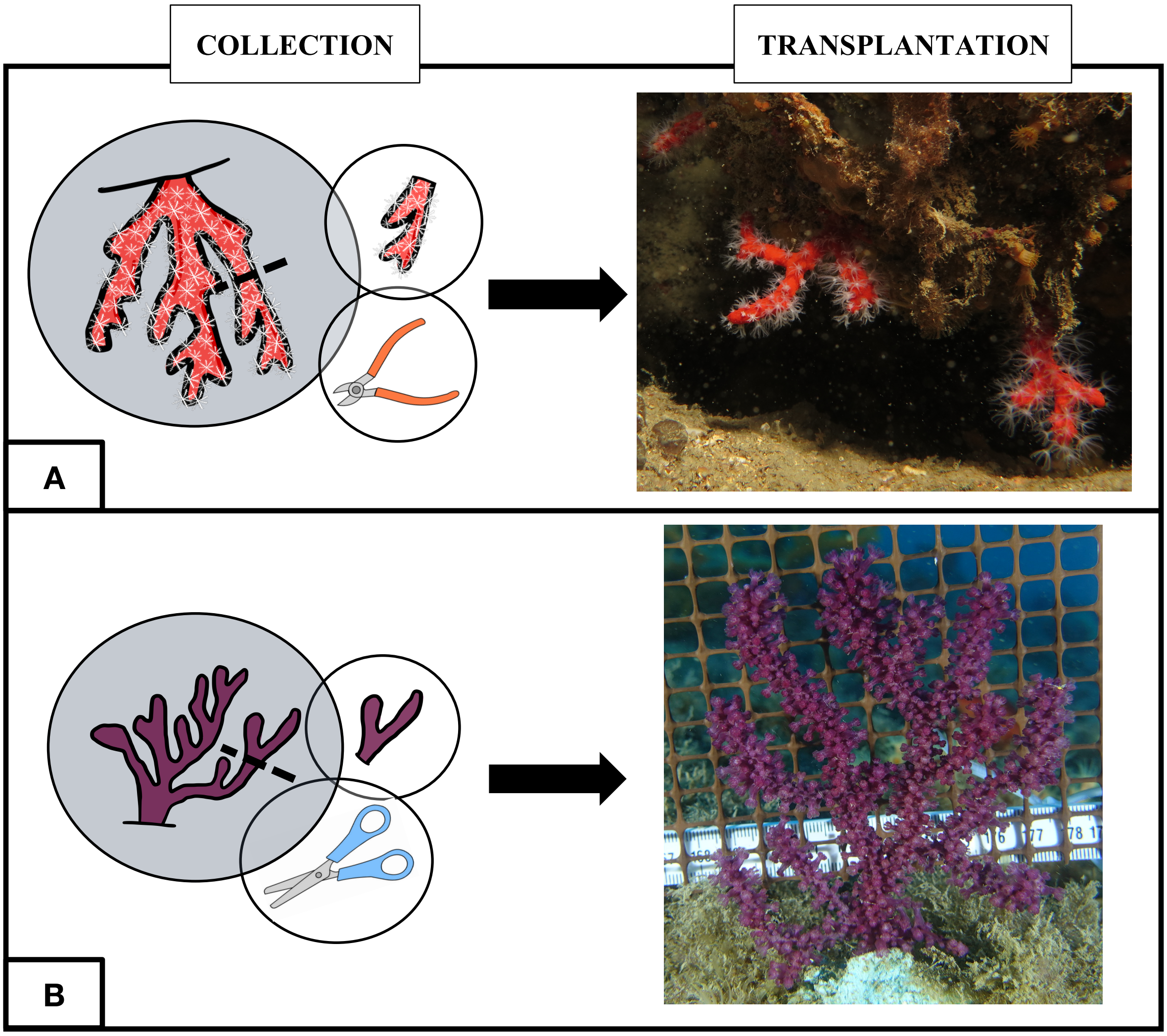

Figure 9: Schematic representation of the collection and transplantation techniques used for (A) the red coral *Corallium rubrum*, and for (B) the red gorgonian *Paramuricea clavata*.

After the transplantation event, photo samplings occurred on 10th April (Ti) and 24th May 2023 (Tf) for *P. clavata*, and on the 7th April (Ti), 24th May and 8th September 2023 (Tf) for *C. rubrum*. Together with the newly transplanted *C. rubrum* fragments, others previously transplanted in 2017, 2018 and 2019 in the framework of the MERCES project were also monitored in this study.

In this project, 3 different techniques have been used to the transplantation (Villechanoux et al., 2022; Roveta et al., 2023) (Figure 10):

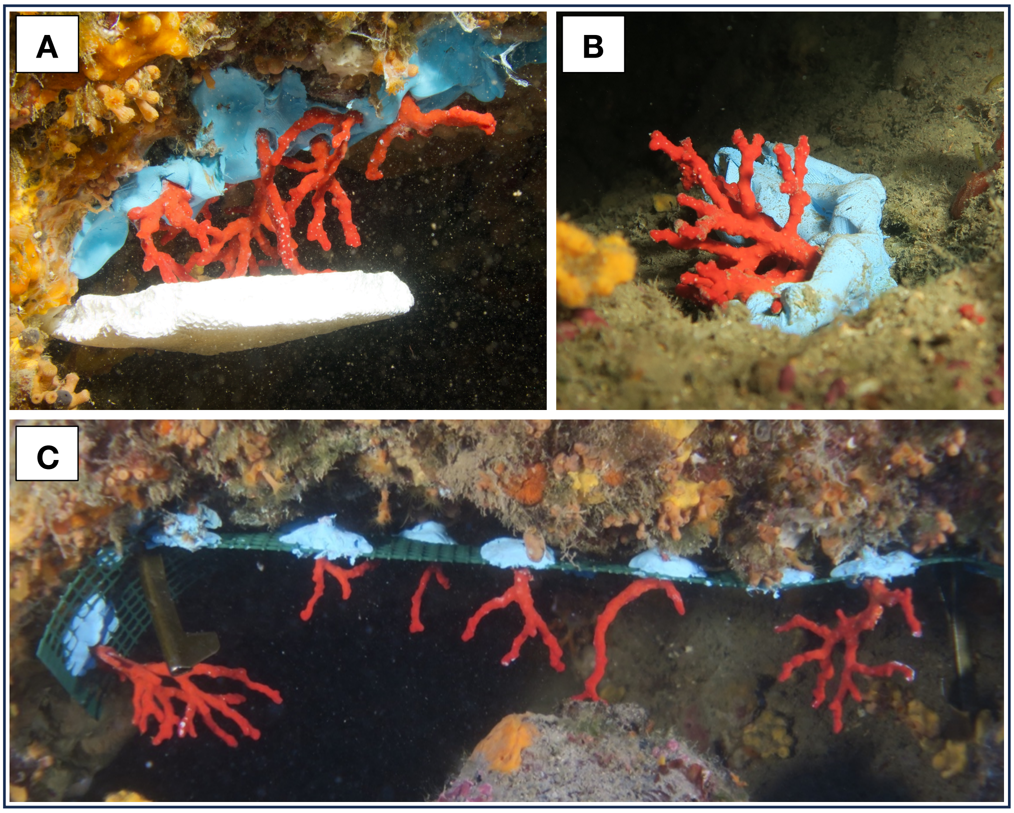

Figure 10: Red coral fragments transplanted using the (A) polystyrene, (B) epoxy and (C) grid techniques.

1. Polystyrene: the floaty material it's been utilized, combined with a resin, to keep the fragments in upside-down position under crevices, instead of an erected position. This approach, should have reduced risk of destruction due to fishing practices, and avoided sedimentation;

2. Epoxy: two-component epoxy putty used as adhesive to attach the fragment to the rock;
3. Grid: PVC-grid fixt to the rock with nails; some corals were trapped in the grid, used as base to sustain the nubbins.

   Since only the grid demonstrated to be successful among the three tested methods (Villechanoux et al., 2022), only fragments transplanted with this technique were considered for the analyses.

2.2.3. Data collection and analysis

After the transplantation, photo surveys of fragments occurred in April, May and September 2023 using a grid or a ruler as metric references. Each fragment of both *P. clavata* and *C. rubrum* has been identified with an alphanumerical code. Photos were analyzed using ImageJ software.

For *C. rubrum*, photos were used to count the apexes of each fragment; the number of apexes was normalized by month for all the fragments obtaining a monthly rate in apexes production. The non-parametric Kruskal-Wallis test was used to test differences in the number of apexes of red coral colonies from the initial transplantation to the last survey occurred in September 2023.

On the other hand, for *P. clavata*, photos were used to measure the height and width of the colony (Figure 11A); these data were then tested for normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test). A paired t-test was to test its potential growth between Ti and Tf. The same photos were also used to make a graphic representation of the colony through its skeletonization (Figure 11B), allowing us to identify 'critical parameters' such as the presence of new branch(es), growth or loss of part(s) of a branch or an entire branch, and tissue damage through time.

All statistical analysis were performed with the free software PAST.

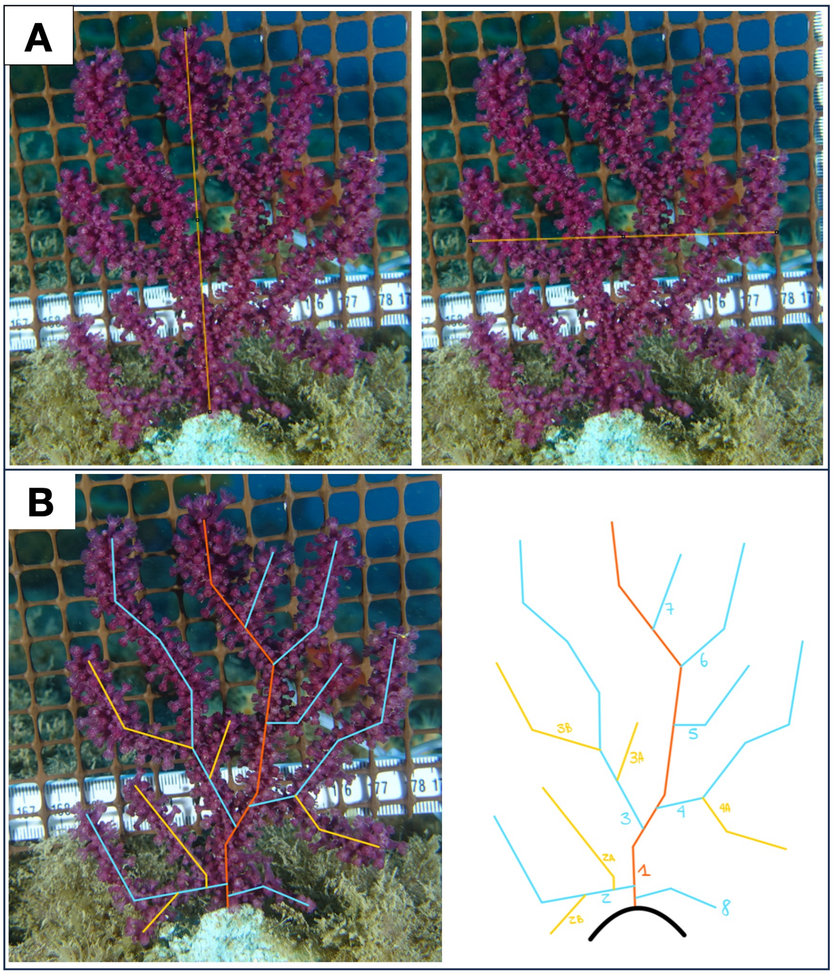

Figure 11: Example of (A) height and width evaluation of a transplanted *Paramuricea clavata* fragment, and (B) its schematic representation.

3. Results

3.1. *Cladocora caespitosa* bleaching, growth and new corallites

During the entire duration of the experiment, all fragments were observed healthy in both conditions (dark and light). Fragments under light conditions did not bleach, keeping a tone of E6-5 of the Australian CoralWatch Coral Health Chart for all the duration of the experiment. On the contrary, except for just one fragment with 1 corallite, all the other fragments placed in dark conditions eventually bleached, starting from a tone of E6-5 and reaching a E2-1 tone (Table 1). Already after a few weeks (T2), 8 colonies started bleaching, while at T3 already an evident bleaching status in most of the fragments (12) was clear (Table 1).

Changing in the total area of each fragment was recorded (Table 2), appearing to be almost null for all the fragments under each treatment.

Apart for 3- and 4- corallites fragments under light conditions, which showed statistically significant differences in their area through the duration of the experiment (repeated measure ANOVA, $p<0.05$), already after 1 month (Tukey post-hoc, $p<0.05$), no statistical changes were found for the other fragments.

Table 1: Bleaching status of fragments of the dark treatment. Colors correspond to the ones present in the Australian CoralWatch Coral Health Chart (CoralWatch - https://coralwatch.org). D = dark treatment; c = corallite. The number in the reference code is referred to the total number of corallites per fragment. Monitoring of the bleaching status in time is expressed by T1, T2, T3, T4, T5 and T6, respectively from the first to the last monitoring activity.

Number of corallites	T1	T2	T3	T4	T5	T6
D-c1	E6	E6	E5	E4	E2	E2
	E6	E5	E3	E2	E1	E1
	E5	E5	E2	E2	E2	E1
	E5	E4	E2	E1	E1	E1
	E6	E6	E6	E6	E5	E4
D-c2	E6	E5	E3	E2	E1	E1
	E6	E6	E5	E4	E2	E1
	E6	E5	E3	E2	E1	E1
	E6	E6	E5	E5	E2	E2
	E6	E6	E3	E3	E2	E1
D-c3	E6	E4	E3	E2	E1	E1
	E5	E5	E3	E3	E1	E1
	E6	E5	E3	E3	E1	E1
	E5	E5	E3	E2	E2	E1
	E6	E6	E3	E2	E1	E1
D-c4	E6	E5	E3	E3	E2	E1
	E6	E4	E2	E2	E1	E1
	E6	E5	E2	E2	E1	E1
	E5	E3	E2	E2	E2	E1
	E5	E3	E1	E1	E1	E1

New corallites were displayed by 11 fragments of the light treatment, and by only 2 fragments of the dark treatment. New corallites were produced by 1 fragment with 1 corallite, for 2 with 2 corallites, for 3 with 3 corallites and for 5 with 4 corallites, within the light treatment; both the 2 new corallites of the dark treatment were produced by fragments with 3 initial corallites (Table 2). The area of the new corallites varied from 0.003 cm^2 to 0.011 cm^2.

Table 2: New corallites produced by fragments of *Cladocora caespitosa*. Appearance of a new corallite is highlighted with an x; the sum of the x represents the total new corallites formed. L = light treatment; D = dark treatment; c = corallite. The number in the reference code is referred to the total number of corallites per fragment. Exhibition of new corallites in time is expressed by T1, T2, T3, T4, T5 and T6, respectively from the first to the last monitoring activity.

	T1	T2	T3	T4	T5	T6	Total new corallites at T6
L-c1						x	1
L-c2		x					1
				x			1
L-c3						x	1
			x				1
				xxx	x		4
L-c4			xxx		x		4
			xxxx				4
					x		1
					x		1
					x		1
D-c3			x				1
			x				1

Table 3: Average area (cm, ± standard deviation) of *Cladocora caespitosa* fragments under light and dark conditions through the two-month experiment. L = light treatment; D = dark treatment; c = corallite. The number in the reference code is referred to the total number of corallites per fragment. Growth status in time is expressed by T1, T2, T3, T4, T5 and T6, respectively from the first to the last monitoring activity.

	T1	T2	T3	T4	T5	T6
L-c1	0.375 ± 0.009	0.320 ± 0.011	0.445 ± 0.013	0.301 ± 0.001	0.402 ± 0.003	0.322 ± 0.008
	0.388 ± 0.012	0.403 ± 0.007	0.340 ± 0.013	0.305 ± 0.003	0.369 ± 0.014	0.301 ± 0.007
	0.558 ± 0.018	0.358 ± 0.012	0.541 ± 0.014	0.322 ± 0.004	0.357 ± 0.009	0.357 ± 0.010
	0.378 ± 0.011	0.276 ± 0.003	0.354 ± 0.009	0.253 ± 0.004	0.305 ± 0.006	0.256 ± 0.007
L-c2	0.428 ± 0.014	0.551 ± 0.022	0.545 ± 0.005	0.477 ± 0.003	0.499 ± 0.007	0.459 ± 0.012
	0.558 ± 0.011	0.474 ± 0.006	0.565 ± 0.023	0.450 ± 0.022	0.534 ± 0.018	0.432 ± 0.011
	0.425 ± 0.022	0.409 ± 0.008	0.541 ± 0.012	0.391 ± 0.010	0.458 ± 0.010	0.396 ± 0.004
	0.630 ± 0.011	0.604 ± 0.014	0.825 ± 0.019	0.515 ± 0.013	0.738 ± 0.005	0.500 ± 0.004
L-c3	0.919 ± 0.039	0.894 ± 0.026	0.961 ± 0.024	0.887 ± 0.017	1.018 ± 0.002	0.880 ± 0.006
	0.865 ± 0.028	0.718 ± 0.014	0.861 ± 0.015	0.764 ± 0.011	0.772 ± 0.011	0.727 ± 0.006
	0.726 ± 0.009	0.717 ± 0.009	0.985 ± 0.007	0.712 ±0.004	0.774 ± 0.007	0.672 ± 0.013
	0.706 ± 0.016	0.746 ± 0.015	0.649 ± 0.025	0.526 ± 0.005	0.525 ± 0.003	0.484 ± 0.005
L-c4	1.094 ± 0.028	1.071 ± 0.008	1.158 ± 0.017	1.055 ± 0.018	1.040 ± 0.010	1.095 ± 0.054
	0.699 ± 0.031	0.741 ± 0.025	0.741 ± 0.025	0.741 ± 0.025	0.741 ± 0.025	0.741 ± 0.025
	0.847 ± 0.017	0.910 ± 0.019	1.026 ± 0.061	1.012 ± 0.015	1.011 ± 0.004	1.001 ± 0.016
	0.527 ± 0.010	0.527 ± 0.010	0.515 ± 0.016	0.503 ± 0.008	0.503 ± 0.008	0.503 ± 0.008
D-c1	0.555 ± 0.024	0.443 ± 0.019	0.384 ± 0.010	0.411 ± 0.022	0.343 ± 0.001	0.397 ± 0.006
	0.291 ± 0.011	0.269 ± 0.020	0.214 ± 0.008	0.201 ± 0.008	0.223 ± 0.006	0.251 ± 0.009
	0.241 ± 0.032	0.220 ± 0.015	0.227 ± 0.017	0.165 ± 0.007	0.180 ± 0.012	0.196 ± 0.008

	0.333 ± 0.009	0.366 ± 0.012	0.377 ± 0.017	0.236 ± 0.009	0.231 ± 0.006	0.222 ± 0.009
	0.263 ± 0.018	0.226 ± 0.008	0.195 ± 0.003	0.157 ± 0.002	0.174 ± 0.008	0.137 ± 0.004
D-c2	0.461 ± 0.041	0.425 ± 0.031	0.464 ± 0.009	0.342 ± 0.008	0.523 ± 0.012	0.470 ± 0.005
	0.375 ± 0.015	0.365 ± 0.032	0.329 ± 0.009	0.252 ± 0.010	0.343 ± 0.005	0.334 ± 0.005
	0.247 ± 0.014	0.348 ± 0.029	0.245 ± 0.006	0.215 ± 0.008	0.319 ± 0.012	0.252 ± 0.007
	0.612 ± 0.017	0.598 ± 0.009	0.640 ± 0.013	0.512 ± 0.010	0.636 ± 0.011	0.483 ± 0.017
	0.470 ± 0.008	0.510 ± 0.015	0.508 ± 0.018	0.414 ± 0.005	0.423 ± 0.014	0.336 ± 0.012
D-c3	0.649 ± 0.028	0.572 ± 0.017	0.564 ± 0.007	0.545 ± 0.014	0.637 ± 0.012	0.623 ± 0.005
	0.640 ± 0.007	0.623 ± 0.024	0.623 ± 0.024	0.480 ± 0.011	0.728 ± 0.010	0.910 ± 0.009
	0.591 ± 0.012	0.562 ± 0.018	0.582 ± 0.020	0.507 ± 0.012	0.357 ± 0.016	0.498 ± 0.012
	0.594 ± 0.013	0.559 ± 0.018	0.551 ± 0.018	0.466 ± 0.014	0.634 ± 0.011	0.522 ± 0.012
	0.847 ± 0.036	0.683 ± 0.007	0.762 ± 0.016	0.608 ± 0.008	0.887 ± 0.004	0.670 ± 0.005
D-c4	0.830 ± 0.029	0.705 ± 0.005	0.705 ± 0.005	0.644 ± 0.023	0.786 ± 0.011	0.616 ± 0.009
	0.854 ± 0.029	0.967 ± 0.013	0.973 ± 0.019	0.596 ± 0.008	0.784 ± 0.009	0.784 ± 0.009
	0.750 ± 0.028	0.839 ± 0.003	0.934 ± 0.014	0.791 ± 0.010	0.919 ± 0.006	0.919 ± 0.006
	0.918 ± 0.030	0.957 ± 0.040	0.875 ± 0.018	0.667 ± 0.122	0.667 ± 0.122	0.667 ± 0.122
	1.048 ± 0.026	1.048 ± 0.026	1.191 ± 0.026	0.943 ± 0.013	0.923 ± 0.008	0.923 ± 0.008

3.2. *Corallium rubrum* survivorship and apexes variation

From the first transplantation activity, back in 2017, until now, a total of 87 fragments have been transplanted, of which 41 died or got lost and 46 survived.

Considering the three transplantation techniques tested in the MERCES project, of the 20 colonies transplanted with the polystyrene, just 9 are still present; only 3 colonies out of the 10 transplanted with the epoxy technique are still on site; while, among the 57 total colonies transplanted with the grid method, 34 colonies are still in place.

Considering the effectiveness of the grid, only the number of apexes of the colonies transplanted with this technique were investigated. Colonies transplanted in 2017, 2018, 2019 and 2023 showed an average monthly decrease of -0.044 ± 0.038, -0.040 ± 0.023, -0.301 ± 0.192 and -0.038 ± 0.054 apexes/month, respectively (Table 4).

Table 4: Average number of apexes per month in the colonies of *Corallium rubrum* transplanted during different transplantation activities.

	Apexes/month
2017	-0.044 ± 0.038
2018	-0.040 ± 0.023
2019	-0.301 ± 0.192
2023	-0.038 ± 0.054
Overall	**-0.106 ± 0.130**

Considering all colonies from Ti to Tf, 15 colonies did not show neither a reduction neither an increment in the total number of apexes, 9 displayed a reduction, while only one colony showed just 1 new apex (Table 5). Overall, no significant differences were found in the variation of apexes between Ti and Tf ($p > 0.05$). Since the lack of significance could have been related to having pooled together colonies transplanted in different years, a second

Kruskal-Wallis test was performed considering only the colonies transplanted in 2017, 2018 and 2019. However, also in this case, no significant differences were recorded ($p > 0.05$).

Table 5: Number of total apexes in the colonies of *Corallium rubrum* transplanted during different transplantation actions. Ti = initial time, corresponding to the year of transplantation; Tf = final time, corresponding to September 2023.

Year of transplantation	Transplanted colony	Ti	Tf	**Tf-Ti**
2017	24	3	3	**0**
	35	4	4	**0**
	126	3	2	**-1**
	46	9	4	**-5**
	49	3	3	**0**
	51	3	4	**1**
	52	2	1	**-1**
2018	55	4	2	**-2**
	56	5	3	**-2**
	95	4	4	**0**
	94	4	3	**-1**
	57	15	11	**-4**
2019	40	10	4	**-6**
2023	101	3	3	**0**
	103	3	2	**-1**
	104	2	2	**0**
	105	3	3	**0**
	108	2	2	**0**
	109	1	1	**0**
	110	1	1	**0**
	111	1	1	**0**
	112	2	2	**0**
	114	3	3	**0**
	115	2	2	**0**
	117	1	1	**0**
	118	1	1	**0**

3.3. *Paramuricea clavata* height, width and critical parameter variation

Of the 25 transplanted colonies of *P. clavata*, 16 are alive while 7 got lost. All colonies were observed healthy during the monitoring activities.

Seven out of the 16 colonies showed an increase in both height and width, while 5 colonies a reduction in both parameters. Instead, four colonies displayed an increase in height and a decrease in width (Table 6). The maximum increase was 2.042 cm in height and 3.977 cm in width, while the highest reduction was -1.543 cm in height and -3.630 cm in width. The paired t-test highlighted a slight significant difference in height ($p = 0.04$) from the Ti to Tf in May 2023, with average increase of 0.595 ± 1.084 cm. Conversely, the paired t-test for the width did not show any statistical difference between Ti and Tf ($p > 0.05$), with an average reduction of -0.905 ± 2.526 cm (Table 6).

Table 6: Height and width (cm) of the transplanted colonies of *Paramuricea clavata*. Ti = initial time, corresponding to the first monitoring (April 2023); Tf = final time, corresponding to May 2023.

	Ti		Tf		**Tf - Ti**	
Transplanted colony	Height	Width	Height	Width	**Height**	**Width**
1	8.953	9.785	10.995	9.674	**2.042**	**-0.111**
2	13.999	18.815	12.456	15.434	**-1.543**	**-3.381**
3	9.201	7.483	10.69	8.685	**1.489**	**1.202**
4	7.996	9.627	8.417	10.035	**0.421**	**0.408**
5	8.26	8.855	7.485	4.791	**-0.775**	**-4.064**
6	12.791	14.731	13.545	9.28	**0.754**	**-5.451**
7	9.503	9.147	11.13	8.942	**1.627**	**-0.205**
8	9.497	16.274	10.268	17.519	**0.771**	**1.245**
10	10.345	1.952	11.341	1.547	**0.996**	**-0.405**
11	10.648	13.814	11.071	14.619	**0.423**	**0.805**
12	7.597	8.405	9.481	12.382	**1.884**	**3.977**
13	12.079	15.551	11.005	12.729	**-1.074**	**-2.822**
14	8.882	14.286	10.487	14.631	**1.605**	**0.345**
16	10.254	15.127	11.447	15.829	**1.193**	**0.702**
17	12.188	13.968	12.051	10.338	**-0.137**	**-3.63**
19	10.622	8.1	10.464	5.009	**-0.158**	**-3.091**
					0.595 ± 1.084	**-0,905 ± 2.526**

Monitoring activities allowed us to observe a variation of some critical parameters, such as the presence of new branch(es), the growth or loss of part(s) of a branch or of an entire branch, and tissue damage. Regarding this last parameter, 2 colonies initially (Ti) displayed a tissue damage, but just one showed an actual recovery at Tf (Figure 12).

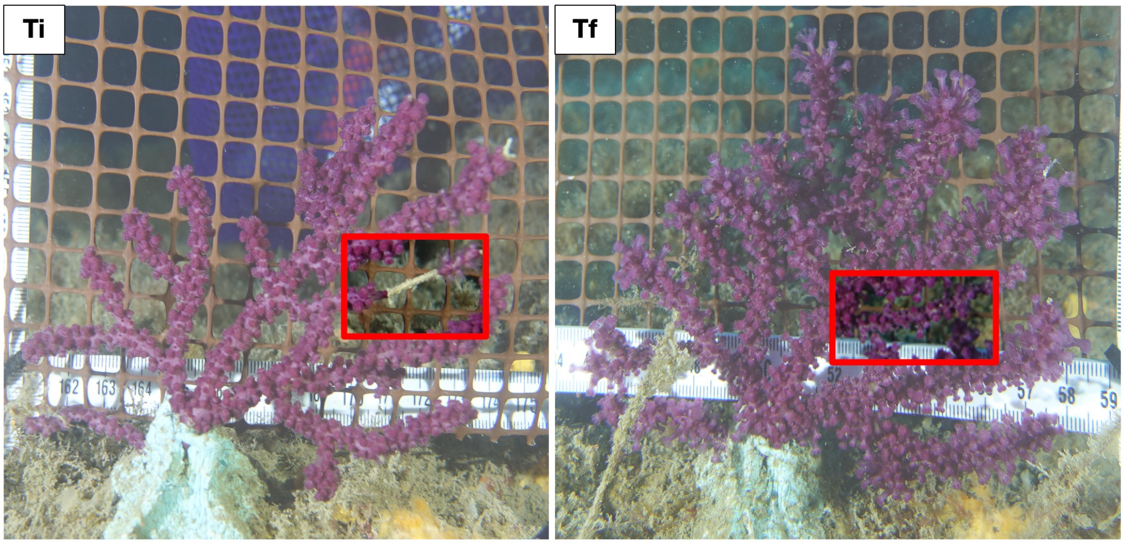

Figure 12: Evidence of tissue damage (Ti) and recovery (Tf) in a transplanted colony of *Paramuricea clavata*. Ti = initial time, corresponding to the first monitoring date (April 2023); Tf = final time, corresponding to May 2023.

Additionally, 5 colonies displayed 1 new branch, while 1 colony exhibit 2 new branches. Conversely, 2 colonies suffered the loss of 2 branches, and 1 colony the loss of 3 branches. Two colonies showed grow of the branches, especially grow of 1 branch in one case and 3 branches in the other, while 4 colonies showed a size reduction, with the loss of 1 or 2 branches (Table 7; Figure 13). Nonetheless, five colonies did not show any structural change.

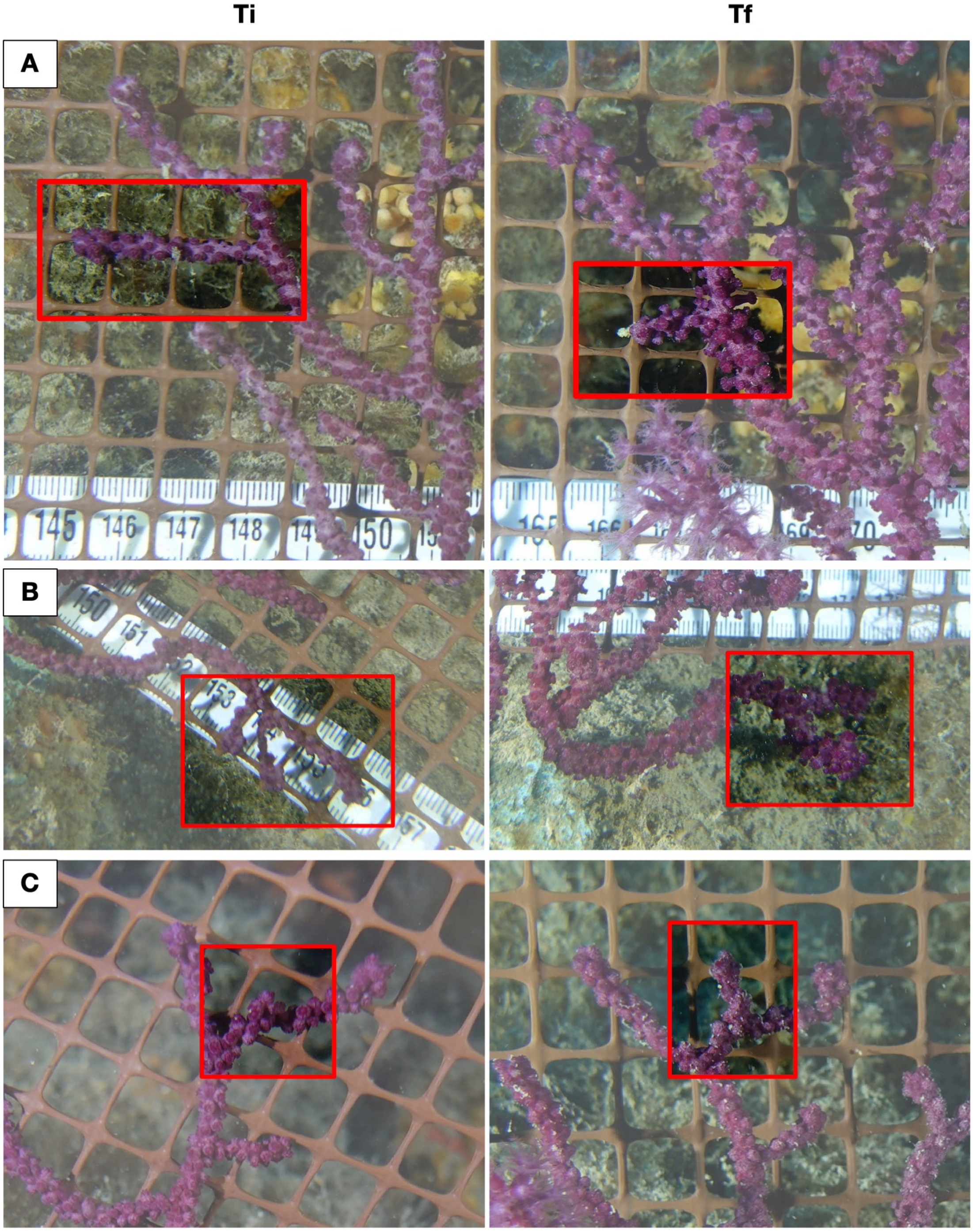

Figure 13: Examples of (A) branch size reduction, (B) branch loss and (C) growth of a new branch between an initial time (Ti, corresponding to the first monitoring in April 2023) and a final time (Tf, corresponding to the monitoring activity conducted in May 2023) in transplanted colonies of *Paramuricea clavata*.

Table 7: Variation of the critical parameter identified for the transplanted colonies of *Paramuricea clavata*.

Transplanted colony	Number of new branches	Number of lost branches	Growth of the branches	Branches size reduction
1	1	2		2
2	1			
6	1	3		
7	1		3	
11	2			
14	1		1	
16				1
17		2		1
19				1

4. Discussion and conclusions

The aim of this study was to investigate the possibility to restore and recover the Mediterranean anthozoans *Paramuricea clavata*, *Corallium rubrum* and *Cladocora caespitosa*. Restoration activities are widely applied all over the world, but to the best of our knowledge, studies dealing with the response of the species involved are still only a few. Being in the *Ecosystem Restoration Decade* (2021-2030), as declared by the United Nations, the results of this study will add information on the topic regarding crucial Mediterranean species, and may represent a reference for future studies.

For what concerns *C. caespitosa*, as previously reported for other cnidarians (Brown 1997; Venn et al., 2006; Hill & Ralph, 2007; Visram & Douglas, 2007), the variation in the tissue color (from E6/5 to E2/E1) in the fragments under dark conditions is a clear evidence of the lower content of zooxanthellae, meaning that a significant reduction in the microalgal density effectively leads to a loss of pigment in the symbiosis (Venn et al., 2006).

Moreover, to fulfil one of the aims of this study, we evaluated in controlled conditions, the coral *C. caespitosa* as a suitable candidate for the development of *ex situ* nurseries for restoration projects. Even if the growth of the colony might not be so evident, the formation of new corallites supports the feasibility of aquaria as a potential nursery for this endemic Mediterranean species.

The production of new corallites seems to follow a pattern: the higher the initial number of corallites (3- and 4-corallite fragments), the higher the number of new corallites produced, in both treatments. Being energetically expensive, budding is usually observed under favorable conditions and high food availability; considering equal amount of food dispensed for the light and dark conditions, a higher presence of zooxanthellae in the light treatment translates into a greater autotrophic contribution, potentially explaining the higher number of new polyps in the fragments exposed to light (Rodolfo-Metalpa et al., 2008). The positive effect of light is also supported by the fact that, in smaller fragments (1- and 2-corallite), the production of new polyps was observed only under this condition.

However, the initial different number of corallites can represent an additional factor influencing the production of new ones. As a matter of fact, a higher number of polyps, increase not only the possibility to catch the prey, but also the total energy available (Sakai et al., 2019). Moreover, the experimental set up must be also taken into account for additional considerations: firstly, the quantity of food given in this study was estimated per corallite, making the food available for 1-corallite fragments more diluted than for fragments with more corallites; secondly, the number of days for food administration (here twice per week) was decided based on previous studies (e.g., Rodolfo-Metalpa et al., 2006, 2008). Since it is known that foraging procedure can affect corals’ growth (Hoogenboom et al., 2015; Huang et al., 2020), the quantity of food calculated and the food administration twice a week may not be ideal to boost the highest growth for this species.

If the light treatment is a clear win-methodology for the production of new corallites, the growth of the colonies is not so evident. In fact, the unclear growth of the colonies can be related with the short period of time in which the experiment was carried out, and the small size of the nubbins, that were not easy to be properly photographed and measured, causing a potential bias in measurement.

Regarding the field activities related with the transplantation, divers always found transplanted colonies of both species with everted polyps, a clear sign that the fragments are alive and seeking for food. Additionally, the transplantation procedures did not cause tissue necrosis in the transplanted fragments, but, on the contrary, the tissue damage present in one of the *P. clavata* fragments fully recovered in 2 months, according with the time-span reported in Bavestrello et al. (1997), suggesting a good health of transplanted colonies. As a matter of fact, a fast coenenchyma regeneration is crucial for preventing presence of epibionts, that, encrusting the coral branches, increase the friction against water current, eventually causing the break of the coral (Mistri, 1994; Bavestrello et al., 1997).

Most of studies on *P. clavata* are mainly focused on the density of the colonies rather than specifically on their size and growth (Cupido et al., 2008; Linares et al., 2008). However, our results showed average higher growth in height, compared to the one reported by Mistri et al. (1994). It needs to be considered that, in the present study, the monitoring has been performed in a restricted time interval, occurred before the summer, while the one performed by Mistri et al. (1994) has been carried out within a year. Additionally, according to Fava et al. (2010), the summer period generally leads to a negative growth of Mediterranean filter and suspension feeders, mainly related to a trophic depletion during this season, thus limiting the available food resources (Coma et al., 1994). A long-term monitoring will help to better understand the growth rate of this transplanted population and to confirm the effectiveness of the transplants and its possible application on grater scale. Not all the transplanted colonies showed positive growth rate, indeed others suffered the complete or partial loss of some branches. Fragment structural modification (e.g., branch length reduction or complete detachment) was previously observed in other cnidarians both tropical and Mediterranean (e.g., *Briareum asbestinum, Plexaura* spp.*, Antipathes dichotoma, Dendronephthya hemprichi, Eunicea flexuosa* and *C. rubrum*) (Lasker, 1983, 1984; Walker & Bull, 1983; Dahan & Benayahu, 1997; Prada et al., 2008; Roveta et al., 2023) and it might be considered a response to stress conditions (Cerrano & Bavestrello, 2008; Roveta et al., 2023). As a matter of fact, transplantation can be considered a stress for coral fragments due to the process of moving transplants to the restoration site, where, even if slightly, different conditions can be found compared to the donor site. This can reduce the natural resistance of the colonies, leading to an increased susceptibility to pathogens (Rinkevich, 2005; Forrester et al., 2012). As observed in some *P. clavata* fragments, the loss of several apexes and therefore a consequent branch and size reduction was also observed in the transplanted colonies of the red coral. Latest transplants (occurred in February 2023) showed a lower average loss of the number of apexes per month compared to the previous ones, suggesting that apexes loss can be related not only to operational procedure during translocation, but also to other stressors, both natural or anthropogenic (Yamashiro & Nishihira, 1994; Dahan & Benayahu, 1997; Acosta et al., 2001; Hoeksema & Waheed, 2011; Coppari et al., 2019). In fact, according to Batson et al. (2020), autotomy can easily occur in cnidarians and the same phenomenon was already documented in this species by previous studies (Russo, 1995; Russo et al., 1997; Roveta et al., 2023). Even though in the past the loss of branches was seen as a potential new asexual reproduction, according to Roveta et al. (2023), we did not observe the reattachment of the lost branches, suggesting that, thanks to the known phenotypic plasticity of *C. rubrum* (Pratlong et al., 2015), this can represent an adaptive behavior to face different stress conditions (e.g., climate anomalies).

Among all the techniques tested for the transplantation of red coral colonies, the grid method unequivocally demonstrated the best performance, allowing the lower colony loss. Effectiveness of the grid technique is highlighted by the results of the present study, as well as by Villechanoux et al. (2022), which however draws attention to the high impact of losing a grid, that would mean loose a whole part of the transplants instead of just one fragment as it would be in case of epoxy detachment. Considering this, grid fixation on the substrate should be optimized by means of an additional fixation method for the nails (e.g., epoxy) potentially reducing the loss of transplants, and increasing the technique effectiveness (Villechanoux et al., 2022).

In conclusion, this thesis has shed light on the urge to focus more attention on coral restoration efforts in the face of growing challenges posed by climate change and anthropogenic impacts. Restoration is possible and it is happening. Future research should improve the validity of this study enhancing restoration effectiveness both in field and in the laboratory. The path ahead may be challenging, but the continued existence of biodiversity and the various benefits it provides to our planet make this endeavor not only necessary but also a moral imperative.

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