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Testing Transplantation Techniques for the Red Coral *Corallium rubrum*

Juliette Villechanoux [1,*], Jan Bierwirth [1], Torcuato Pulido Mantas [2] and Carlo Cerrano [2,3,4]

[1] Faculty of Sciences, Ghent University, 9000 Ghent, Belgium; jan.bierwirth@imbrsea.eu
[2] Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona, Italy; t.pulido@pm.univpm.it (T.P.M.); c.cerrano@staff.univpm.it (C.C.)
[3] Stazione Zoologica Anton Dohrn, 80122 Napoli, Italy
[4] Fano Marine Center, 61032 Fano, Italy
* Correspondence: juliette.villechanoux@imbrsea.eu

Abstract: *Corallium rubrum* has been exploited by humankind for centuries. The long-term exploitation dynamics of this species make it even more important today to increase protection and restoration efforts as it provides a significant range of ecosystem services. This becomes even more important in areas where natural recovery is hindered or unlikely. So far, only very few experiments have been carried out in the past, investigating suitable techniques for the successful transplantation of this species. For this reason, a review was conducted in order to synthesize previous results and identify the most promising methodologies. Additionally, six different transplantation techniques were tested and discussed in the context of the review. Five techniques used fragments for transplantation, while one used newly settled larvae on PVC-tiles. Shallow *C. rubrum* colonies often grow upside down under crevices and rims as well as in caves, making the transplantation of fragments comparatively challenging. Here, *C. rubrum* was transplanted upside down under crevices using a PVC-grid in combination with epoxy putty to hold fragments in place, and the results indicated the potential benefits of this technique. In a novel approach, shallow colonies, and larvae on settling plates were also transferred to deeper areas, suggesting that mesophotic populations can be restored to reconstruct pre-exploitation conditions. Attaching the colonies to the roof of crevices provided a level of survivorship consistent with conventional erect transplantations of colonies on rock bottom but had the advantage of being more removed from sedimentation and anthropogenic disturbance. Future work must develop permanent grid-mounting methods for use in the crevices before this approach can be further explored for large-scale restoration efforts.

Keywords: Octocorallia; Mediterranean Sea; mesophotic; red coral; transplantation; restoration

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1. Introduction

Coralligenous reefs are temperate bioconstructions mainly formed by coralline algae at low light levels hosting many taxa [1,2]. In Mediterranean coralligenous assemblages, one of their main epibenthic components are gorgonian corals. They contribute to providing three-dimensional habitat complexity, usually enhancing local biodiversity [1,3–7].

Coralligenous accretions, when growing on vertical walls, can generally shape rocky cliffs with parallel rims, creating substrates exposed to light and sedimentation on their upper side, and a lower substrate sheltered from sediments and light exposition. While the upper part of the concretions generally hosts photophilous organisms with flexible habits, the lower portions normally host sciophilous species characterized by hard skeletons. Among the latter, a peculiar species is the red coral *Corallium rubrum* (Linnaeus, 1758) [8–10] endemic to the Mediterranean Sea living on vertical cliffs, crevices, and caves from 20 to 200 m depths and can be found down to 1000 m depth on open walls, rocky outcrops and seamounts [11,12]. Most knowledge of *Corallium rubrum* mainly comes from shallow populations (20 to 50 m depth), highlighting a bias towards relatively shallow populations

in the literature. It is a slow-growing, gonochoric species with a time-limited sexual reproduction in summer; low polyp fecundity, with less than one larvae produced per polyp [13–15]; and high recruitment densities [16–20]. Colonies can already be fertile at 2 cm tall, but optimum fertility is reached at 6 cm [14,21]. Its initial growth rate can be averaged to 0.6 mm/year in basal diameter and 1 cm/year in height, taking decades for a colony to reach an economically valuable size (current market value around USD 1000 per gram) and hundreds of years for a forest to form [22,23].

The red coral has been exploited for millennia for its hard red calcium carbonate skeleton used for precious artifacts production (jewelry, sculptures, amulets) [24,25], making it one of the most ancient and valuable harvested living marine resources [26]. Its extensive harvesting resulted in a dramatic size and structural shift in shallow water populations from banks previously shaped as a "forest-like" structure, dominated by large branched colonies of 30–50 cm height (10–30 mm diameter) to populations shaped like a "grass plain-like" structure, dominated by small colonies of 3–5 cm height (5–7 mm diameter) [14,27,28]. A reversal trend has been documented in some French, Spanish, and Italian Marine Protected Areas, where red coral fishery has been definitively forbidden (Cerbère-Banyuls Marine Natural Reserve (1974), Scandola Natural Reserve (1975), Côte Bleue Marine Park (1982), Bouches de Bonifacio Natural Reserve (1999), Calanques National Park (2012), and Portofino marine Protected Area [28–30]). To prevent overfishing and ensure long-term yields of this resource in the Mediterranean Basin, a minimum diameter of 7 mm was established for harvesting purposes (1983) and a ban on dredge use (ingegno) at Mediterranean scale was implemented in 1994. Nowadays, due to the depletion of shallow banks and the establishment of harvest regulations, the harvesting interest is turning towards deeper banks (50–200 m depth) despite the fact their ecology is still poorly understood [31–33]. An additional consideration has to be made regarding deep populations, which may be considered as a genetic refuge, enhancing species resilience in the case of thermal anomalies on the upper layers of the water column, and therefore should be also considered for conservatory measures [30,34,35].

Ancestral humans' fishing activity completely changed the structure of this species' shallow populations, as well as its original distribution. Based on data available for comparison, which originates from already impacted populations, *C. rubrum* is not under imminent extinction risk, but its populations are undoubtedly decreasing. It is considered an endangered species by the International Union for Conservation of Nature but is not included in Appendix II of the Convention on the Trade in Endangered Species (CITES) despite two proposals in the past years from the US and EU to list it [36,37].

Therefore, efforts are needed to identify populations affected by harvesting and to implement restoration actions in order to repopulate and maintain the ecosystem services provided by this species, such as the consolidation of coralligenous substrate or the establishment of a three-dimensional framework that act as a habitat for a wide range of species including microorganisms, algae, invertebrates, or fish [1]. In addition, *Corallium rubrum* health can be negatively affected by rapidly growing stressors such as ocean acidification [23] and microplastic pollution [38], but its resilience seems to be enhanced when colonies grow in a high-biodiversity environment [39]. Research efforts on Mediterranean octocoral restoration techniques increased during the past decade including the investigation of transplantation methods [40–43] to define the best techniques to restore damaged coralligenous outcrops, one of the most vulnerable and fragile marine habitats in the Mediterranean Sea.

However, a comprehensive and synthesized work of transplantation methods on *C. rubrum* is currently lacking in today's literature. Here, we provide an overview and guidance on transplantation techniques of *C. rubrum* to help future restoration projects based on an initial literature review paired with a case study testing six transplantation techniques of *C. rubrum* including one of larval enhancement in the Ligurian Sea. The main objective is to analyze the effectiveness of each method tested. For this purpose, we analyzed:

(i). The survival rate and colony loss rate;
(ii). The appearance of new branches;
(iii). The variations in the pattern of growth.

Among the methods executed, we transplanted *C. rubrum* in an upside-down position under crevices and compared the performance to fragments transplanted in an erected position. Using this approach, long-term survival may be increased by reducing the risk of destruction due to commercial and artisanal fishing practices, as well as constraints due to sedimentation. Furthermore, shallow colonies and settlers were transferred to deeper waters in a novel approach testing whether mesophotic populations can be restored to reconstruct pre-exploitation conditions.

2. Materials and Methods

2.1. Literature Review

A precise literature research was conducted on transplantation experiments of *C. rubrum* in order to identify the state-of-the-art knowledge on its transplantation techniques. Online libraries (Web of Science, Google Scholar, and Scopus) were consulted using specific terminology related to transplantation techniques. Scientific papers were selected according to the criteria of having transplantation, recruitment or settlement experiments mentioned in their abstract. Due to poor results on finding online literature older than 2000, research was extended on grey literature using books and reports. It allowed us to extend the study to Italian and French publications from the 1990s that are not available online.

2.2. Transplantation Experiments

The six different *C. rubrum* transplantation experiments were conducted in the Ligurian sea at Gallinara Island (where the red coral has disappeared) and at Portofino Marine Protected Area (Figure 1) (where the red coral is still present, although colonies are of altered population structure) [29].

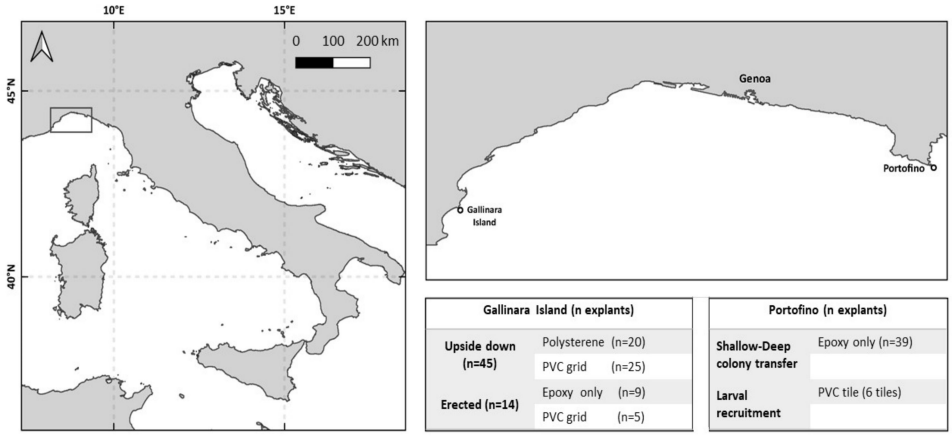

Figure 1. Location sites of the transplantations experiments with the number of fragments transplants (explants) at each location for each method.

Collection of *C. rubrum* was performed by scientific divers at a depth of 25 m in Portofino, as previous research has shown that colonies of the upper layer of distribution are potentially more resistant to thermal stress. Since thermal anomalies and climatic extreme events are only expected to increase in frequency and severity during the following decades [44], selecting donor colonies with the highest potential tolerance to hyperthermia may promotes a higher genetic diversity and the dispersal of thermo-tolerant genotypes.

Transplants were obtained by fragmenting the apical branches of randomly chosen adult colonies with a minimum height of 10 cm and placed into sealed plastic bags. Apical branches of 2–6 cm were collected without altering the original population density

(250–300 col/m^2) [22]. On the boat, plastic bags were placed in coolers (16–21 °C) for transportation. The protocol of fragment collection was identical for each transplantation experiment, apart from of the donor site for experiments carried out at Portofino MPA.

At Gallinara Island, 59 fragments were transplanted at 30 m depth with a two-component epoxy putty used as adhesive: 45 fragments were placed upside down at the basal side of a coralligenous rim (08/2017 and 10/2017), while 14 were transplanted erected on the rocky substrate (03/2018). To hold the fragments in place, four different techniques were tested (Figure 2A–D). Under the crevices, either polystyrene packaging (Polystyrene method; n = 20) or a PVC-grid (Grid under crevices method, n = 25) was used. For the erected transplants, either only the epoxy putty (Epoxy putty on rocks method; n = 9) or a PVC-grid as well (Grid on rocks method; n = 5) was used. In total, 5 grids were installed on top of the epoxy and fixated with metal pegs (3 under crevices and 2 on rocks) with 3 to 10 transplants per grid. Using the polystyrene and epoxy putty techniques, transplants were attached in small patches of 3 to 5.

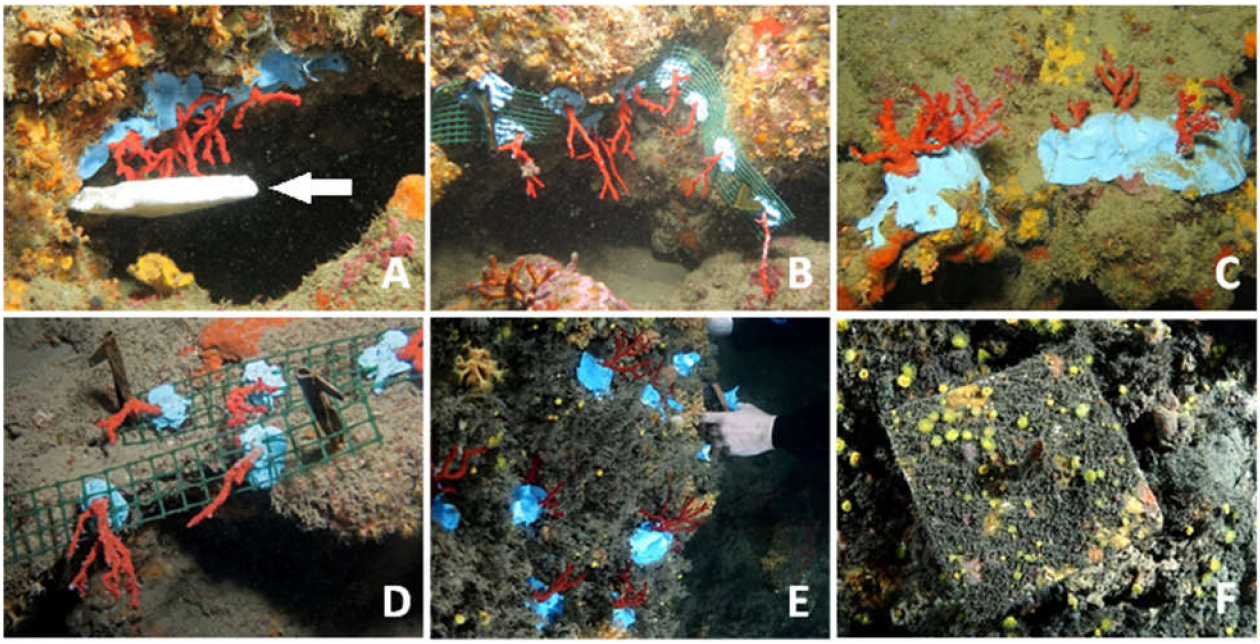

Figure 2. Images of the different transplantation techniques in shallow water directly after transplantation: (**A**) Polystyrene method, the arrow points at the polystyrene sheet; (**B**) Grid under crevices method; (**C**) Epoxy putty on rocks method; (**D**) Grid on rocks method; (**E**) Shallow colonies transplanted to deep water method; (**F**) Larval enhancement experiment on PVC tiles.

In Portofino MPA, there is a red coral population with a wide depth range, with the shallowest colonies smaller than the deeper ones. These differences are still to be clarified and there are multiple hypotheses. Deeper colonies are found in a recess in the wall and appear to have never been damaged by fishing. They are therefore older. The more superficial colonies begin to reproduce earlier due to the warmer waters on average. By investing first in sexual reproduction, they do not reach the size of the deepest colonies.

To assess the level of plasticity of the shallower colonies, two transfer experiments (see Figure 2E,F) were carried out: 39 large colonies from an open wall at 30 m depth were transplanted on a vertical open coralligenous wall at 70 m depth, and larval recruits on PVC tiles were transferred from 30 m to 70 m depth (both 04/2018). For the first, fragments were fixed using two-component epoxy putty. Larvae were recruited on six 20 × 20 cm white PVC tiles previously fixed with steel screws on the ceiling of Colombara cave in Portofino at 34 m depth as described by Costantini et al. [20] in 2016. PVC was chosen due to previous success in larval recruitment experiments [19,45]. The tiles were left for two

years in order to collect two cohorts of recruits (summer 2016 and summer 2017). In April 2018, they were transferred by scientific divers to 70 m depth at the same location as for the large colonies' transplantation. PVC tiles were fixed vertically on the wall using steel screws to avoid sedimentation on top. To limit larvae loss, the tiles were transferred in a framed plastic box, where they were maintained distant from each other and remained stable during the descent.

2.3. Data Collection

Underwater visual census surveys and photographic samplings were carried out by scientific divers at each location after transplantation to collect data on transplants and recruits settled on the PVC tiles. A digital camera (Canon G16) equipped with light was used to photograph each colony. At Gallinara Island, data were collected in August 2017, October 2017, November 2017, July 2018, September 2018, January 2019, May 2019, August 2019, June 2021, September 2021, and November 2021. In Portofino, surveys were conducted in April 2017, June 2018, September 2018, and October 2019. No surveys could be carried out in 2020 and early 2021 due to the COVID-19 pandemic.

2.4. Data Analysis

Photographic samples were used to analyze survival, detachment, death, height growth, and changes in the number of branches of transplants. The detachment or death of transplants was recorded separately to identify if the loss was related to the transplantation methodology or to natural mortality. A transplant was considered dead when it was completely affected by necrosis or by epibiosis. If there were no photographic samples for three consecutive surveys, the transplant was considered lost. The branches were counted in each sample for each transplant. Software ImageJ [46] was used to measure fragment height. However, this technique could only be applied to both methods, including a grid as for the others no reference was included in the photos. Height was measured from the base to the highest point of the coral in a vertical plane.

Photographic samples of the PVC tiles were used to identify the initial number of shallow *C. rubrum* settlers (e.g., larvae settled in the Colombara cave at 35 m depth from 2 cohorts present on the tile on day 1 at 70 m depth), the number of deep *C. rubrum* settlers, their survival, and their density. It was not possible to estimate the age of each settler since their height could not be measured. Density was defined as the number of settlers per decimeter square.

2.5. Statistical Analysis

Survival rate of transplants and settlers at a given survey was defined as the ratio between the number of living and attached transplants/settlers and the total number of transplants/settlers transplanted/present in the beginning.

Detachment rate of transplants at a given survey was defined as the ratio between the number of detached transplants and the total number of explants transplanted in the beginning.

Branching difference (change in the number of branches) of one transplant was defined as the ratio between the number of branches present in the last survey where the transplant was alive and the initial number of branches. Transplants detached on the second day of the experiments were not considered, as the timespan was too short. To test if there was a significant difference between the different transplantation methods in Gallinara, a Kruskal–Wallis test was performed (data did not follow a normal distribution).

Height growth of transplants at a given survey was defined as the ratio between the measured height and the initial transplant measured height in the first survey. Transplants detached on the second day of the experiment were not considered. Due to the low number of samples for the grid on rocks method, no statistical test was performed to test the presence of a significant difference between the two different grid transplantation methods for height growth.

A Spearman's correlation test was performed between the branching difference and the height growth of transplants.

All statistical tests and most of the graphical content were performed on RStudio (R Development Core Team 2008), the others on Microsoft Excel.

3. Results

3.1. Review on Past C. rubrum Transplantations and Larval Enhancement Experiments

A total of 10 scientific publications were found for transplantation experiments and 6 for larval recruitment experiments [Table A1, Appendix A] conducted either in France, Italy, or Spain, all of them in shallow waters (25–40 m depth). The first trial was carried out in situ in 1979 with very poor results (0% of survival) due to method failure, causing the transplants' deaths [47]. In the next decade, four other attempts followed, of which one method was found not appropriate, while data are missing for the others [48–51]. In 1992, the first large-scale experiment on artificial substrate obtained survival rates of 40%, with loss only due to detachment and not to the death of the organism. It was the first experiment using some sort of resin to fix transplants on the porphyry substrate [52]. After this study, using resin became the main technique to attach *C. rubrum* transplants. Cerrano et al. performed a series of transplantation experiments in 1997 [53] and 2000 [54] testing different substrates obtaining good results for coralligenous environments. Since then, red coral transplants were scarce, with only two further studies being conducted (2015 [54] and 2018 [42]). After a few months, survival rates were usually high with 100% (n = 4) survival, but after 2 or 4 years, it dropped to 71.5% (n = 6) on average. No previous experiment transplanted red coral in an upside-down position under crevices; it was always transplanted in an erected position.

The first larval recruitment experiment on tiles was carried out in 2000 [54], and since then it has become a recurrent method to study settlement and recruitment density for *C. rubrum* [Table A2, Appendix A]. Different materials were used such as limestone, cement, marble (n = 4) [16,28,54,55], PVC (n = 1) [19], and even one with electrical currents [18]. The average settlement density is 4.71 $\pm$ 5.17 settlers·dm^{-2} with a minimum of 0 settlers·dm^{-2} and a maximum of 19.12 settlers·dm^{-2}. Only one study examined tiles over a long time period (21 years), which resulted in a considerably lower density of 0.9 $\pm$ 0.3 colonies·dm^{-2}.

3.2. Unpublished Transplantation Experiments Techniques

3.2.1. Survival, Detachment in Shallow Waters

Apart from the transplants that showed clear signs of necrosis (5%), the survival rate is always referred to transplants that stay in situ. A reduction in this rate is mainly a consequence of detachment from the substrate.

The survival rate after four years of *C. rubrum* transplanted under crevices was 30% (n = 6) using the polystyrene method and 32% (n = 8) using the grid under crevices method (Figure 3). For the colonies transplanted in an erected position on rocks, 33% (n = 3) survived using the epoxy putty on rocks method and 60% (n = 3) using the grid on rocks method after three years and six months (Figure 3).

For the polystyrene method (Figure 3), a major decline was observed at the beginning of the experiment with 15% of loss occurring already after one day and 40% after two months due to detachment (Figure 4). Another decline of 25% was recorded after 2020, when no surveys could be conducted.

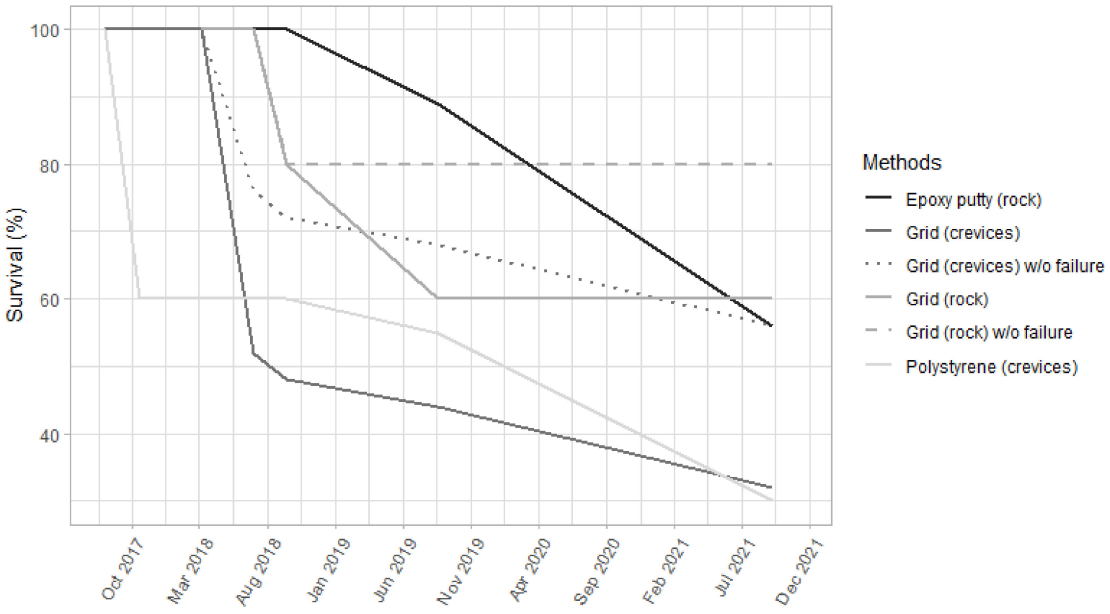

Figure 3. Survival (expressed in percentage) of *C. rubrum* transplants from the beginning of the experiments to November 2021 for each transplantation method at Gallinara Island. Dotted lines represent survival of transplants without taking the detachment of the grid into account. There is a total period of 4 years for transplants under crevices and 3 and a half years for transplants on rocks.

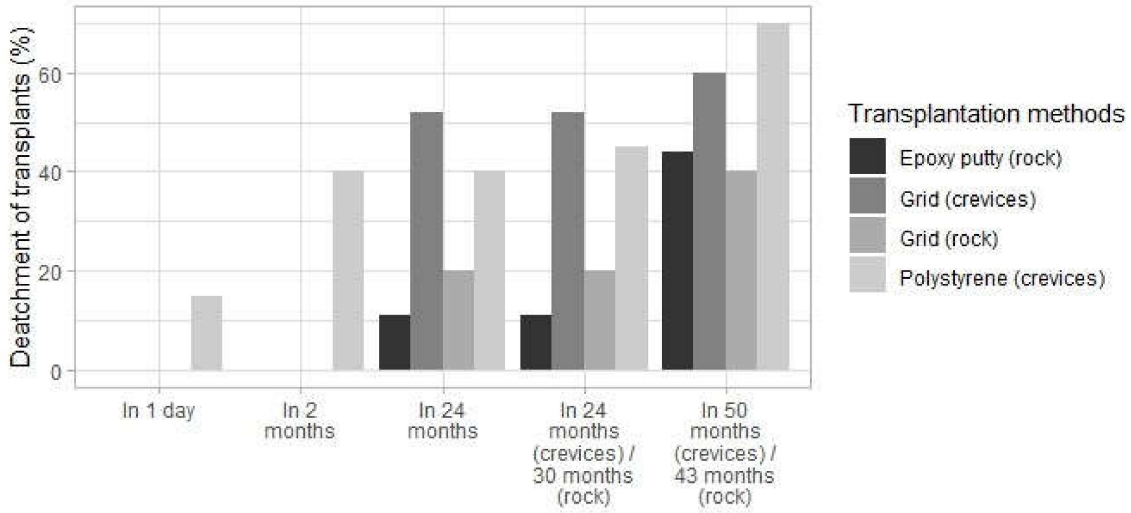

Figure 4. Detachment of *C. rubrum* transplants (expressed in percentage) from the beginning of the experiments to November 2021 for each transplantation method at Gallinara Island.

For the grid under crevices method, the first loss of transplants was observed only after one year with a sharp decline of 52% due to one of the grids falling, resulting in the loss of 24% of all transplants (Figure 3). The decline persisted in the following years, with a total loss of 12% in 2020. As for the grid on rocks method, the first loss was observed after five months (20%), while after three years, one of the grids detached, leading to a loss of 20% of all transplants (Figure 3). Finally, the epoxy putty on rocks method showed the first loss of transplants after one year (11%) (Figure 3).

The loss of transplants was mostly due to method failure, either because of the detachment of transplants from the epoxy putty, the detachment of the epoxy putty from the substrate, or because of grids falling (Table 1). Indeed, method failure affected 70% of transplants using the polystyrene method, 60% using the grid under crevices method (with

24% because of grid detachment), 40% using the grid on rocks methods, and 44% using the epoxy putty method (Figure 4). On the other hand, necrosis affected only 5% (n = 3) of all transplants: 4% (n = 1) of transplants using the grid under crevices method and 22% (n = 2) of transplants using the epoxy putty method. The cause of death was due to full necrosis for the latest (covered by coralline algae after 3 years) and to full epibiosis cover for the first (its size was extremely small) (Figure 5). Two other transplants from the same epoxy putty method patch were affected by partial necrosis and survived. In total, epibiosis affected 10% (n = 6) of transplants, all located under crevices of which 3% used the grid method and 7% used the polystyrene method. Sediment cover affected 7% of all transplants. They were in an erected position using the epoxy putty method, but it did not affect their survival.

Table 1. The percentage of transplant loss due to the different method failure reasons for each method.

Methods	Detachment of Epoxy Putty	Detachment from Epoxy Putty	Detachment of Grid from Substrate
Polystyrene	10	60	-
Grid under crevices	0	46	24
Grid on rocks	0	20	20
Epoxy putty on rocks	0	44	-

Figure 5. *Corallium rubrum* transplanted using the grid method under a crevice. The first day of the experiment with all their branches and initial sizes (**A**), one year after transplantation with lost branches and decreased size (**B**), and the last survey with one transplant covered by epibiosis (**C**).

3.2.2. Branching Patterns of Transplants

In total, 39 transplants were considered for branching difference calculation: 12 from the polystyrene method, 5 from the grid on rocks method, 13 from the grid under crevices method, and 9 from the epoxy putty on rocks method. For all transplantation methods, most of the transplants either lost branches in the first 2 years of the experiment or the number of branches remained stable from the moment they were transplanted to the last survey in which they were alive (Figure 5). Using the polystyrene method and the grid on rocks method, 50% and 60% of transplants lost branches, respectively. Fewer transplants lost branches using the grid under crevices method (46%) and the epoxy putty on rocks method (44%). Transplants gained branches only using the polystyrene method (17%) and the grid under crevices method (8%).

3.2.3. Height Growth of Transplants

In total, only 13 transplants from both grid methods could be measured: 10 under crevices and 3 on rocks. By November 2021, transplants' height decreased on average by 24% for the ones under crevices and by 1% for the ones in an erected position (Figures 6 and 7). Most of the transplants under crevices (80%, n = 8) decreased in size

within one year after transplantation and 20% (n = 2) continued to decrease after that. Only one transplant decreased in size (18%) after one year using the grid on rocks method while the two others may have increased in size. Spearman's correlation test results show a very poor and non-significant correlation (p-value = 0.4689, rho = 0.024) between branching difference and height growth. This test was only performed on 13 samples.

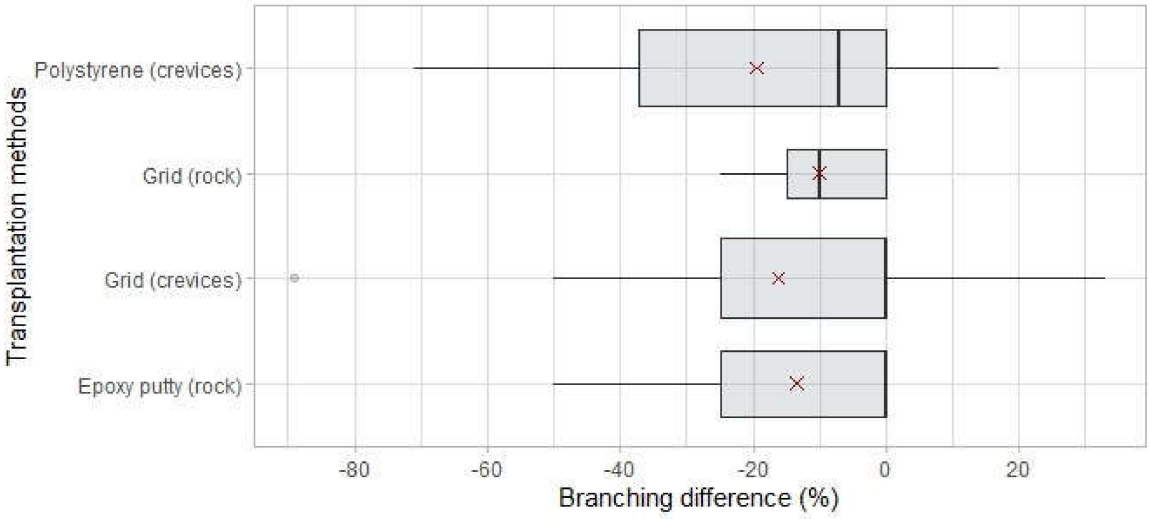

Figure 6. Change in the number of branches (branching difference, in percentage) of *C. rubrum* transplants for each transplantation method in Gallinara Island. The red cross represents the average. Boxes' width is relative to the number of transplants.

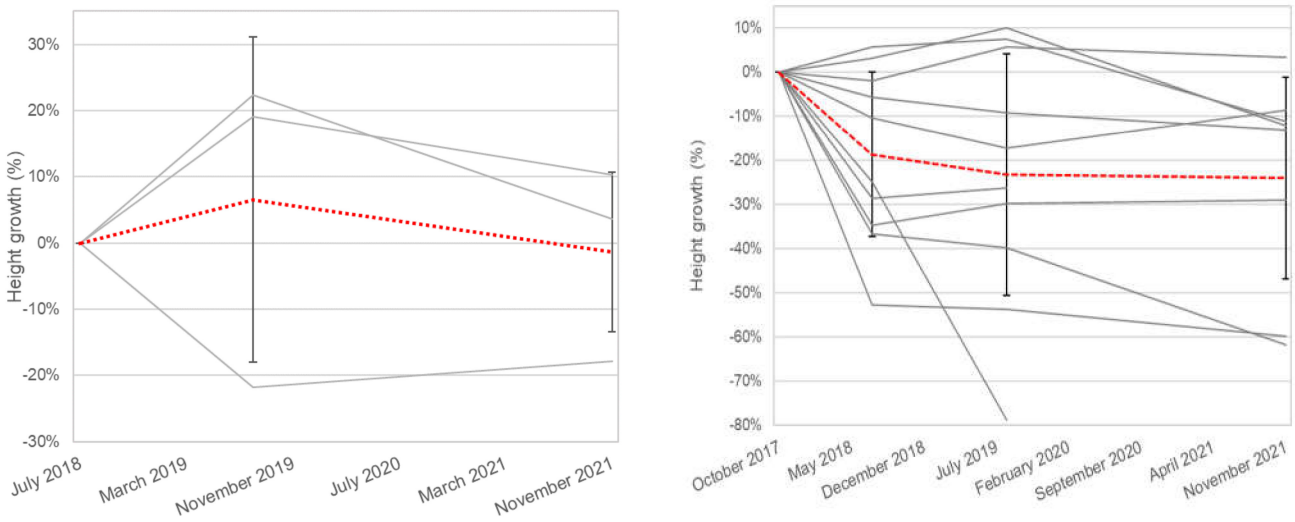

Figure 7. Height growth (expressed in percentage) of *C. rubrum* transplants using the grid method under crevices (**left**) and on rock (**right**) from the beginning of the experiment until November 2021 with the average (red line) and standard deviations. A growth stopping translates to a transplant loss.

3.3. Transfer of Colonies and Settlers from Shallow to Deep Waters

3.3.1. Survival and Detachment of Transplants

The survival rate of transplanted shallow water colonies to deeper waters was 82% (n = 30) after one and a half years. The 18% loss is exclusively due to the detachment of transplants from the epoxy putty, and no death was observed. In the last survey in October 2019, 59% of transplants were affected by sediment cover, but explants seemed healthy to visual observation with predominant polyps. Of the surviving transplants, 3% (n = 1)

were affected by branching decrease by losing 12.5% of their branches. Otherwise, all other surviving transplants did not experience any branching difference.

3.3.2. Survival of Shallow Settlers and Settlement of Deep Settlers

Three PVC tiles were identified from the survey's sampling (tile A, tile B, tile C) of which one picture sample is missing in the first survey (tile C). Shallow and deep settlers could then not be identified in tile C. The survival rate of shallow settlers after 1 and a half years in deep waters was 31% on tile A and 50% on tile B (Figure 8). The number of shallow settlers on tile B decreased by 50% after 2 months and remained stable, while for tile A, it decreased continuously until October 2019. Tile A had 3.25 shallow settlers·dm^{-2} at the beginning, which decreased to 1 shallow settler·dm^{-2} by October 2019 (Figure 9). Although tile B showed a higher survival rate of shallow settlers, it showed smaller density at first (0.5 shallow settler·dm^{-2}) that decreased by half by October 2019.

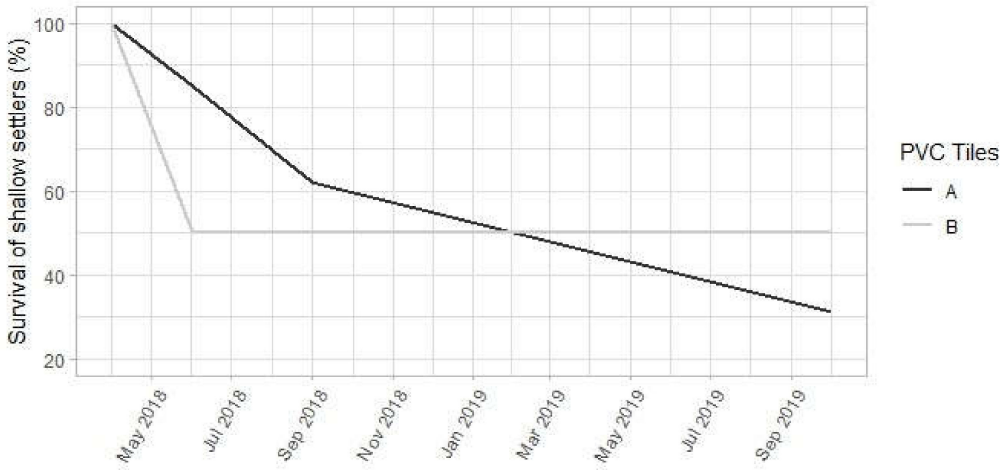

Figure 8. Survival (expressed in percentage) of *C. rubrum* shallow settlers on PVC tiles from the beginning of the experiment in deep water to October 2019 at Portofino MPA.

Larvae from deep *C. rubrum* colonies were observed to settle on both tiles during the spawning events of 2018 and 2019 (Figure 9). In 2018, settlers appeared in June and September on both tiles, while in 2019, settlers only appeared on tile A. Tile A had 2.25 deep settlers·dm^{-2} in June 2018, which decreased to 0.75 deep settlers·dm^{-2} by October 2019. Tile B had 1 deep settler·dm^{-2} in June 2018, which decreased to 0.75 deep settlers·dm^{-2} by October 2019. Settlers from the 2018 cohort were lost on tile A (22%) and B (75%). In October 2019, shallow settlers dominated tile A (57%) while they were the least abundant on tile B (25%), which was dominated by deep settlers from the 2018 cohort (75%). The average settlement density of shallow settlers is 1.28 ± 1.14·dm^{-2} and 1.33 ± 0.59·dm^{-2} for deep settlers (from April 2018 to October 2019). Including tile C, the total average settlement density of all settlers confounded is 1.98 ± 1.52·dm^{-2}, with tile A having the highest one (3.63 ± 1.14 settlers·dm^{-2}).

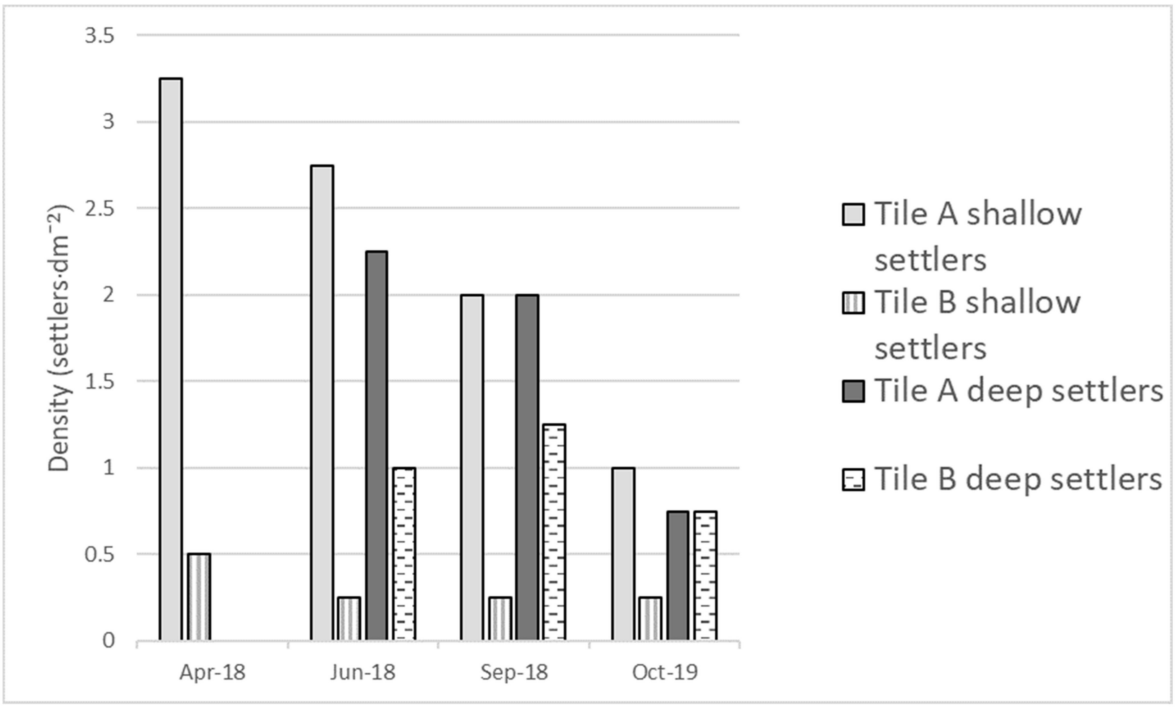

Figure 9. Settlement density (expressed in settlers·dm^{-2}) of *C. rubrum* shallow and deep settlers from the beginning of the experiment in deep water to October 2019 on each tile at Portofino MPA.

4. Discussion

The bibliographic analysis on methodologies to transplant red coral colonies and the description of original pilot activities to test different approaches provide useful suggestions for future *Corallium rubrum* restoration strategies. The peculiarity of red coral with respect to other gorgonians is its hard tree-like skeleton, allowing the colony to be handled easily during transplant. This has generally addressed previous experience, generally adopting putty or structures holding colonies (e.g., pinches in Monte Carlo artificial caves).

In terms of survival, we can affirm that the current knowledge suggests that the best methods are using a two-component epoxy putty as glue to attach transplants on coralligenous substrate in an erected position (71.5% of survival after 2–4 years). The fact that loss of transplants was always due to method failure or detachment highlights the resistance and adaptive abilities of shallow red coral colonies.

In terms of reproduction, it seems that the best results are those recorded under roofs with numerous small colonies (0.1–0.15 colonies·dm^{-2}) settling on the roof of artificial caves (e.g., Monte Carlo artificial caves) and numerous juveniles and colonies (2.25 $\pm$ 1.70 juveniles·dm^{-2}, 0.9 $\pm$ 0.3 colonies·dm^{-2}) settling on PVC tiles in natural caves (e.g., Colombara cave and Marseilles).

In this study, we present the first *in situ* experiment of transplanting *C. rubrum* under crevices with two promising techniques to maintain the fragments: polystyrene and PVC grids. After four years, they showed a similar survival rate (30% and 32%), but the polystyrene method seemed less effective at the beginning with transplant detachment recorded in the first week and months. The disadvantage of the grid method is the risk of grid detachment from the substrate, causing 24% of transplant loss. While detachment was the main cause of loss and occurred in both methods, it was earlier but slower with the polystyrene method and later but suddenly with the grid method. The grid method seems therefore more reliable to transplant *C. rubrum* under crevices.

We also tested another technique to attach transplants on coralligenous substrate in an erected position by using a PVC grid as an extra holding material. Even though it showed the best survival rate (60%) of all techniques and the lowest detachment rate (40%), loss

was also caused by grid detachment (20%). This method failure goes against the initial intention of the technique to avoid transplant loss. In total, from under crevices and on rocks, two grids fell out of five originally. It is evident that for better results, grid fixation on the substrate should be improved in further experiments and thus improve the survival of transplants and technique effectiveness. Suggestions would be a second material ensuring immobility of the metal pegs or screw them deeper and tighter without compromising the substrate.

The survival rates of the presented techniques are similar to the ones from transplantation experiments carried out with Mediterranean gorgonian species (30–98% of survival) [40–43,56,57]. However, they are lower than the average reported for previous *C. rubrum* experiments (71.5%), all conducted in an erected position, suggesting the inclination of the substrate can strongly affect the survival of transplants. Additionally, one must take into account that the sample size used in each technique, especially for the grid on rocks method ($n = 5$), were considerably smaller compared to previous *C. rubrum* transplantation experiments (average sample size of 141.5 ± 67.3). Therefore, we strongly recommend increasing the sample size, especially for promising techniques using PVC grids, to obtain more accurate results. For better results, grid fixation on the substrate should be improved in further experiments and thus improve the survival of transplants and technique effectiveness.

The loss of branches and height decrease observed at the beginning of the experiments, independently of the technique used, suggests an adaptive behavior from *C. rubrum* to adapt to new environmental conditions and respond to stress. The lack of correlation between the branching difference and variations in height growth may be due to the small sample size, or it could reveal that the branches lost were not only the apical branch but also side branches. It is like an autotomy procedure which was only observed in this species in laboratory conditions [58]. These observations plus the low mortality rate (5%) of transplants support and go in line with the high resistance and adaptive capacity of shallow *C. rubrum* previously seen in other transplantation experiments and under marginal environmental conditions [42,51–54,59–61].

It is expected in the following years to observe an increase in the total height of the colonies, in the ramification number, and in the development of a basal plate that would re-attach the explant directly to the substrate, covering the epoxy/grid and ensuring its resilience even after the plastic is degraded. All of this assuming no extreme event occurs on the restoration site/depth. We would also expect the production of larvae that could enhance genetic diversity and promote thermo-tolerant genotypes. Finally, to verify long-term survival once the grid detachment problem is fixed, continuous monitoring of the area to assess if, over time, the "forest effect" and its diversity associated returns to the restored area would be needed in order to support restoration efforts.

Despite the genetic diversity threshold found at 40–50 m depth [12], shallow colonies also seem able to survive in deeper environments according to our successful transfer experiment (82% of survival) with no sign of suffering: On the opposite of transplantations in shallow waters, transplants did not lose branches and were not affected by mortality. We also introduce the fact that shallow settlers can survive at 70 m depth after one and a half years.

While previous larval enhancement experiments focused on shallow environments, this study presents the ability of *C. rubrum* deep colonies larvae to settle on tiles for two consecutive spawning seasons, despite the fact of bryozoans and turf algae colonizing the surface. The deep settlement density observed (1.33 ± 0.59 settlers·dm^{-2}) was smaller than recruitment densities reported for shallow populations, probably explained by the low density of deep red coral colonies [35] and the limited connectivity in this species [62]. The inclination of the tiles can also affect the number of newly settled larvae [19].

The optimal density and sex ratio to guarantee a self-sustaining red coral population are still unknown. The possibility to work with recruiting coral larvae tiles offer the possibility to transfer juveniles that can gradually adapt to the new environmental conditions,

avoiding the loss of branches. Moreover, bringing among shallow populations juvenile colonies coming from large mesophotic colonies will let us know if the smaller size of shallow colonies is an environmental constraint or a genetic bottleneck due to the collection of the biggest colonies. The transfer of shallow juveniles to the mesophotic population can increase the red coral population density, facilitating brooding and limiting the risk of the Allee effect [63].

5. Conclusions

Historical data on red coral fishing activity can offer a good picture of the distribution of red coral in the past, suggesting where restoration actions should be prioritized.

The data on survivor rate reported here suggest the geomorphology of the cliff is an important variable to be considered when restoration plans are designed, and the inclination of the substrate can strongly affect both the survival of transplants and the eventual pattern of recruitment. It is also an important factor to protect transplanted colonies from net-impacts caused by fishing activities and thus increase restoration results if shallow *Corallium rubrum* restorations are focused in these areas.

Corallium rubrum is a very resistant species, as shown with the low mortality rate of transplants in the shallow transplantation techniques reported here. However, these techniques are waiting to be further improved and assessed to reach a higher percentage of survivors and guarantee an effective restoration, with reproductive colonies and the recruitment of larvae coming from transplants. This can be achieved by controlling additional loss from grid failure and focusing on restoring reduced populations under crevices to avoid entanglement in nets, thus improving reduced populations.

With deeper populations being harvested, the successful transfer methods reported here offer a way to reconstruct pre-exploitation conditions before banks become completely depleted. Finally, this study offers a summary of the restoration tools for *C. rubrum* currently lacking in in the current literature.

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Appendix A

Table A1. List of articles considered to summarize *C. rubrum* transplantation experiments.

Reference	Location	Technique	Depth (m)	Period Length	Substrate Type	Survival Rate	Detachment Rate	Mortality	Number of Colonies
Weinberg 1979 [47]	Banyuls-sur-Mer	Hard PVC-tube with 2 incisions + screws to hold the fragment fixed on cement blocks	10	few weeks	Photophilous algae	0%	0%	100%	NA
Bianconi et al., 1988 [48]	Scandola (Corsica)	Fixed on panels with metal wire	36–39	NA	Coralligenous	0%	0% (whole panel fell)	100%	NA
Giacomelli et al., 1988 [49]	Naples Zoologic station	Hanging colonies facing down, tied with a copper wire	NA	NA	Aquarium	Used a few colonies	NA	NA	NA
Arosio et al., 1989 [50]	Alghero (Sardegna)	Resin and stainless-steel tweezers	NA	NA	Coralligenous	NA	NA	NA	NA
Catteneo-Vietti et al., 1992 [51]	Montecarlo Marine Reserve	(i) Fixed with bolts to polypropylene panels (ii) held with plastic tweezers (iii) glued into porphyry bar wholes with Devcon resin	27/35	2 years	Artificial cement cave	40%	60%	0%	NA
Pais et al., 1992 [52]	Alghero (Sardegna)	Quick-settling cement as glue in holes on small tiles.	25	NA	Small concrete pipe with metal base	100%	2 colonies	0	NA
Chessa et al., 1997 [56]	Alghero (Sardegna)	Small boards with ready-to-set concrete	NA	NA	Cement tube	100%	0	0	NA
Cerrano et al., 1997 [53]	Gallinara island	Oily putty normally used for glass panes applied in the crevices as glue	20–24	NA	Coralligenous	0%	100%	0%	35
Cerrano et al., 1997 [53]	Gallinara island	Two-component epoxy putty	20–24	1 month	Coralligenous	100%	0%	0%	50
Cerrano et al., 2000 [54]	Portofino, Punta del Faro	Two-component epoxy putty	35	3 years	Coralligenous	10%	NA	NA	100
Cerrano et al., 2000 [54]	Portofino, Mohawk Deer wreck	Two-component epoxy putty	34	3 years	Metal sheets of wreck	0%	NA	NA	100
Cerrano et al., 2000 [54]	Gallinara island	Two-component epoxy putty	24	3 years	Coralligenous	50%	NA	NA	300

Table A1. *Cont.*

Reference	Location	Technique	Depth (m)	Period Length	Substrate Type	Survival Rate	Detachment Rate	Mortality	Number of Colonies
Cerrano et al., 2000 [54]	Gallinara island, Umberto I wreck	Two-component epoxy putty	40–50	3 years	Metal sheets of wreck	0%	NA	NA	140
Cerrano et al., 2000 [54]	Sorrentina peninsula, Scoglio del Vervece	Two-component epoxy putty	45	3 years	Coralligenous with *A. cavernicola*	80%	NA	NA	100
Cerrano et al., 2000 [54]	Sorrentina peninsula, Punta Campanella	Two-component epoxy putty	38	3 years	Biocenosis of *A. cavernicola* and *E. singularis*	80%	NA	NA	100
Cerrano et al., 2000 [54]	Sorrentina peninsula, Secca della Vetara	Two-component epoxy putty	38	3 years	Biocenosis	80%	NA	NA	100
Ledoux et al., 2015 [59]	Riou Island and Palazzu Island	Disks with 8 holes (1 hole = 1 colony), no putty used.	20/40	few months	Plates made of PVC disks	100%	0%	0%	192
Montero-Serra et al., 2018 [42]	Parc Natural del Montgri, Illes Medes i Baix Ter	Two-component epoxy putty	15–17	4 years	Coralligenous	99.10%	NA	NA	300

Table A2. List of articles considered to summarize *C. rubrum* larval enhancement experiments on tiles.

Reference	Location	Technique	Depth (m)	Density (Settlers/dm^2)	Number of Settlers	Period Length
Cerrano et al., 2000 [54]	Punta del Faro and Colombara cave, Portofino (Italy)	10 fiber cement panels fixed on vertical and sub-horizontal walls	NA	Vertical: 1.6 (Punta del Faro); 0 (Colombara)/Sub-horizontal: 5 (Puntal del Faro); 11.7 (Colombara)	NA	2 years
Garrabou et al., 2002 [28].	Marseilles (France)	12 Urgonian limestone (same geological nature as the local coast) panels attached together within a frame roped off to the lateral wall of the cave	27	0.9 ± 0.3 colonies	36 (colonies)	21 years
Bramanti et al., 2005 [16]	Calafuria coast (Italy)	20 white marble tiles fixed with a central steel screw onto the vault of crevices	25/35	19.12 ± 4.97 (25 m); 9.75 ± 2.87 (35 m)	388	4 years
Bramanti et al., 2007 [55]	Calafuria, Elba MPA and Medes Islets MPA (Spain)	54 white marble tiles fixed via a central Fisher's screw to the substrate onto the vaults of crevices	25–35	Calafuria 2.77 ± 3.04; Medes 1.6 ± 1.96; Elba 1.1 ± 1.4	138	1 year
Benedetti et al., 2011 [18]	Calafuria coast (Italy)	3 CaCO3 substrata (lithogenic CaCO3 (marble tiles), electro-accreted CaCO3 in the absence and in the presence of cathodic currents) fixed on the vault of crevices with central Fisher's screws	35	3 ± 2.5 (marble tiles); 2.7 ± 1 (electro-accreted CaCO3 plates without cathodic current); 0.6 ± 0.4 (electro-accreted CaCO3 plates with cathodic current)	NA	1 year
Costantini et al., 2018 [19]	Colombara cave, Portofino (Italy)	16 white PVC tiles drilled in the center fixed inside the cave by steel screws	34–39	8.69 ± 5.96 (recruits); 2.25 ± 1.70 (juveniles)	372	2 years

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