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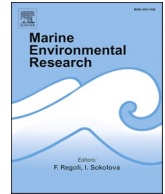
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Investigating the outcomes of a threatened gorgonian *in situ* transplantation: Survival and microbiome diversity in *Paramuricea clavata* (Risso, 1827)

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ABSTRACT

Gorgonian octocorals are threatened by global and local stressors that can act synergistically to affect their health. In recent years, mass mortality events triggered by marine heatwaves have caused demographic declines in Mediterranean gorgonian populations that may lead to their collapse. Potential changes in microbiome composition under stressful conditions may further increase the susceptibility of the gorgonian holobiont to disease. Given the low recovery capacity of gorgonians, restoration approaches using transplantation are becoming an increasingly attractive option to counteract their decline. Here, we compared the survival and microbiome diversity of *Paramuricea clavata* colonies transplanted to sites differing in depth and local environmental conditions. Gorgonians sampled at a greater depth than the transplantation site were more likely to suffer necrosis after 1 year of monitoring. Gorgonian transplantation into environments disturbed by an anthropogenic source of pollution resulted in an imbalance of the microbiome with potential consequences on the success of restoration initiatives.

1. Introduction

In the Mediterranean Sea, coralligenous reefs host high levels of benthic species diversity, including habitat-forming invertebrates such as gorgonian octocorals, which provide structural complexity and contribute to maintaining the whole assemblage (Ballesteros, 2006; Ponti et al., 2014). However, as for the vast majority of coastal marine ecosystems, this unique biodiversity is increasingly threatened by multiple human-induced pressures that could have cumulative effects (Bevilacqua et al., 2018; Piazzi et al., 2012; Ponti et al., 2018). In the last decades, coralligenous assemblages have been affected by mass mortality events triggered by the occurrence of marine heatwave events associated with climate change (Garrabou et al., 2022). Among a wide range of invertebrate taxa affected, up to 80 % of gorgonian colonies have shown partial or total necrosis in populations from several areas in North-western Mediterranean. The most severe impacts are observed in the shallow waters (0–30 m depth), in relation with the higher warming of this depth range (Estaque et al., 2023; Garrabou et al., 2009). Due to their large geographical scale and accelerating frequency, these

mortality events severely threaten future trajectories of coralligenous ecosystems by modifying community composition and dynamics (Gómez-Gras et al., 2021; Verdura et al., 2019). In addition to warming, gorgonians also face multiple local stressors associated with human activities such as fishing, boat anchoring, recreational scuba diving and coastal pollution that can result in increased mortality rates (Coma et al., 2004; Ferrigno et al., 2021; Ponti et al., 2018). Bearing in mind the on-going anthropisation of the Mediterranean coastline (Wolff et al., 2020), these local pressures are expected to increasingly contribute to the demographic decline of gorgonian populations driven by climate change (Gissi et al., 2021; Linares and Doak, 2010).

Like reef-building scleractinian corals, gorgonian octocorals are holobiont entities comprising the cnidarian host and its microbial associates (van de Water et al., 2018a). The microbiome is thought to play important roles in the fitness of holobiont, participating in nutrient acquisition and defence against pathogens (Krediet et al., 2013). Environmental stressors can induce changes in microbiome composition that may impair host health and increase the susceptibility to opportunistic infections (McDevitt-Irwin et al., 2017). In line with this, local

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anthropogenic disturbances correlate with alteration of the microbial community in the red gorgonian *Paramuricea clavata* (Vezzulli et al., 2013), and colonisation by pathogenic *Vibrio* strains could be involved in mass mortality events during marine heatwaves (Bally and Garrabou, 2007). The composition of the microbiome could therefore serve as an indicator of the level of stress suffered by the gorgonians and help predict their resilience in a disturbed environmental context.

Due to their slow growth and low recruitment rate (Coma et al., 1998; Linares et al., 2008a; Santangelo et al., 2012), the regression of gorgonian populations may induce long-term effects by reducing habitat complexity. Lower colony densities also favour the growth of erect algae, including non-indigenous species, which in turn reduce the recruitment of benthic invertebrates by substrate occupancy and hinder the recovery of the coralligenous assemblage through a cascading effect (Ponti et al., 2014). Given their habitat-forming role in coralligenous assemblages, the collapse of gorgonian populations would have major consequences at the ecosystem level, with the loss of the services they provide (Gómez-Gras et al., 2021; Ponti et al., 2018).

These concerns about the decline of gorgonians are prompting the development of effective measures for their conservation and recovery. Along with conservation efforts based on increasing marine protected areas, active restoration approaches based on transplantation have been proposed as a possible way of speeding up the recovery of affected populations by assisted gene flow (Garrabou et al., 2021). The aim of transplantation is to introduce new individuals in order to increase the reproductive capacity and the adaptive potential of the target population. Transplants could be generated from colonies of deeper populations spared from heatwaves (Bramanti et al., 2023), which are also less affected by human activities such as diving or boat anchoring according to the “deep refugia” hypothesis (Bongaerts et al., 2010). However, the introduction of individuals from an external population raises the question of their tolerance to new environmental conditions, particularly if the aim is to restore areas subject to thermal anomalies or persistent anthropogenic pressures. The local adaptation of source populations may lead to differential stress responses and survival rates in transplanted colonies, thereby undermining restoration strategies. For example, populations of gorgonians from greater depths may have lower levels of thermotolerance (Pivotto et al., 2015; Torrents et al., 2008), which could compromise their ability to be transplanted to shallow areas. However, it is difficult to assess the state of health of transplants based solely on the symptoms of tissue necrosis, as these are representative of the final stage of stress-induced disease. In this respect, studying the effects of transplantation on the microbiome could provide a useful tool for detecting an early state of disease before physical signs occur (Egan and Gardiner, 2016).

Although transplantation-based restoration practices for scleractinian corals have been implemented for many years (Boström-Einarsson et al., 2020; Ferse et al., 2021), gorgonian transplantation trials have been the subject of relatively little work, mainly aimed at defining suitable methodologies and assessing the survival of transplants. Pilot experiments have been carried out on different species of Mediterranean gorgonians within shallow habitats (e.g., *P. clavata* (Linares et al., 2008b), *Eunicella cavolini* (Montseny et al., 2020), *Corallium rubrum* (Villechanoux et al., 2022)) or in the mesophotic zone (Montseny et al., 2019). Although the survival rates of the transplants are relatively high (around 30%–98% survival for the different species), it is apparent from these studies that the feasibility of future restoration actions on a larger scale will depend heavily on the longevity of the fragments attached to the substrate, in addition to the cost and logistical effort required underwater. Due to its ease of use, the “raw transplantation” with marine epoxy putty (Linares et al., 2008b) appears to be the most realistic method for implementing restoration of entire populations, yet it would clearly be desirable to consider the still high cost of these operations and to limit anthropogenic impacts on gorgonians before resorting to such a strategy. Encouraging results have recently been obtained from relatively small-scale initiatives involving

several gorgonian species (Casoli et al., 2022; Topçu et al., 2023). However, local oceanographic variations such as the intensity of water currents or irradiance level appear to influence the loss of epoxy-attached transplants (Linares et al., 2008b), but the extent of these effects is still poorly documented. Overall, more detailed investigations of the effects of relocation of gorgonians between sites or depths are needed to guide the development of future restoration operations, with the aim of gaining a better understanding of the factors affecting the adaptability and survival of transplants, including those at the microbiome level.

In this study, we investigated the fate of *P. clavata* transplants by monitoring their survival and microbiome variations in different environmental contexts. Firstly, fragments of *P. clavata* colonies were reciprocally transplanted between depths at the same site to test the feasibility of restoring shallow populations using individuals from deeper populations. Secondly, we compared the response of transplants placed in their original location or moved to another coastal site more exposed to local anthropogenic pollution.

2. Methods

2.1. Study area

The study was carried out in the Calanques National Park (Marseille, France) from October 2020 to November 2021. Two experimental sites were selected, approximately 4 km apart, where groups of transplants were installed to test their response to different environmental conditions (Fig. 1). The *Impérial du Large* site (Riou archipelago; 43.1696° N, 5.3948° E), where we transplanted 4 transplants groups (i.e., IMP20, IMP40, IMP20_40, and IMP40_20), is relatively unaffected by anthropogenic disturbances and harbours dense *P. clavata* populations (approximately 30–40 colonies per m²) across a wide depth range, from 15 m to 75 m depth. The *Podestat* site (43.2096° N, 5.3844° E), where we transplanted 2 transplants groups (i.e., POD and FRA), is located at the coast and is exposed to the anthropogenic pollution caused by the proximity of the Cortiou outfall (43.2131° N, 5.4032° E), which discharges effluents from the Marseille wastewater treatment plant. Despite successive improvements in wastewater treatment, pollution from urban effluents continues and has caused significant damage to marine ecosystems since the outfall was built in the 19th Century (Oursel et al., 2013; Savriama et al., 2015). At this site, an experimental restoration project is underway, involving the immersion of an artificial reef series designed to encourage the repopulation of this heavily affected area (REXCOR project; <https://www.seaboost.fr/>). These concrete artificial reefs were immersed in February 2018 and, at the time of our study, were already colonised by encrusting and bushy cover composed mainly of serpulid worms, algal turf, hydrozoans and bryozoans. Along with the presence of populations of the yellow gorgonian *E. cavolini* in the dim-light habitats of the *Podestat* site, a few young colonies of this species have also been observed on a number of reef modules. However, to our knowledge there have been no reports of *P. clavata* colonies on either the artificial reefs or the nearby natural substrate at *Podestat*.

We took advantage of the existence of these structures to test the response of *P. clavata* transplants attached to an artificial substrate compared with their attachment to the natural substrate at the same site of *Podestat*.

2.2. Experimental design

To determine the effect of the environment on the survival and the bacterial communities of *P. clavata* transplants, we started at the end of October 2020 (T0) a series of transplantation experiments on the two sites of *Impérial du Large* and *Podestat* (Fig. 1).

First, at the *Impérial du Large* site, unbranched apical fragments from colonies dwelling at 20 m and 40 m depth were reciprocally

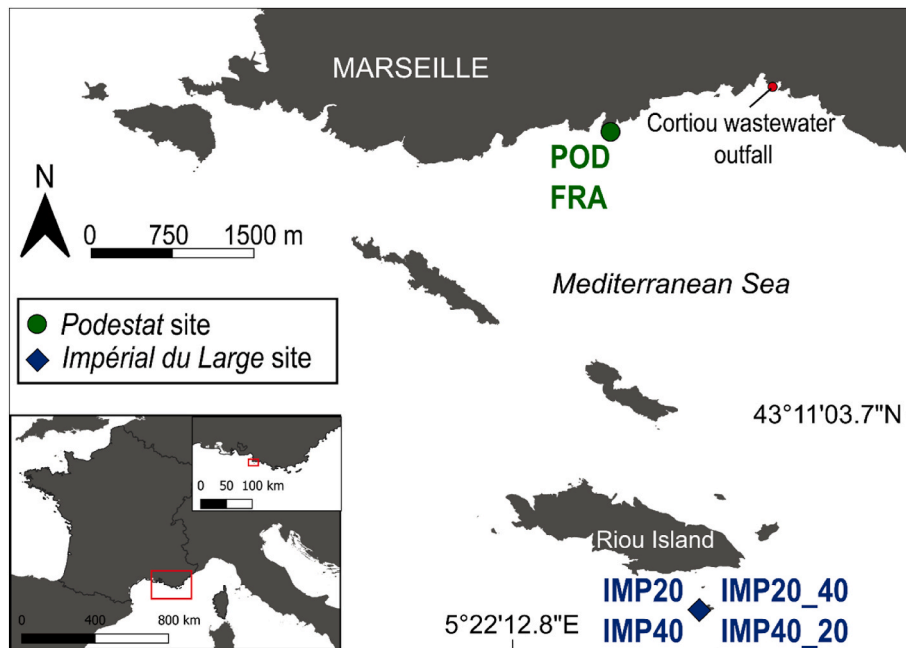


Fig. 1. Map of the transplantation sites for *Paramuricea clavata* in the area of Marseille (France). The location of the transplants groups at the *Impérial du Large* (IMP20, IMP40, IMP20_40 and IMP40_20) and *Podestat* (POD, FRA) sites is indicated. The Cortiou outfall located close to the *Podestat* site is shown. The inset maps show the geographical location of the study area in the Calanques National Park on the South-eastern coast of France (North-western Mediterranean).

transplanted between the two depths (Fig. 2). For the survival survey, a total of 240 fragments were collected from 30 colonies at each depth (4 fragments per colony). Two fragments of each colony were back-transplanted to their respective depth of origin at 20 m or 40 m as controls (IMP20 and IMP40 transplants groups, respectively), and 2 fragments were cross-transplanted from their initial depth to the different depth at 40 m or 20 m (IMP20_40 and IMP40_20 transplants groups, respectively).

For the analysis of bacterial communities, 80 fragments were

collected from 10 colonies chosen randomly among the 30 colonies at each depth (4 fragments per colony). The fragments from each colony were transplanted following the previous procedure (2 transplants attached at their depth of origin and 2 transplants relocated to the different depth). For 4 colonies at 20 m and 40 m depth, an additional fragment was sampled and stored at -20°C in RNAlater (Thermo Fisher Scientific Inc., Waltham, MA, USA) for the analysis of baseline bacterial communities, raising the total to 88 collected fragments.

Thus, each transplants group at the *Impérial du Large* site (i.e., IMP20,

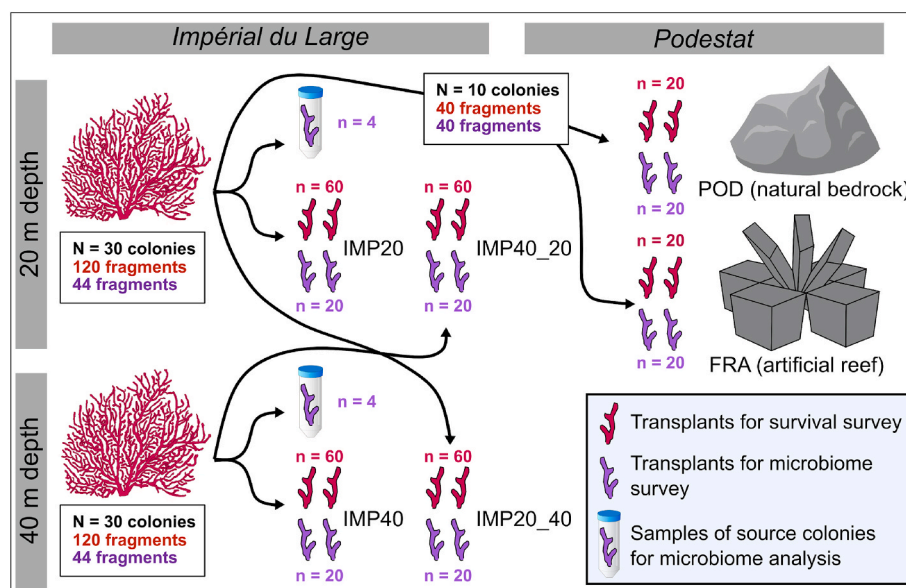


Fig. 2. Design of the transplantation experiment. The sampling design of the reciprocal transplantation of *Paramuricea clavata* colony fragments between depths (IMP20_40 and IMP40_20 transplants groups) and back-transplanted controls (IMP20 and IMP40 groups) at the *Impérial du Large* site is shown (left). At the *Podestat* site (right), colony fragments sampled at the *Impérial du Large* site were transplanted at their initial depth (20 m) onto bedrock (POD group) and an artificial reef structure (FRA group). The number of source colonies is indicated (N), as well as the number of fragments (n) dedicated to the survival (red) and microbiome (purple) studies at each site and depth. The 10 colonies used for the transplantation at *Podestat* were taken among the 30 source colonies from the *Impérial du Large* site at a depth of 20 m.

IMP40, IMP20_40, and IMP40_20) comprised 80 fragments, including 60 transplants dedicated to monitoring survival and a pool of 20 transplants from which samples were taken for microbiological survey (see paragraph 2.5 below for details on the final number of transplants processed for microbiome analysis).

On the other hand, at the *Podestat* site, we transplanted a total of 80 unbranched apical fragments collected from 10 colonies originating from the 20 m depth population at the *Impérial du Large* site (8 fragments per colony). These colonies were the same as those sampled for the reciprocal transplantation experiment between depths. Four fragments per colony were attached to the natural bedrock at 20 m depth (POD transplants group) for a total of 40 transplants. The four other transplants were attached at the same depth on the concrete structure of a “fractal” type artificial reef of the REXCOR project (FRA transplant group) for a total of 40 transplants. Among the 40 transplants settled on each substrate, 20 were dedicated to monitoring survival and 20 provided a pool of samples for the microbiological survey (see paragraph 2.5 below).

2.3. Transplantation method

Fragments of around 10 cm in size were taken from large, healthy source colonies (i.e., >50 cm high) using scissors, and collected in containers in which the fragments from each colony could be stored separately. Transplantation was then carried out using IVEGOR® two-component epoxy putty, after exposing the hard substrate with a wire brush. The fragments were then inserted into a suitably sized ball of putty and pressed against the substrate with the fingers. The divers wore latex gloves to avoid any potential transfer of contaminating microbes. During transplantation, an order of attachment from left to right and top to bottom was maintained to allow identification of transplants according to their original source colonies.

2.4. Monitoring of transplanted gorgonians

The monitoring of transplants was carried out by scuba diving over 4 time periods: 1 month (T1 - December 2020), 3 months (T2 - March 2021), 6 months (T3 - May 2021) and 1 year (T4 - November 2021) after transplantation. To estimate the survival of the transplants, the response variables measured were the presence or absence of each transplant from each group at each time point and the percentage of their surface affected by tissue necrosis. The loss of transplants due to detachment of the putty from the substrate or due to detachment of the fragments from the putty was not considered separately.

2.5. Microbiome sampling and sequencing

For microbial analyses, 4 transplants were sampled from each group IMP20, IMP40, IMP20_40, IMP40_20, POD and FRA after 6 months (T3) and 1 year (T4), resulting in a total of 48 samples. In addition, analyses were performed on 4 fragments taken in October 2020 (T0) from different colonies at 20 m and 40 m depth in the source populations of the *Impérial du Large* site, which provided 8 samples for determination of baseline microbiomes.

As a number of transplants were lost by detachment during the course of the experiment, the transplants sampled at each time point T3 and T4 were randomly selected from the survivors in the initial pool of 20 transplants per group (Fig. 2). Transplants with more than 20 % necrosis were excluded from the sample set and, in accordance with the experimental design for distribution and attachment, the transplants sampled in a group at each time point were from different source colonies.

The apical part (4–5 cm in length) of the transplants was harvested with previously sterilised scissors, and immediately preserved in RNA-later. The tubes were kept at 4 °C overnight and then moved to –20 °C for long-term storage. DNA was extracted from a small portion (0.5 cm

in length) of each fragment with the DNeasy PowerSoil Pro kit (Qiagen GmbH, Hilden, Germany) following the manufacturer’s protocol. When partial necrosis was observed on the transplants, only tissue taken from a living part was used for extraction. Extracted DNA was quantified and quality checked using a NanoDrop One spectrophotometer (Thermo Fisher Scientific).

To determine the composition of the bacterial communities, the V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 341F (5'-CCTACGGGNGGCWGCAG) and 805R (5'-GAC-TACHVGGGTATCTAATCC) (Klindworth et al., 2012) and Illumina MiSeq paired-end sequencing (2 x 300 bp length) was performed at the Génome Québec Innovation Centre (Montréal, QC, Canada) using the v3 chemistry according to the manufacturer’s protocol. Sequences have been submitted to the NCBI Sequence Read Archive (SRA) repository under the project number PRJNA1038685.

2.6. 16S rRNA gene amplicon data analysis

Sequence analysis was performed with the package “dada2” v.1.22 (Callahan et al., 2016) in RStudio v. 2021.09 (Rstudio Team, 2020). The sequences were filtered, dereplicated, denoised, and merged. Chimeric sequences were excluded and the representative sequences were then classified against the SILVA v.138 database (Quast et al., 2013) for the taxonomic assignment of amplicon sequence variants (ASVs). Rare sequences ($n < 10$ reads over all samples) and sequences assigned to mitochondria, chloroplasts and archaea were removed before subsequent analyses. A total of 2,224,744 high-quality sequences were obtained for the entire dataset generated from the 56 *P. clavata* samples. The ASV table was rarefied to 18,840 reads per sample to allow for comparisons across samples, transplant sites and time points.

Alpha diversity analyses, including the calculation of diversity indices (Chao1 richness, Shannon and inverse Simpson diversity) (Chao and Lee, 1992; Shannon, 1948; Simpson, 1949), were computed using the “phyloseq” R package (McMurdie and Holmes, 2013). In addition, barplots and heatmaps showing composition of communities were generated with the “ggplot2” package (Wickham, 2011) through the Rstudio suite. Beta diversity differences for bacterial assemblages were visualised using principal coordinate analyses (PCoA) based on Bray-Curtis dissimilarity matrix generated in “phyloseq”.

2.7. Statistical analyses

All statistical analyses were computed in R language performed in the RStudio suite v. 2021.09 (Rstudio Team, 2020). Tests were considered significant for p -values < 0.05 .

2.7.1. Attachment and necrosis of the transplants

In the survey design, the presence/absence and necrosis data for each transplant in each group and time point were considered as multivariate assemblages. To test for differences in presence/absence data between transplants groups during the 1-year study, a permutational multivariate analysis of variance (PERMANOVA) was performed. The analysis model included a single explicative factor which was the treatment applied to the transplants (see paragraph 2.2), described here under the variable “Groups” fixed with 6 levels: IMP20, IMP40, IMP20_40, IMP40_20, POD and FRA. PERMANOVA was performed with 999 permutations based on the Jaccard similarity matrix between groups, calculated from the data frame containing the presence/absence information for each transplant at each time point.

The same analysis model was used to test for differences in the occurrence and amplitude of necrosis between transplants groups. The data frame containing the percentage of necrosis for each transplant at each time point was modified by adding a dummy variable (+1) and performing a square root modification. A PERMANOVA was then carried out on the fixed factor “Groups” with 999 permutations based on Bray-Curtis dissimilarity matrix between groups, and pairwise comparisons

were performed using a Bonferroni p -value correction.

For the analysis of attachment and necrosis, the multivariate dispersion of the samples was therefore calculated using the “betadisper” function in the “vegan” package (Oksanen et al., 2018) and the homogeneity of group dispersions at each sampling time was tested using the “permutest” function with 999 permutations, followed by pairwise comparisons where applicable.

2.7.2. Alpha diversity of gorgonian microbiomes

Non-parametric tests were used to statistically evaluate the differences in alpha diversity metrics between the microbiomes, since the data did not have a normal distribution, as verified by Shapiro-Wilk tests. To test for alpha diversity differences between source colonies and transplants and between time points, we performed a Kruskal-Wallis rank sum test for each alpha diversity metric (Observed ASVs, Chao1, Shannon, and InvSimpson) and with each of the two explicative factors “Groups” (fixed with 8 levels: source colonies at 20 m, source colonies at 40 m, IMP20, IMP40, IMP20_40, IMP40_20, POD and FRA) and “Time” (fixed with 3 levels: T0, T3 and T4).

In order to explore differences in alpha diversity at each time point (T0, T3 and T4), further independent tests were performed for each alpha diversity metrics. To test for differences between the alpha diversities of the source populations at T0, we performed non-parametric Wilcoxon tests on the explicative factor “Groups”, fixed with 2 levels (source colonies at 20 m and source colonies at 40 m). To test for differences in the alpha diversity metrics between transplants groups at each time point T3 and T4, we performed independent Kruskal-Wallis rank sum tests for each metric on the explicative factor “Groups” (fixed with 6 levels: IMP20, IMP40, IMP20_40, IMP40_20, POD and FRA). When Kruskal-Wallis tests were significant, post hoc Dunn tests were carried out with Benjamini-Hochberg p -value correction for pairwise comparisons between group levels (package “FSA”; Ogle et al., 2023).

2.7.3. Bacterial community composition

To test for differences in the bacterial community composition during the study, a two-factor PERMANOVA was carried out. The analysis model included two crossed factors: “Groups”, fixed with 6 levels (IMP20, IMP40, IMP20_40, IMP40_20, POD and FRA), and “Time”, fixed with 2 levels (T3 and T4). The PERMANOVA was performed with 9,999 permutations based on Bray-Curtis dissimilarity matrix computed from

the data frame containing the relative abundance of each ASV at T3 and T4, to test for microbiome differences between transplants groups and/or time points, and for the interaction between these two factors. Pairwise comparisons were then carried out (package “pairwiseAdonis”; Martinez Arbizu, 2020) using a Benjamini-Hochberg p -value correction. The multivariate dispersion of samples was calculated with the “betadisper” function and the homogeneity of group dispersions was tested using the “permutest” function with 9,999 permutations. To determine the average contributions of ASVs to the overall average Bray-Curtis dissimilarity, a Similarity Percentage analysis (SIMPER) was performed using the “simper” function of the “vegan” package with 999 permutations.

3. Results

3.1. Survival of the *P. clavata* transplants

Overall, the number of transplants remaining attached decreased considerably over the survey time points for each treatment (Fig. 3). The attachment of transplants varied significantly between the transplants groups during survey (PERMANOVA, $F = 3.191$, $p = 0.003$). These differences were mainly due to variations in dispersion (Permutest, $F = 3.048$, $p = 0.013$) resulting from a more homogeneous response for the POD transplants group over the whole survey (Permutest, pairwise comparisons, $p < 0.05$; Supplementary Table S1). The transplants belonging to the POD group showed the best lifespan with 80 % of the transplants (16 out of 20) remaining in place at the end of the 1-year study period. The least successful transplants were those in the FRA group, with only 25 % of the transplants still in place after 1 year (5 remaining out of 20). The loss of transplants was caused either by detachment of the epoxy putty from the substrate or by detachment of the colony fragment from the putty support.

Considering only the attached transplants, the occurrence and the severity of tissue necrosis varied significantly between the transplants groups during surveys (PERMANOVA, $F = 6.890$, $p = 0.001$) (Fig. 3). These differences were only due to a difference in the barycentre since the dispersion of the data did not differ between transplants groups (Permutest, $F = 2.012$, $p = 0.092$). Necrosis rates were low during the first 6 months of the experiment (ranging from 0 % to 5.5 % for the IMP20_40 and POD transplants groups, respectively) and increased markedly after this period. Transplants transferred from 40 m to 20 m

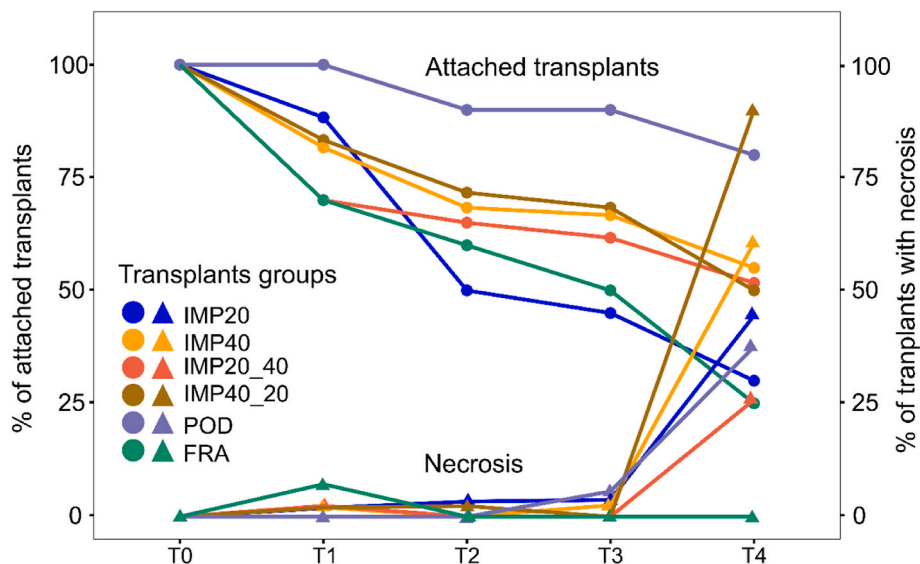


Fig. 3. Fate of transplants during the 1-year survey. The percentage of transplants remaining at each time point (T0: start of experiment; T1: 1 month; T2: 3 months; T3: 6 months; T4: 1 year) relative to the initial transplants is shown on the left y-axis (circles). The percentage of transplants affected by necrosis compared to the number of transplants remaining at each time point is shown on the right y-axis (triangles).

depth (IMP40_20 group) showed more frequent and extensive necrosis (90 % of necrosed transplants after 1 year; Table 1; Supplementary Fig. S1) than all the other transplants groups, with the exception of the IMP40 control group consisting of colony fragments back-transplanted at their initial depth of 40 m (pairwise PERMANOVA, $p < 0.05$; Supplementary Table S2). These transplants from the source population at 40 m depth (IMP40 and IMP40_20 groups) were also more likely to show total necrosis of their tissue and thus be considered dead (Table 1). In contrast, none of the still-attached transplants taken from the source population at 20 m depth and transferred to 40 m had died after 1 year of the experiment.

The health of the source colonies at the *Impérial du Large* site was also visually inspected during surveys to monitor the transplants, and none were found to show any signs of disease.

3.2. Microbial diversity of the gorgonian transplants

The alpha diversity of bacterial communities associated with each *P. clavata* sample was assessed through the measurements of Chao1, Shannon and inverse Simpson indices (Table 2). When comparing microbiomes by time points, the observed and predicted richness of communities (number of unique ASVs and Chao1 index, respectively) differed between 6 months and 1 year after transplantation (Kruskal-Wallis, post hoc Dunn test, $p < 0.001$; Supplementary Tables S3 and S4). For source colonies and transplants sampled at 6 months, there were no significant differences in alpha diversity metrics between sample groups at any time point (Supplementary Table S5). Considering samples taken after 1 year, Chao1 richness was higher in the transplants from POD and FRA groups at the *Podestat* site compared to the control fragments back-transplanted at the *Impérial du Large* source site at 20 m (IMP20 group) or 40 m depth (IMP40 group) (Dunn test, $p < 0.05$; Supplementary Table S5). Shannon's diversity index for the POD and FRA groups was also higher compared with the IMP20 control transplants, as was the inverse Simpson's index for FRA but not for POD transplants groups (Dunn test, $p < 0.05$).

The overall composition of the bacterial communities varied significantly between transplants groups and between sampling times, and two-factor PERMANOVA revealed a significant interaction between the factors "Groups" and "Time" ($F = 5.7444$, $p = 1e-04$; Supplementary Table S6). Pairwise comparisons detected significant differences between the microbiomes of all transplants groups, except between the POD and FRA groups at the *Podestat* site and the IMP40 and IMP20_40 groups at 40 m depth. In the case of the POD and FRA transplants groups compared to all other groups, an interaction between the effect of location and time was observed, indicating temporally distinct changes in the microbiomes of these two groups.

Ordination analyses showed no consistent pattern of clustering for the bacterial communities associated with the transplants after 6 months (Fig. 4A). In contrast, differences were observed for the microbiomes of the POD and FRA transplants groups at *Podestat* after 1 year of transplantation, which clustered separately from the transplants at the *Impérial du Large* site at 20 m or 40 m depth (Fig. 4B). These two groups at *Podestat* showed a more dispersed pattern of samples in the PCoA than the *Impérial du Large* groups, suggesting different levels of microbiome

Table 1

Necrosis and mortality rates of transplants after 1 year of the experiment (time point T4), expressed as a percentage of the remaining transplants for each group. Transplants affected by 100 % necrosis are counted as dead.

Transplants groups	% of necrosed transplants	% of dead transplants
IMP20	44	11
IMP40	61	21
IMP20_40	26	0
IMP40_20	90	27
POD	38	0
FRA	0	0

Table 2

Alpha diversity metrics (mean \pm SD) for *Paramuricea clavata* samples from the source colonies at 20 m or 40 m depth (T0) and the transplants groups after 6 months (T3) and 1 year (T4).

Time	Sample groups	Alpha diversity metric			
		Unique ASVs	Chao1	Shannon	InvSimpson
T0	Source colonies (20 m)	62 \pm 21	65 \pm 24	0.77 \pm 0.19	1.36 \pm 0.11
	Source colonies (40 m)	38 \pm 8	39 \pm 8	0.73 \pm 0.09	1.40 \pm 0.07
T3	IMP20	48 \pm 25	49 \pm 25	0.74 \pm 0.18	1.37 \pm 0.05
	IMP40	33 \pm 21	34 \pm 21	1.11 \pm 0.42	1.72 \pm 0.39
	IMP20_40	30 \pm 10	30 \pm 10	1.00 \pm 0.21	1.60 \pm 0.18
	IMP40_20	36 \pm 9	37 \pm 9	0.79 \pm 0.31	1.42 \pm 0.18
	POD	47 \pm 13	47 \pm 13	0.92 \pm 0.18	1.49 \pm 0.14
	FRA	58 \pm 20	60 \pm 19	1.13 \pm 0.44	1.72 \pm 0.56
T4	IMP20	47 \pm 13	49 \pm 13	0.60 \pm 0.06	1.27 \pm 0.02
	IMP40	51 \pm 20	53 \pm 19	0.78 \pm 0.06	1.38 \pm 0.02
	IMP20_40	74 \pm 17	78 \pm 17	1.06 \pm 0.21	1.55 \pm 0.11
	IMP40_20	105 \pm 90	108 \pm 87	1.34 \pm 0.88	2.07 \pm 1.01
	POD	217 \pm 87	218 \pm 85	3.31 \pm 1.85	24.73 \pm 18.15
	FRA	204 \pm 25	207 \pm 24	3.08 \pm 0.63	9.66 \pm 8.54

stability across the transplantation sites. This observation was supported by the differences in dispersion between groups in pairwise comparisons (Permutest, $p < 0.01$; Supplementary Table S7).

3.3. Bacterial community composition

Overall, the sequence dataset represented 29 bacterial phyla, with 10 phyla detected at > 1 % relative abundance in the microbiomes (Supplementary Table S8). *Proteobacteria* dominated the microbiome across all samples, although their proportion varied considerably between the transplants groups at different time points (56.9 %–99.4 % relative abundance). The other phyla present in smaller proportions but detected in all groups of samples were *Bacteroidota* (0.02 %–21.7 %), *Verruimicrobiota* (0.09 %–2.3 %), *Cyanobacteria* (0.07 %–5.7 %), and *Firmicutes* (0.02 %–3.8 %).

At a lower taxonomic level, 54 bacterial classes were identified, with 12–44 classes detected in each group of samples. In *P. clavata* source colonies from the *Impérial du Large* site, the microbiome was largely dominated by *Gammaproteobacteria* at 20 m and 40 m depth (> 98.4 % relative abundance) (Fig. 5A). Most of the *Gammaproteobacteria* belonged to the *Endozoicomonadaceae* family which represented on average > 97.2 % of the sequences identified at both depths (Fig. 5B).

Six months after transplantation, *Gammaproteobacteria* and *Endozoicomonadaceae* remained the most abundant class and family in the microbiome of transplants from each group (accounting for > 89.9 % and > 80.3 % of the sequences, respectively) (Fig. 5). Noticeably, *Alphaproteobacteria* represented 2.1 %–5.8 % of the microbiome in POD, FRA and IMP40_20 transplants groups, whereas they comprised less than 0.3 % in all other samples at that time.

After 1 year of experimentation, POD and FRA transplants showed major changes in the composition of their microbiome. The relative abundance of *Gammaproteobacteria* decreased markedly (32.9 % and 21.1 % of the retrieved sequences in POD and FRA transplants,

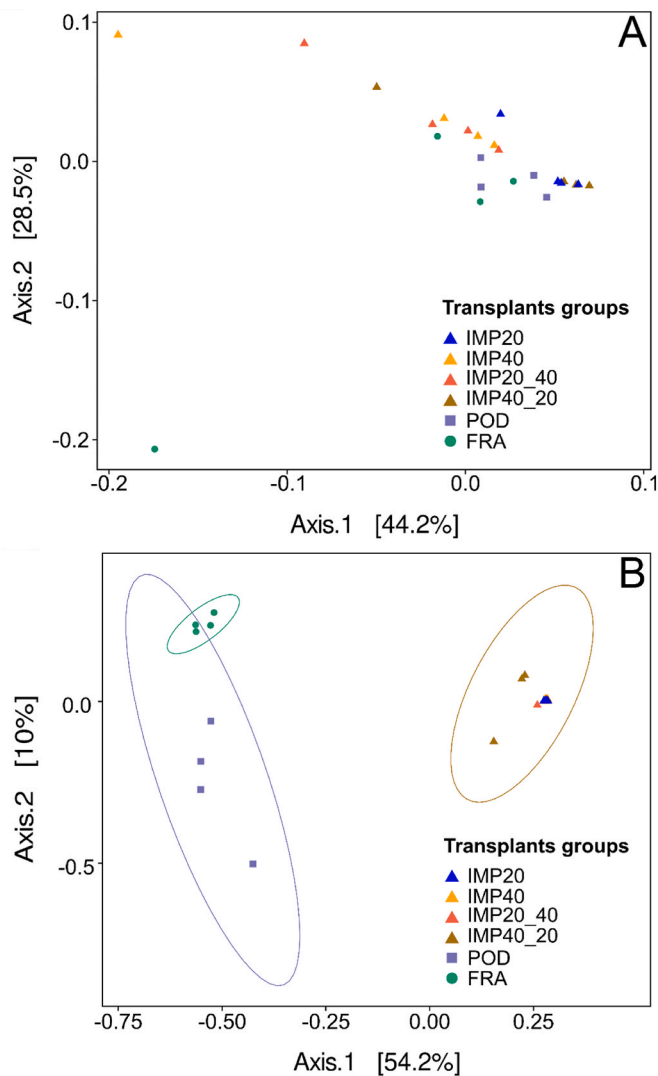


Fig. 4. Principal Coordinate Analysis (PCoA) plots based on Bray-Curtis dissimilarity of the composition of bacterial communities in the transplants groups after 6 months (A) and 1 year (B) of transplantation. The microbiomes of $n = 4$ transplants per group are shown for each time point. (A) PCoA plot showed no clear pattern of clustering. (B) The ellipses (95 % confidence intervals) represent distinct clustering of transplanted gorgonian microbiomes from the POD and FRA groups compared to the other transplants groups.

respectively) and *Alphaproteobacteria* was the most abundant class in both groups (41.8 % and 35.8 % relative abundance) (Fig. 5A). The microbiomes of POD and FRA transplants included other classes in varying proportions depending on the transplants group, the most represented being *Bacteroidia* (4.7 %–21.7 %), *Cyanobacteriia* (4.2 %–5.8 %), *Clostridia* (2.1 %–3.4 %), *Campylobacteria* (2.3 %–2.5 %) and *Planctomycetes* (1.9 %–2.0 %). *Alphaproteobacteria*, *Bacteroidia* and *Cyanobacteriia* were also detected in greater abundances in the IMP40_20 transplants (8.6 %, 3.3 % and 1.6 %, respectively) compared with the microbiomes of the other transplants from the *Impérial du Large* site (i.e., IMP20, IMP40 and IMP20_40 groups) which were constituted of >96.9 % *Gammaproteobacteria*.

At the family level, the abundance of *Endozoicomonadaceae* was drastically reduced in the microbiome of the FRA and POD transplants and accounted for only 0.5 % (FRA) to 2.1 % (POD) at 1 year (Fig. 5B). These microbiomes included greater abundance of several families of *Alphaproteobacteria*, including *Kiloniellaceae* (2.1 %–2.6 %), *Kordiimonadaceae* (1 %–7.9 %), *Rhodobacteraceae* (1.6 %–19.6 %) and *Terasakiellaceae* (1.8 %–25.6 %). Among *Gammaproteobacteria*,

Francisellaceae (1.0 %–8.6 %) and *Pseudoalteromonadaceae* (0.05 %–2.9 %) families were identified in increased proportions, and *Vibrionaceae* represented over 3 % of the community, compared with less than 0.1 % in all other transplants groups.

To identify the bacterial ASVs that contributed most to the overall difference in communities between time points, we performed a SIMPER analysis comparing the microbiomes at 6 months and 1 year (Supplementary Table S9). Among the most influential ASVs, members of the *Endozoicomonadaceae* (ASV1, ASV2, ASV4 and ASV8) were responsible for 45 % of the dissimilarity. In addition, members of *Terasakiellaceae* (ASV3), *Rhodobacteraceae* (ASV5), *Saprospiraceae* (ASV6), *Kordiimonadaceae* (ASV15) and *Cyanobiaceae* (ASV21) each accounted for 1 %–5.7 % of the dissimilarity, with an increased mean abundance after 1 year.

3.4. Diversity and distribution of the *Endozoicomonadaceae*

Within *Endozoicomonadaceae*, all the retrieved ASVs ($n = 54$) were affiliated to the genus *Endozoicomonas* except for FRA transplants which harboured an ASV (ASV261) affiliated to the genus *Kistimonas* after 1 year of experimentation. A heatmap was constructed to visualise the distribution of the *Endozoicomonadaceae* ASVs identified in all groups of transplants (Fig. 6). Transplants sampled after 6 months and colonies from source populations at 20 m and 40 m depth harboured similar assemblages of *Endozoicomonadaceae*, with ASV1 and ASV2 being the most abundant ASVs (78.2 %–90.1 % and 5.1 %–9.3 % relative abundances, respectively). A total number of 18–27 individual *Endozoicomonadaceae* ASVs were found in each of these groups of transplants.

By contrast, a different distribution pattern of *Endozoicomonadaceae* ASVs was observed after 1 year. Although there was no clearly discernible change in the ASV assemblages associated with the reciprocally transplanted fragments at 20 m and 40 m depth, transplants from the POD and FRA groups showed the loss of a majority of these ASVs (including ASV1 and ASV2) and harboured distinct *Endozoicomonadaceae* communities. The number of *Endozoicomonadaceae* ASVs was greatly reduced in POD ($n = 13$) and FRA transplants ($n = 8$) compared with the other samples (ranging between 23 and 30 ASVs). Nine and 2 ASVs were exclusively detected in POD or FRA transplants, respectively. Three ASVs (ASV174, ASV414 and ASV1390) were shared between POD and FRA transplants but absent in the other groups, and a fourth ASV (ASV142) was shared only with the IMP40_20 transplants.

4. Discussion

According to the transplantation experiment conducted in this study, the loss of *P. clavata* transplants due to technical failure could strongly affect the effectiveness of future restoration actions. Of all the transplants installed for the survival survey ($n = 280$), more than half (53 %) were lost as a result of detachment from the substrate or detachment of the colony piece from the epoxy putty. We observed significant variations in the attachment efficiency between groups of transplants at different sites and depths, which may have multiple causes linked to the characteristics of the substrate (e.g., roughness, crumbling) or local environmental conditions. Among the latter, different hydrodynamic regimes at the coast (*Podestat* site) and near the Riou archipelago (*Impérial du Large* site) could have caused a higher loss rate at the second site due to its increased exposure to currents. Our frequent scuba monitoring surveys at this site have enabled us to observe rapid water movements during easterly wind regimes, in addition to the permanent Liguro-Provençal-Catalan Current flowing eastward (Millot, 1999). Nevertheless, at the *Impérial du Large*, the two groups of transplants placed at 20 m depth showed different behaviour, with a higher percentage of loss for the IMP20 back-transplanted transplants compared to IMP40_20 transplants moved from 40 m to 20 m. Although there is no definitive explanation for this difference, it is conceivable that local variations in the level of water current exposure caused by the bedrock relief at a scale of meters or less could have influenced the performance

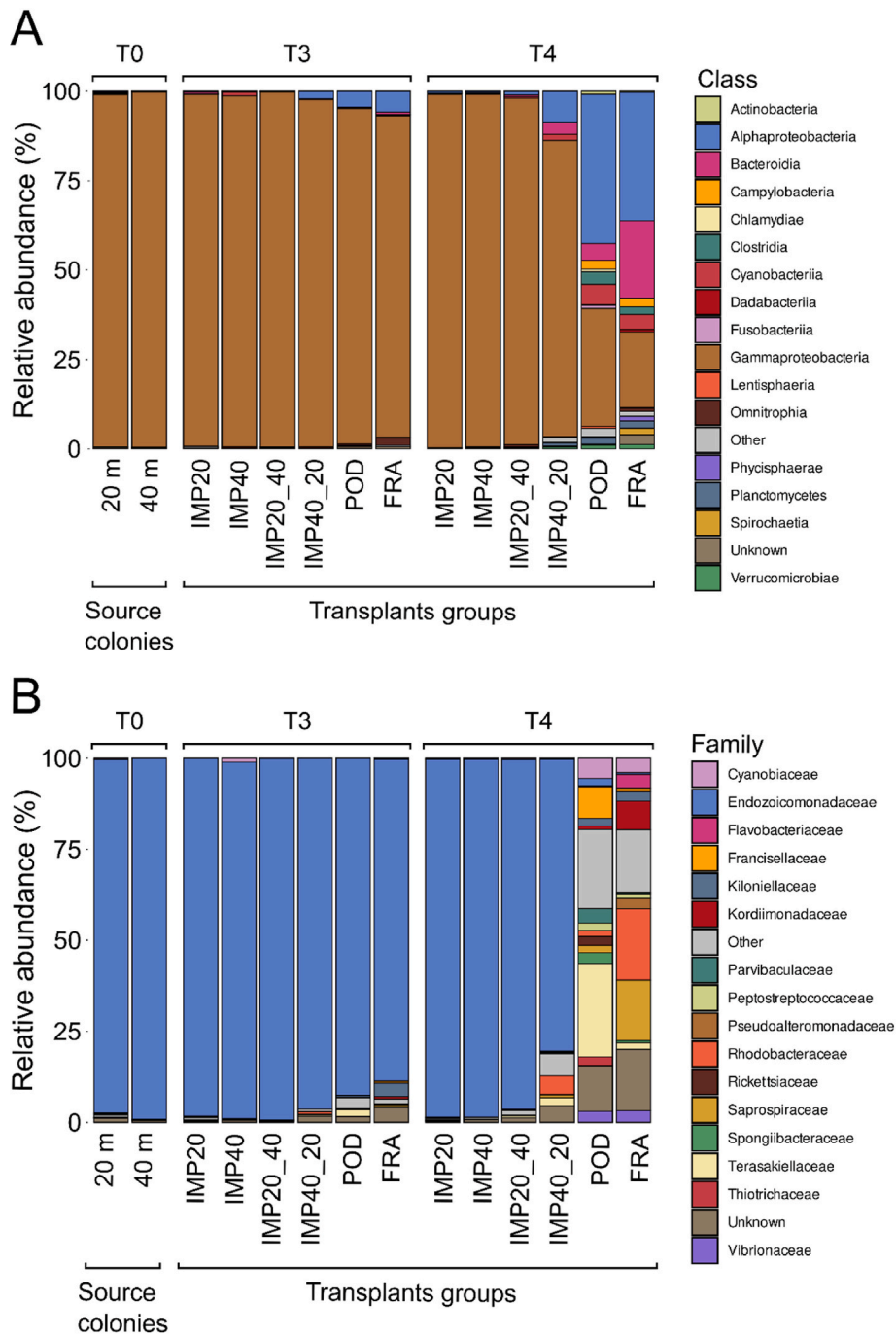


Fig. 5. Bacterial community composition (mean relative abundance %) of transplants groups across time points at class level (A) and family level (B). Each column represents data from n = 4 source colonies at T0 or n = 4 transplants after 6 months (T3) and 1 year (T4) of the experiment, accounting for 75,360 sequence reads. Taxa with a mean relative abundance of less than 0.5 % (class level) or 2 % (family level) are combined in the ‘Other’ category.

of the transplants at 20 m depth. However, with the exception of the IMP20 group, the performance of the transplants at 20 m and 40 m depth did not show any marked difference on the same site *Imperial du Large*.

At *Podestat*, 75 % of the transplants attached to the artificial reef (FRA group) were lost after 1 year, whereas only 25 % detachment from the natural substrate (POD group) was observed at the same site. The relatively smooth surface of the concrete forming the reef, although recently colonised by encrusting organisms, may explain the poorer durability of the transplants over time compared with the natural rocky substrate. As underlined by previous studies on gorgonian transplantations (Linares et al., 2008b; Villechanoux et al., 2022), improving the method for the long-lasting installation of transplants is an essential

objective in order to achieve survival rates compatible with restoration plans aiming to strengthen reproductive capacity and recruitment. Taking environmental conditions, such as local hydrodynamics, into account when selecting sites and populations for restoration will be a step towards greater effectiveness, even if it will require more systematic field studies.

In addition to the loss of transplants, poor health or disease can be another source of failure in restoration efforts by affecting the survival and reproductive potential of gorgonians. While the necrosis rates in the different transplants groups were low during the first 6 months of the experiment, the proportion of necrotic transplants increased sharply during the final survey period after the summer. A similar influence of

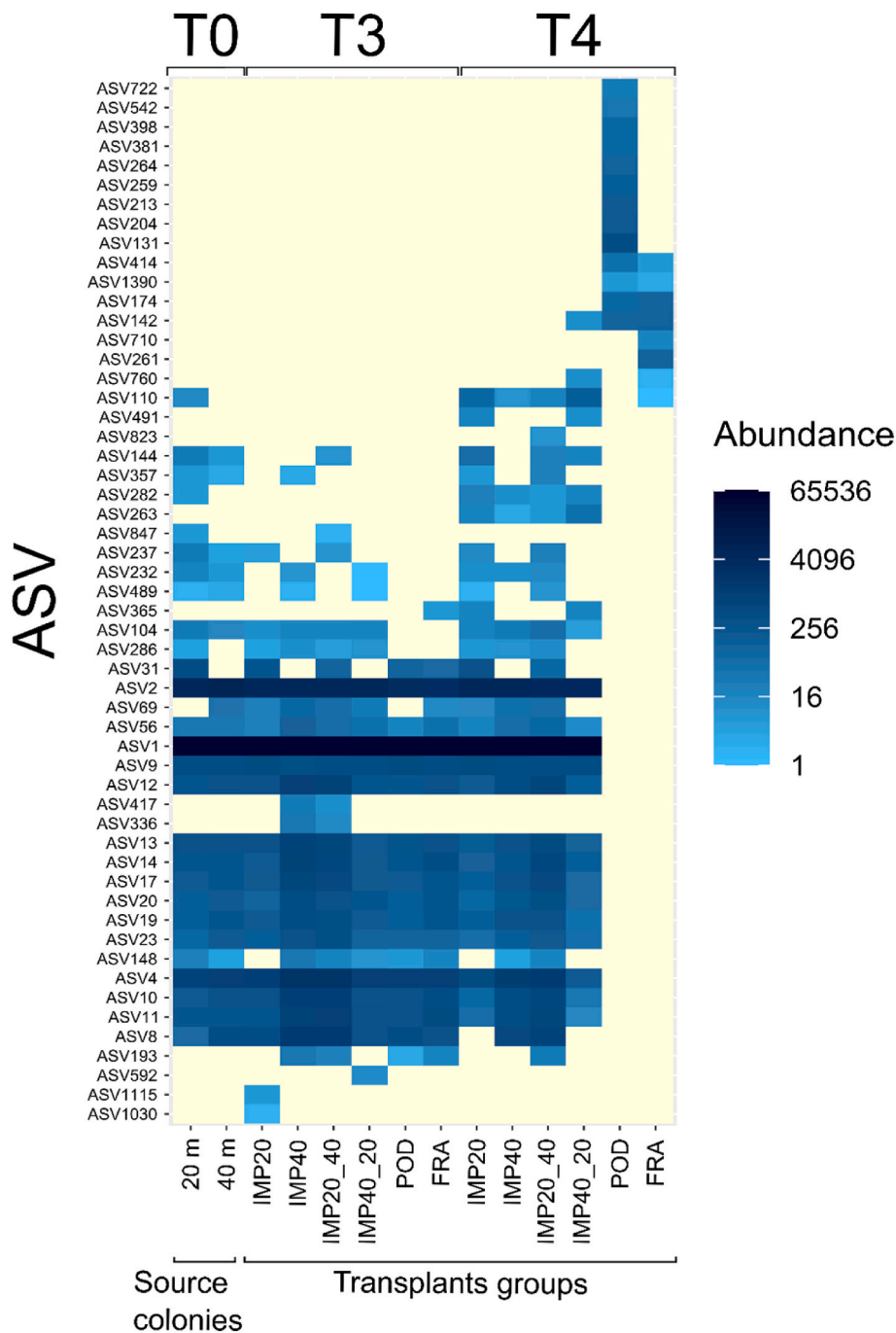


Fig. 6. Heatmap of the abundances of *Endozoicomonadaceae* ASVs in the microbiome of the source colonies and transplants groups over time. Each column contains the cumulative abundances of $n = 4$ source colonies at T0 or $n = 4$ transplants after 6 months (T3) and 1 year (T4) of the experiment, with the abundance of the total bacterial community of each group of transplants normalised to 75,360 sequence reads. All ASVs represented belong to the genus *Endozoicomonas*, except ASV261, which belongs to the genus *Kistimonas*.

the time of year on the onset of necrotic signs has already been reported in transplants of several gorgonian species, including *P. clavata* and *Eunicella* spp. (Fava et al., 2010). Consistently, a decline in the health of transplanted colonies of the Mediterranean reef-building coral *Cladocora caespitosa* has also been attributed to stress caused by high summer temperatures (Roveta et al., 2023). The increase in seawater temperature in summer may have triggered necrosis in transplants already stressed by handling, although the temperature profiles recorded during our experiment (Supplementary Fig. S2) showed that the potential threshold of thermotolerance of *P. clavata* at 25 °C was not exceeded (Crisci et al., 2017). However, other studies in controlled conditions

have indicated that longer exposure to 23 °C may be sufficient to trigger partial mortality (Coma et al., 2009), thus supporting a possible effect of temperature on the rate of necrosis in transplants. More generally, the late onset of signs of disease observed in our experiment and in other studies highlights the value of a long timeline (i.e., at least 1 year) to explore the potential ability of gorgonians to withstand pronounced changes in their environment.

Overall, only a few studies have investigated the effect of reciprocal transplantation between shallow and deep habitats on the survival of corals and gorgonians. A range of abiotic and biotic parameters, such as temperature, light, sedimentation or food availability, vary with depth

and may thereby contribute to divergent selection in the adaptive ability of depth-associated populations. Although variation in response can be observed from one species to another, survival rates were generally highest when scleractinian corals were transplanted back to the original depth rather than to a different depth (Bongaerts et al., 2011; Laverick and Rogers, 2018; Tamir et al., 2020). In addition, transplants of a coral species collected from the deeper waters showed complete mortality when transplanted to the shallower habitat, but survived at their original depth (Bongaerts et al., 2011). These contrasting survival rates of transplants suggest that local adaptation of coral populations to their environment may result in poor performance of transplants moved from one depth to another.

In the case of gorgonians, reciprocal transplantations have been carried out between colonies of the Mediterranean red coral *C. rubrum* originating from populations at 20 m and 40 m depth (Ledoux et al., 2015). These studies demonstrated local adaptation of shallow populations, possibly related to the fine-scale genetic structure of this species. However, all transplants at both depths survived without necrosis over the relatively short duration of the experiment (6 months). In *P. clavata*, a low level of genetic differentiation along depth ranges occurs at the site scale, however variation in survival of potentially depth-adapted populations has not been explored through reciprocal transplantation experiments (Pilczynska et al., 2019). In the present study, we found that transplants moved from 40 m to 20 m depth at the *Impérial du Large* site showed the highest rates of necrosis (90 % of injured transplants) and mortality (27 % of dead transplants), whereas all transplants from the 20 m depth population moved to 40 m survived after 1 year. This result indicates that local adaptation of the deeper source population of *P. clavata* may affect its survival in shallow habitats. A low capacity of deep populations to cope with depth-related environmental variation is of paramount significance for restoration, as it may challenge the potential role of deep populations as refuges to supply shallower populations most affected by anthropogenic disturbance (Bongaerts et al., 2010). Further research to determine whether the observed differential effect of depth reflects a general pattern of local adaptation in *P. clavata* populations will be key to developing future restoration strategies for this species.

While a number of studies have investigated the effect of transplantation on coral colony survival (Boström-Einarsson et al., 2020), little attention has been paid to the outcome on the associated microbial communities. Based on research on reef-building scleractinian corals, there is some evidence that transplantation can induce changes in the composition of the associated microbiome, with potential implications for the strategies that could be used for ecological restoration (Corinaldesi et al., 2023). To our knowledge, the present study is the first to investigate the effects of transplantation on the microbiome of gorgonian octocorals. Transplantation to the initial site and depth of the source population at *Impérial du Large* did not change the overall composition of the bacterial communities after 1 year. In contrast, significant changes in the microbiomes occurred after transplantation to *Podestat*, a site differing from the original site of the source population.

At the *Impérial du Large* site, *Gammaproteobacteria* affiliated to the *Endozoicomonas* genus were dominant in the microbiome of source colonies and transplants, in agreement with other studies on *P. clavata* (van de Water et al., 2017; Vezzulli et al., 2013). *Endozoicomonas* is a key symbiont of corals and gorgonians (Neave et al., 2016), although also associated with a number of other marine invertebrates such as sponges (Nishijima et al., 2013) and ascidians (Schreiber et al., 2016). Previous studies suggest a role of *Endozoicomonas* in host nutrient provision through synthesis of carbohydrates and amino acids (Ding et al., 2016; Neave et al., 2017a). They may also have a role in chemical defences by the production of antimicrobial activities (Rua et al., 2014).

Transplanted gorgonians at the *Podestat* site showed a very strong decrease in *Endozoicomonas* and an increase in overall bacterial diversity with a range of new taxa which mostly affiliated with *Alphaproteobacteria*. An increase in diversity of the microbiome has previously been

found in stressed corals (Cróquer et al., 2013; Sunagawa et al., 2009; Vega Thurber et al., 2009). The enrichment in *Alphaproteobacteria* was driven by the appearance or a higher abundance of several families which have previously been associated with diseases of marine organisms. For example, an increase in *Rhodobacteraceae* is observed in multiple scleractinian coral diseases (Pollock et al., 2017; Sunagawa et al., 2009) and in the necrotic tissues of octocorals (Keller-Costa et al., 2021), probably indicating uncontrolled growth of this opportunistic commensal in weakened hosts. Bacteria affiliated to *Terasakiellaceae* are also enriched in stressed corals (Deignan et al., 2023; Li et al., 2021). Moreover, members of *Rickettsiaceae*, a group of obligate intracellular parasites that may induce diseases in marine invertebrates (Friedman et al., 2000), were identified in the *Podestat* transplants group. In addition, *Gammaproteobacteria* families which have been associated to coral diseases, such as *Pseudoalteromonadaceae* (Sunagawa et al., 2009) and *Vibrionaceae* (Cervino et al., 2008) were also found in increased proportions in the *Podestat* transplants, as well as members of *Bacteroidia* (e.g., *Flavobacteriaceae* and *Saprospiraceae*) and *Campylobacteria* classes possibly involved in the colonisation of dying coral (Gignoux-Wolfsohn et al., 2017; Keller-Costa et al., 2021). The increased diversity was accompanied by a higher degree of beta-dispersion between community compositions, thereby indicating unstable microbiomes. The observation of large taxonomic shifts and a greater variability likely reflect a major disruption of the balance within the microbiome leading to a dysbiotic state and an impaired host health (Egan and Gardiner, 2016; Zaneveld et al., 2017).

Despite evidence of dysbiosis, the transplants collected at the *Podestat* site for the microbial analysis showed no visible signs of necrosis or disease. This suggests that the increase of opportunistic taxa could result from the loss of control of the microbiome structure rather than being a secondary consequence of the necrosis. There is some evidence for a potential role of the dominant symbiont *Endozoicomonas* in regulating the microbial communities by antagonistic effects against opportunistic taxa (Morrow et al., 2015; Neave et al., 2016; Rua et al., 2014). Since a decrease in *Endozoicomonas* has been correlated to environmental stress (McDevitt-Irwin et al., 2017; Roder et al., 2015; van de Water et al., 2018b; Ziegler et al., 2016), their decline in *Podestat* transplants driven by unfavourable conditions could be a major cause of host colonisation by disease-associated bacteria. Interestingly, the initial *Endozoicomonas* phylotypes were replaced by a less diverse assemblage of new phylotypes after 1 year. It has been found that multiple genotypes of *Endozoicomonas* are often associated with an individual host (Neave et al., 2017b), which may differ in their metabolism and biosynthetic capacities (Neave et al., 2017a). The occurrence of such a variation in *Endozoicomonas* communities from *Podestat* transplants could result from a process of “symbiont shuffling” leading to the selection of genotypes that are better adapted to changing environmental conditions (van de Water et al., 2018a), while their reduced abundance may have facilitated the establishment of foreign bacteria.

In addition to the dysbiosis observed for transplants located at *Podestat*, a less pronounced shift in microbiome composition was also apparent for transplants transferred from 40 m to 20 m depth at the *Impérial du Large* site. Notably, the taxa involved in this shift are among those affected in the microbiomes of the *Podestat* transplants, with an increased proportion of *Rhodobacteraceae*, *Terasakiellaceae* and *Saprospiraceae*, and a marked decrease in *Endozoicomonadaceae*. This suggests that similar processes are involved in the imbalance of microbiomes following a change in location or depth, but that they lead to different levels of dysbiosis. However, the extent of the changes in microbiome composition did not correlate with the disease symptoms. Transplants that have been moved from 40 m to 20 m depth showed the greatest proportion of necrosis and mortality, whereas those moved to *Podestat* were less affected and did not die. One explanation for these different responses may be linked to the nature of the stresses suffered by the two groups of transplants. Transplants from colonies at 40 m depth and relocated to 20 m depth are confronted with a change in thermal regime

which, in addition to the stress of handling and implanting transplants, may have exceeded their tolerance capacity. In support of this hypothesis, a clear difference between the temperature profiles at 20 m and 40 m depth was observed during the summer season (Supplementary Fig. S2), which may have favoured the necrosis of transplants from a deep population potentially less thermotolerant than that originating at 20 m depth (but see Crisci et al., 2017). At *Podestat*, the thermal regime is unchanged compared to the *Impérial du Large* source site at a depth of 20 m, but the proximity of the discharge from the Cortiou wastewater outfall is undoubtedly a stress factor that may contribute to the destabilisation of the microbiomes, despite few signs of necrosis after 1 year. It should be noted that a relatively high rate of necrosis (44 %–61 %) was also observed for the control groups back-transplanted to their initial location and depth at *Impérial du Large*, thus supporting the fact that transplantation itself is a source of stress in the absence of any change in the environmental context.

Taken together, our data indicate that transplantation can result in disruption of the gorgonian microbiome and colonisation by opportunistic bacteria that overtake the *Endozoicomonas* symbionts. The shift towards a dysbiotic state, however, was not necessarily associated with visual symptoms of disease and a longer survey would be useful to study the fate of transplants. Yet, regardless of the onset of disease symptoms, the increase of pathogen abundance in transplanted gorgonians constitutes a potential source of contamination that could spread to nearby colonies by direct contact or water-borne transmission (Shore and Caldwell, 2019), thereby compromising the health of surrounding populations. At a time of increasing interest in emerging conservation approaches (Anthony et al., 2017), such as assisted gene flow, which may involve the relocation of foreign individuals within natural populations, it is essential to better assess the disease risks induced by transplantation (Moriarty et al., 2020). Given the arguably critical role of the gorgonian microbiome in host fitness, understanding the factors that determine its response in a changing or degraded environment will be a key issue in anticipating the success or failure of active management projects based on transplantation.

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CRedit authorship contribution statement

Tristan Estaque: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Solène Basthard-Bogain:** Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Olivier Bianchimani:** Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Vincent Blondeaux:** Investigation. **Adrien Cheminée:** Writing – review & editing, Visualization, Methodology. **Margaux Fargetton:** Investigation. **Justine Richaume:** Investigation. **Marc Bally:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2024.106384>.

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